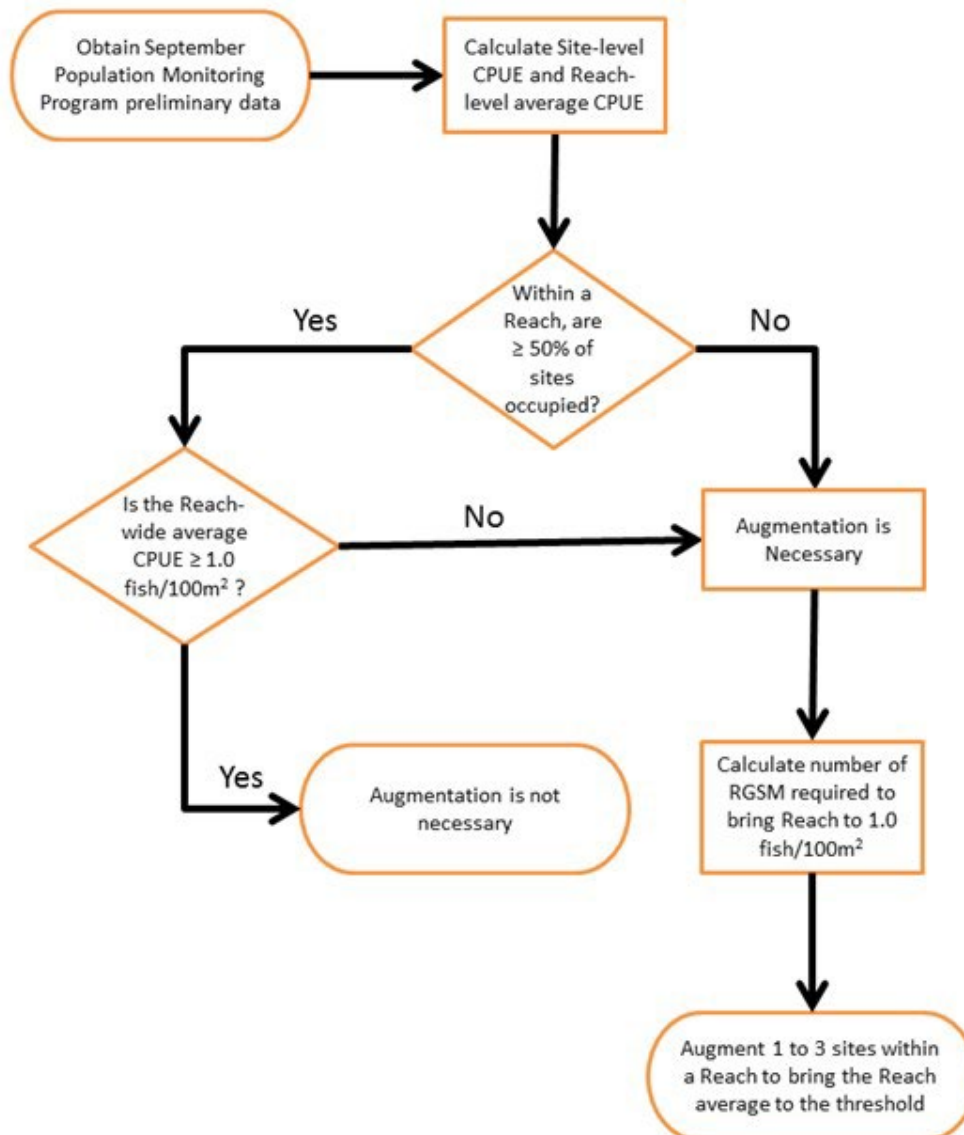


FINAL RIO GRANDE SILVERY MINNOW ANNUAL AUGMENTATION PLAN 2023-2028



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Executive Summary

Rio Grande Silvery Minnow (*Hybognathus amarus*; hereafter RGSM) is an endangered, small-bodied fish now found only in the Middle Rio Grande (MRG) of central New Mexico. Rio Grande Silvery Minnow was listed as endangered in 1994 primarily due to extirpation from 90-95% of its former range. In 2000, the U.S. Fish and Wildlife Service (USFWS) developed policy for the controlled propagation of endangered species. In 2001, the first RGSM Augmentation Plan (hereafter Augmentation Plan) was introduced to guide the USFWS's efforts to propagate and augment the wild population and contribute towards recovery of the species.

The purpose of augmentation is to increase the resilience of RGSM and improve the species' ability to persist through time. This is achieved by increasing overall abundance and distribution of RGSM following years of low recruitment by augmenting wild populations with genetically diverse hatchery-reared fish. Since 2001, over 3 million hatchery raised RGSM have been released into the MRG. Initially, stocking and monitoring efforts focused on the Angostura Reach where catch rates were low and the benefit of augmentation was expected to be higher than other reaches. Eventually, all reaches received hatchery fish and hatchery constraints have limited production for the Middle Rio Grande to 300,000 or less per year.

Rio Grande Silvery Minnow densities decline during years of low spring runoff. Recovery from population bottlenecks is hindered by lack of spawning adults in some years. While hatchery released individuals cannot count directly towards recovery goals, their presence and successful reproduction can contribute to recovery through increased demographic resilience by increasing spawner numbers and preventing loss of genetic diversity. Long-term population and genetics monitoring confirm augmentation increases the number of potential spawners in spring and genetic diversity has been maintained in the wild population.

The revised augmentation plan was effective 2018-2022 and requires revision for 2023-2028. Minor changes from the 2018 Augmentation Plan include:

- Updated early spring planning calculation to reflect inclusion of additional years of long-term population monitoring data
- Comparison of autumn release calculations based on September monitoring and October monitoring to determine if adjustments to fish releases are required

Table of Contents

Executive Summary	3
Table of Contents	4
List of Tables	5
List of Figures	6
Introduction	7
Objectives of the Augmentation Plan	10
Study area	11
Sources of Data	13
Relationship to Recovery Plan	14
Reach Level Target Densities and Stocking Requirements	15
Source of Fish	18
Timeline of Yearly Activities	18
Early Season Production Target	19
Final Calculation for Releases	21
Fish Marking Techniques	23
Release Sites and Timing of Releases	23
Adaptive Management and Evaluation	23
Risk	27
Data Availability Statement	28
Literature Cited	29
Appendix A	37
Appendix B	42

List of Tables

Table 1- Annual streamflow forecast (thousands of acre-feet [KAH]), estimates of Rio Grande Silvery Minnow needed in autumn based on various criteria used prior to the 2018 Augmentation Plan, under the current Augmentation Plan using September RGSM Population Monitoring Program numbers, and actual numbers of RGSM released. Approximately 37,000 PIT tagged fish have been released and included in the actual released column from 2018 to 2022. *No augmentation occurred in the Angostura Reach during these years. **Additional RGSM were released in the winters of 2017/2018, 2018/2019, and 2019/2020 to examine the effects of season on survival... 16

Table 2- American Southwest Ichthyological Researchers Rio Grande Silvery Minnow population monitoring program sites, reach, river mile (RM), distance from next downstream site, area between sites, and the maximum RGSM needed for augmenting each site. River miles (RM) are measured from Elephant Butte Dam. 22

List of Figures

Figure 1-Simplified life cycle of wild and hatchery-reared Rio Grande Silvery Minnow in the Middle Rio Grande, New Mexico.	9
Figure 2-Map of the Middle Rio Grande, New Mexico, river reaches, diversion dams, and where wild populations of Rio Grande Silvery Minnow are augmented with hatchery-reared fish. The Cochiti Reach (not shown) occurs upstream of the Angostura Diversion Dam. Circles represent standard population monitoring sites.	12
Figure 3-Decision matrix for determining if, where, and how many Rio Grande Silvery Minnow are required for augmenting wild populations of in the Middle Rio Grande, New Mexico, based on catch-per-unit-effort (CPUE) obtained during standard population monitoring surveys in September each year.	17
Figure 4-Generalized linear regression of April 1st streamflow forecast at the Otowi gage (USGS gage 08313000), March through July, in New Mexico predicting the number of Rio Grande Silvery Minnow needed for autumn augmentation in the Middle Rio Grande, New Mexico, based on September catch-per-unit-effort from the Rio Grande Silvery Minnow Population Monitoring Program.....	20

Introduction

The Rio Grande Silvery Minnow *Hybognathus amarus* (RGSM) was historically found in the mainstem Rio Grande and its larger tributaries (the Pecos, Chama and Jemez Rivers) from near Española, NM, to the Gulf of Mexico, and in the Pecos River from Santa Rosa, NM, downstream to at least the Texas border (Treviño-Robinson 1959; Bestgen and Platania 1991). Currently, RGSM occur in the Middle Rio Grande of central New Mexico (MRG), between Cochiti Dam and Elephant Butte Reservoir, representing 340 km (210 mi) or 5-7% of the historical range (Bestgen and Platania 1991). As a result, RGSM was listed as federally endangered in 1994 (USOFR 1994).

The decline of RGSM throughout its range can be attributed to various factors including modification of stream discharge patterns and sediment loads (Dudley and Platania 2007), channel desiccation (Archdeacon 2016), obstructions to upstream movement (Dudley and Platania 2007; Archdeacon et al. 2018), and extreme drought (Archdeacon et al. 2020a), in addition to channel alterations and channelization (Cowley 2006; Swanson et al. 2011). Modification of stream discharge patterns may be the most important factor in the decline of RGSM: the autumn RGSM population responds favorably to increased volume and duration of spring snow-melt runoff (Archdeacon 2016; Walsworth and Budy 2021; Yackulic under review). This suggests habitats created during high spring flows are important for eggs and larvae to develop and recruit to the adult population (Pease et al. 2006; Mortensen et al. 2020).

Rio Grande Silvery Minnow belong to a unique reproductive guild of freshwater fishes collectively known as pelagic broadcast spawning minnows (Balon 1975, 1981). This mode of reproduction is more common in marine species and hypothesized to maximize distribution of eggs among suitable habitats more efficiently than fish visiting each individual suitable habitat (Hoagstrom and Turner 2015). Neutrally buoyant, non-adhesive eggs are spawned directly in the water column (Platania and Altenbach 1998). Eggs and larvae can passively drift up to 340 km downstream in the MRG before being able to move to lower velocity areas (Dudley and Platania 2007). Drift distance is dependent on temperature-dependent larval development rates (Platania 2000) and local habitat features that may retain eggs.

Nearly three times as many RGSM larvae are found along main channel shoreline habitats than restored backwater habitats (Valdez et al. 2021). Higher flows likely create more slow-water habitats as floodplain or other off-channel areas become inundated, retaining eggs and larvae closer to the spawning areas (Widmer et al. 2012). Decreased spring runoff and changes in channel morphology have likely increased contemporary drift distances of eggs and larvae through channelization resulting in faster water velocities (Dudley and Platania 2007). Recruitment is poor in years with little flooding (Archdeacon 2016; Walsworth and Budy 2021), as many eggs and larvae

are likely lost downstream (Dudley and Platania 2007). Because the species is short-lived¹ (Horwitz et al. 2018), low or failed recruitment in even a single year can significantly affect demographic resilience, which is the inherent ability of a population to resist and recover after a disturbance (Capdevila et al. 2020).

In the wild, RGSM are a typical *r*-selected, opportunistic species (Winemiller 2005). Rio Grande Silvery Minnow are relatively fecund with age-1 females producing approximately 500 fertile eggs in spawning experiments (Osborne et al. 2013), with 1,000 to 9,000 total mature eggs per female (Platania and Altenbach 1998; Caldwell et al. 2019), generally live through only a single spawning season (Horwitz et al. 2018), and exhibit a type-III survival curve (i.e., low survival to adulthood). The life cycle of a wild RGSM begins with a pre-spawn standing stock of adults which may spawn during elevated spring flows (generally mid-April through mid-June, Archdeacon et al. 2020a), releasing eggs directly into the water column, which quickly develop through larval forms to juveniles, and reach adult size by late autumn (Figure 1). Elevated gonadosomatic indices, suggesting ovary maturation and spawning, begins in early April and peaks in May or early June depending on conditions, which vary year (Archdeacon et al. 2020a). During low spring runoff years, eggs are relatively easily collected in the main channel (Dudley et al. 2021b).

Captive propagation is an important tool for conservation and recovery of endangered fishes (Osborne et al. 2020). Implementation often involves difficult and controversial decisions that require balancing the risks associated with small population sizes and risks associated with introducing captive-reared individuals to wild populations (Anders 1998; USFWS 2000). Captive propagation can be used to preserve genetic diversity (Meffe 1987; Osborne et al. 2012), provide unique natural history or behavioral observations (Rakes et al. 1999; Platania and Altenbach 1998), stave off extinction (Paragamian and Beamesderfer 2004) or provide individuals for reintroduction efforts without harming wild source populations (Shute et al. 2005; Lamothe et al. 2021).

The objectives of propagation and augmentation are based on three key ideas, described in detail below, involving collection of eggs from the wild: 1) raising wild-caught eggs in captivity to supplementing wild stock in years when enough eggs are collected, 2) raising wild-caught eggs to serve as broodstock with low levels of direct hatchery ancestry, and 3) returning wild-caught (as eggs) or progeny to supplement wild fish during years of poor natural recruitment (Figure 1). When eggs are not available due to river conditions or other factors, juveniles, when available, may be taken in the autumn. These ideas are instrumental in maintaining captive populations of RGSM that

¹ There is on-going debate on the longevity of wild RGSM (Cowley et al. 2006). However, no repeatable data from aging hard structures has suggested RGSM reach greater than age 3, either in contemporary or historical collections (Horwitz et al. 2018).

are genetically diverse. The goals of the RGSM propagation and augmentation program are to help recover the species by reducing the threat of extinction by bolstering wild populations, buffering against genetic losses during years of failed wild recruitment, and providing a pool of adult broodstock from wild-caught eggs (USFWS 2010), while minimizing risks associated with augmenting wild populations with hatchery fish by following recommended guidelines (George et al. 2009).

Simplified life cycle of wild and hatchery-reared Rio Grande Silvery Minnow in the Middle Rio Grande, New Mexico.

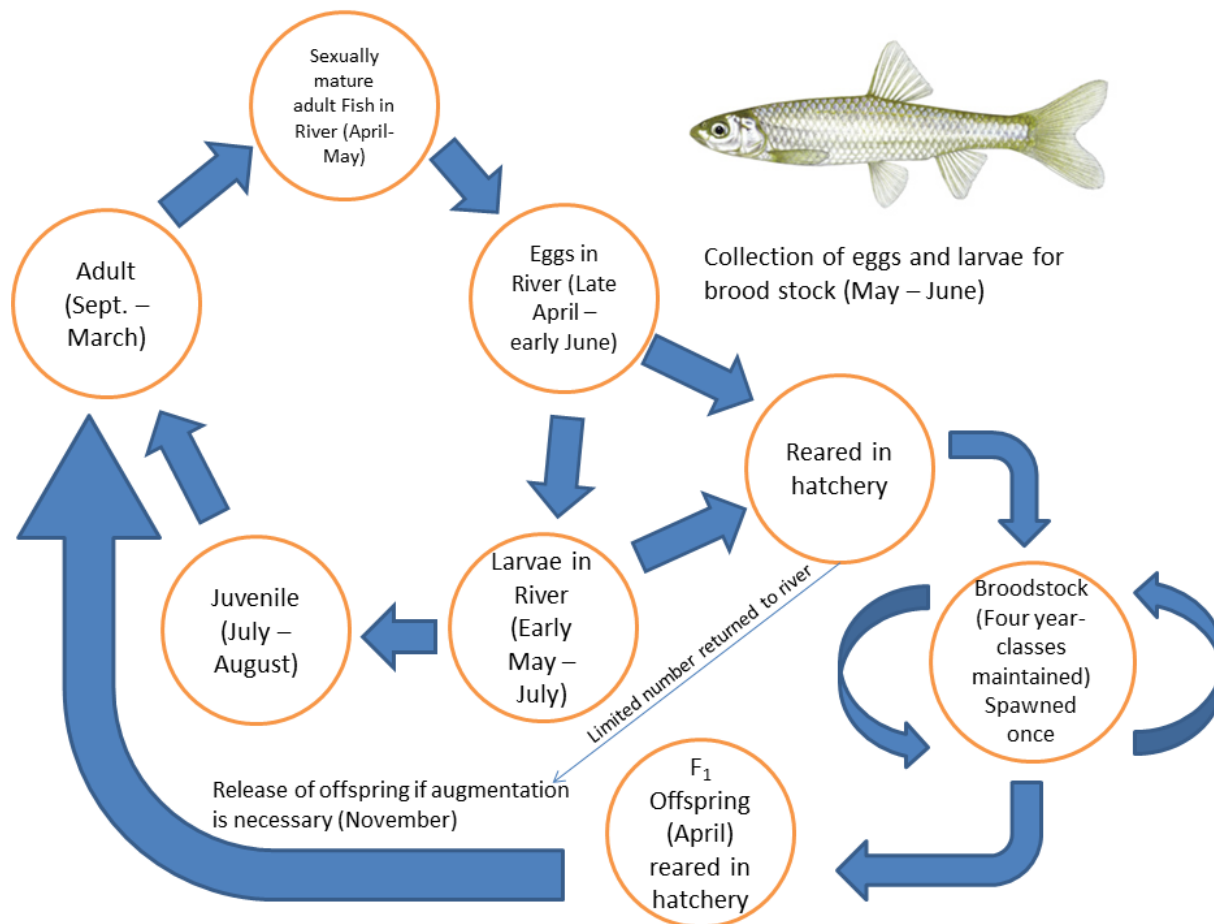


Figure 1-Simplified life cycle of wild and hatchery-reared Rio Grande Silvery Minnow in the Middle Rio Grande, New Mexico.

Objectives of the Augmentation Plan

Augmentation activities for RGSM are considered one part of the solution to prevent extinction of the species. The intention of captive propagation and augmentation of RGSM is to improve the demographic resilience of the species, in turn improving the likelihood of persistence. Propagation and augmentation activities cannot replace the need for long-term habitat restorations and adequate flow conditions in the MRG. The goal of this augmentation plan is to maintain populations of RGSM in the MRG and to preserve the genetic diversity of the species in the wild in the face of population bottlenecks. The specific objectives of this augmentation plan are to:

- 1) Define the calculation for early spring hatchery production of age-0 fish
- 2) Estimate the number of RGSM to be stocked each year after irrigation season
- 3) Summarize data and research that may inform augmentation practices
- 4) Outline suggested research to improve the beneficial effects of augmentation

Study area

The MRG is separated into four reaches, each designated by its upstream surface water diversion structure: Cochiti (~20 miles), Angostura (~ 40 miles), Isleta (~ 50 miles), and San Acacia (variable depending on the elevation of Elephant Butte Reservoir, but ~ 65 miles to the reservoir pool, Figure 2). Cochiti Reach has been monitored infrequently since 1994, when RGSM were present on Santo Domingo and San Felipe Pueblos. The most recent limited surveys in this reach near Peña Blanca, New Mexico, did not result in any RGSM collections (Torres et al. 2008). The Angostura Reach contains a drinking water diversion dam that incorporates a fish passage (e.g., fishway) with the intention of allowing RGSM to pass upstream. While the fishway does allow passage of RGSM, it may not always be functional depending on water levels (Archdeacon and Remshardt 2012), but the Angostura Reach is considered continuous. The general characteristics of the MRG in the Angostura, and to a greater extent the Isleta and San Acacia Reaches, consists of sand-bar braided channel with sand being the dominate substrate in the downstream segments (Massong and Tashjian 2006; Massong et al. 2010; Swanson et al. 2011).

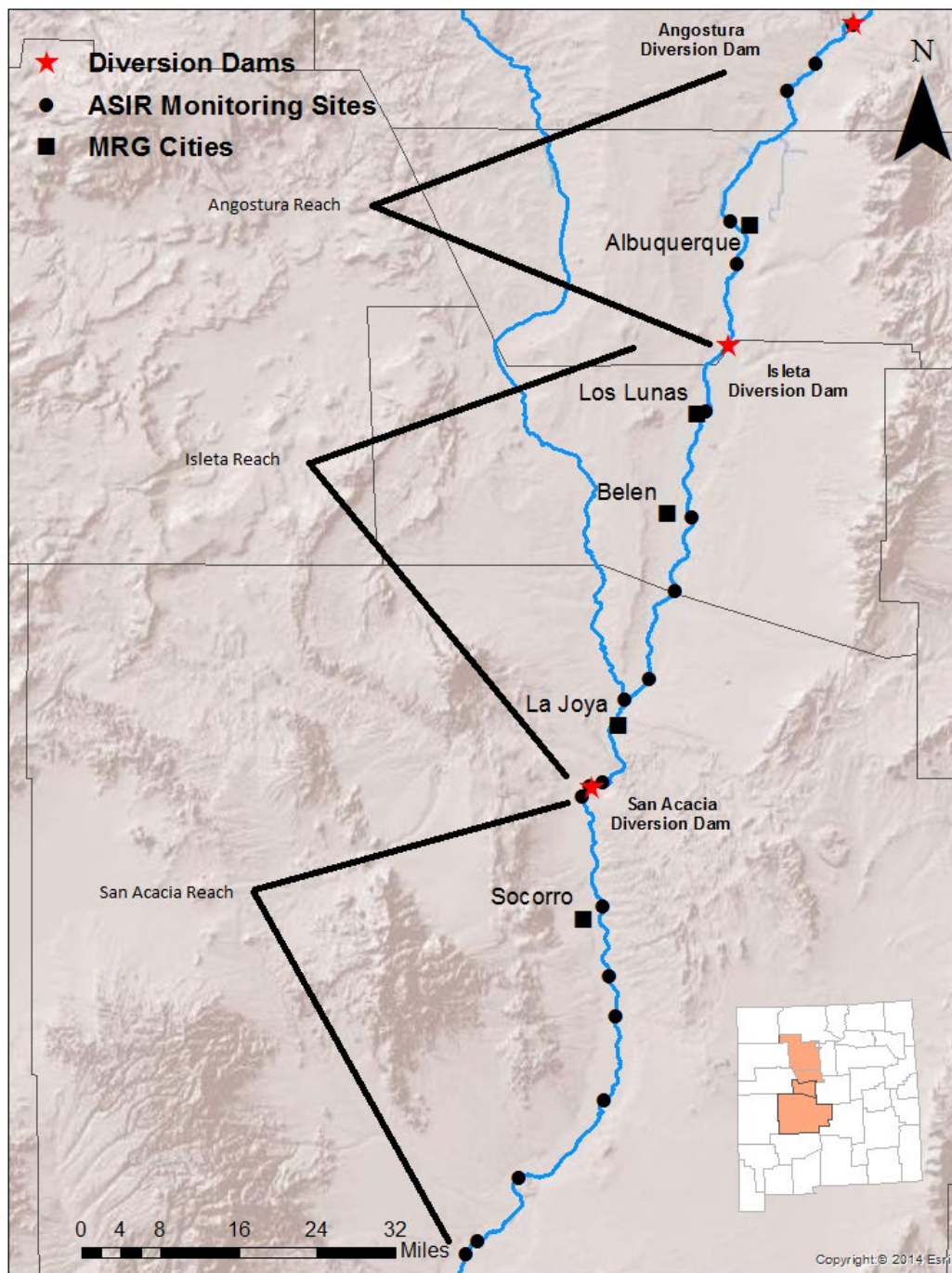


Figure 2-Map of the Middle Rio Grande, New Mexico, river reaches, diversion dams, and where wild populations of Rio Grande Silvery Minnow are augmented with hatchery-reared fish. The Cochiti Reach (not shown) occurs upstream of the Angostura Diversion Dam. Circles represent standard population monitoring sites.

Sources of Data

Rio Grande Silvery Minnow

Catch-rates of RGSM in September are taken from the RGSM Population Monitoring Program (e.g., Dudley et al. 2021a). September data are used in lieu of the October data (available at <https://webapps.usgs.gov/MRGESCP/data/rio-grande-silvery-minnow-population-monitoring>) as the October data is not available early enough to allow fish tagging and site selection. Surveys are performed at 20 standardized sites (Figure 2) and site-level catch-per-unit-effort is (CPUE) expressed as the total number of RGSM collected at a site divided by the total area sampled at a site. These CPUEs are used to calculate any shortfalls of the reach target CPUEs, described below.

River Area

A geographic information system with aerial imagery was used to calculate the surface area between each of the 20 standardized sites in the RGSM Population Monitoring Program. The width of the river was measured from aerial photography in 2012. Wetted width can change with flow, changing the actual surface area of the river between sites. Thus, the width was measured from vegetated bank to vegetated bank (i.e., base winter flows and pre-snowmelt runoff flows) to obtain a likely liberal estimate of surface area.

Forecast Data

Streamflow forecast data are taken from the Natural Resources Conservation Service's (NRCS) April 1 "Most Probable" Streamflow Forecast (generally available by April 7) available at: <https://www.nrcs.usda.gov/wps/portal/wcc/home/snowClimateMonitoring/snowpack/basinDataReports/>

The specific location for the forecast used in analysis is the Rio Grande at Otowi Bridge in the Rio Grande Basin for the March – July period and the 50% exceedance forecast (in thousands of acre-feet; KAF) is used for the planning prediction. The 50% exceedance is the predicted volume of water passing the Otowi Bridge gage that has equal chances of the observed volume being higher or lower. That is, if the 50% exceedance is 500 KAF, there is a 50% chance the observed volume of water will be greater and a 50% change the volume will be lesser than forecasted. The Otowi Bridge forecast is used for augmentation predictions because there no other relevant forecast downstream except San Marcial, which is highly correlated with forecasts for the San Marcial station. In prior Augmentation Plans, the percent of average was used; however, the NRCS changed to using median values from a different period or record. Thus, using the 50% exceedance forecasts instead of percent of average or median allows standardization of predictor values among all years.

Relationship to Recovery Plan

The USFWS's New Mexico Fish and Wildlife Conservation Office in conjunction with the USFWS's Southwestern Native Aquatic Resources & Recovery Center oversees management activities associated with propagation and augmentation, including development of propagation and augmentation plans, monitoring activities, collection of broodstock for propagation activities, transfer of fish between propagation facilities, and coordination and release of hatchery fish into selected locations.

The priority for RGSM recovery related to augmentation is to maintain and enhance populations in the MRG as identified section 3 of the Recovery Actions and Narrative in the Rio Grande Silvery Minnow Recovery Plan, First Revision (USFWS 2010):

3.0 Ensure the survival of the Rio Grande Silvery Minnow in its current habitat and reestablish the species into suitable habitats in its historical range

3.2 Continue Rio Grande Silvery Minnow augmentation activities.

Augmentation of the existing population of the Rio Grande Silvery Minnow has already taken place. The captive breeding program must be continued in order to provide fish for future augmentation, as necessary.

3.2.1 Annually review and revise the Rio Grande Silvery Minnow augmentation plan for the Middle Rio Grande.

The need for augmentation of populations and sub-populations will spatially and temporally vary. In 2001, the Rio Grande Silvery Minnow augmentation plan was created (Remshardt 2001). This plan identifies augmentation locations and identifies population numbers needed to achieve goals in a timely manner. The plan was revised in 2008 (Remshardt 2008), and should continue to be refined, as new information becomes available and the species moves toward recovery.

3.2.2 Coordinate augmentation needs with propagation activities.

Based upon annual population estimates, determine the number of Rio Grande Silvery Minnow needed to augment each population (or subpopulation) to enable timely achievement of long-term population goals. Based upon estimates of populations and sub-populations, augmentation plans will be developed for each reach. Annual population estimates should be used to refine each augmentation plan.

3.2.3 Improve our understanding of the effects of various stocking conditions and release sites on Rio Grande Silvery Minnow

A formal augmentation program (Remshardt and Davenport 2003) has been implemented since June 2002. Releases have occurred at several sites and dates throughout all reaches. Monitoring of the augmentation efforts has provided information on effective stocking conditions and release sites. Research efforts should continue.

Reach Level Target Densities and Stocking Requirements

During the early years of RGSM augmentation (2002-2007), release calculations and sites were experimental and varied from year to year. Beginning in 2008, predicted stream flows were used to estimate numbers of hatchery fish to produce each spring, and numbers of fish released were based on September catch-rates from the RGSM Population Monitoring Program (Table 2). Until 2018 stocking occurred on the site level. That is, if any single RGSM Population Monitoring Program site had a CPUE below the threshold of 1.0 fish/100m² during September Population Monitoring Program surveys, the site was augmented with hatchery fish at levels needed to achieve the target CPUE for that site, described below. However, this could result in one of two situations that are difficult to resolve and are addressed by switching to a reach-based stocking approach.

First, in years 2012 to 2014 for example, every site fell below the threshold of 1.0 fish/100m² (Dudley et al. 2021a). It was not feasible to visit and stock all 20 sites over a short period of time. Instead, fish were spread among 3 to 5 sites within a reach. Second, in 2017 for example, one site was dry in September and required stocking under the previous augmentation plan. However, the remainder of the sites in the reach had extremely high densities and stocking a single site during one of the years of highest observed CPUE did not make sense as rapid colonization was expected after continuous flows returned.

The intent of augmentation is to improve the abundance and distribution, and thus demographic resilience, of RGSM within the MRG. Thus, future stocking calculations will focus on the reach level, and will consider both observed CPUE and observed occupancy within a reach. A reach will be stocked if <50% of sites are occupied, addressing low distribution, or if the average CPUE of a specific reach is below 1.0 fish/100m², addressing low abundance (Figure 2). When stocking is necessary, fish will be released in the upstream areas of each reach, as hatchery fish tend to disperse downstream immediately (Platania et al. 2020) but further research on hatchery fish dispersal is warranted.

Table 1- Annual streamflow forecast (thousands of acre-feet [KAH]), estimates of Rio Grande Silvery Minnow needed in autumn based on various criteria used prior to the 2018 Augmentation Plan, under the current Augmentation Plan using September RGSM Population Monitoring Program numbers, and actual numbers of RGSM released. Approximately 37,000 PIT tagged fish have been released and included in the actual released column from 2018 to 2022. *No augmentation occurred in the Angostura Reach during these years. **Additional RGSM were released in the winters of 2017/2018, 2018/2019, and 2019/2020 to examine the effects of season on survival.

Year	Forecast (KAF)	Pre-2018 Estimate	September Estimate	Actual Released
2002	170	257,000	257,000	55,482
2003	465	291,000	291,000	83,384
2004	NA	149,000	94,000	175,798
2005	NA	28,000	0	259,077
2006	NA	112,000	0	418,851
2007	NA	16,000	0	133,154
2008	1170	45,000	0	0*
2009	650	24,000	0	21,218*
2010	750	143,000	113,000	135,990*
2011	370	149,000	0	194,594*
2012	335	261,000	261,000	274,557*
2013	235	293,000	293,000	293,069
2014	230	274,000	274,000	268,318
2015	395	226,000	184,000	200,549
2016	435	20,000	0	69,002
2017**	920	11,000	0	60,366
2018**	141	182,000	123,000	198,560
2019**	1020	15,000	0	94,455
2020**	385	171,000	171,000	316,334
2021	415	165,000	124,000	208,722
2022	375			
Total				3,462,480

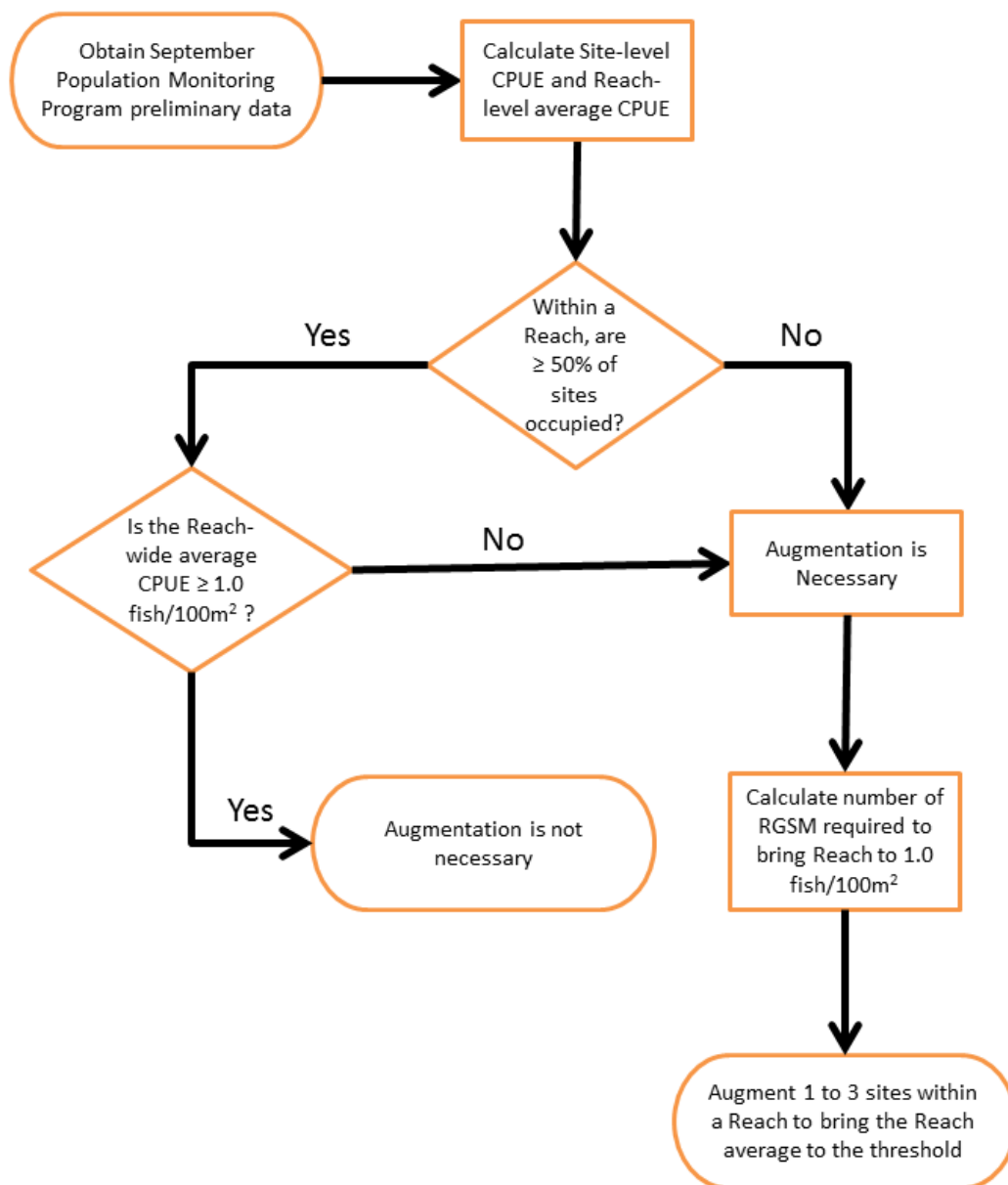


Figure 3-Decision matrix for determining if, where, and how many Rio Grande Silvery Minnow are required for augmenting wild populations of in the Middle Rio Grande, New Mexico, based on catch-per-unit-effort (CPUE) obtained during standard population monitoring surveys in September each year.

Source of Fish

Released fish can originate from several sources from this priority list: 1) wild captured eggs reared in hatchery; 2) captive-spawned eggs of wild stock; and 3) captive-spawned eggs of domestic stock. Maintaining stocks at several facilities reduces the risk of extinction due to stochastic or catastrophic events and serves to minimize the impact of any one facility on the genome of the RGSM. No specific percentage is expected to come from one facility or another and relative contributions from each facility can vary substantially from year to year.

Stocked fish must survive to spawn the following spring to contribute to the next generation. Population models suggest low initial survival of hatchery fish (Yackulic et al. in press). To maximize this survival from the time of release to spawning, hatchery RGSM should meet minimum health requirements. Fish raised by captive propagation will be stocked out (age-0 or 1) at a minimum of 35 mm standard length (45 mm total length) and a condition factor (K_{tl} ; Froese 2006) of > 0.80 as evaluated by the New Mexico Fish & Wildlife Conservation Office staff performed on a representative sample of no less than 50 fish. Although data are lacking for RGSM, K_{tl} and fish length can significantly impact apparent survival after release (Franssen et al. 2021). Thus, to improve post-release survival, batches of RGSM with mean TL < 45 mm TL or mean K_{tl} < 0.80 (as estimated from samples) will be held in captivity until they reach the minimum length and condition requirements. Fish that are < 45 mm TL or visually in poor condition (e.g., emaciated or with skeletal deformities) should not be tagged for release in order to improve the batch estimates of length and condition.

Approximate Timeline of Yearly Activities

- April—Spring meeting of the Captive Propagation workgroup; early season production targets
- September—Health and genetic sampling
- October — Population monitoring results from September are used for final release calculations; autumn meeting of the Captive Propagation workgroup,
- November — Mark all fish, transport and release when the river is continuous and following RGSM Population Monitoring, typically after November 10th

Early Season Production Target

Predicting the number of captive reared RGSM needed for autumn augmentation from early spring flow estimates has been difficult. Spawning in the hatchery facilities must occur early in the year before spring runoff and summer drying have occurred. From available predictive variables, including the April 1 streamflow forecast², the March 15 Rio Grande Upper Basin snowpack, and the previous October CPUE, the April 1 streamflow forecast predicts September release calculations moderately well (Figure 4). To estimate this relationship, the forecasted streamflow from April 1 each year was used to predict actual numbers of fish needed based on autumn CPUE, by using a generalized linear regression assuming a quasibinomial error structure. However, at least two years (2011 and 2016) had recruitment high enough that no augmentation was required, despite low forecasted flows (Figure 4). Conversely, in 2010, the flow forecast was relatively high but over 100,000 RGSM were needed for augmentation.

Because of the uncertainty in both forecasted streamflow and measuring abundance of fish, there is much variability in actual release number. However, broad categories are evident: years where the forecast is < 300 KAF and many hatchery fish are needed to reach the minimum threshold of 1.0 fish/100m²; years where the forecast is > 800 KAF and hatchery augmentation is not needed; finally, years where the forecast is between 300 and 800 KAF in which numbers of fish needed are highly variable. The upper 95% confidence interval from this relationship will be used for planning early season production targets to account for this uncertainty and a minimum of 45,000 fish will be produced in all years, regardless of predicted streamflow.

² Streamflow forecast data for the Rio Grande at Otowi Bridge are available from 2002, 2003, and 2008 to present

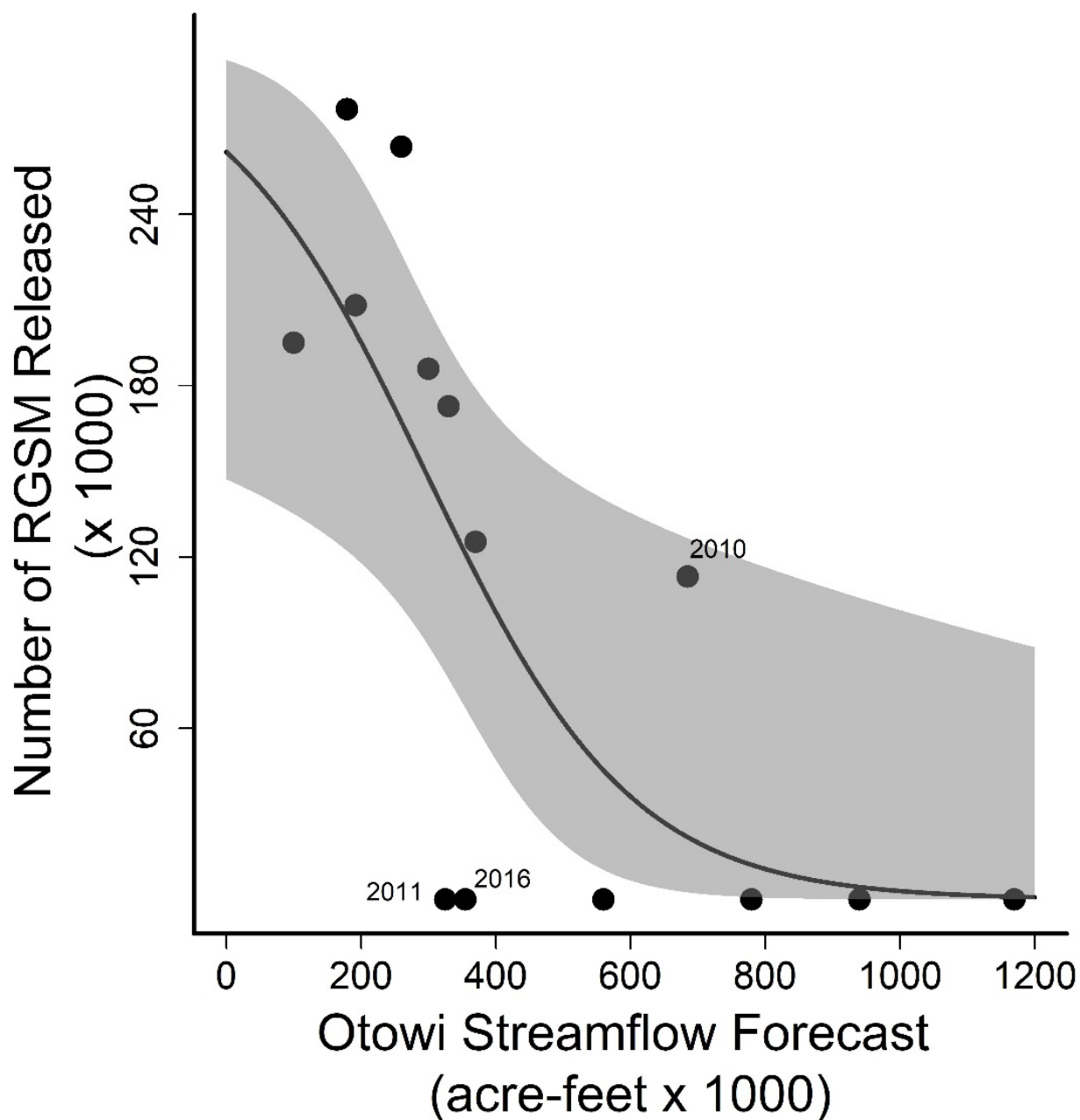


Figure 4-Generalized linear regression of April 1st streamflow forecast at the Otowi gage (USGS gage 08313000), March through July, in New Mexico predicting the number of Rio Grande Silvery Minnow needed for autumn augmentation in the Middle Rio Grande, New Mexico, based on September catch-per-unit-effort from the Rio Grande Silvery Minnow Population Monitoring Program.

Final Calculation for Releases

Between January and October additional modifications to the augmentation targets will be made and discussed with propagation facilities for planning purposes. On or about September 20th, the final augmentation targets and stocking locations will be made available to each of the propagation facilities. The augmentation targets will be calculated based on the September CPUE reported from the RGSM population monitoring program data. Typically, October catch rates are the standard for determining status of fish populations, but the time and preparation needed to determine specific stocking rates makes using the October numbers problematic (e.g., October monitoring data may not be available until mid-November). September catch rates will be used as a surrogate. Using the equation:

$$R_i = (1 - C_o) \times (\text{total measured area } m^2 \text{ for each Reach})$$

where; R_i = Number of fish to release at Reach i

C_o = Observed CPUE at each Reach in September

a reach-specific number of fish is calculated based on observed CPUE and the area in a reach (Table 2). This reach-specific total number of fish is then distributed among one to three sites with the lowest September CPUE. Numbers and locations of release sites will vary from year to year depending on the overall number of fish to be released and availability of habitat suitable for release.

Table 2- American Southwest Ichthyological Researchers Rio Grande Silvery Minnow population monitoring program sites, reach, river mile (RM), distance from next downstream site, area between sites, and the maximum RGSM needed for augmenting each site. River miles (RM) are measured from Elephant Butte Dam.

Site	Reach	RM	Distance (km)	Area (m²)	Maximum RGSM
1	Angostura	209.7	9.5	1,656,597	17,000
2	Angostura	203.8	6.1	726,075	7,000
3	Angostura	200.0	26.7	4,251,209	43,000
4	Angostura	183.4	8.2	1,418,164	14,000
5	Angostura	178.3	27.2	4,281,428	43,000
NA	Angostura Total	NA	77.7	12,333,473	124,000
6	Isleta	161.4	15.9	2,803,754	28,000
7	Isleta	151.5	13.4	1,488,942	15,000
8	Isleta	143.2	20.3	2,353,636	24,000
9	Isleta	130.6	5.8	408,354	4,000
10	Isleta	127.0	16.4	1,489,804	15,000
11	Isleta	116.8	1.0	203,869	2,000
NA	Isleta Total	NA	72.8	8,748,359	88,000
12	San Acacia	116.2	2.6	154,690	2,000
13	San Acacia	114.6	24.3	2,189,178	22,000
14	San Acacia	99.5	12.6	1,674,833	17,000
15	San Acacia	91.7	7.4	819,332	8,000
16	San Acacia	87.1	12.9	978,072	10,000
17	San Acacia	79.1	16.9	1,071,123	11,000
18	San Acacia	68.6	13.0	705,887	7,000
19	San Acacia	60.5	4.5	155,784	2,000
20	San Acacia	57.7	16	778,815	8,000
NA	San Acacia Total	NA	110.2	8,527,714	87,000
NA	MRG Total	NA	260.7	29,609,546	299,000

Fish Marking Techniques

All released fish should be externally batch-marked with Visible Implant Elastomer (VIE) tags for identification as hatchery fish. This method provides the level of detail needed for readily distinguishing hatchery from wild fish. These tags produce low mortality < 5% and have high tag retention > 95% in the similar Western Silvery Minnow *H. argyritis* (Neufeld et al. 2015). All fish are tagged anterior to the dorsal fin, on the left in even-numbered years and on the right in odd-numbered years. Each year's release will be given a different mark (color and/or location) to the extent possible. Tag marks should be a minimum of 3 mm and readily visible, non-fluorescing VIE colors should be avoided when possible. Fish that are released that are not age-0 should be given a different identifying mark when possible to facilitate cohort growth analyses. Tag color combinations will be chosen to help minimize year-class misidentification on recaptured fish. However, additional research projects that require unique marks may limit the number of color choices available for distinguishing augmented fish (Curtis 2006).

Release Sites and Timing of Releases

All fish will be stocked after water diversions for irrigation have stopped, continuous flows are present, and Rio Grande Population Monitoring has concluded (generally by November 15th). Stocking in the autumn will allow captive-reared individuals to reach maximum size while avoiding predation, competition, and habitat degradation and loss during summer low-flow periods when age-0 fish are most susceptible. Autumn release also allows the stocked fish several months to acclimate to the river before higher flows (and spawning) occur the following spring.

Fish will be transported from the production facilities and tempered with river water. Tempering times will vary depending on the size of the transport tank and river temperature and will proceed at no more than 2° C per hour until transport tanks are within 1° C of the river temperature. After acclimating, fish will be released into appropriate slow-water habitats. Because habitats can shift from year to year, exact release locations may shift, but without interfering with the overall goal of distributing fish within the reach. Cages or block nets may be used to create low-velocity habitats when none are available within a reasonable distance from release locations.

Evaluation of Augmentation

Periodic review of augmentation strategies and methods is useful for ensuring augmentation is beneficial and cost effective. For example, repeated over-calculation of numbers for release could increase risk of domestication selection, labor-intensive, and potentially costly to house and feed unneeded fish. Conversely, maintaining or releasing too few RGSM may not buffer the population against further demographic or genetic losses. Third, adjusting and evaluating release strategies to improve survival of

hatchery fish could justify releasing fewer fish, as well as changes to the release locations or the timing of releases. Yet, captive breeding programs for fish and mussels rarely evaluate effectiveness in three key areas: broodstock collection, rearing and release methods, and post-release monitoring (Rytwinski et al. 2021). The captive propagation and augmentation program for Rio Grande Silvery Minnow has been reviewed since its inception and includes evaluation of long-term genetic diversity of both wild fish and hatchery broodstock and offspring, genetic effects of different hatchery spawning strategies, formulation of hatchery diets, determination of age-specific fecundities, and comparison of survival to spawning from differing release times. Here, existing evaluations of hatchery techniques are summarized under the categories suggested by Rytwinski et al. (2021) and new experiments are suggested.

Broodstock collection

Collection of genetically diverse and representative broodstock is an important consideration for all conservation hatchery programs (Osborne et al. 2020). Traditional hatchery programs, typically dealing with salmonid fish species, can cause a decline in fitness of wild stocks (Araki et al. 2007) or even replacement with hatchery stocks (Quiñones et al. 2014). Broodstock for RGSM rely on collection of wild-caught eggs during the spawning period in April through June, though most occur in May (Dudley et al. 2021a). Here, all broodstock have spent a portion of their lives in the river, captured as either eggs during spring spawning events, or in years of high abundance as juveniles in autumn when egg collections are not effective. While broodstock collection strategies for RGSM have not been evaluated, the genetic diversity of the broodstock themselves has been monitored since almost the beginning of the augmentation program (e.g., Osborne et al. 2006). Because of the drifting nature of RGSM eggs, many are expected to drift to unsuitable habitat and have high mortality, and only a small fraction will recruit to the adult population (Alò and Turner 2005). The genetic makeup of wild caught RGSM eggs differs over time within the spawning period, and egg samples collected at a limited temporal and spatial scale are not necessarily a random sample of breeding adults (Osborne et al. 2005). Thus, wild-caught eggs used as broodstock from single locations and times during spawning may not be genetically representative. Nonetheless, levels of genetic diversity can vary among captive broodstock cohorts but have generally been representative of wild fish (Osborne et al. 2012; Osborne and Turner 2021). Release of individuals from captive rearing should be used sparingly because of the inherent risk of domestication selection. More recently, broodstock and their offspring have been sampled and analyzed for a variety of genetic diversity indices (Osborne and Turner 2021). Future studies could focus on the maximizing genetic diversity in actual collections, primarily comparing diversity among locations of egg collections, as the timing and duration of spawning is difficult or impossible to control.

Rearing and release methods

Several studies have examined rearing methods, beginning with spawning strategies to maximize diversity and equalize contributions among potential parents. Captive spawning of RGSM is typically induced by injecting females with carp pituitary extract. Over 400,000 have been spawned and released in a single year with this method (Archdeacon 2022), with age-0 females usually producing 500 to 1000 eggs with >60% fertilization (Osborne et al. 2013; Caldwell et al. 2019). Spawning in a naturalized, outdoor environment has been induced through flow and water stage manipulations during spring; however, 720 and 750 broodstock produced only 172 and 254 fish for augmentation, respectively, but had exceptionally high growth rates (Hutson et al. 2018). Osborne et al. (2013) compared three captive spawning strategies (monogamous pair, hormone-induced communal spawning, and environmentally cued communal spawning) and found no differences in genetic diversity or egg production among methods and recommended communal spawning over monogamous pairs. Osborne et al. (2013) also determined that environmentally cued spawning, induced through rapid increase in turbidity, resulted in more viable eggs, but was the most difficult strategy to implement.

The majority of RGSM are raised in grow-out ponds or tanks and fed commercial fish feed. Feeding and diet of hatchery reared RGSM has also been examined. Gut contents of RGSM raised in hatchery ponds suggests they consume a variety of foods, primarily insects, formulated fish feed, and diatoms (Watson et al. 2009). Caldwell et al. (2010) optimized hatchery feeds to balance cost and growth, identifying a less expensive growth option. Rio Grande Silvery Minnow raised exclusively on or supplemented with hatchery feed had higher whole-body percent lipids and higher Fulton condition factor compared to fish without access to hatchery feed (Powell et al. 2017). Rio Grande Silvery Minnow raised in a naturalized conservation aquaculture unit had Fulton condition factors varying from poor (< 0.80) to excellent (> 0.90) in multiple years (Tave et al. 2019; Archdeacon 2022). Post-release survival of fish raised in the naturalized unit has not yet been evaluated.

Rigorous evaluations of release methods for RGSM are lacking. Soft-release methods (e.g., holding a pen for several hours before release) have been used previously, but areas of low current velocity large enough to install holding pens are infrequent and the practice was abandoned. A comparison of autumn and late winter releases was implemented in 2019, finding both groups were present in nearly equal numbers the following April (Appendix C of Archdeacon et al. 2020b).

Post-release monitoring

Both population dynamics (e.g., Dudley et al. 2021a) and genetics (e.g., Osborne and Turner, 2021) of wild RGSM are monitored post-release. Within the MRG basin,

augmentation is fulfilling the primary purpose of improving demographic resilience by increasing abundance and distribution of RGSM. In low water years, augmentation with hatchery fish has increased the number of potential spawning fish in the following spring since the program began in 2002 (Appendix A). A short-term, high-coverage sampling (e.g., 40 sites within the San Acacia Reach sampled in April 2019) revealed the impact of augmentation on the number of potential spawners may be greater than observed from the RGSM Population Monitoring program, finding over 4 times as many hatchery fish as wild fish (Appendix C of Archdeacon et al. 2020b). Following low spring runoff years, hatchery individuals can make up > 90% of all fish rescued during summer irrigation season (Archdeacon 2016). Taken together, it is evident hatchery augmentation is performing as intended, improving the distribution and abundance of potential spawners. Determining if hatchery fish are spawning concurrent with wild fish is on-going (e.g., Archdeacon et al. 2020a).

Although Osborne et al. (2006) caution that reliance on hatchery fish may erode genetic diversity, augmentation with hatchery fish has buffered the wild population from losses of diversity despite large changes in population size (Osborne et al. 2012). Average number of alleles and heterozygosity have been maintained since the beginning of the augmentation program (Osborne et al. 2020). Continued genetic monitoring of broodstock, refuge populations, and hatchery-produced offspring will help reduce likelihood and extent of transferring any negative effects to wild populations (Osborne and Turner 2021).

Future studies

Integration of RGSM Population Monitoring, fish rescue, and augmentation data suggests post-release survival may be lower than desired and the effectiveness of augmentation could be improved with better survival of hatchery fish (Yackulic et al. in press). Apparent survival (e.g., mortality not distinguished from emigration from release site) may be higher for fish released during late winter compared to autumn. However, this was not observed during experimental releases; fish from both autumn and late winter releases appeared equally in surveys (Appendix C of Archdeacon et al. 2020b), suggesting mortality occurs quickly after release. Season of release does affect apparent survival of some salmonids (Karppinen et al. 2014; Zeug et al. 2020), and further research is warranted.

Enrichment of fishes' physical, sensorial, occupational, social, and dietary experience can improve well-being and is particularly well-studied in ornamental species (Brown and Day 2002; Arechevala-Lopez et al. 2021). For example, occupational enrichment such as flow conditioning (Franssen et al. 2021) or addition of substrate to grow-out habitats (Näslund and Johnsson 2016; Jones et al. 2021) may improve fish welfare and increase post-release survival. Other options including pro- or

pre-biotic treatments (Rohani et al. 2022) and soft-release techniques (Jonsson et al. 1999; Mokdad et al. 2022) may improve post-release survival to spawning but most research is related to commercially or recreationally important species.

Excess fish

In years where hatchery fish are produced exceeding of requirements for autumn augmentation, those fish will be released in the reach of lowest abundance. Additionally, some excess fish may be used for USFWS-approved research efforts. Although there is risk of replacing wild fish with hatchery stock (Araki & Schmid 2010), this is unlikely for RGSM. First, the numbers of excess fish have been relatively low since release calculations were standardized in 2008 (Table 2). Second, it is likely that most hatchery fish die shortly after release, with only 10 to 20% surviving to the following spawning season (Yackulic et al. in press). Third, wild and captive-spawned fish have similar levels of genetic diversity, and in some years augmented fish have greater haplotype richness compared to wild fish (Osborne and Turner et al. 2021). Fourth, future genetic analyses may be able to provide results prior to releasing hatchery fish, thus avoiding transferring any detrimental effects to wild populations.

Risk

Releasing captive-reared fish into the wild is not without risks. Genetic and ecological risks must be evaluated against the possible benefits of the augmentation efforts (Osborne et al 2006; Araki et al. 2007). These genetic risks are described in detail in the draft Rio Grande Silvery Minnow Genetics Management and Propagation Plan. Other ecological concerns associated with stocking captive reared RGSM in the wild include passive downstream movement, pathogen and parasite transmission, intra- and interspecific competition, and predation. By allowing fish to reach minimum size and condition (35 mm SL; $K_H = 0.80$) before stocking, VIE tagging is more effective and over-winter survival is expected to be higher. Samples from all sources of stocked fish will be analyzed for presence and extent of pathogens and parasites before transfer or stocking into the Rio Grande.

Choosing not to augment endangered species with hatchery-reared fish also has inherent dangers, such as extinction and further loss of genetic diversity (Anders 1998; Paragamian and Beamesderfer 2004). Given the increasing number of threats to western United States ecosystems, development of sound management strategies is necessary. Other flow and nonflow management options are possible, and in most years, strategically adding water to the system would result in the most benefit for the species (Yackulic et al. in press). However, such flow management options are not always possible, and given the reality that habitat improvement and restoration are not going to happen at the scale or timeframe necessary to serve as a viable alternative to augmentation. Following specific guidelines and providing a detailed decision-making

process for augmenting wild populations with hatchery raised fish will help minimize these risks (George et al. 2009).

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Disclaimer

The findings and conclusions in this plan are those of the author and do not necessarily represent the views of the U.S. Fish and Wildlife Service. The use of tradenames does not imply endorsement from the U. S. Fish and Wildlife Service or the U. S. Government.

Data Availability Statement

Data are available at <https://www.doi.org/10.17632/nwc7k6rm47>

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Appendix A

Comparison of September and October catch-rates for calculating Rio Grande Silvery Minnow augmentation needs

Introduction

Rio Grande Silvery Minnow augmentation is intended to improve the abundance and distribution of potentially spawning stock each spring. Rio Grande Silvery Minnow are added each autumn to bring reach-wide catch-rates up to 1.0 fish per 100 m². These calculations are based off the September Rio Grande Silvery Minnow Population Monitoring Program numbers as October numbers are not available early enough to make decisions on how many RGSM to stock. Significant channel drying is possible after September monitoring is completed and likely reduces the abundance and distribution of wild fish, increasing the need for augmentation. Here, stocking calculations based on both September and October Rio Grande Silvery Minnow Population Monitoring are compared to determine if there is a need for an adjustment to September calculations.

Methods

Fish catch data and effort during September and October of each year were obtained from standard RGSM Population Monitoring Program results. Numbers of RGSM needed for autumn augmentation were calculated separately for September and October of each year by using the decision tree and calculations given above. Streamflow forecast data was obtained from the NRCS 50% exceedance streamflow forecast for the Otowi Gage for the March through July period. Numbers of RGSM needed were transformed to a percentage of maximum (299,000) and modeled as a logistic regression assuming a quasibinomial error structure. A generalized linear regression model was used to predict numbers of RGSM needed from the forecasted streamflow and month RGSM catch data was collected. Model predictions were back-transformed and multiplied by 299,000 to estimate numbers of RGSM needed.

Results

Use of October RGSM catch rates in lieu of September data resulted in equal or higher estimates of fish in most years, but numbers varied by year and no consistent correction factor could be applied (Table 1). Annual differences in the calculations ranged from -8,000 to 205,000. Four years, 2011, 2018, 2020, and 2021 had differences of 97,000 to 205,000. The average difference was 35,900; however, excluding the four years with the largest difference reduced the mean to < 10,000. All years with large differences were when September catch-rates were ~2 fish per 100m² or lower. The largest average differences between September and October were in intermediate years with predicted streamflow between 400 and 600 KAH, resulting in differences of about 60,000 fish (Figure 1).

Table 1-Forecasted 50% exceedance streamflow (thousand acre-feet, KAF) for March through July at the Otowi gage, New Mexico, by year, and the estimated number of Rio Grande Silvery Minnow required for augmentation based on observed catch-rates in September and October, the difference between those estimates, and the observed mean discharge (cfs) at the San Acacia, New Mexico, gage.

Year	Forecast (KAF)	September Estimate	October Estimate	Difference	Mean discharge
2002	170	257,000	266,000	9,000	464.6
2003	465	291,000	283,000	-8,000	192.1
2004	NA	94,000	114,000	20,000	2.37
2005	NA	0	0	0	156.6
2006	NA	0	35,000	35,000	279.5
2007	NA	0	0	0	186.3
2008	1170	0	0	0	259.4
2009	650	0	0	0	252.6
2010	750	112,000	102,000	-10,000	201.2
2011	370	0	205,000	205,000	134
2012	335	261,000	299,000	38,000	26.1
2013	235	293,000	295,000	2,000	1392
2014	230	274,000	299,000	25,000	145.6
2015	395	184,000	230,000	46,000	74.8
2016	435	0	0	0	63.9
2017	920	0	0	0	229.6
2018	141	123,000	269,000	146,000	44.6
2019	1020	0	0	0	144.3
2020	385	171,000	268,000	97,000	26.5
2021	415	124,000	237,000	113,000	43.9
2022	375				

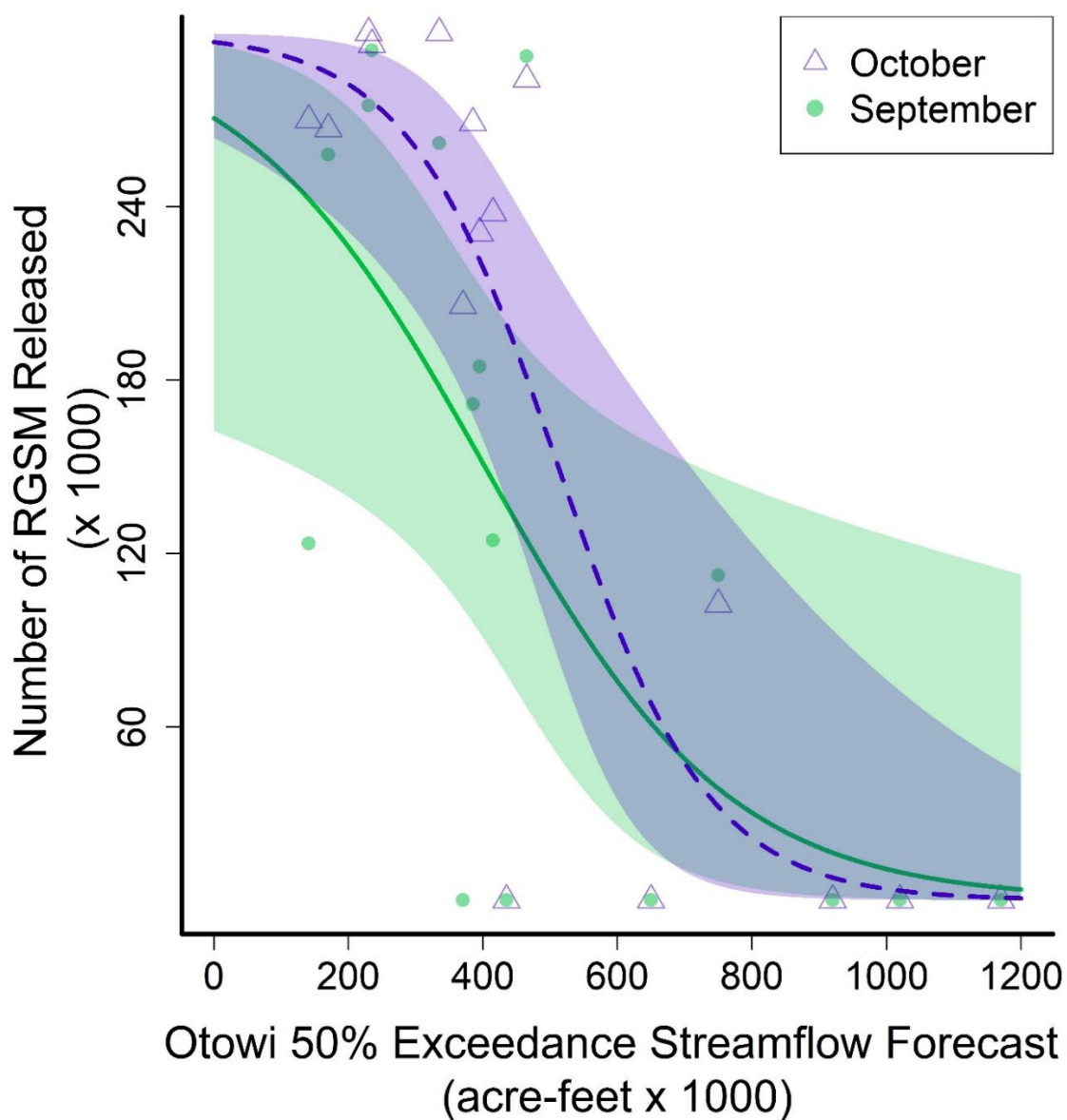


Figure 1 – Predicted number of hatchery Rio Grande Silvery Minnow in September (solid green line) and October (dashed purple line) that need to be released based on expected streamflow at the Otowi, New Mexico, gage (USGS gage 08313000).

Discussion

There was generally good agreement ($< 50,000$ difference) between September and October calculations except in 4 of the 20 years (2011, 2018, 2020, and 2021). Three of those four years (2018, 2020, 2021) had low CPUE (~ 1.0 or lower) in September followed by < 50 cfs average discharge in September, which suggests channel drying plays at least some role in the differences between September and October. Some differences may be attributable to sampling error and variability in the CPUE estimates. The average difference in the augmentation calculation was 35,900. However, the average difference in years where the September mean discharge was below 100 cfs was $\sim 66,000$, and $\sim 20,000$ when the mean was over 100 cfs. The year with the largest difference was 2011, with no clear explanation why the change was so large. Overall, October estimates generally suggested more fish needed to be released compared to September estimates. Any excess fish produced could be released to make up for this difference.

Appendix B

Evaluation of augmentation on numbers of potential spawning Rio Grande Silvery Minnow from long-term population monitoring

Introduction

Captive breeding has been an integral part of Rio Grande Silvery Minnow recovery *Hybognathus amarus* (Osborne et al. 2020). The captive breeding program for Rio Grande Silvery Minnow began in the early 2000s, shortly after being listed as an endangered species in the United States (USFWS 1994). Because the species is short-lived with most of fish being age-1 each spring (Horwitz et al. 2018), the demographic resilience of the species can be greatly reduced even after a single year of poor recruitment (Archdeacon et al. 2020). Augmentation with wild fish is intended to restore demographic resilience by increasing the potential spawning stock each spring and preventing losses of genetic diversity (Osborne et al. 2012).

Captive breeding programs are frequently used to assist in recovery of freshwater fishes and mussels, but many are not evaluated for effectiveness in several key areas (Rytwinski et al. 2021). Post-release monitoring is important for determining captive programs are working as intended and helping to reach conservation goals. Rio Grande Silvery Minnow are ideal for these evaluations, as both long-term programs monitoring genetic indices and population indices have been in place nearly two decades. Long-term genetic monitoring revealed that diversity has been maintained since genetic monitoring began (Osborne et al. 2020), but it is difficult to establish a causal relationship with augmentation. No formal analysis of the effects of the augmentation on spawner abundance have been conducted, but this is greatly simplified because nearly all hatchery-reared Rio Grande Silvery Minnow receive visible implant elastomeric tags (VIE) to distinguish them from wild-spawned fish. Here, pre-spawning abundance and distribution of wild fish is compared to the sum of wild and hatchery fish to determine if augmentation is improving the abundance and distribution of Rio Grande Silvery Minnow prior to spawning.

Methods

The Middle Rio Grande is defined here as the Rio Grande from Cochiti Dam downstream to Elephant Butte Reservoir, approximately 300 km depending on the Elephant Butte Reservoir water elevation. Standard monitoring for Rio Grande Silvery Minnow occurs pre-spawning in April each year at 20 sites until 2017, when the number of sites was increased to 30. Sites are placed from the Angostura Diversion Dam downstream to the confluence with the Low-Flow Conveyance Channel (Figure 1) and were selected as representative sites but have similar numbers of Rio Grande Silvery Minnow compared to randomly selected sites (Archdeacon et al. 2015).

Fish are collected by using a 3.0 m x 1.8 m beach seine (mesh size = ~5 mm). Fish were collected in 18 to 20 seine hauls in discrete mesohabitats (riffle, run, pool, backwater) either in the main channel or along a shoreline. Seine hauls were standardized to specific numbers of habitats and enough hauls were performed to limit

within-site sampling variability (Archdeacon et al. 2020). From 2008 to 2019, all hatchery-raised Rio Grande Silvery Minnow were given a VIE mark to distinguish them from wild fish. The total number of both wild and hatchery Rio Grande Silvery Minnow was summed for a site and the total area seined was recorded as a measure of effort within a site. Data from April of year, from 2009 to 2020, were used for analysis as all wild Rio Grande Silvery Minnow could be distinguished from hatchery fish.

Catch-per-unit-effort (CPUE) and naïve occupancy (uncorrected for detection probability) were used as proxies for abundance and distribution. Generalized linear mixed-effects models were used to estimate April expected (e.g. average) CPUE and occupancy.

Raw counts of wild Rio Grande Silvery Minnow and all Rio Grande Silvery Minnow were modeled separately as functions of prior year May discharge at the Central gage (gage #) as a measure of cohort strength, $\log_e$ of effort used as an offset, and site as a random effect. Naïve occupancy was modeled similarly using a logistic regression where detections were coded as 1 and non-detections as 0.

Results

Both CPUE and occupancy were increased in April by addition of hatchery fish (Table 1). Although the interaction term of hatchery fish and discharge as not significant, it is apparent the effect is greater when wild fish CPUE is low following years of low May discharge (Figure 2). Occupancy was similarly affected by the addition of hatchery fish, with an increase in occupied sites more apparent following years of low May discharge (Figure 2).

Table 1 – Parameter estimates, standard error (SE) and P-value for Rio Grande Silvery Minnow models estimating catch-per-unit-effort (CPUE) and occupancy in April (pre-spawn) of wild (Intercept) and hatchery fish based on average daily discharge during the preceding May (Q) and their interaction.

Model	Parameter	Estimate	SE	P-value
CPUE				
	Intercept	-7.641	0.230	<0.0001
	Q	0.0009	0.0001	<0.0001
	Hatchery	0.677	0.235	0.004
	Q*Hatchery	-0.0002	0.0001	0.054
Occupancy				
	Intercept	-1.80	0.261	<0.0001
	Q	0.001	0.0002	<0.0001
	Hatchery	0.693	0.299	0.02
	Q*Hatchery	-0.0002	0.0001	0.17

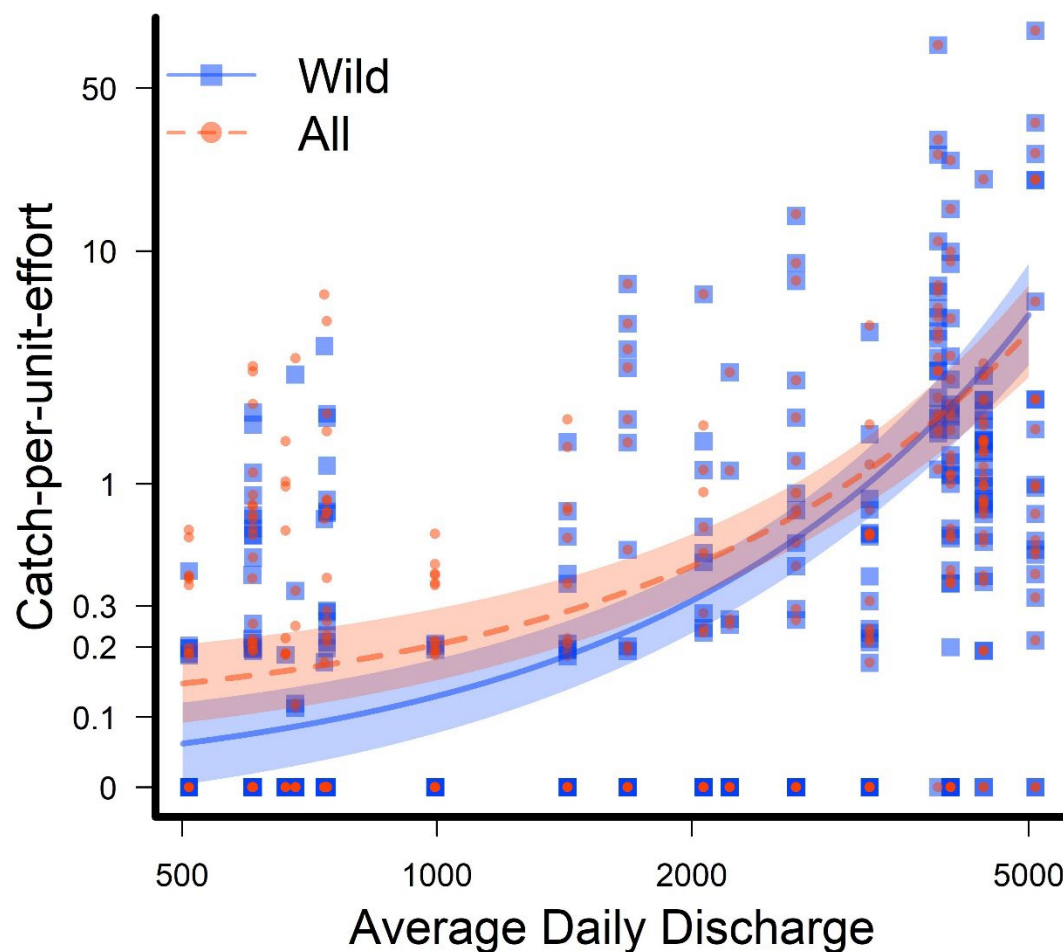


Figure 1 – Estimated catch-per-unit-effort (CPUE, fish per 100 m²) of potential spawning stock for wild (solid purple line) and all (wild and hatchery combined, dashed orange line) Rio Grande Silvery Minnow Potential in April (pre-spawn) at 20 sites in the Middle Rio Grande, New Mexico from 2002 – 2019. Average daily discharge (cfs) is from the month of May in the year prior at the Central gage. Points represent site-specific observations, lines the estimated mean, and the shaded area represents the 95% prediction interval.

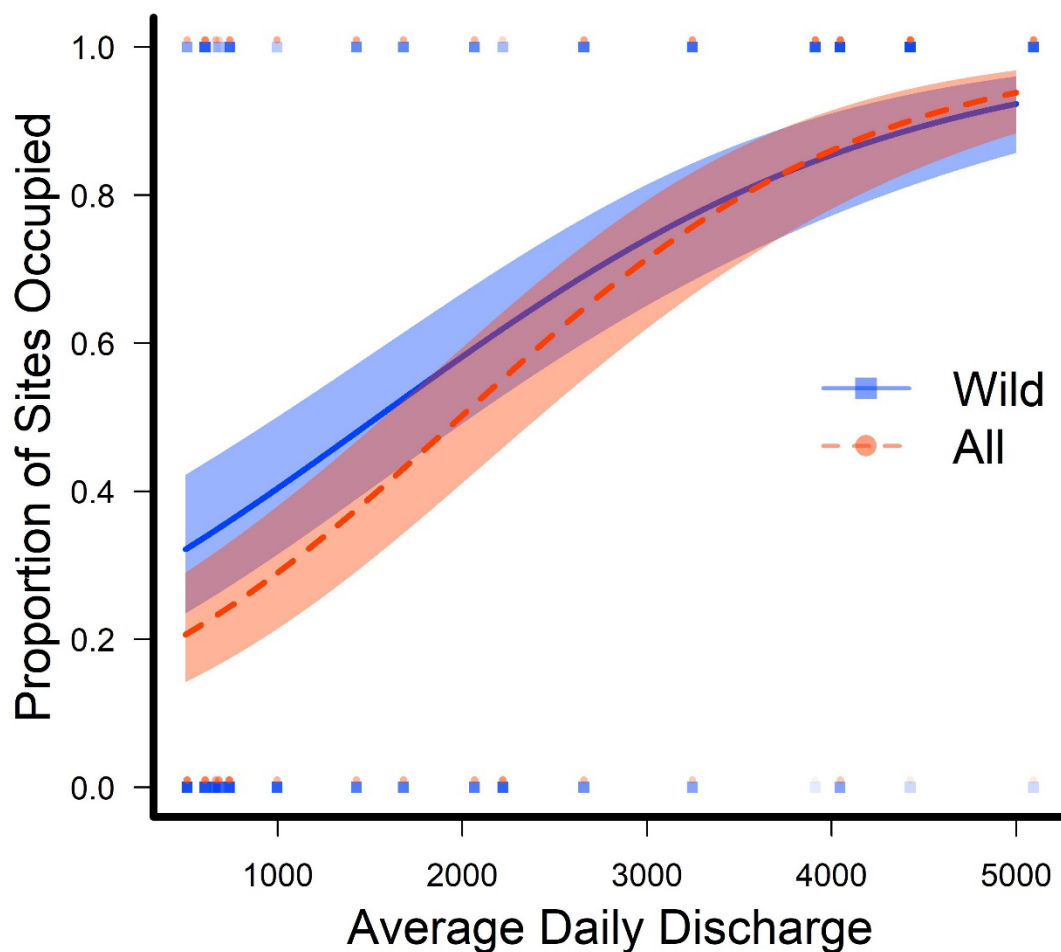


Figure 2 – Estimated proportion of sites occupied by potential spawning stock for wild (solid purple line) and all (wild and hatchery combined, dashed orange line) Rio Grande Silvery Minnow Potential in April (pre-spawn) at 20 sites in the Middle Rio Grande, New Mexico from 2002 – 2019. Average daily discharge (cfs) is from the month of May in the year prior at the Central gage. Points represent site-specific observations, lines the estimated mean, and the shaded area represents the 95% prediction interval.

Discussion

Stocking with hatchery fish following years of poor recruitment improved both the abundance and distribution of potential spawning stock of Rio Grande Silvery Minnow. The effect was greater following years with poor runoff, nearly doubling the CPUE and occupancy compared to that of only wild fish in years < 1,000 cfs mean daily discharge. However, the effect of adding hatchery fish is diminished as average daily discharge

approaches or exceeds 2,000 cfs, as fewer hatchery fish are released in those years. While genetic diversity has been maintained (Osborne and Turner 2021), improvements can still be made to the augmentation program. Particularly, releasing more fish or improving post-release survival could have a significant positive effect on the number of potential spawning fish following poor recruitment years, which in turn can allow Rio Grande Silvery Minnow to recover rapidly after population bottlenecks. Overall, long-term population monitoring combined with marking hatchery-reared Rio Grande Silvery Minnow demonstrates that augmentation of wild populations

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