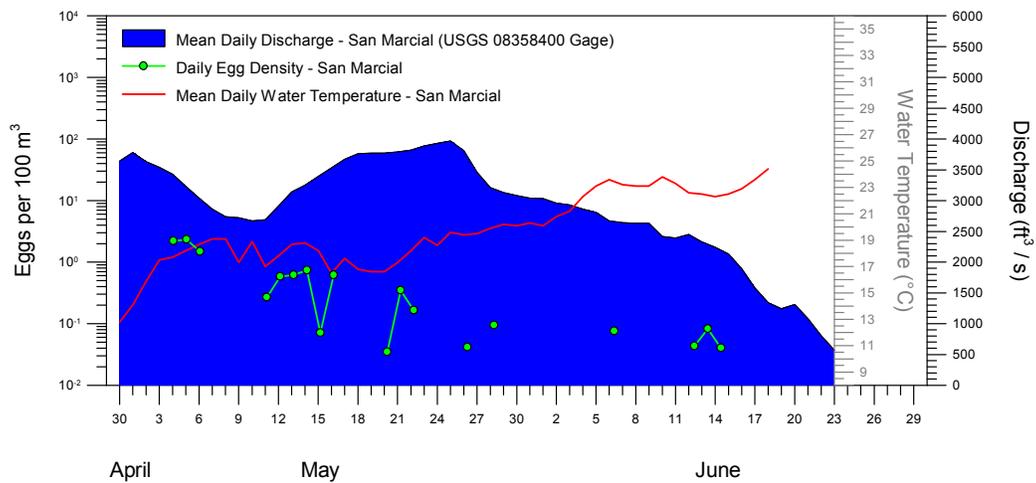
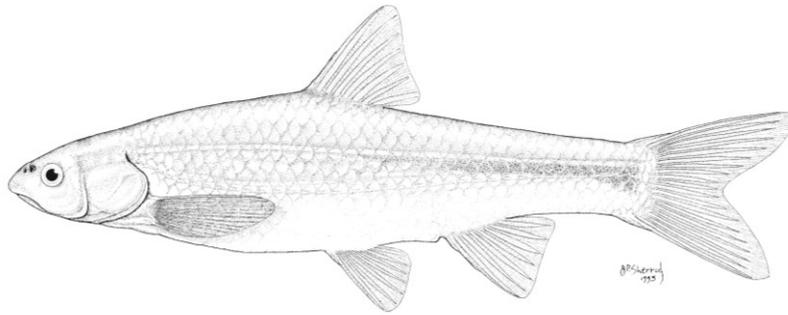


**RIO GRANDE SILVERY MINNOW REPRODUCTIVE MONITORING DURING 2017**

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



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19 October 2017

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## EXECUTIVE SUMMARY

Systematic monitoring of the reproduction of Rio Grande Silvery Minnow has been conducted annually since 2001. Previous studies demonstrated mid-April to mid-June as the primary period of spawning activity. The 2017 study was a continuation of the long-term monitoring effort in the lower portion of the San Acacia Reach, just upstream of Elephant Butte Reservoir. Two additional sites (one in the Angostura Reach and one in the Isleta Reach), which had been sampled periodically from 2006 to 2011, were also sampled intensively in 2017.

Rio Grande Silvery Minnow mixture-model estimates ( $E(x)$ ), using standardized egg passage rate data ( $E_p = \text{eggs} / \text{s}$ ) from 2003 to 2017, were highest in 2011 ( $6.05 \times 10^1$ ) and lowest in 2004 ( $1.36 \times 10^{-3}$ ). Values of  $E_p$  are indicative of the relative downstream transport of eggs across years, corrected for annual differences in flow magnitude. There was a steady decline in estimated egg passage rates from 2011 to 2013, followed by an increase in 2014. Egg passage rates declined ( $P < 0.05$ ) from 2015 ( $7.75 \times 10^{-1}$ ) to 2016 ( $6.12 \times 10^{-2}$ ), but increased slightly in 2017.

Ecological models revealed that changes in the density and occurrence of Rio Grande Silvery Minnow eggs were reliably predicted by seasonal differences in river flows over time (2003–2017). Out of 129 models considered, we found that high flows during spring were crucial (i.e., > 70% of model weight) in explaining why some years had substantially lower egg passage rates (i.e. reduced downstream transport of eggs) than others. In summary, we found that low egg passage rates were most common during years with elevated and extended spring flows, whereas high egg passage rates occurred most frequently during years with lower and more abbreviated peak spring flows.

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 ( $X^2 = 28.44$  and  $P < 0.001$ ). The probability of collecting eggs was predicted to increase rapidly up to about 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 and during a 200% increase was 0.97.

Rio Grande Silvery Minnow egg presence-absence data also revealed associations with water temperatures, though not as robust as the discharge relationships, during the study period ( $X^2 = 11.98$  and  $P < 0.001$ ). The probability of collecting eggs ranged from 0.64 (temperature = 14°C) to 0.27 (temperature = 26°C). The trend in the probability of collecting eggs showed a steady decrease as a function of elevated water temperatures.

Sampling was reinitiated at the Albuquerque and Sevilleta sites in 2017, which allowed for historical comparisons of longitudinal egg passage rates from 2006 to 2017. The annual trends in egg passage rates for all three sites were relatively similar over time. Overall, the estimated egg passage rates at Sevilleta and San Marcial were consistently higher than at Albuquerque. The mixture-model was used to estimate and compare longitudinal egg passage rates in 2017 at the Albuquerque (0.06), Sevilleta (0.50), and San Marcial (0.27) sampling sites. While the 2017 egg passage rates at Sevilleta were significantly higher ( $P < 0.05$ ) than at Albuquerque, there were no significant differences between the Albuquerque and San Marcial estimates or the Sevilleta and San Marcial estimates.

Despite the seemingly large number of Rio Grande Silvery Minnow eggs transported downstream every year, some portion remains upstream. 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. 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. As successful growth and survival of Rio Grande Silvery Minnow, from the egg through the early larval stages, requires about one month, the persistence of these nursery habitats is essential during this crucial developmental phase. The future conservation status of Rio Grande Silvery Minnow appears strongly dependent on reliably ensuring appropriate seasonal flow and habitat conditions to support the crucial spawning and early recruitment phases of this imperiled species.

## INTRODUCTION

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 this area (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 Denis, 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 for 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 would have been characterized as a dynamic semiarid river ecosystem.

The reduced species diversity typical of semiarid 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 from large portions of their range (Speckled Chub, *Macrhybopsis aestivalis* and Rio Grande Shiner, *Notropis jemezanus*) or have become extinct (Phantom Shiner, *Notropis orca* and Rio Grande Bluntnose Shiner, *Notropis simus simus*) 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 key life-history characteristics. All were small (generally < 90 mm SL) and 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 pelagic-spawning fishes (Platania and Altenbach, 1998). These fishes spawn non-adhesive eggs that swell rapidly with water and become nearly neutrally buoyant. Spawning is generally associated with increases in discharge, such as spring runoff or summer rainstorms. The eggs expand from about 1.6 mm to 3.0 mm in diameter shortly after spawning and are passively transported by water currents, to some extent, during development. Egg hatching time is temperature dependent; it usually occurs in 24 to 48 hours between 25°C and 30°C and within 72 hours at 20°C (Platania, 2000). Recently hatched larval fish may be subject to additional passive transport for several days (ca. 3–5 days) until development of the gas bladder.

The time 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 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. However, two large dams (Cochiti and Elephant Butte), along with three smaller dams (Angostura, Isleta, and San Acacia), now prevent the upstream movement of fishes and fragment the once continuous range of Rio Grande Silvery Minnow.

Systematic monitoring of the reproduction 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 preliminary, yet extensive, monitoring effort involved quantifying the occurrence and passage of eggs from nine sites; spawning was documented from late March to late June of 1999. Limited 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.

A long-term sampling effort was initiated in 2001 to document spawning by Rio Grande Silvery Minnow in the San Acacia Reach, near the downstream terminus of its range (Platania and Dudley, 2002). Monitoring also occurred at this site in 2002 (Platania and Dudley, 2003), 2003 (Platania and Dudley, 2004), and 2004 (Platania and Dudley, 2005). Additional monitoring efforts were conducted from 2006 to 2008 (Platania and Dudley, 2006, 2007, 2008) and resulted in the intensive sampling of the Angostura, Isleta, and San Acacia reaches of the Middle Rio Grande. Recent monitoring efforts (2009–2016) occurred in all three reaches but at a reduced intensity (e.g., fewer days and shorter sampling times). More intensive reproductive monitoring in the Angostura, Isleta, and San Acacia reaches was reinitiated in 2017.

The primary objectives of this study were to characterize the timing, duration, and magnitude of spawning by Rio Grande Silvery Minnow in the Angostura, Isleta, and San Acacia reaches of the Middle Rio Grande. Additional objectives included assessing differences in Rio Grande Silvery Minnow egg passage rates across years; examining the relationships between flow, temperature, and spawning; and characterizing spatial spawning patterns in the Angostura, Isleta, and San Acacia reaches. This long-term monitoring study provides insight into the key environmental factors affecting trends in the temporal and spatial spawning patterns of Rio Grande Silvery Minnow and can assist managers in developing successful strategies for its recovery.

## STUDY AREA

The principal area of interest for this study is the reach between the outflow of Cochiti Reservoir and inflow to Elephant Butte Reservoir; this area encompasses the range of Rio Grande Silvery Minnow in the Middle Rio Grande (Figure 1). Several large dams and numerous irrigation diversion dams regulate flow in the Middle Rio Grande. Cochiti Dam has been operational since 1973 and is the primary flood control structure that regulates flows in the Middle Rio Grande. Reach names were taken from the diversion structure at the upstream boundary of each fragmented river reach. There was one sampling site in the Angostura Reach (Angostura Diversion Dam to Isleta Diversion Dam), one site in the Isleta Reach (Isleta Diversion Dam to San Acacia Diversion Dam), and one site in the San Acacia Reach (San Acacia Diversion Dam to the head of Elephant Butte Reservoir).

The reproductive effort of Rio Grande Silvery Minnow has been periodically monitored at a wide variety of collecting localities in the Middle Rio Grande from 1996 to 2017. However, consistent and long-term sampling efforts (2001–2017) have only been conducted in the downstream-most portion of the San Acacia Reach. The San Acacia Reach of the Middle Rio Grande is about 64 miles (102 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/silt substrata, 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 reach of the Rio Grande downstream of San Marcial Railroad bridge crossing is confined to a channel that is frequently less than 50 m wide. Braiding of the channel is uncommon except under conditions of relatively low flow.

Given the downstream drift of eggs, long-term collecting activities have consistently been conducted near the terminus of the San Acacia Reach (San Marcial [UTM: 305552 E; 3711984 N; NAD83]), just upstream of Elephant Butte Reservoir, to maximize the number of eggs collected and to inform local egg rescue efforts. This site was downstream of a U.S. Geological Survey stream gaging station located near San Marcial, New Mexico (# 08358400). In addition to easy accessibility and favorable river conditions (e.g., current being carried through a single river channel, gently sloped banks, and moderate gradient), the only means of vehicle access to this site was gated. This area has been sampled annually from 2001 to 2004 and from 2006 to 2017.

Two additional sites were sampled in 2017, which had been sampled periodically in the past (2006–2011). These sampling sites were located in the downstream portions of the Angostura and Isleta reaches. In the Angostura Reach, the sampling site (Albuquerque [UTM: 346277 E; 3874723 N; NAD83]) was located in the same area that was consistently sampled from 2006 to 2008. In the Isleta Reach, the sampling site (Sevilleta [UTM: 330304 E; 3796524 N; NAD83]) was located in the same area that was consistently sampled from 2006 to 2011. These additional sampling sites in the Angostura and Isleta reaches not only allowed for a more detailed assessment of spatial spawning patterns but also enabled direct comparisons across monitoring sites over time.

Diel and seasonal discharge varied greatly during 2016 and 2017, especially in southern reaches of the Middle Rio Grande (Figure 2). There was a general trend of lower flow at downstream locations (e.g., U.S. Geological Survey (USGS) San Acacia Gage [#08354900] and USGS San Marcial Gage [#08358400]) compared to upstream locations (e.g., USGS Albuquerque Gage [#08330000]). During May and June 2017, flows increased to unusually elevated levels that persisted for many weeks throughout the study area. Peak flows in 2017 occurred in late May. Flow conditions in 2016 and 2017 included periods of very low discharge after June, particularly in the southern reaches. As compared with the generalized historical spring runoff (based on mean daily discharge values from 1973 [Cochiti Dam operational] to 2016), the timing of this event was relatively typical in 2016 and 2017. While the spring flow magnitude was modest and truncated in 2016, it was elevated and extended in 2017.

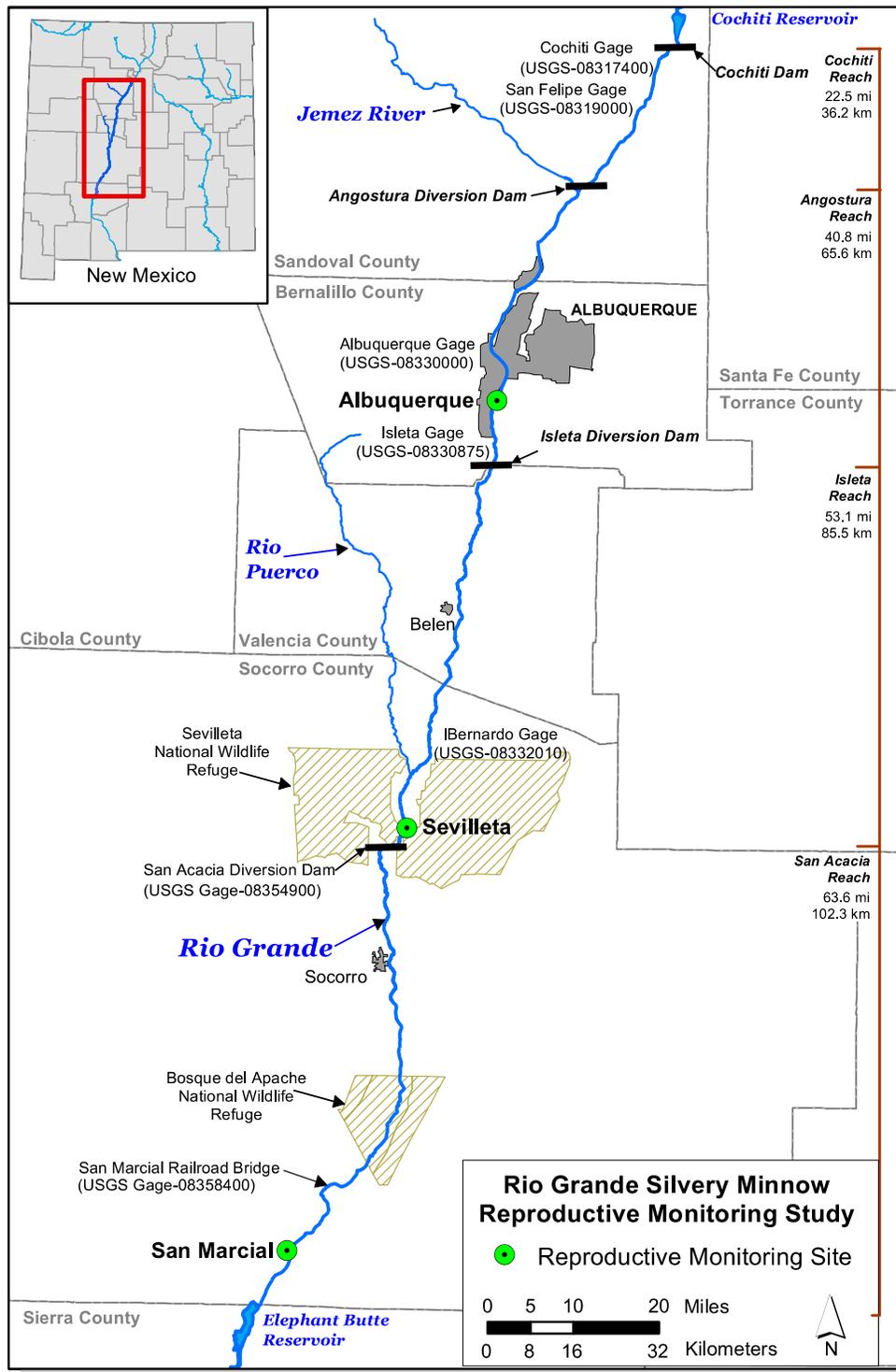


Figure 1. Map of the Middle Rio Grande, New Mexico, and the Rio Grande Silvery Minnow reproductive monitoring sites.

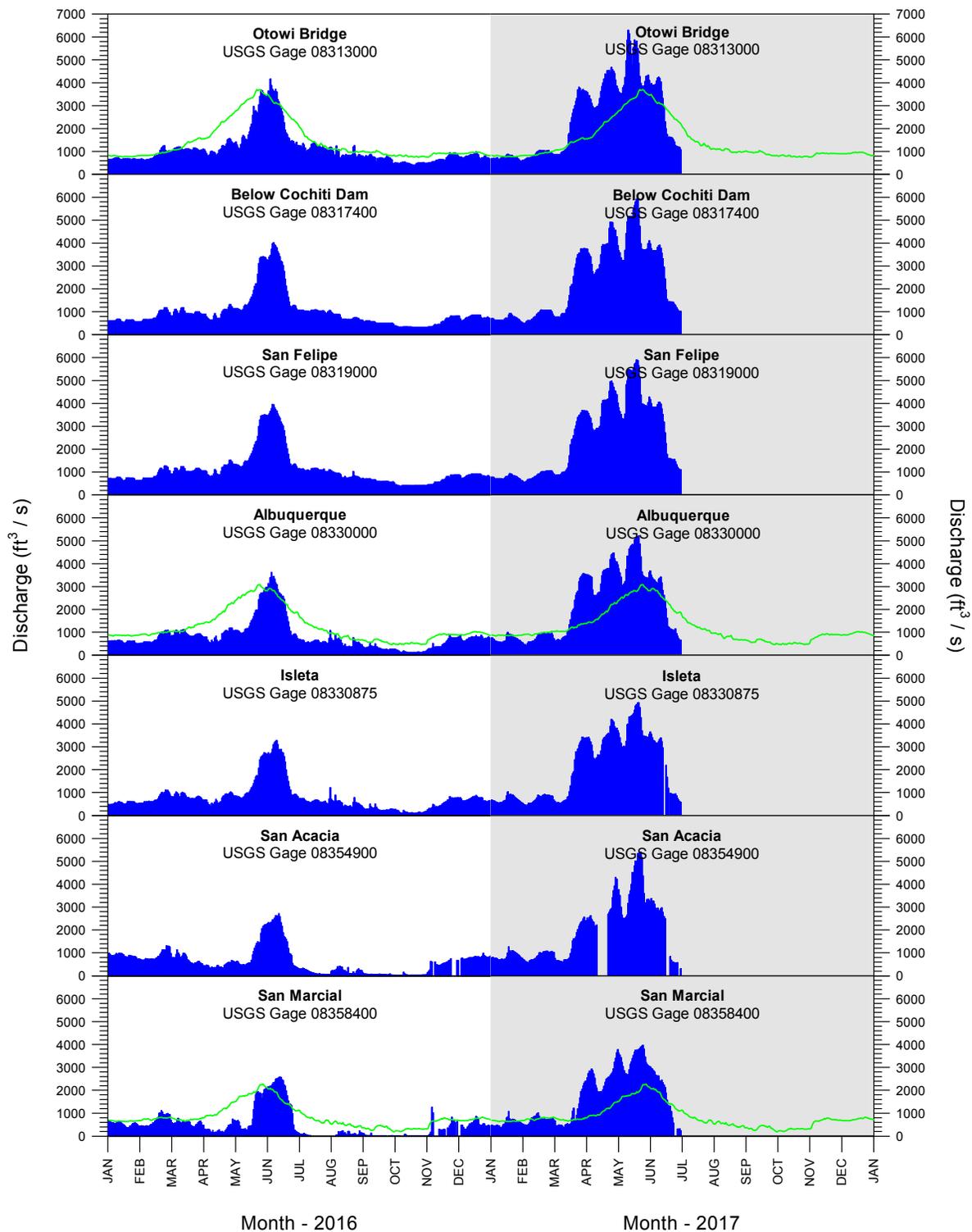


Figure 2. Rio Grande discharge from January 2016 through June 2017 at U.S. Geological Survey (USGS) gaging stations. Green lines are historical mean daily discharge values (from 1973 [Cochiti Dam operational] to 2016). Discharge data are provisional and subject to change.

## MATERIALS AND METHODS

Temperature-logging devices (Onset [Hobo TidbiT v2]) were deployed at each study site to record hourly water temperatures. These data loggers have a high level of accuracy ( $\pm 0.2^\circ\text{C}$ ), from  $0^\circ\text{C}$  to  $50^\circ\text{C}$ , and their stability (drift) is about  $0.1^\circ\text{C}$  per year (Onset Computer Corporation, 2017). We limited their use to five years. If data loggers became buried in the substrata 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 the monitoring sites were presented graphically for comparative purposes.

The egg-collecting device, developed specifically for the collection of large numbers of live and undamaged semibuoyant fish eggs (Moore Egg Collector; MEC [Altenbach et al., 2000]), was the only sampling apparatus used in this project. We determined the volume of water sampled by using a mechanical flow meter, which was attached to the MEC. The total number of eggs collected ( $n$ ), relative to the total volume of water sampled ( $V$ ;  $\text{m}^3$ ), was used to calculate the density of drifting eggs ( $D$ ; eggs per  $100 \text{ m}^3$ ), using the formula:  $D = ((n / V) \cdot 100)$ .

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), which have resulted in increased efficiency of the original MEC (i.e., greater volume of water sampled). 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.

When the number of eggs collected during any 15-minute period was too numerous to count in the field, those samples were preserved in 95% ethanol, labeled with the appropriate field number, and accessioned into the Division of Fishes (Museum of Southwestern Biology, UNM). Eggs were not staged (i.e., determining approximate time from spawning), as this would require substantial laboratory work outside of the current objectives of this study. However, all egg samples were sorted and enumerated in the laboratory after the field portion of the project, and data were entered into electronic spreadsheets.

Rio Grande Silvery Minnow egg density values are dependent on flow conditions, thereby precluding unadjusted comparisons 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  $E_p$  (eggs / s) are indicative of the relative passage rate of eggs across years, corrected for inter-annual differences in flow magnitude.

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 rigor of the data acquired from this 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 less suitable for computing a valid estimate of  $E(x)$  (described below). These issues precluded the use of data from 2001 or 2002 for quantitative or statistical comparison with data from subsequent years.

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 (2003–2017) were analyzed using PROC NLMIXED (SAS, 2017), 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 commonly sampled period (1 May to 10 June from 2003 to 2017), 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 ( $\delta$  = probability of egg occurrence,  $\mu$  = mean of the lognormal  $E_p$  distribution,  $\sigma$  = standard deviation of the lognormal  $E_p$  distribution, and  $E(x)$  = estimate of  $E_p$ ). The number of eggs passing a sampling site, during an average 30-day period ( $E(x)_{30d}$ ), was estimated with  $E_p$  data by using the formula:  $E(x)_{30d} = E(x) \cdot 86,400 \text{ s} \cdot 30 \text{ d}$ .

General linear models were used to incorporate covariates to model  $\delta$ ,  $\mu$ , and  $\sigma$  where a logit link was used for  $\delta$  and log links were used for  $\mu$  and  $\sigma$ . 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 hydrological variables at USGS Gage #08358400 (SAN; Rio Grande Floodway at San Marcial, NM). Maximum discharge (SANmax), mean discharge (SANmean), and days exceeding threshold discharge values in 1,000 cfs increments (days > 500 [SAN>500], 1,500 [SAN>1,500], and 2,500 [SAN>2,500] cubic feet per second, cfs) were covariates that represented different spring runoff conditions (1 May to 10 June). Two drought years (2006 and 2013) were excluded from this analysis, as flows never exceeded 100 cfs; more suitable spawning flows occurred only after the termination of sampling. 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  $\delta$ , there is no over-dispersion or extra-binomial variation, and for  $\mu$ , no extra variation provided beyond the constant  $\sigma$  model. Random effects models ( $R$ ) were also considered for  $\delta$  and  $\mu$  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. Random effects were integrated out of the likelihood (Pinheiro and Bates, 1995) during fitting of the model.

Goodness-of-fit statistics (logLike =  $-2[\log\text{-likelihood}]$  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 across all sampling years. 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 ( $\delta$  or  $\mu$ ), 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 was used to determine how the probability of collecting eggs, based on presence-absence data, changed as a function of different river flows or water temperatures. The percent change in mean daily discharge from two days to one day prior to egg collection, using long-term sampling data (2003–2017), was used in the first analysis. This duration was chosen to allow time for the discharge changes occurring at the San Marcial gage to reach the San Marcial site. This metric best represented the approximate change in mean daily discharge that occurred just prior to spawning. Similarly, the long-term sampling data (2003–2017) were used in a second analysis to assess how the probability of collecting eggs changed as a function of mean daily water temperature during the sampling period. For both analyses, the associated 95% confidence intervals of the modeled regression lines were constructed using inverse predictions (SAS, 2007) of discharge or temperature across the range of modeled egg collection probabilities.

## RESULTS

### *Temporal Spawning Patterns (2001–2017)*

Despite substantial inter-annual differences in Rio Grande Silvery Minnow spawning metrics at San Marcial (Table 1), there were many similarities regarding intra-annual patterns in reproduction over time (2001–2017; Figures 3–8). While the timing, duration, magnitude, and frequency of spawning varied somewhat across years, the highest numbers of eggs were typically collected during a relatively short period in May. During 2017, mean daily water temperature at San Marcial fluctuated from about 14°C to 20°C in May but consistently remained above 20°C during June.

Mean daily water temperatures during the initial and peak spawning events were relatively similar across 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. Mean daily water temperatures ranged between 16.5°C and 23.6°C during days when eggs were collected at San Marcial in 2017.

Rio Grande Silvery Minnow mixture-model estimates ( $E(x)$ ), using standardized egg passage rate data ( $E_p$ ) from 2003 to 2017, revealed notable differences across sampling years (Figure 9). Standardized egg passage rates were highest in 2011 ( $6.05 \times 10^1$ ) and lowest in 2004 ( $1.36 \times 10^{-3}$ ). There was a steady decline from 2011 to 2013, followed by an increase in 2014. Egg passage rates declined ( $P < 0.05$ ) from 2015 ( $7.75 \times 10^{-1}$ ) to 2016 ( $6.12 \times 10^{-2}$ ), but increased slightly in 2017. Simple estimates of mean egg passage rates, using the method of moments, were similar to mixture-model estimates.

General linear models of Rio Grande Silvery Minnow mixture-model estimates revealed that variation in the mean of the lognormal distribution of  $E_p$  ( $\mu$ ) and the probability of occurrence ( $\delta$ ) was reliably predicted by changes in hydrological variables over time (2003–2017). The top model ( $\delta(\text{Year}) \mu(\text{SANmax}+R)$ ) received about 73% of the  $AIC_c$  weight ( $w_i$ ) and had an  $AIC_c$  value of 1,137.60 (Table 2). The next three models, which accounted for about 25% of the cumulative  $w_i$ , were related to the interaction among  $\delta$ ,  $\mu$ , and hydrological variables representing elevated spring flows (e.g., SANmean). The global model ( $\delta(\text{Year}) \mu(\text{Year})$ ) received < 2% of  $w_i$ , and all remaining model combinations (124 out of 129) each received < 0.2% of  $w_i$ . In summary, we found that low egg passage rates were most common during years with elevated and extended spring flows, whereas high egg passage rates occurred most frequently during years with lower and more abbreviated peak spring flows.

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 ( $X^2 = 28.44$  and  $P < 0.001$ ; Figure 10). Flows used to calculate the percent change in discharge ( $\Delta$ ) ranged from < 50 cfs to > 3,500 cfs. The probability of collecting eggs ranged from 0.22 ( $\Delta$  discharge = - 50%) to 0.42 ( $\Delta$  discharge = 0%) during periods of declining or stable flows, respectively. The probability of collecting eggs was predicted to increase rapidly up to about 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 and during a 200% increase was 0.97.

Rio Grande Silvery Minnow egg presence-absence data also revealed associations with water temperatures, though not as robust as the discharge relationships, during the study period ( $X^2 = 11.98$  and  $P < 0.001$ ; Figure 11). The probability of collecting eggs ranged from 0.64 (temperature = 14°C) to 0.27 (temperature = 26°C). There was less certainty in predicted values (i.e., broader confidence intervals) at the coolest and warmest water temperatures. However, the trend in the probability of collecting eggs showed a steady decrease as a function of elevated water temperatures.

Table 1. Rio Grande Silvery Minnow spawning summary data by year, from 1 May to 10 June, at San Marcial.

Year <sup>1</sup>	Sampling Effort (days)	Eggs Present (days)	Eggs Absent (days)	Occurrence <sup>2</sup> (% freq.)	Abundance (eggs)
2001	38	16	22	42.1	89,542
2002	41	6	35	14.6	150,327
2003	41	18	23	43.9	13,292
2004	41	3	38	7.3	5
2006	41	10	31	24.4	6,039
2007	41	39	2	95.1	10,995
2008	41	3	38	7.3	155
2009	41	9	32	22.0	346
2010	38	15	23	39.5	364
2011	41	36	5	87.8	95,421
2012	41	18	23	43.9	12,398
2013	41	13	28	31.7	1,745
2014	41	24	17	58.5	9,726
2015	39	30	9	76.9	6,356
2016	41	13	28	31.7	175
2017	38	15	23	39.5	125

<sup>1</sup> = Reproductive monitoring was not conducted in 2005.

<sup>2</sup> = Values based on the percentage of days when eggs were present relative to the sampling effort (days).

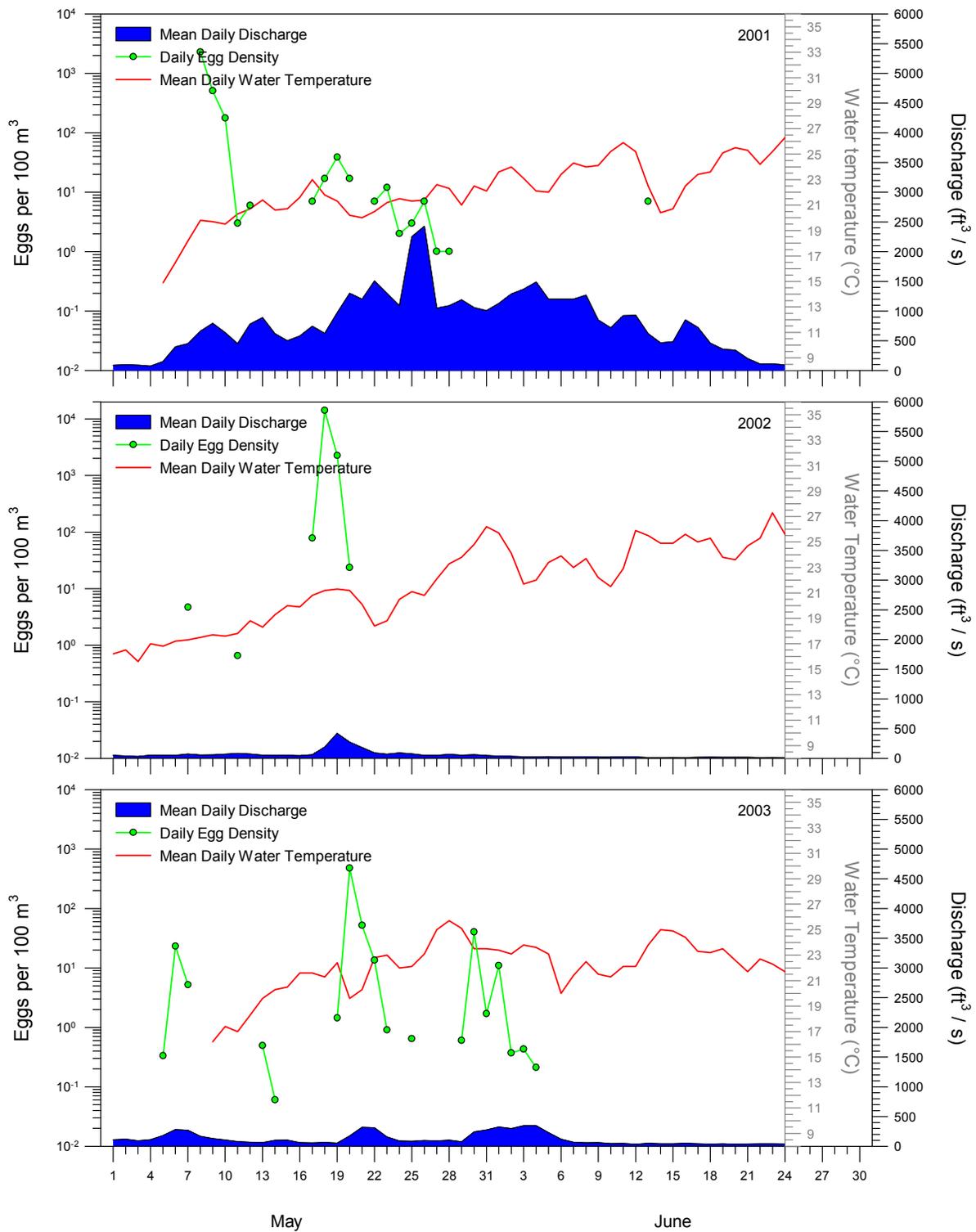


Figure 3. Mean daily discharge, daily egg density, and mean daily water temperature during the 2001–2003 Rio Grande Silvery Minnow reproductive monitoring study periods at San Marcial.

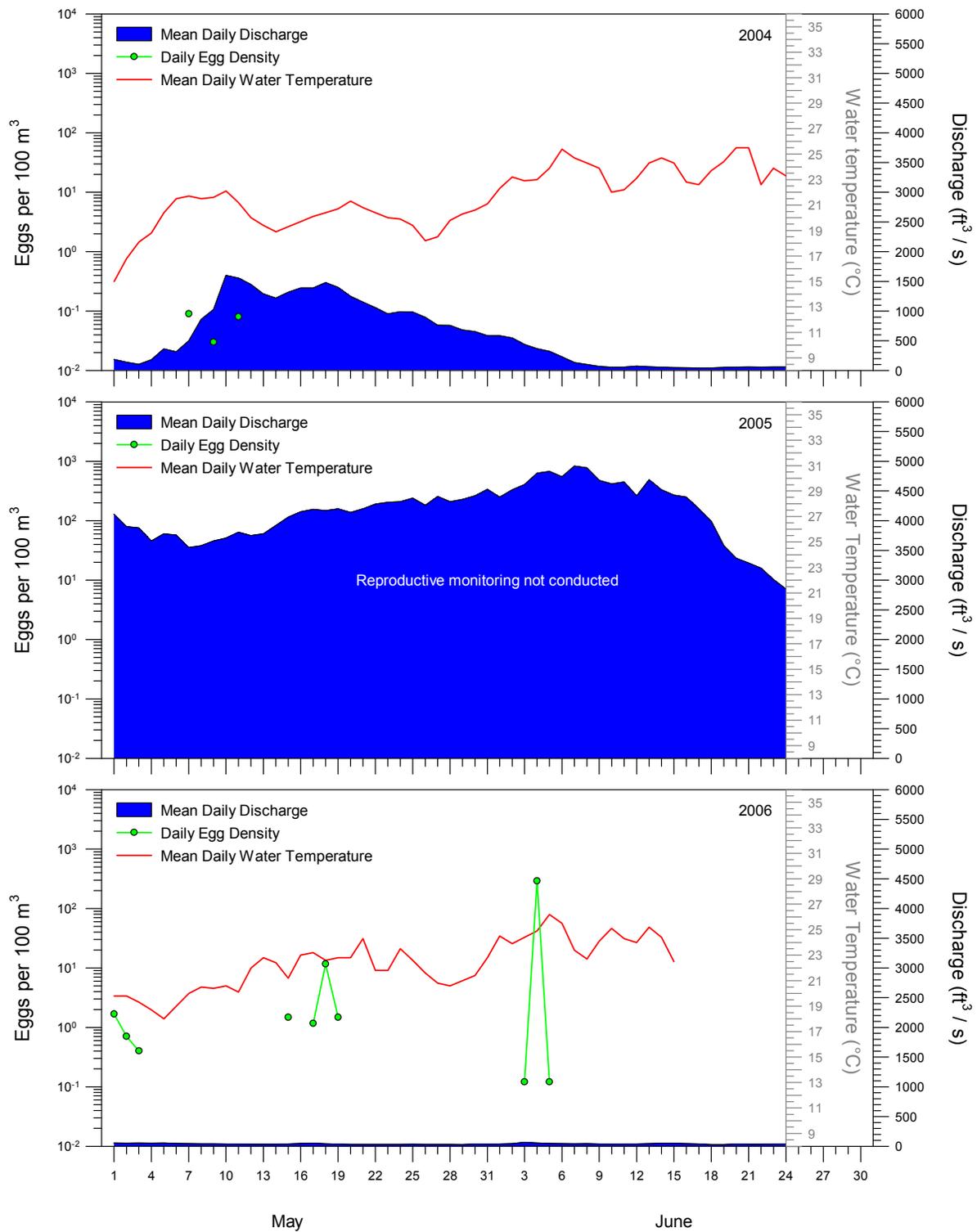


Figure 4. Mean daily discharge, daily egg density, and mean daily water temperature during the 2004–2006 Rio Grande Silvery Minnow reproductive monitoring study periods at San Marcial.

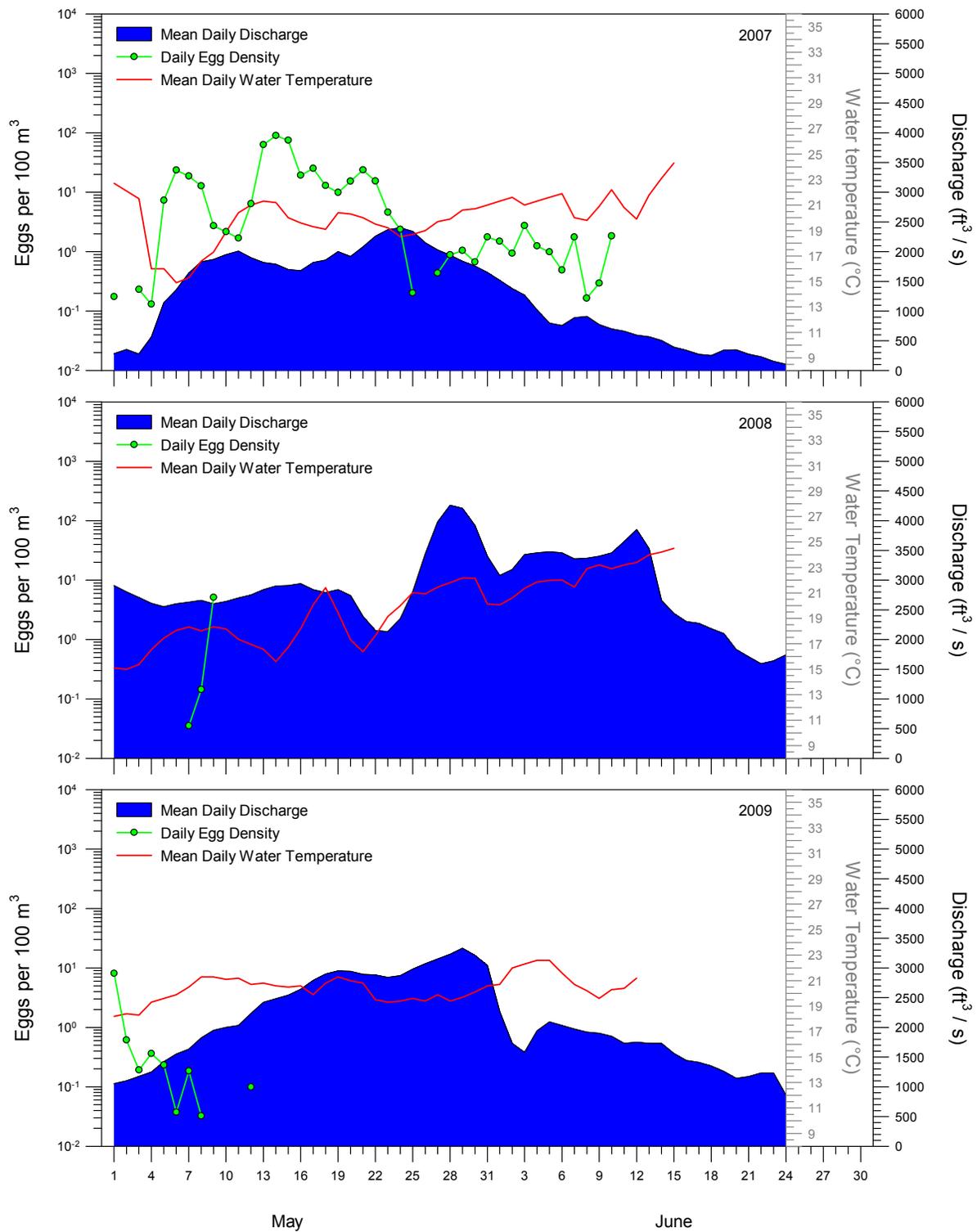


Figure 5. Mean daily discharge, daily egg density, and mean daily water temperature during the 2007–2009 Rio Grande Silvery Minnow reproductive monitoring study periods at San Marcial.

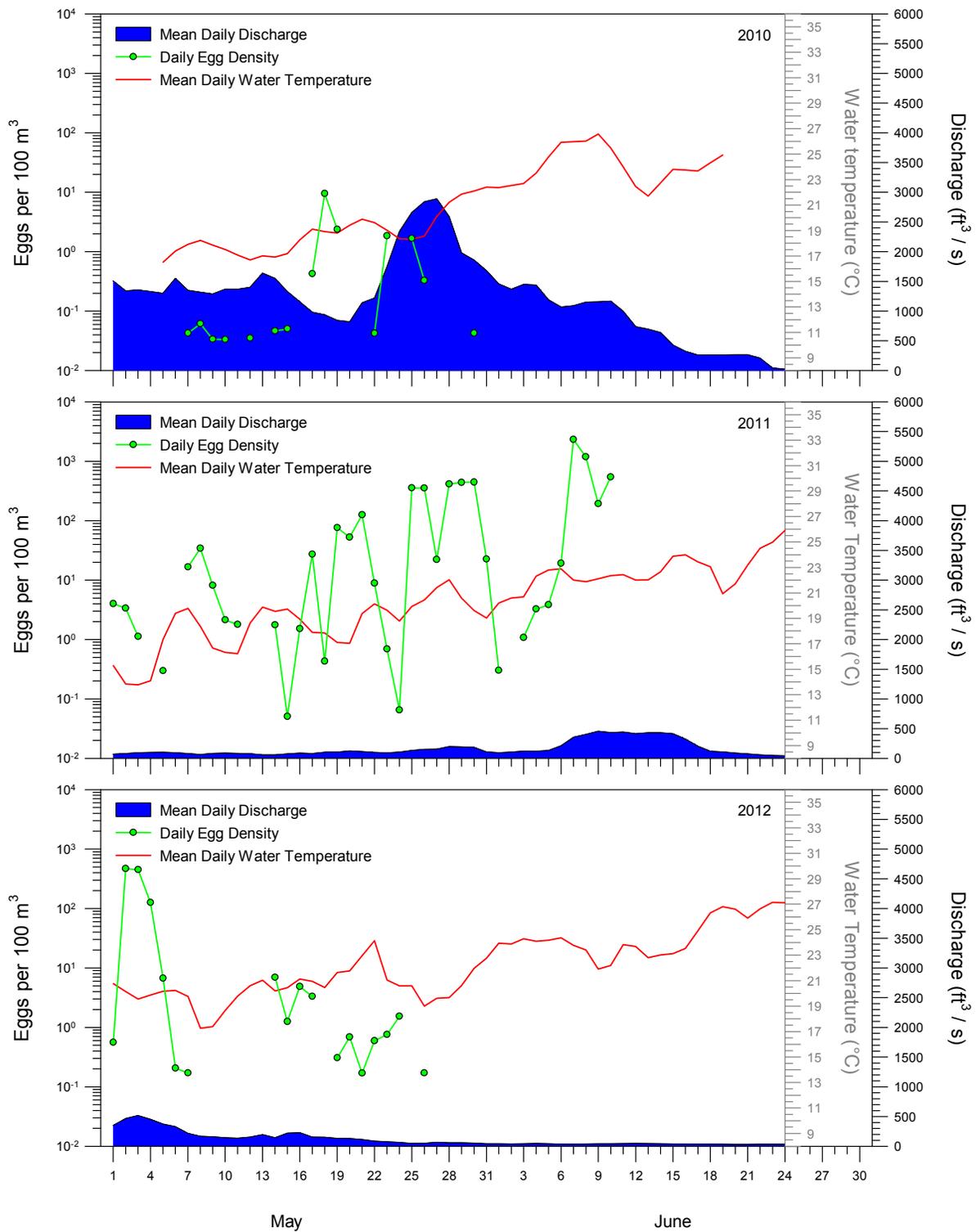


Figure 6. Mean daily discharge, daily egg density, and mean daily water temperature during the 2010–2012 Rio Grande Silvery Minnow reproductive monitoring study periods at San Marcial.

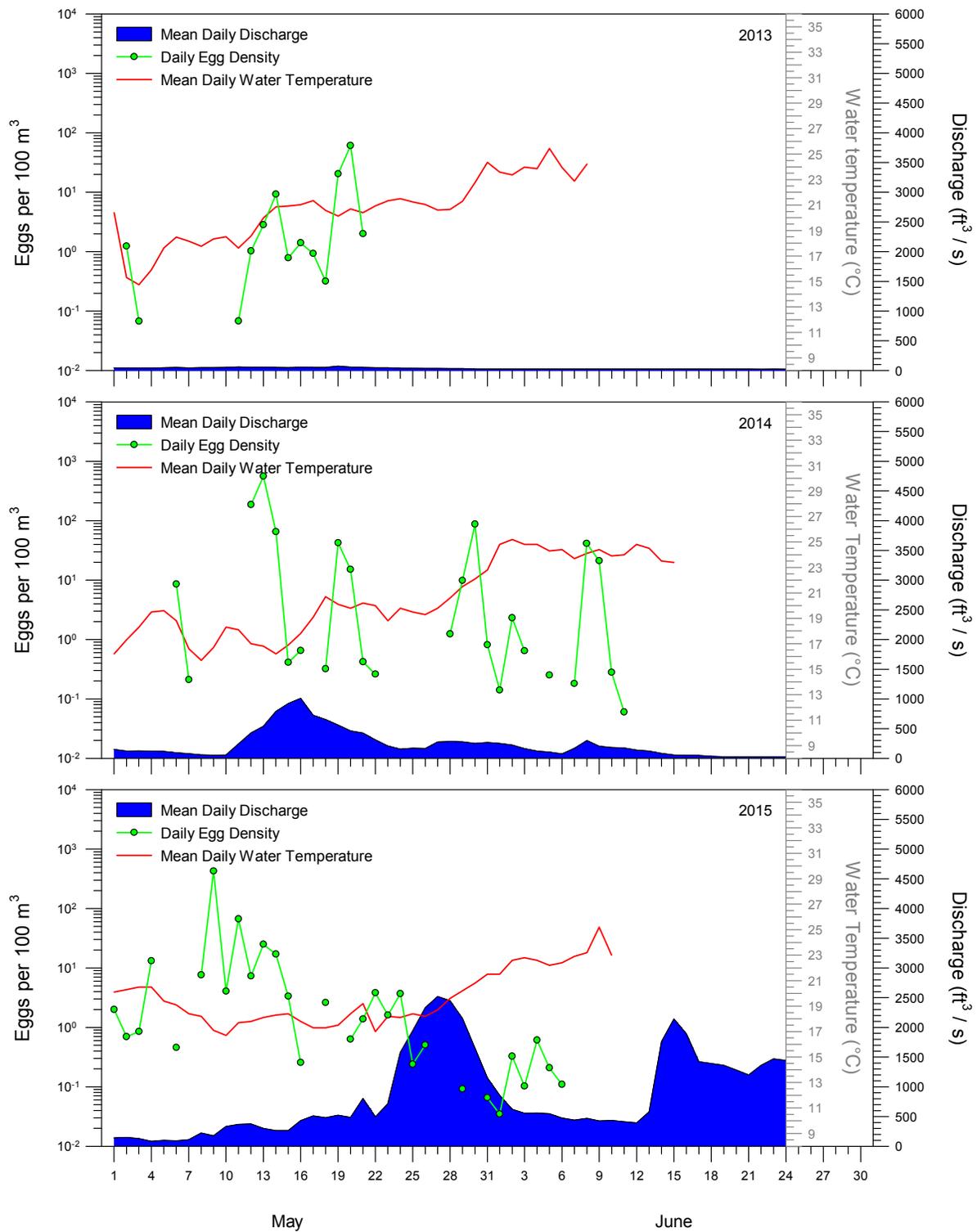


Figure 7. Mean daily discharge, daily egg density, and mean daily water temperature during the 2013–2015 Rio Grande Silvery Minnow reproductive monitoring study periods at San Marcial.

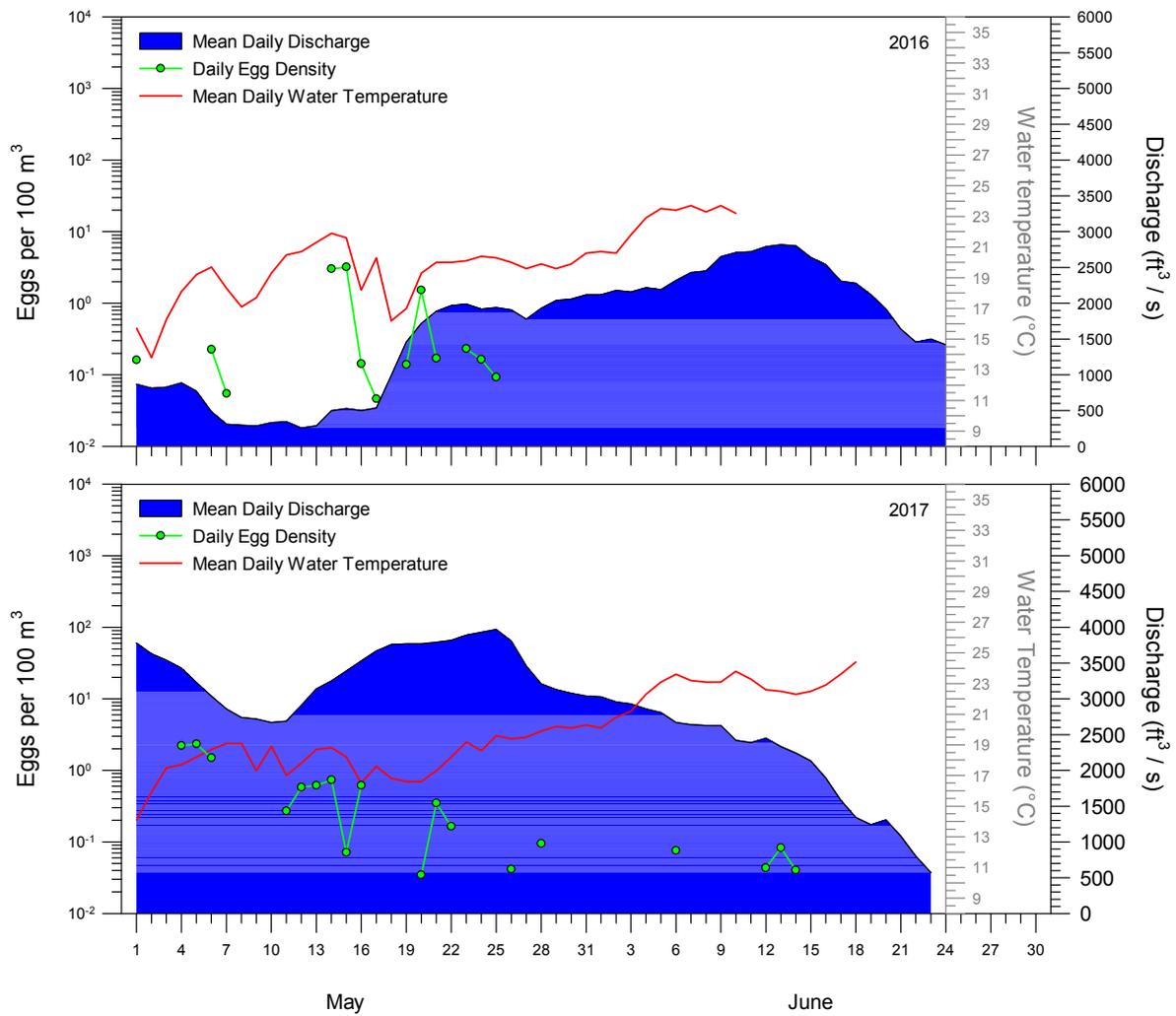


Figure 8. Mean daily discharge, daily egg density, and mean daily water temperature during the 2016–2017 Rio Grande Silvery Minnow reproductive monitoring study periods at San Marcial.

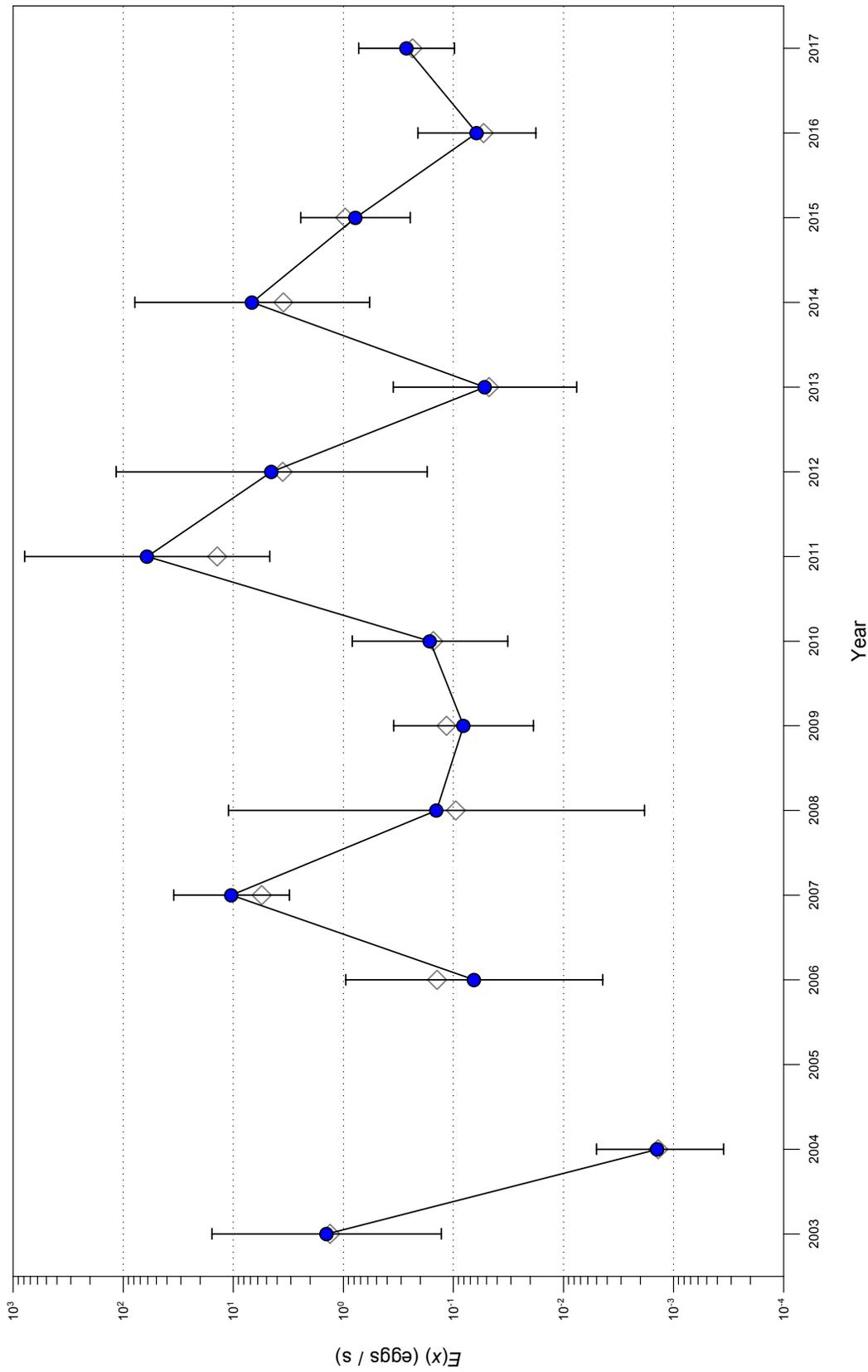


Figure 9. Rio Grande Silvery Minnow mixture-model estimates ( $E(x)$ ) of egg passage rates at the San Marcial site, from 1 May to 10 June, across years. Modeled estimates (circles), 95% confidence intervals (bars), and simple estimates using the method of moments (diamonds) are illustrated.

Table 2. General linear models of Rio Grande Silvery Minnow mixture-model estimates using egg passage rate data, from 1 May to 10 June, at San Marcial (2003–2017).

Model <sup>1</sup>	logLike <sup>2</sup>	K <sup>3</sup>	AIC <sub>c</sub> <sup>4</sup>	w <sub>i</sub> <sup>4</sup>
$\delta(\text{Year}) \mu(\text{SANmax}+R)$	1,102.29	17	1,137.60	0.7305
$\delta(\text{Year}) \mu(\text{SANmean}+R)$	1,105.90	17	1,141.21	0.1200
$\delta(\text{Year}) \mu(\text{SAN}>500+R)$	1,106.87	17	1,142.19	0.0737
$\delta(\text{Year}) \mu(\text{SAN}>1,500+R)$	1,107.57	17	1,142.88	0.0521
$\delta(\text{Year}) \mu(\text{Year})$	1,067.04	36	1,145.00	0.0180
$\delta(\text{Year}) \mu(\text{SAN}>2,500+R)$	1,115.34	17	1,150.65	0.0011
$\delta(\text{SANmean}+R) \mu(\text{SAN}>500+R)$	1,133.23	9	1,151.61	0.0007
$\delta(R) \mu(\text{SANmean}+R)$	1,135.53	8	1,151.84	0.0006
$\delta(\text{Year}) \mu(\text{SANmax})$	1,118.78	16	1,151.95	0.0006
$\delta(\text{SANmean}+R) \mu(\text{SANmean}+R)$	1,133.64	9	1,152.02	0.0005

<sup>1</sup> = Model variables included year (2003–2004, 2007–2012, 2014–2017) and hydrological variables at USGS Gage #08358400 (SAN; Rio Grande Floodway at San Marcial, NM), allowing for random effects (*R*).

<sup>2</sup> = Likelihood (-2[log-likelihood]) was estimated for each model.

<sup>3</sup> = Higher numbers of parameters indicate higher model complexity.

<sup>4</sup> = Top ten models were ranked by Akaike's information criterion (AIC<sub>c</sub>) and include the AIC<sub>c</sub> weight (*w<sub>i</sub>*).

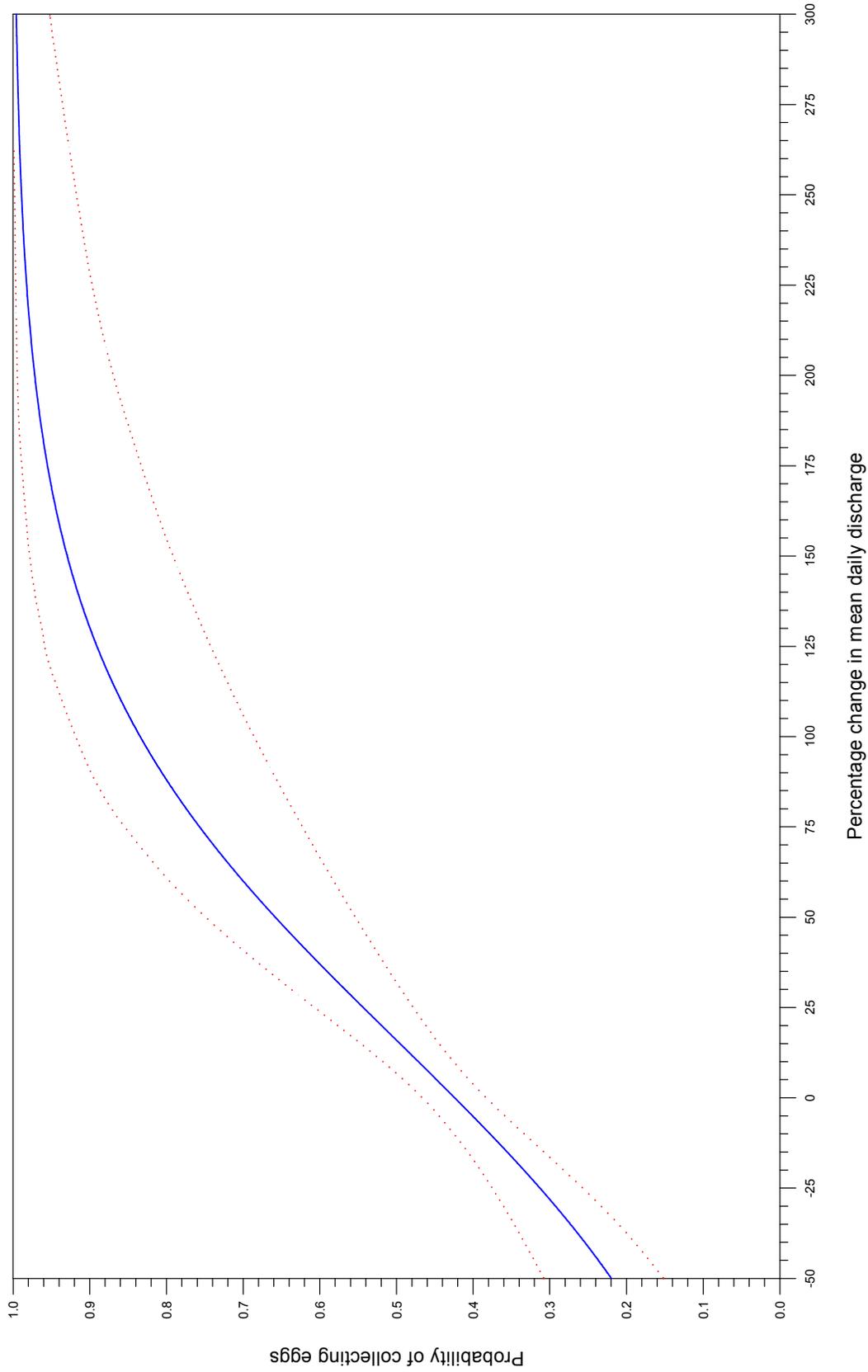


Figure 10. Logistic regression plot, using San Marcial data from 1 May to 10 June (2003–2017), illustrating the probability of collecting eggs as a function of the percentage change in mean daily discharge from two days to one day prior to egg collection. Graph shows logistic regression line (solid) and 95% confidence intervals (dotted).

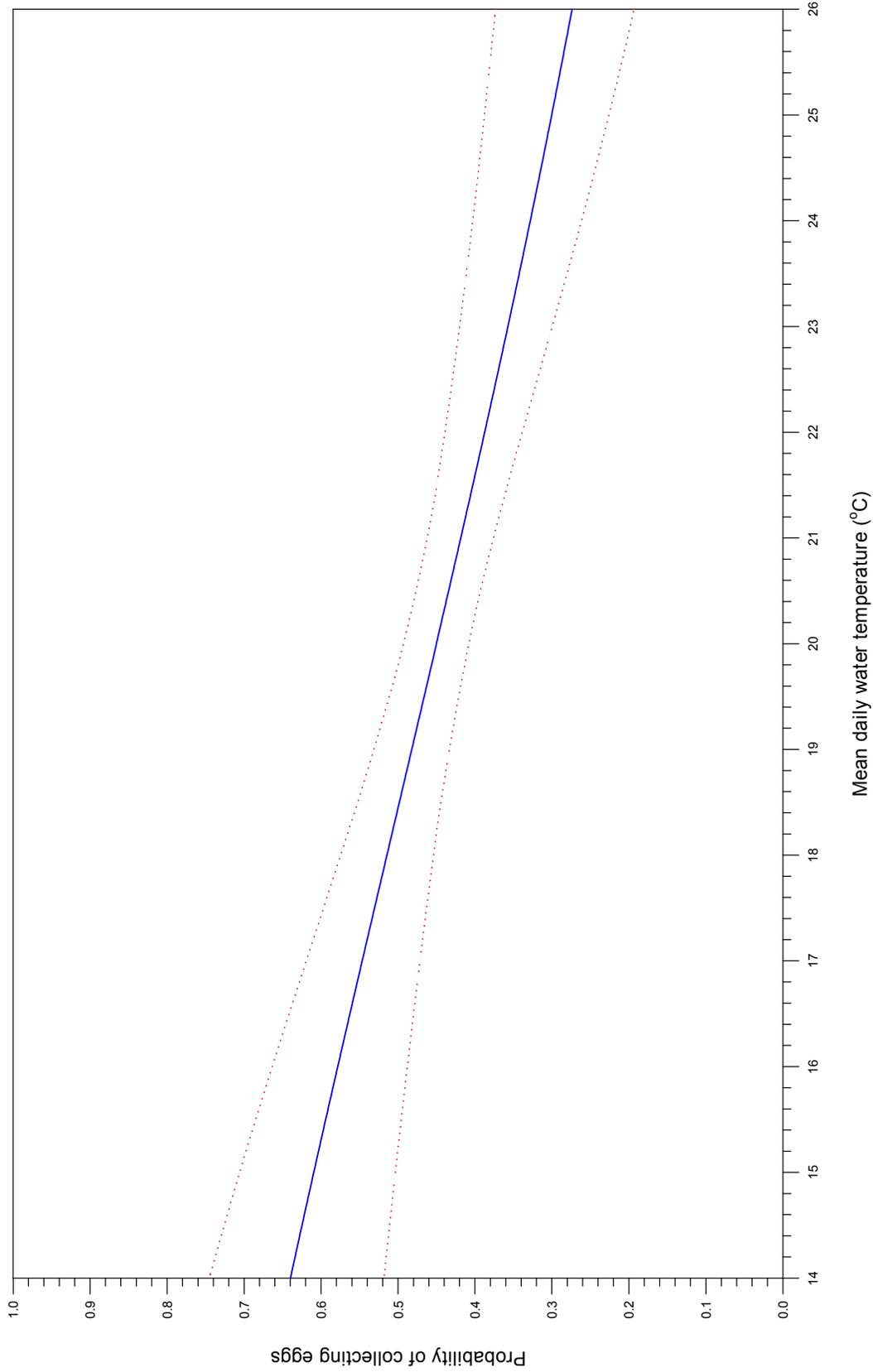


Figure 11. Logistic regression plot, using San Marcial data from 1 May to 10 June (2003–2017), illustrating the probability of collecting eggs as a function of mean daily water temperature. Graph shows logistic regression line (solid) and 95% confidence intervals (dotted).

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## *Spatial Spawning Patterns (2006–2017)*

### *Monitoring sites*

Sampling at Albuquerque and Sevilleta was conducted from 2 May through 21 June, whereas sampling at San Marcial was conducted from 4 May through 21 June, because of project logistics. The cumulative volume of water sampled was similar at the Albuquerque, Sevilleta, and San Marcial sampling sites (82,289.2 m<sup>3</sup>, 67,628.2 m<sup>3</sup>, and 95,933.9 m<sup>3</sup>, respectively). Rio Grande Silvery Minnow spawning was documented at all three sites throughout the study period (Table 3 and Figure 12). The three sites cumulatively yielded 450 eggs; the majority was collected at Sevilleta (n = 249). The number of eggs estimated to be transported downstream, during an average 30-day period, was 149,818 at Albuquerque, 1,286,669 at Sevilleta, and 689,472 at San Marcial.

### *Comparisons across sites*

Mixture-models were used to estimate and compare longitudinal egg passage rates in 2017 at the Albuquerque (0.06), Sevilleta (0.50), and San Marcial (0.27) sampling sites (Figure 13). While the 2017 egg passage rates at Sevilleta were significantly higher ( $P < 0.05$ ) than at Albuquerque, there were no significant differences between the Albuquerque and San Marcial estimates or the Sevilleta and San Marcial estimates. Also, there was no significant difference between the egg passage rates at San Marcial in 2016 as compared with 2017.

Sampling was reinitiated at the Albuquerque and Sevilleta sites in 2017, which allowed for historical comparisons of longitudinal egg passage rates from 2006 to 2017. The annual trends in egg passage rates for all three sites were relatively similar over time, with some exceptions. For example, the estimates were significantly higher ( $P < 0.05$ ) in 2007, as compared with 2006, at San Marcial but not at Albuquerque or Sevilleta. After a multiyear decline, estimates of egg passage rates, at both Sevilleta and San Marcial, were significantly higher in 2011 than in 2010. Overall, the estimated egg passage rates at Sevilleta and San Marcial were consistently higher than at Albuquerque.

Table 3. Number of Rio Grande Silvery Minnow eggs collected per day at the three sampling sites.

Sampling Date <sup>1</sup>	Albuquerque	Sevilleta	San Marcial
02-May-17	0	38	NS
03-May-17	1	17	NS
04-May-17	0	22	18
05-May-17	0	2	17
06-May-17	0	0	26
07-May-17	1	2	0
08-May-17	0	4	0
09-May-17	0	11	0
10-May-17	0	1	0
11-May-17	0	6	4
12-May-17	0	1	11
13-May-17	0	10	13
14-May-17	0	19	14
15-May-17	0	14	1
16-May-17	0	1	6
20-May-17	0	0	1
21-May-17	0	0	8
22-May-17	0	4	2
24-May-17	0	1	0
25-May-17	0	2	0
26-May-17	3	0	1
27-May-17	1	0	0
28-May-17	2	65	2
29-May-17	0	3	0
30-May-17	2	7	0
31-May-17	3	1	0
01-Jun-17	1	1	0
03-Jun-17	2	0	0
04-Jun-17	0	10	0
05-Jun-17	7	0	0
06-Jun-17	5	1	1
07-Jun-17	0	4	0
08-Jun-17	4	0	0
10-Jun-17	10	0	0
11-Jun-17	4	2	0
12-Jun-17	5	0	1
13-Jun-17	12	0	2
14-Jun-17	2	0	1
16-Jun-17	5	0	0
17-Jun-17	2	0	0
<b>TOTAL (EGGS)</b>	<b>72</b>	<b>249</b>	<b>129</b>

<sup>1</sup> = Table does not include dates that eggs were not collected at any of the sampling sites (NS = Not Sampled).

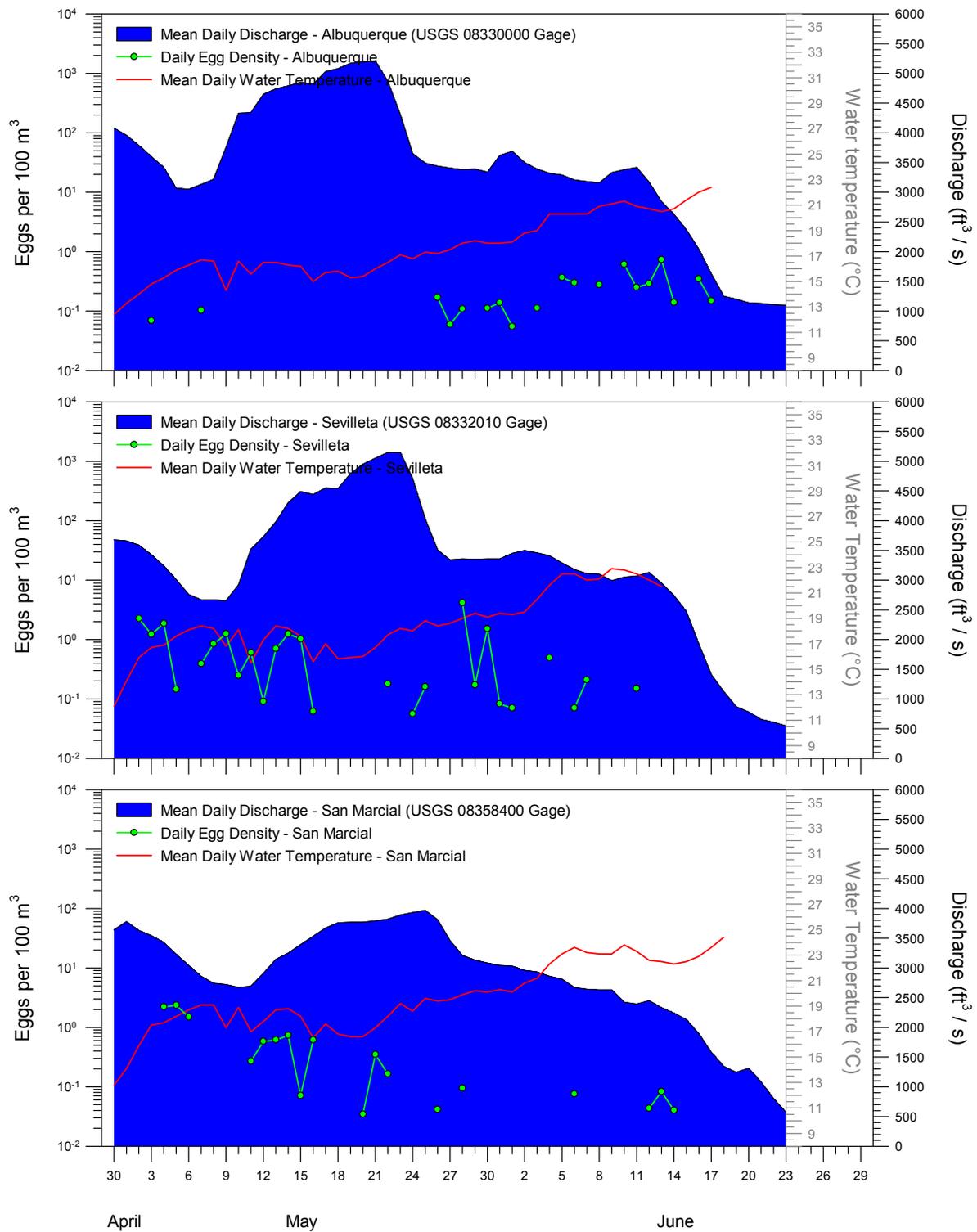


Figure 12. Mean daily discharge, daily egg density (Albuquerque and Sevilleita [2 May to 21 June]; San Marcial [4 May to 21 June]), and mean daily water temperature during the 2017 study at the three reproductive monitoring sites.

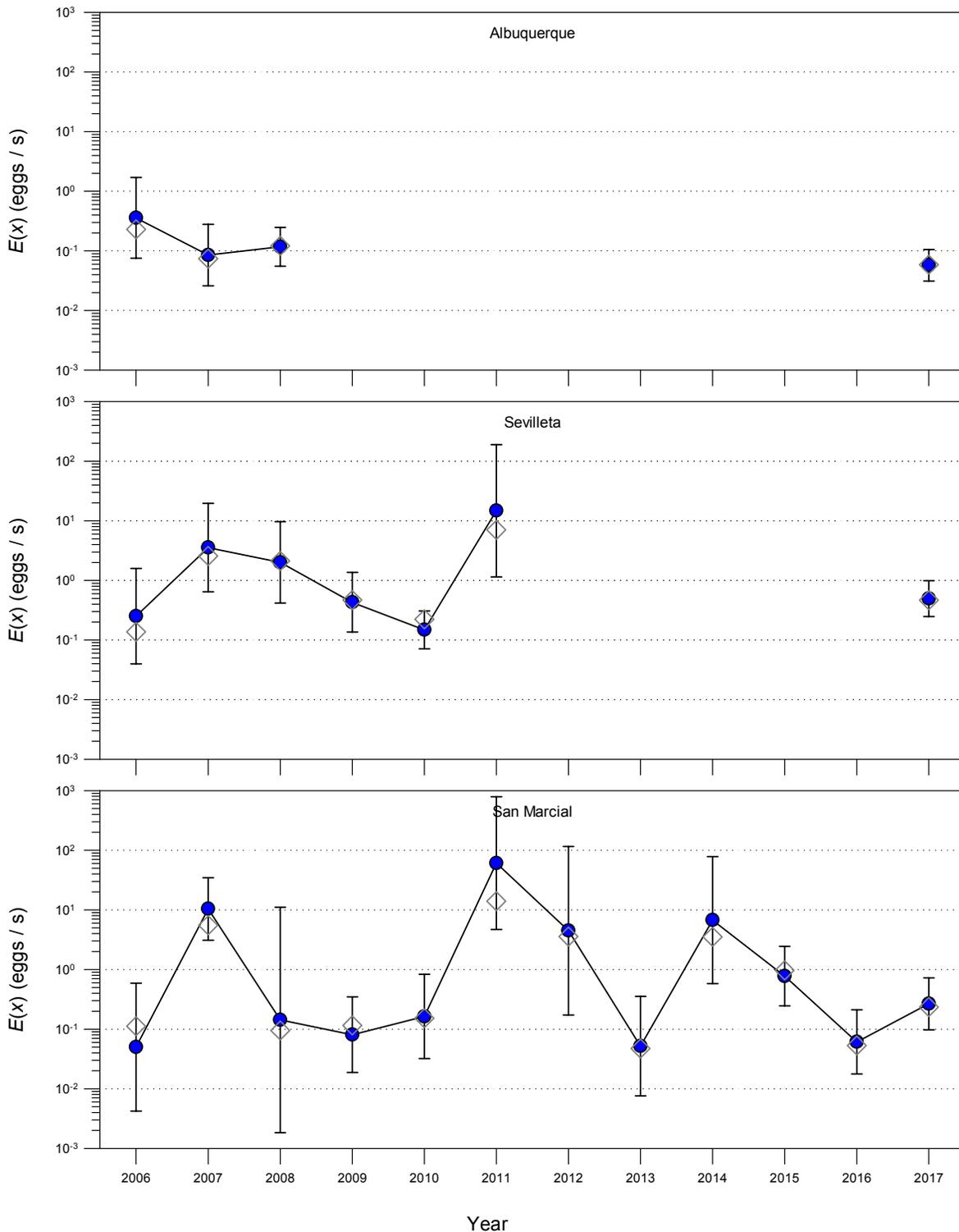


Figure 13. Rio Grande Silvery Minnow mixture-model estimates ( $E(x)$ ) of egg passage rates, from 1 May to 10 June, across sites and years. Modeled estimates (circles), 95% confidence intervals (bars), and simple estimates using the method of moments (diamonds) are illustrated.

## DISCUSSION

The negative effects of dam-related modifications on the native fishes of the Great Plains and American Southwest 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). Flow regulation and river fragmentation in these regions have led to the decline or extirpation of several pelagic-spawning cyprinids, whose reproductive propagules frequently drift downstream of instream barriers or into unsuitable reservoir habitats (Dudley and Platania, 2007). The downstream transport of eggs and larvae likely contributed to the loss of this species from the Cochiti Reach and to its decline in the Angostura Reach (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 Rio Grande Silvery Minnow eggs. The closure of Cochiti Dam resulted in the vastly reduced passage of fine sediments that has, in turn, contributed to channel degradation, armoring, and narrowing (Lagasse, 1985). While arroyos, backwaters, and other “nursery habitats” may result in increased upstream retention of eggs (Porter and Massong, 2004a, 2004b; Pease et al., 2006), these low velocity mesohabitats are relatively rare, particularly in incised portions of the river.

Spawning by Rio Grande Silvery Minnow, and other members of its reproductive guild, is triggered by specific environmental cues (Platania and Altenbach, 1998). These fishes typically spawn shortly after increases in flow during the late spring and early summer. Elevated flows result in increased water velocities/depths in some areas and inundated habitats in other areas. Additionally, there are changes in water chemistry that accompany flow increases, particularly when large amounts of soil 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 likely that Rio Grande Silvery Minnow are spawning as a result of some combination of these altered 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 activity occurs over a relatively wide range of mean daily water temperatures (ca. 14 to 26°C), but most eggs are collected over a more narrow range of temperatures (ca. 17 to 22°C). This interaction, however, is complex and varies across reaches and years. 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 physiological, and perhaps behavioral, readiness to spawn. Field collections (1993–1995) indicated that elevated GSI values corresponded with increased water temperatures during spring (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 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., March) or later in the year (e.g., July), when water temperatures are excessively cool or warm, have an increased rate of mortality. However, individuals spawned slightly earlier in the year might have an increased chance of survival as compared to those spawned later in the year, since there would presumably be reduced competitive pressure from other larval fishes for food resources (Pease et al., 2006), which become widely available shortly after the seasonal inundation of floodplain habitats (Junk et al., 1989).

The recruitment of Rio Grande Silvery Minnow, through the spring and summer, is likely affected by both abiotic (e.g., flow, temperature, water quality) and biotic (e.g., food availability, competitive interactions, predation pressure) factors. Genetic analyses of wild eggs and adults suggest that survival is 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). The broad range of conditions that result in Rio Grande Silvery Minnow reproduction could indicate that there is no single ideal spawning cue (i.e., combination of abiotic/biotic conditions) that would consistently result in their increased survival and recruitment success. The closest combination of favorable conditions, based on the last two decades of spawning studies, appears to be increased flows that occur with appropriately warm water temperatures.

While increased flows can lead to expanded larval fish nursery habitats and presumably higher recruitment success, there is no guarantee that flows will continue to rise or be sustained 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 subjected to biotic and physical conditions that may preclude their successful survival and growth, particularly during the warmer 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 eggs and survival of larvae (Platania, 2000). In addition to high water temperatures and possibly poor water quality, the likelihood of negative biotic interactions (e.g., predation or competition) would likely increase as suitable habitats contract during summer low flows.

The top ecological models, based on the long-term spawning data (2003–2017), indicated that high and extended flows during spring were associated with decreased egg passage rates, whereas lower and more abbreviated peak spring flows were associated with increased egg passage rates. 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 retention and successful recruitment of early life history stages of many freshwater fish species throughout the world (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). It is likely that higher numbers of eggs are retained upstream during years with sustained high flow events, which would account for their reduced displacement past our sampling sites (i.e., reduced egg passage rates). In contrast, short-duration and low-magnitude flow events, which typically occur during years with extended low flows, appear to result in the elevated downstream transport of eggs (i.e., increased egg passage rates). Differences between downstream egg passage rates during high and low flow years, and their presumed effects on survival and recruitment, could help explain the increased autumnal density of Rio Grande Silvery Minnow during years with elevated and sustained flows during spring (Dudley et al., 2017).

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 part of a day approximately represented the rate at which eggs were transported downstream of the site during that day, and 3) 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 spatial differences in the densities of drifting fish eggs across the river channel (Worthington et al., 2013a, 2013b). However, the number of eggs estimated to be transported downstream during spawning events would still be quite high in most years even with notable violations of these assumptions.

The total number of Rio Grande Silvery Minnow eggs collected was generally obtained through direct counting of eggs in the field. This direct counting method was used for nearly all sampling days during the spawning season across years. However, we needed to occasionally preserve egg samples when the total number of eggs collected exceeded our ability to accurately count them, while also operating the MECs. This threshold was exceeded when more than about 1,000 eggs were collected every hour. While these intense spawning events have only occurred a few times since this study began, the need to accurately quantify the number of eggs was particularly crucial during these important events.

Since 2002, we have only used actual eggs counts because we found that volumetric estimation of egg counts lacked the rigor necessary to obtain an accurate count. Based on several trials conducted in 2011, we also determined that time-based estimates of the number of eggs collected were even less accurate than volumetric estimates (Dudley and Platania, 2011).

Since Rio Grande Silvery Minnow is the only extant species remaining within the original reproductive guild of pelagic-spawning cyprinids 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 during this and previous investigations were those of the Common Carp, *Cyprinus carpio*. Fortunately, there are numerous differences between the eggs of this, and similar, species that aid in its 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, Rio Grande Silvery Minnow eggs 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 within a day, was combined for the purposes of this report. The variation in egg densities among MECs, and different sequential periods in a day, was minimal compared to the variation across days. The primary purpose in sampling with two MECs over an extended duration was to both detect the presence of eggs and to obtain an accurate estimate of egg densities 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 egg passage rates occur in the Isleta and San Acacia reaches. 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 events (Dudley et al., 2017). 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 during years when flows were exceptionally low in the San Acacia Reach, resulting in substantial drying and loss of fish from portions of that reach (Dudley et al., 2017).

Despite the seemingly large number of Rio Grande Silvery Minnow eggs transported downstream every year, some portion remains 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. 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; Widmer et al., 2012; Gonzales et al., 2014). As successful growth and survival of Rio Grande Silvery Minnow, from the egg through the early larval stages, requires about one month (Platania, 1995), the persistence of these nursery habitats is essential during this crucial developmental phase. The future conservation status of Rio Grande Silvery Minnow appears strongly dependent on reliably ensuring appropriate seasonal flow and habitat conditions to support the crucial spawning and early recruitment phases of this imperiled species.

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