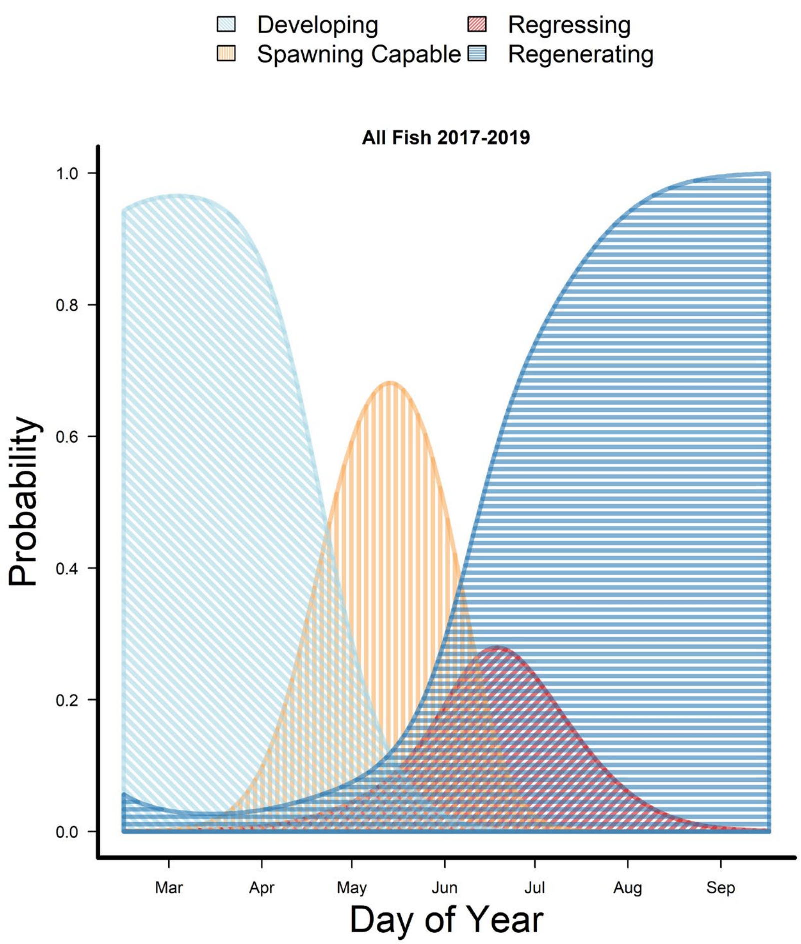
REPRODUCTIVE PHENOLOGY OF WILD AND HATCHERY-REARED RIO GRANDE SILVERY MINNOW



Thomas P. Archdeacon, Tracy A. Diver, and Rachel E. Grey
United States Fish and Wildlife Service

New Mexico Fish and Wildlife Conservation Office
3800 Commons N.E.
Albuquerque, New Mexico, 87109

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DRAFT

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Prepared by:

Thomas P. Archdeacon, Tracy A. Diver, and Rachel E. Grey

U.S. Fish and Wildlife Service
New Mexico Fish and Wildlife Conservation Office
3800 Commons N.E.
Albuquerque, New Mexico 87109

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DISCLAIMER

The findings and opinions in this report are the authors and do not necessarily represent the views of the U.S. Fish & Wildlife Service or U.S. Government. The data here are considered preliminary and subject to change under future examinations.

DATA AVAILABILITY STATEMENT

The data used in this report are available from the lead author and will be curated in a public data repository upon completion of histological tissue examination.

EXECUTIVE SUMMARY

The study of reproductive phenology through ovarian development can help answer a wide range of questions needed for the management of imperiled species. For fishes, baseline reproductive timing may be used to guide water management strategies to enhance recruitment or stimulate spawning. The reproductive cycle in gonochoristic fish is categorized into five ovarian phases within a continuum: immature fish that are not and have never been reproductively active; the developing phase signals the beginning of the reproductive season and is characterized by gonadal growth and development in preparation for spawning; the spawning capable phase characterized by advanced gamete development and oocyte maturation; the regressing phase signaling the end of the reproductive season, and the regenerating phase (e.g., resting) where mitotic proliferation occurs prior to the next spawning cycle.

The peak of Rio Grande Silvery Minnow spawning is generally the middle of May and has been inferred from the appearance of larvae and eggs in the river. Our specific objectives were to document the reproductive and spawning seasons of both wild and hatchery-stocked female Rio Grande Silvery Minnow through macroscopic and histological examination of ovarian tissue to improve water and other management decisions. We used a time-series of weekly to monthly ovary collections made from February through September, 2017-2019, to describe the reproductive cycle of both wild and hatchery-reared Rio Grande Silvery Minnow.

The proportion of spawning-capable females was low before April and after mid-June. The highest proportion of spawning-capable females occured during the month of May. A subsequent peak in regressing females followed in July and August, with most females in the regenerating phase by July. We observed few differences between hatchery-reared and wild fish. Instead, inter-annual variation among years was more significant and likely reflected environmental conditions and management actions. Channel drying in the San Acacia Reach in 2018 began before Rio Grande Silvery Minnow were fully capable of spawning and assured a year-class failure in that reach. Water management decisions should consider the intended effects (stimulate spawning or enhance recruitment), the reproductive readiness of the population, and antecedent environmental and water storage conditions to achieve the desired management outcome.

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INTRODUCTION

The study of reproductive processes is central to conservation biology and can help answer a wide range of questions in the management of imperiled species (Comizzoli et al. 2019). Anthropogenic alterations to freshwater ecosystems have resulted in population declines of many native fishes in the United States and around the world (Strayer and Dudgeon 2010; Costigan and Daniels 2012; Radinger et al. 2016). To mitigate declines, species recovery plans have been implemented to offset anthropogenic changes. Baseline information on reproductive cycles may be useful for identifying ultimate and proximal drivers of spawning. In turn, this information may be used to guide water management strategies or develop designer environmental flow regimes (*sensu* Acreman et al. 2014) that enhance spawning, mitigate threats to reproductive success or understand the effects of climate change on species reproduction, particularly in flow regulated rivers. Thus, understanding variation in reproductive cycles in temperate fishes is important for mitigating population declines.

The reproductive cycle in gonochoristic fishes is comprised of five phases: one gonadotropin-independent phase of immature fish and four gonadotropin-dependent phases of mature fish (Figure 1; Brown-Peterson et al. 2011). Immature fish are in the gonadotropin-independent phase, have never reproduced and are considered outside the reproductive cycle. As fish undergo gonadotropin-dependent gonadal growth and gamete development they become sexually mature and enter the developing phase of the reproductive cycle. Once a fish has reached sexual maturity, they can never go back to being immature. The reproductive cycle is comprised of four different phases: developing, spawning capable, regressing, and regenerating. The developing phase is characterized by gonadal growth and development in preparation for spawning. Fish enter the spawning capable phase when they can spawn due to advanced gamete development and oocyte maturation (but this does not imply the fish is actively spawning). The end of the reproductive season is known as the regressing phase and includes fish that have spawned and fish that were capable of spawning but did not and are actively resorbing ova. Fish then move into the regenerating phase where mitotic proliferation occurs prior to the next spawning cycle. The reproductive season encompasses the fish in developing, spawning capable, and regressing phases, whereas the spawning season is only fish in the spawning capable phase (Heins and Brown-Peterson 2022).

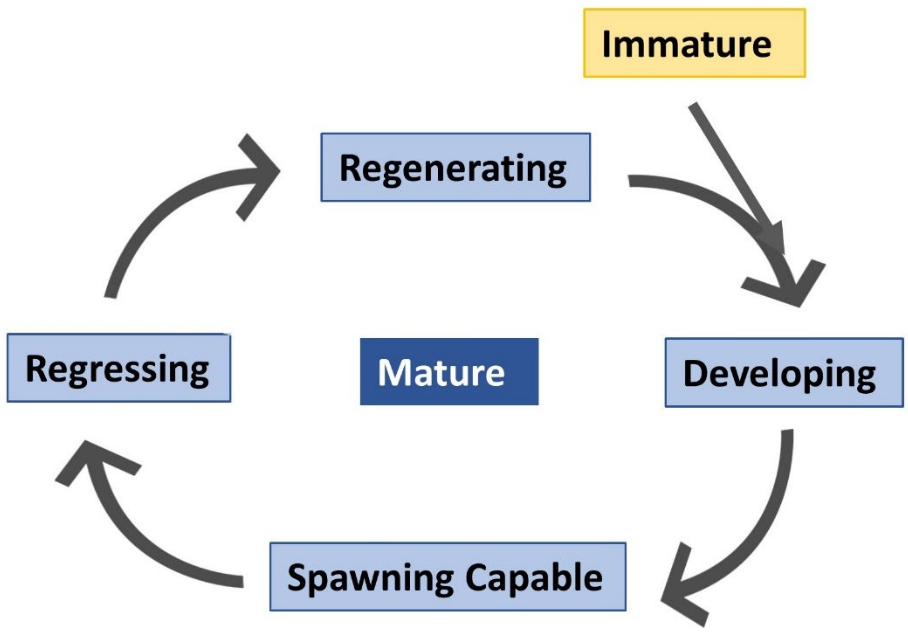

Figure 1-Conceptualized reproductive cycle of female gonochoristic teleost fishes broken by reproductive phase. The reproductive season encompasses the developing, spawning capable, and regressing phases, and the spawning season occurs when fish are in the spawning capable phase.

Understanding how management actions aid or impede the natural reproductive ecology of a species is critical information for designing population supplementation programs. Release of hatchery-reared individuals must be appropriately timed to improve reproductive output and the ecological effectiveness of the propagation program. Recovery plans for many endangered fishes often include the production and release of hatchery-reared individuals to restore self-sustaining populations (Mueller 2003; Ryden 2005; Archdeacon et al. 2023). Supplementation programs (*senus* Claussen & Philipp 2023) have been implemented with the objective to increase population size and avoid unnatural loss of genetic diversity (Miller and Kapuscinski 2003; George et al. 2011; Osborne et al. 2020). Yet, to meet recovery goals, survival and reproduction of hatchery released individuals must occur. However, trade-offs exist in management of endangered species, and it is important to assess the costs and benefits of management actions. A successful propagation program must evaluate both the economic (i.e., survival and cost-benefit) and ecological (i.e., life history traits, genetic diversity) effectiveness to ensure recovery efforts are maximized. Many supplementation programs fail in key areas, including post-release monitoring (Rytwinski et al. 2021).

The Rio Grande Silvery Minnow *Hybognathus amarus* (RGSM) was once abundant and widespread throughout the Rio Grande watershed (Treviño-Robinson 1959; Bestgen and Platania 1991). Rio Grande Silvery Minnow belong to a unique reproductive guild of pelagic broadcast spawners (Balon 1981; Platania and Altenbach 1998). Many of these fishes occur in freshwater streams of the western United States and are imperiled (Dudley and Platania 2007; Hoagstrom and Turner 2015; Worthington et al. 2018). For RGSM, reproductive success is closely tied to the magnitude and duration of the snowmelt driven spring runoff in the Middle Rio Grande (MRG) basin (Yackulic et al. 2022). Extensive, anthropogenic modifications to the MRG, flow regime, and multi-year droughts have contributed to the decreasing range and abundance of RGSM since the mid-20th century (Bestgen and Platania 1991; Dudley and Platania 2007). In response, RGSM was listed as endangered in 1994 (USFWS 1994) and hatchery supplementation began in the early 2000s (Archdeacon et al. 2023).

Supplementation of wild populations with hatchery fish is a critical component of recovery of Rio Grande Silvery Minnow, ideally maintaining or improving genetic diversity (Alò and Turner 2005) but more realistically slowing genetic erosion following multiple population collapses (Osborne et al. 2023). Currently, autumn fish releases are the standard protocol of the RGSM Augmentation Plan (Archdeacon 2023). Fish are released in November and early December, after irrigation diversions have ceased for the year and flows have stabilized. Benefits of this strategy include reduced maintenance costs and reduced time in captivity, which reduces the risk of domestication selection. Pre-spawning demographic surveys in April suggested fish released in November appeared to have similar survival as fish released in February (Archdeacon et al. 2023), likely because mortality occurs nearly immediately after release in the river (Yackulic et al. 2022). However, there may be differences in reproductive readiness between wild and hatchery fish, or between different release seasons of hatchery fish. Evaluating the trade-off between reproductive readiness and costs of production is critical for effective management and should be evaluated based on the species and system. After survival, reproduction of hatchery released individuals in the wild is the second step to reestablishment, and cost-benefit analyses should ideally be based on stocked fish that have begun reproducing (Durst 2009).

Spawn timing of Rio Grande silvery minnow is generally the middle of May and has been inferred from first appearance of larvae in collections (Krabbenhoft et al. 2014) and observations of eggs in the drift (Dudley et al. 2023). However, the full reproductive cycle is best described by examining reproductive tissue of females. A better understanding of intra- and interannual variation in the reproductive cycle would not only inform supplementation practices, but also help water managers make decisions on the best uses for limited water supplies. Our specific objectives were to document the reproductive and spawning seasons of both wild-spawned and hatchery-reared female Rio Grande Silvery Minnow through macroscopic and histological examination of ovarian tissue. We used a series of monthly to weekly tissue collections made from late winter through early autumn to describe the reproductive cycle of Rio Grande Silvery Minnow.

METHODS

Study Site

The MRG extends from downstream of Cochiti Dam to Elephant Butte Dam (Figure 2), approximately 337 km, of which approximately 300 km is upstream of Elephant Butte Reservoir. The MRG is divided into four reaches by diversion dams: Cochiti Reach, Angostura Reach, Isleta Reach, and San Acacia Reach (Figure 1). The MRG in the Angostura, Isleta, and San Acacia reaches is a large, sand-bottomed stream (Swanson et al. 2011). Currently, the only surviving population of RGSM occurs in the MRG (Bestgen and Platania 1991). Areas below the San Acacia and Isleta diversion dams frequently dewater or have substantially reduced flows between mid-June and the end of October each year, up to nearly 82 km in 2012 (Archdeacon 2016). We selected four locations for release of Rio Grande Silvery Minnow in the San Acacia Reach. Releases were split between late autumn (November or early December) and late winter (February) each year. Fish collection locations and release locations of hatchery fish are shown in Figure 2. The flow regimes during the study varied greatly among years; 2017 and 2019 were extremely high spring runoff years, while 2018 was one of the lowest spring runoff years on record (Figure 2).

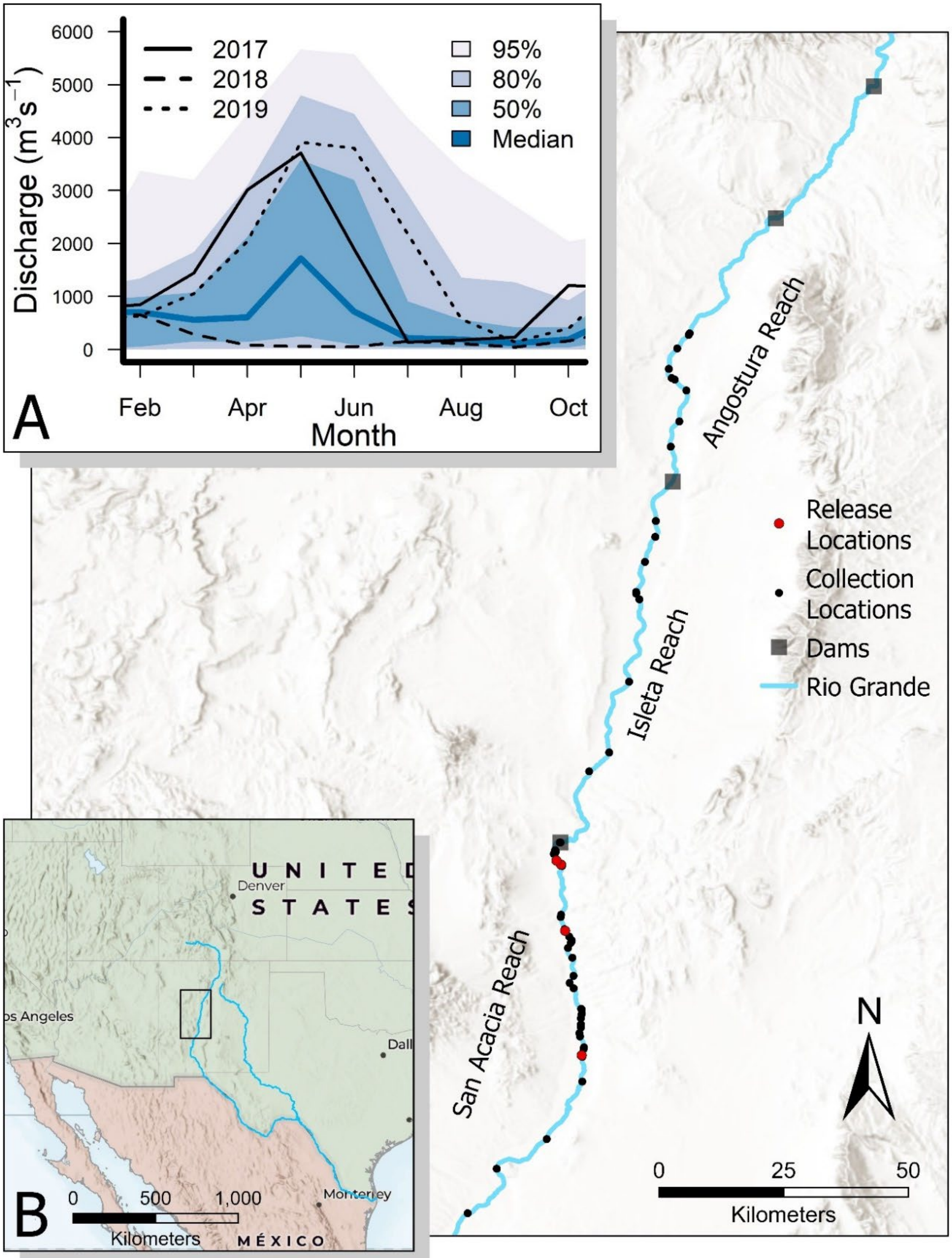

Figure 2-Hatchery fish release and ovarian tissue collection locations for Rio Grande Silvery Minnow in the Middle Rio Grande of New Mexico, 2017-2019. Observed flow during the study period and monthly flow quantiles (1970-2020) is shown in inset A; the study area is shown in inset B.

Fish Collection

We used a variety of fish collection methods depending on discharge and conditions at a site. Primarily we used beach seines, but supplemented seining with fyke nets during high flows and backpack electrofishing during low flows. In 2017 we collected only wild fish, from all three reaches (Figure 1) to compare inter-reach differences. In 2018 and 2019, we focused only on the San Acacia Reach and the comparison among two groups of hatchery fish and wild fish. Collections were made biweekly from March through September. In 2019, we focused on the suspected spawning season, with approximately monthly collections February-April and July-September, with weekly collections beginning in late April through early June.

Fish Preservation

Rio Grande Silvery Minnow were euthanized with MS-222 and preserved in 10% zinc-buffered formalin during field collections. Abdomens of fish were slit to allow the formalin to penetrate the body cavity to ensure tissues are properly fixed for histological examination. After 5 to 7 days, fish were transferred to a water bath for 5 to 7 days to remove formalin prior to handling, followed by subsequent dehydration in 35% ethanol, and 50% ethanol for 5 to 7 days. Specimens were placed in 70% ethanol for long-term storage until laboratory examination, described below.

Laboratory Examination

All preserved specimens were measured (standard length; SL) to the nearest 0.1 mm and blot-dried prior to being weighed to the nearest 0.01 g. Sex was determined by examination of gonads; paired ovaries were removed, dry-blotted and weighed to the nearest 0.001 g for gonadosomatic index calculations ([GSI]; gonad weight/body weight*100). Ovarian phase of development was classified for all ovaries based on a standardized scheme (Table 1; Brown-Peterson et al. 2011; Heins and Brown-Peterson 2022): immature (IM), developing (DE), spawning capable (SC), regressing (RN), and regenerating (RG). These classifications may change after future histological examination. Examples of each phase are shown in Figure 3.

Table 1-Descriptions of reproductive phases observed in ovaries of gonochoristic teleost fishes.

Phase	Description
Immature	Small, thin ovaries with little space between oocytes. Ovaries are easily torn with forceps. Small oocytes give the appearance of a smooth, uniform ovarian tissue, lacking graininess or distinct oocytes. Often colorless or with very faint color. Posterior portion of ovary very thin, very wispy, almost transparent so you can see the peritoneum; not filled out. Hollow space in the posterior
Developing	Enlarging ovaries that appear more turgid; ovaries become larger and more rotund in shape with less interstitial space between oocytes. Earlier phases of developing may appear grainier due to presence of primary growth oocytes among larger, more developed oocytes. Ovary coloration is mostly homogeneous and becomes more opaque/semi-translucent (during earlier developing phases with primary growth oocytes) or somewhat cream-yellow in color. Oocyte size is variable; however, developing oocytes generally look more turgid.
Spawning Capable	Ovaries will have a speckled or granular appearance due to hydrated oocytes; dark, external ovarian wall is thinner and more transparent. Ovaries are large, robust and may appear swollen. Oocytes are easily visible macroscopically and are variable in color; most appear more peach in color.
Regressing	Ovaries become more flaccid and are generally smaller. Oocytes appear grainer in texture and coloration is widely variable, with remnant peach and/or cream-yellow oocytes mixed in with small, translucent oocytes. Oocyte size is also highly variable.
Regenerating	Smaller ovaries relative to SC or DV with oocytes that appear similar to the immature phase; however, ovaries are larger. The cells are not as transparent with the color being more vivid and varied. Small oocytes give the appearance of a smooth, uniform ovarian tissue with little space between oocytes; do not contain macroscopically distinct oocytes, which are more uniform in size and are very small. Posterior portion is more ‘full’ than immature fish; oocytes are relatively consistent throughout ovary. Posterior oocytes are very small.

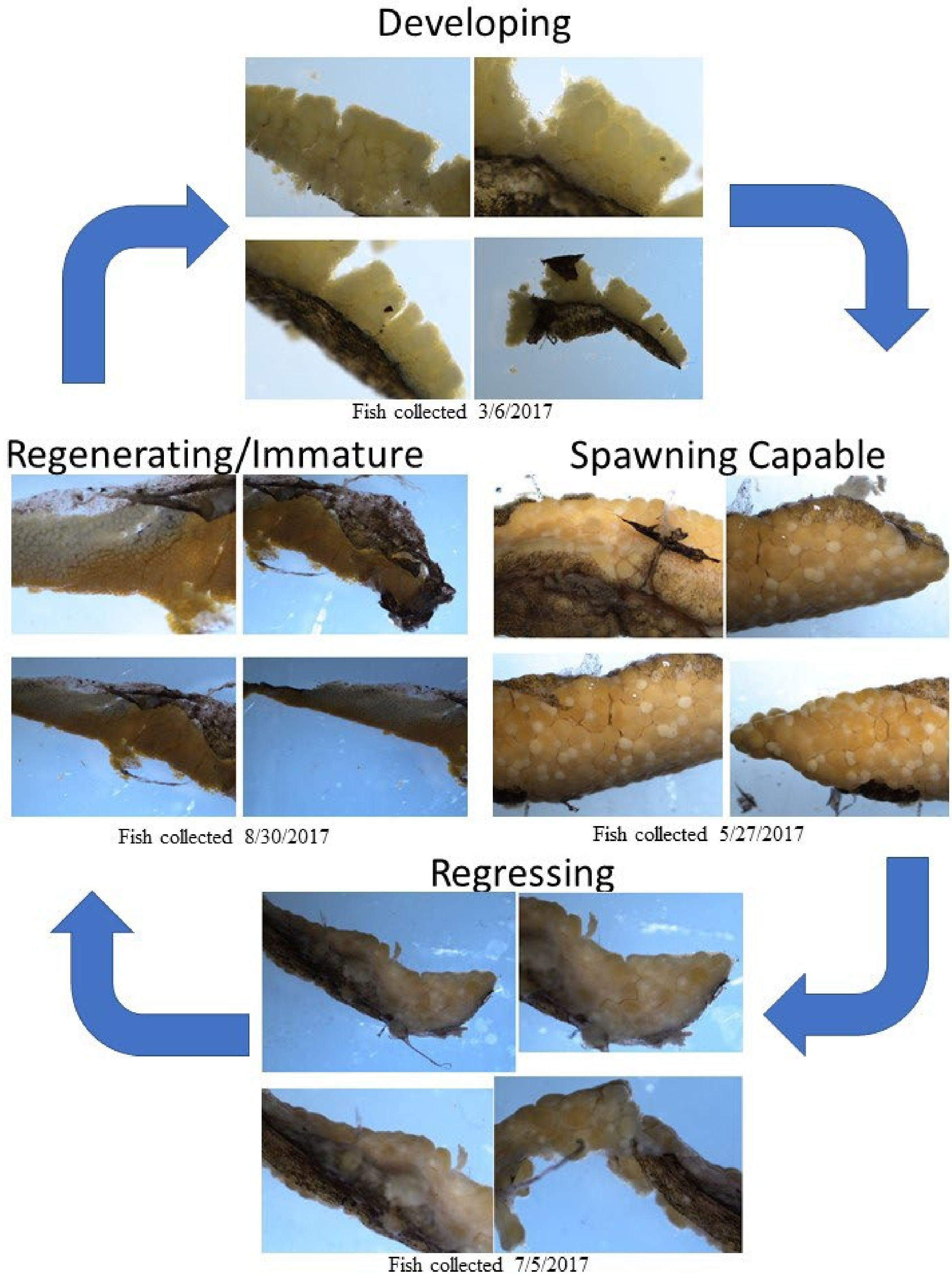

Figure 3-Representative images of ovaries in each reproductive phase of Rio Grande Silvery Minnow reproductive development.

Analyses

We predicted reproductive phase based on ordinal day of the year. We used multinomial categorical regressions to predict the percent of the population in each phase for each day of the year for all fish, hatchery and wild fish, and each year separately. Multinomial logistic regression is an extension of binary logistic regression and that allows for more than two categorical outcomes. We fit the model:

$$\text{logit}(Count_p) = \beta_1(day\ of\ year) + \beta_2(day\ of\ year^2)$$

Where the count per reproductive phase has a binomial distribution with a mean of y and variance of σ^2

$$\text{logit}(Count_p) \sim B(y, \sigma 2)$$

We used a quadratic term in the regression to account for the non-linear relationship between day of year and reproductive phase. Models were fit in R (version 4.1.1) using package nnet (version 7.3-18).

RESULTS

We collected 2,789 individual Rio Grande Silvery Minnow from 2017-2019. Of those, we were able to remove usable ovaries from 1,010 females among all groups. Adult fish, whether of hatchery origin or wild, were difficult to obtain after the spawning season in 2018 and 2019, resulting in low sample sizes, particularly in 2019 (Figure 4).

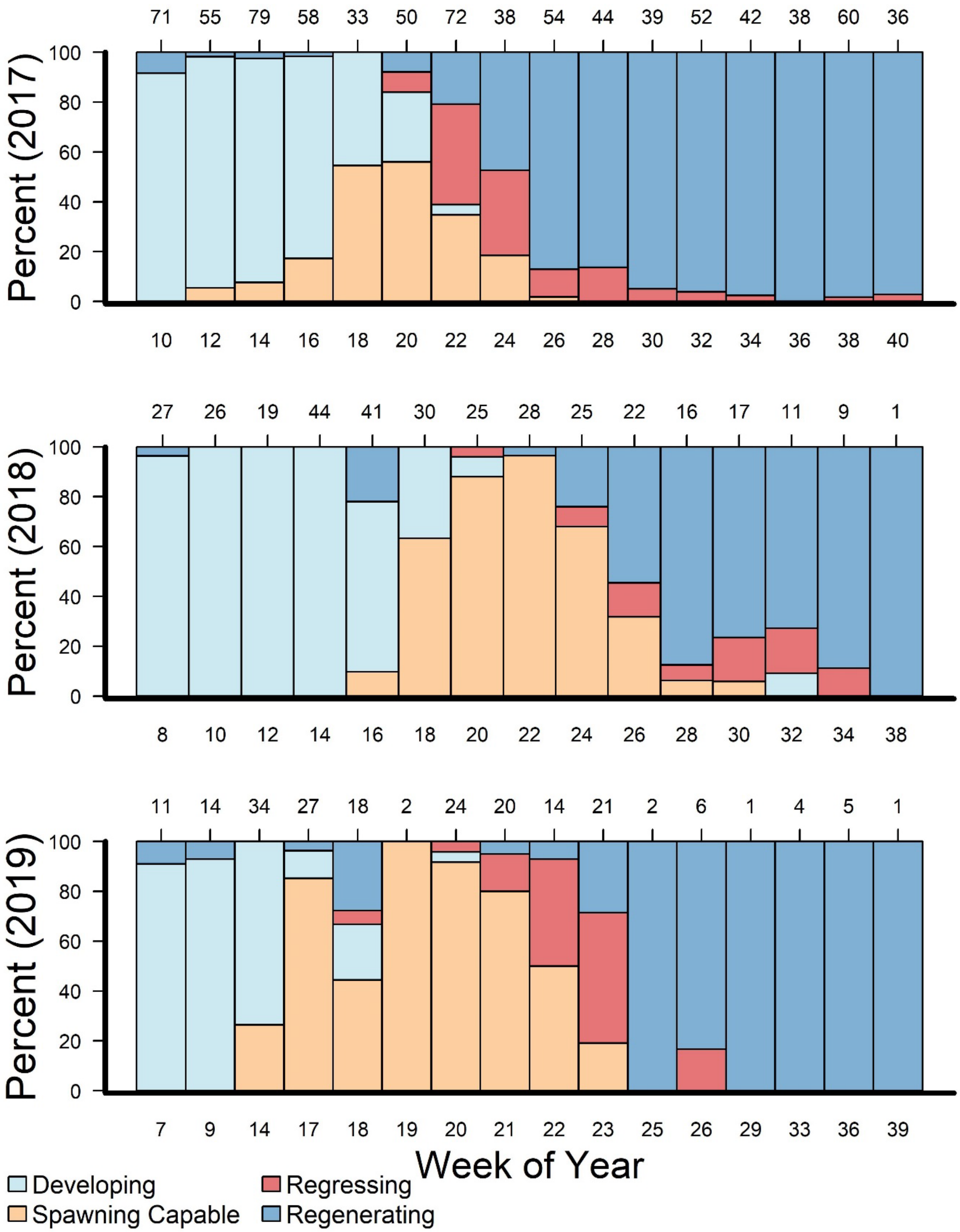

Figure 4-Frequency bar plot of each reproductive phase by week of year for Rio Grande Silvery Minnow in the Middle Rio Grande, New Mexico. Sample sizes for each collection are shown above the bars.

The reproductive season spanned the entire sampling period, whereas the spawning season spanned the middle of March through early July (Figure 5). However, the proportion of spawning-capable females was very low before April and after mid-June. The highest proportion of spawning-capable females occurs during the month of May. A corresponding peak in regressing females follows in June and July, with the majority of females in the regenerating phase by mid-June.

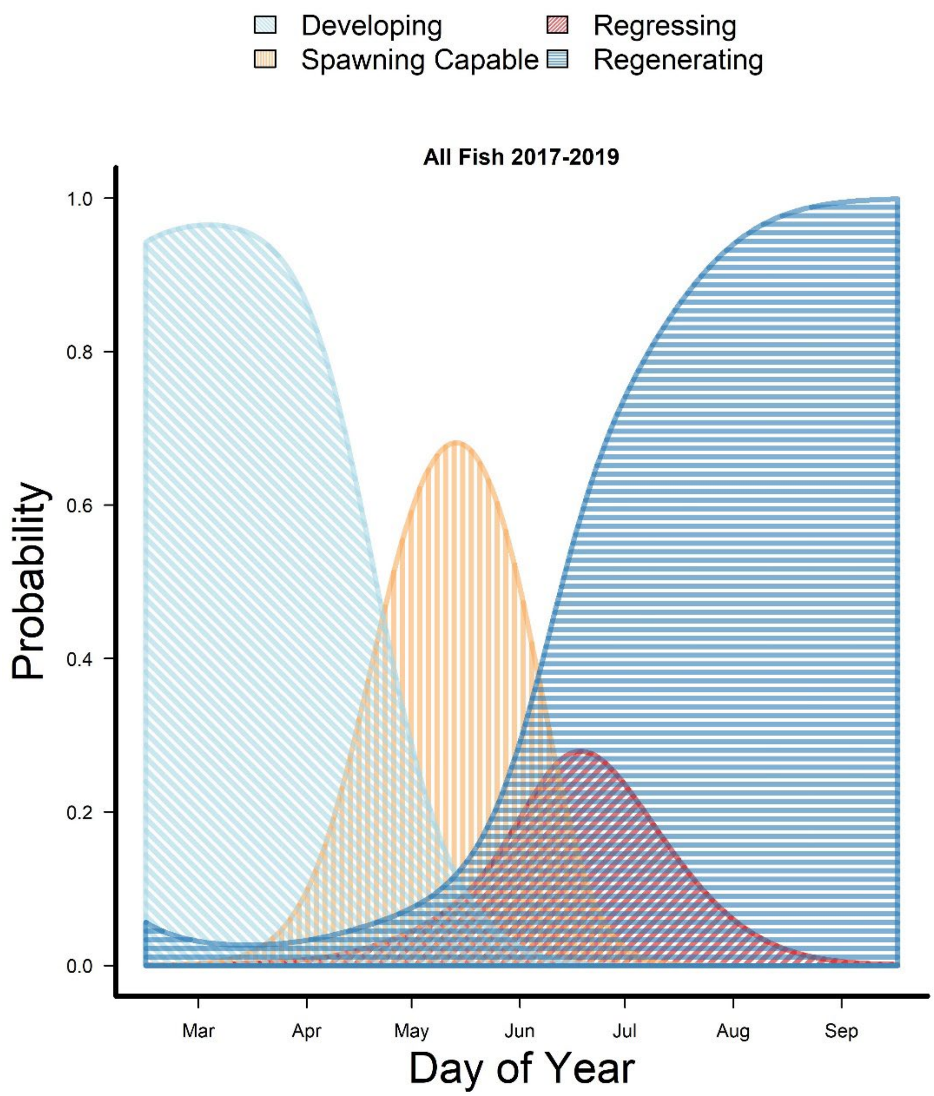

Figure 5-Estimated probability of each reproductive phase for Rio Grande Silvery Minnow by day of year, averaged over three years and combining hatchery and wild fish.

Comparison of reproductive timing of hatchery and wild females suggests there are few meaningful differences between the groups, although the higher peak in probability of spawning capable hatchery females during May and June may indicate hatchery fish have less plasticity in spawning season (Figure 6).

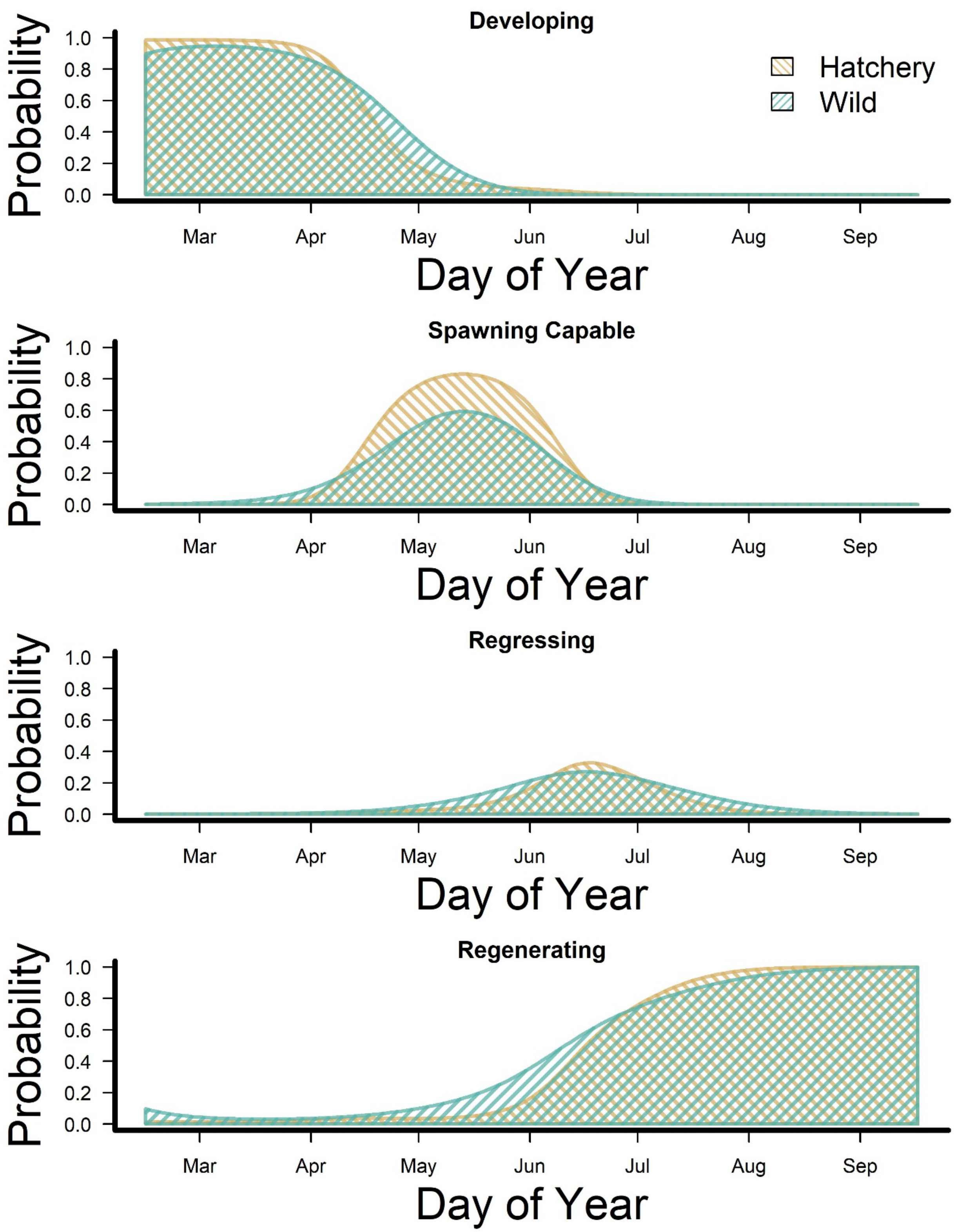

Figure 6-Comparison between hatchery and wild Rio Grande Silvery Minnow probability of reproductive phase over time, averaged over two years.

Within hatchery fish, there were very little differences in timing of reproductive phases based on season of release (Figure 7). Conversely, there was considerable variation among years with respect to each reproductive phase (Figure 8), indicating environmental conditions may be driving the reproductive season. Peak spawning appeared to occur later in 2018, which was an extremely low flow year (Figure 1), with spawning-capable and regressing fish present throughout the summer into autumn (Figure 4). Spawning was extended temporally in 2019, although this may have been due to low sample sizes later in the year. Nonetheless, regressing individuals began appearing as early as week 17 in 2019, three weeks earlier than 2017 or 2018, suggesting spawning began earlier in 2019 than the other years.

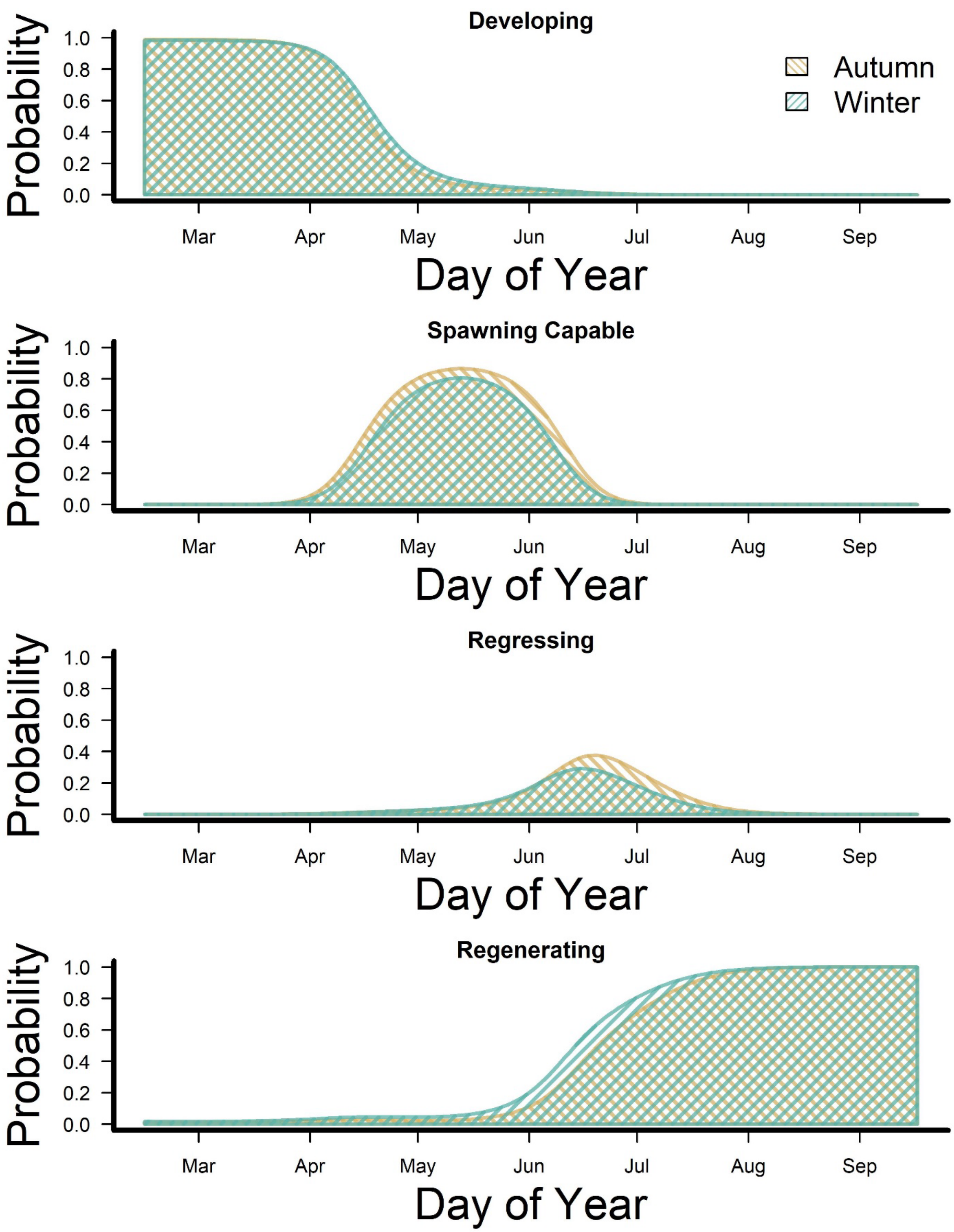

Figure 7-Comparison between season of release of hatchery Rio Grande Silvery Minnow probability of reproductive phase over time, averaged over two years.

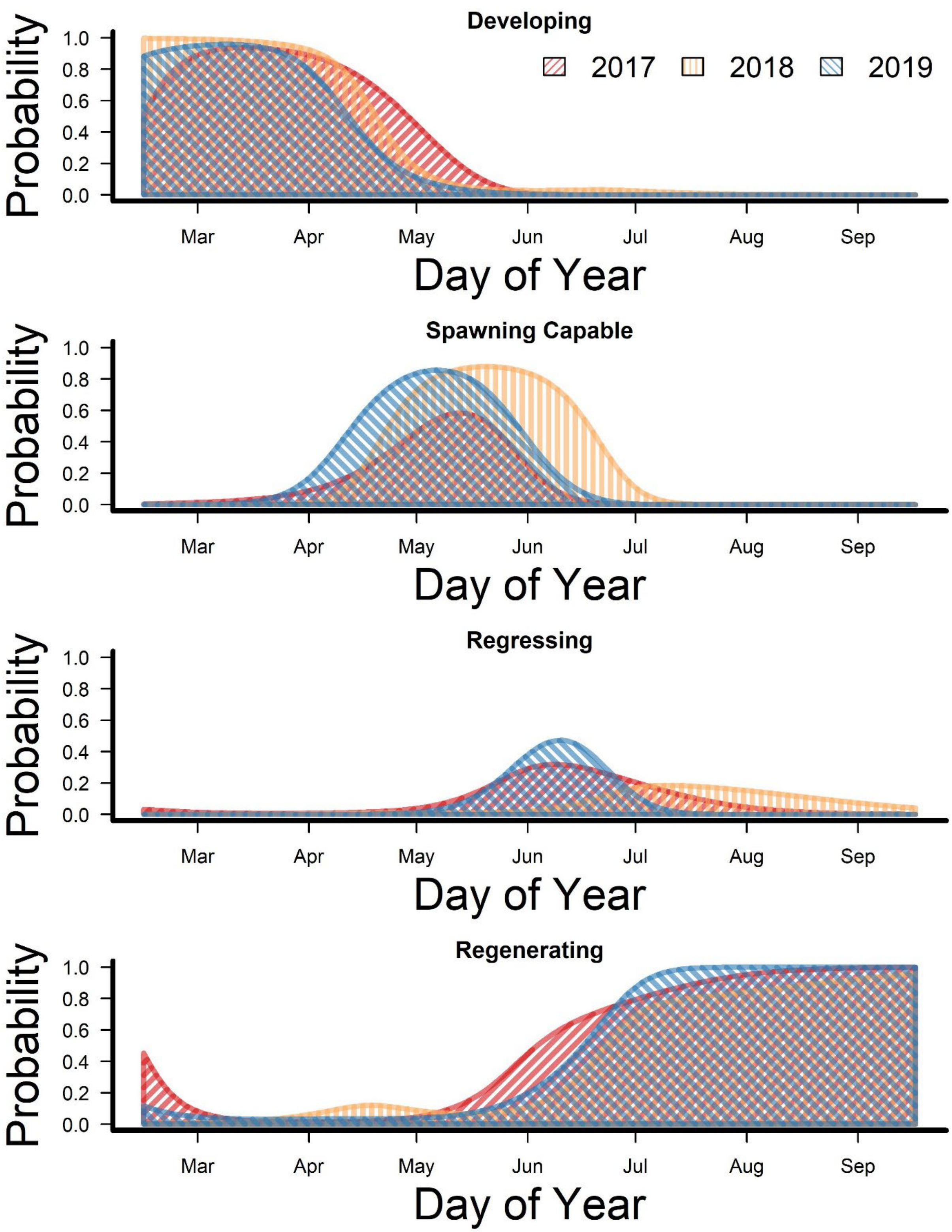

Figure 8-Comparison of Rio Grande Silvery Minnow probability of reproductive phase over three years.

DISCUSSION

Past comparisons of reproductive performance between hatchery and wild fish suggest hatchery fish are less fit than their wild counterparts, but nearly all studies have been conducted on salmonids (e.g., Araki et al. 2007, Araki and Schmid 2010; Bertnson et al. 2011). We employed an experimental release of hatchery fish during two different seasons, while also gathering baseline information on reproductive development timing of wild Rio Grande Silvery Minnow. While we are not able to directly compare reproductive contributions to subsequent generations, we were able to compare the reproductive phenology of hatchery and wild Rio Grande silvery minnow and determine that reproductive development was similar between hatchery and wild fish. Post-release monitoring is an important, and often ignored, aspect of hatchery supplenentation programs (Rytwinski et al. 2019) that allows for evidence-based changes to conservation programs. Here, we performed post-release monitoring of hatchery-reared fish to determine if hatchery fish had reproductive development timing like wild fish.

We observed few meaningful differences between reproductive timing of wild and hatchery Rio Grande silvery minnow. Small sample sizes hindered strong inferences in 2019, but overall differences in reproductive timing between years suggested annual variation, likely driven by local flow and climatic conditions, was greater than variation between hatchery and wild fish. One noticeable difference was that a higher proportion of hatchery fish appeared to be in the spawning-capable phase during peak reproductive periods. Possibly, these fish were more advanced because they were in better physical condition prior to release, receiving supplemental food, and held in warmer water during winter months. It would be expected that, if condition prior to release was an important factor, fish released in winter would be more like wild fish than hatchery fish released in the spring with respect to proportion of fish spawning and timing of spawn. However, comparisons between hatchery fish released in two different seasons did not support this, as both seasons had similar peaks in spawning-capable fish. Comparisons among different release seasons of hatchery fish also had minimal differences, suggesting that if a hatchery fish survives until spring following release, it likely enters the spawning-capable phase. Similarly, numbers of fish surviving to spawn appeared similar between the autumn and winter release groups (Archdeacon et al. 2023), suggesting efforts to improve supplementation practices may be better allocated to other considerations. For example, further research should be conducted to improve low survival rates of hatchery Rio Grande Silvery Minnow (Yackulic et al. 2022) to improve the effectiveness of the supplementation program and potentially reduce costs associated with fish production and rearing.

Overall, our work generally agrees with work based on larval collections (Krabbenhoft et al. 2014) and long-term egg collection data (Dudley et al. 2023). Back-calculated hatch dates of RGSM from 2016 and 2017 based on length suggest there is limited spawning and recruitment in early April, but the peak of successful recruitment to the larval stage occurs in early or mid-May and declines sharply in early June (Valdez et al. 2019; 2021). Note that this study and the work of Valdez et al. (2019; 2021) measure different aspects of reproduction and recruitment: we have directly measured reproductive readiness independent of recruitment, whereas Valdez et al. (2019; 2021) are estimating hatch dates of fish that successfully recruited to the larval or post-larval stage. These survivors have already been filtered through many ecological processes and may not necessarily reflect the reproductive status of the population, given the species has high variance in reproductive success (Osborne et al. 2005).

Management of water to improve recruitment should target mid-May through early June to coincide with the largest number of spawning-capable Rio Grande Silvery Minnow. In low water years, spawning appears to be delayed. Thus, smaller water pulses designed to stimulate spawning for collection of large numbers of eggs for broodstock, but not large enough to enhance recruitment, are better timed for the end of May into mid-June to trigger synchronized spawning events (Durham and Wilde 2008) and facilitate large collections of eggs for broodstock. Channel drying in 2018 in the San Acacia Reach occurred prior to. Females delayed spawning because there was no spawning cue. This is clearly observed in the later peak in spawning capable females in 2018 compared to 2017 and 2019, and the extended period we observed regressing females in 2018. Although recruitment is poor during low runoff years (Archdeacon et al. 2020), channel drying prior to spawn virtually ensured recruitment failure of that year-class in the San Acacia Reach.

The relatively short spawning season (April through June) is somewhat surprising when compared to other North American freshwater minnow species. Most minnows are batch-spawning, releasing multiple clutches of eggs throughout spring and summer (Heins and Rabito 1986; Quinn 2019; Heins and Brown-Peterson 2022). Even within the pelagic-broadcast spawning guild of freshwater minnows, RGSM appears to have a truncated spawning season. Plains Minnow *H. placitus*, Pecos River Bluntnose Shiner *Notropis simus pecosensis* in the Pecos River, New Mexico, and Smalleye Shiner *N. buccula* in the Brazos River, Texas, appeared to have a spawning season extending from April through September (Taylor and Miller 1990; Durham and Wilde 2008; Archdeacon et al. 2014). The short spawning season observed here may be due to the contemporary flow regime, where high spring-flows are followed by low flows and channel drying (Archdeacon & Reale 2020). Channel drying has historically occurred during extreme droughts but has become more frequent and regular in the 20th and 21st centuries after water abstraction and dam construction (Scurlock 1998). Rio Grande Silvery Minnow have been restricted to the Middle Rio Grande since the early 1960s (Bestgen and Platania 1991) and subjected to declining spring flows and total annual water volumes (Blythe and Schmidt 2018). Possibly, the truncated spawning season is an adaptation to changing flow regimes. However, four other species of pelagic broadcast-spawning minnows were extirpated from the Middle Rio Grande mid-20th century; the truncated spawning season of RGSM compared to the other species, tied strongly to spring runoff, may have staved off extinction of RGSM in the MRG.

Continuing work

A subset of all ovaries will be used for histological examination to refine macroscopic criteria. All gonads used for histological examination were infiltrated and embedded using the protocols for JB-4 resin as described in Sullivan-Brown et al. (2011). After infiltration, the gonad was cut dorso-ventrally into three subsections which were individually placed in separate molds. Once fully cured, the most posterior subsection was sectioned using an automated microtome at a thickness of 5 micrometers for a minimum of 20 sections with five sections being mounted per slide. Only odd number slides were stained using Hematoxylin and Eosin, even numbered slides are stored for alternative stains or future research needs. Each stained slide will be assessed using light microscopy and the section with most visible nuclei is used for determining gonad development phase. The developmental phase will be assigned using phases of oocyte development and oocyte size following Brown-Peterson et al. (2011) and Heins and Brown-Peterson (2022). Use of histological examination typically provides more accurate assignment of reproductive phase to ovaries and more robust conclusions than use of gonadosomatic index or macroscopic investigation alone but is more time consuming and costly (Brewer et al. 2008).

Thus, results presented here are preliminary and may be revised after histological examination. Although the major patterns are not likely to change, individuals in the tails of the distributions may change and influence statistical estimates, particularly in 2019 when sample sizes were small. Finally, categorical state-space models may better describe the distributions instead of multinomial regression models and may be used for future analyses of environmentally driven variation. State-space models are flexible hierarchical models that allow the separation of processes and observation error, better estimating the parameters of interest, and becoming more commonly applied to model time-series data (Auger-Méthé et al. 2021).

Conclusions

While we have not directly measured the relative contribution of hatchery fish to recruitment, we have shown that hatchery fish are developing similarly to wild fish. Genetic analyses that identify parent-offspring pairs are necessary to truly determine the contribution of hatchery fish to future generations of RGSM. However, we have demonstrated that hatchery fish follow similar reproductive phenology as wild fish. Further, we have provided evidence of delayed spawning in extreme low-water years, an important finding for water managers wishing to trigger a synchronous spawning event. Future efforts should focus on improving survival of hatchery fish to improve the effects of supplementation on populations and reduce costs. Inter-annual variation in reproductive timing is apparent and water management decisions should consider the intended effects (stimulate spawning or enhance recruitment), the reproductive readiness of the population, and antecedent environmental conditions.

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