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SEASONAL VARIATION IN REPRODUCTIVE CONDITION OF THE PECOS BLUNTNOSE SHINER (*NOTROPIS SIMUS PECOSENSIS*)

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ABSTRACT.—Reproductive strategies vary among freshwater fishes. Information on reproductive characteristics is important for water managers' efforts to protect and recover imperiled species. We describe aspects of the reproductive ecology of Pecos Bluntnose Shiner (*Notropis simus pecosensis*). We determined gonadosomatic index, examined ovarian condition, and counted mature ova to determine the seasonal reproductive timing and duration of Pecos Bluntnose Shiner in the Pecos River, New Mexico. Monthly changes in gonadosomatic index, ovarian stage, and number of mature ova per female suggest that Pecos Bluntnose Shiner has a reproductive season extending from April through September, with a peak occurring in June and July. In July, 93% ($n = 44$) of female Pecos Bluntnose Shiner were in reproductive condition, regardless of size. The highest number of mature ova counted (1498) was observed in a 59.5-mm (standard length) female from June 2009. Peak spawning activity coincides with sustained water releases from reservoirs; these releases have higher peaks and longer duration than natural rainfall events. Results of this study provide important insight on the reproductive biology of a threatened fish and may be useful in long-term conservation planning.

RESUMEN.—Las estrategias reproductivas varían entre los peces de agua dulce. La información sobre las características reproductivas es importante para los gestores de los ambientes acuáticos con el fin de proteger y recuperar especies en peligro. Describimos aspectos de la ecología reproductiva de la carpa (*Notropis simus pecosensis*). Determinamos el índice gonadosomático, las condiciones del ovario, y contamos los óvulos maduros para determinar la temporada de reproducción y la duración de las carpas en el Río Pecos, Nuevo México. Los cambios mensuales en el índice gonadosomático, la etapa de ovario y el número de óvulos maduros por hembra sugirieron que las carpas tienen una temporada reproductiva desde abril hasta septiembre, con un pico entre junio y julio. En julio, el 93% ($n = 44$) de las hembras se encontraron en condiciones reproductivas, independientemente de su tamaño. El mayor número de óvulos maduros contabilizados (1498) en una hembra de 59.5 mm (longitud estándar) fue en junio del 2009. El pico máximo de actividad de desove coincide con una liberación prolongada de agua de los embalses, con picos mayores y mayor duración que los eventos de lluvia naturales. Los resultados de este estudio proporcionan información importante sobre la biología reproductiva de un pez amenazado y pueden ser útiles en la planificación de su conservación a largo plazo.

Describing life history characteristics is critical for recovery of federal trust species. Reproductive information, such as seasonal timing and duration of reproductive activity, is required for managing imperiled species confined to rivers that are heavily managed for human water consumption. Determining the timing and duration of spawning for Great Plains riverine fishes is especially critical in areas subject to perennial low flows and summer drying, or that use reservoir releases to move water from one irrigation district to another. These reservoir releases may have negative effects on reproductive efforts of some reproductive guilds of fishes (Dudley and Platania 2007). Examining the reproductive biology and timing of imperiled fish species in streams subject to annual low flows and potential drying can inform water managers on methods to mitigate negative effects of water management on fish populations.

The range of Bluntnose Shiner (*Notropis simus*) has declined significantly since the species was described in 1875. Historically found in the Rio Grande and its major tributaries, the nominate species is extinct (Chernoff et al. 1982). The extant subspecies, the Pecos Bluntnose Shiner (*N. s. pecosensis*) now occurs only in the Pecos River of New Mexico, between Taiban Creek and Brantley Reservoir (Sublette et al. 1990, Propst 1999). Pecos Bluntnose Shiner was listed as federally threatened in 1987. Causes of the decline of Pecos Bluntnose Shiner include store-and-release flow manipulation, habitat loss, channel straightening, and groundwater pumping

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(U.S. Fish and Wildlife Service 1987, Robertson 1997, Propst 1999).

Pecos Bluntnose Shiner belongs to a reproductive guild of fishes characterized by buoyant, nonadhesive eggs that are broadcast-spawned directly into the water column (i.e., pelagic, broadcast-spawning minnows; Platania and Altenbach 1998). As with other pelagic, broadcast-spawning minnows, increasing stream flow prompts spawning activity, and eggs travel downstream for approximately 2 days before hatching (Platania and Altenbach 1998). Hatched larvae swim to low-velocity, stream-margin habitats (Wootton 1998). Pecos Bluntnose Shiner has a prolonged spawning season, based on the wide range of lengths within an age group and the presence of recently hatched individuals late in the season (Hatch et al. 1985). Discovering further histological evidence of a protracted spawning season would help interpret monthly length-frequency histograms and length-based growth analysis and age assignments (i.e., modal progression) in the absence of determination using hard parts. Further, determining the commencement and peak of spawning activities will inform water managers of possible risks to threatened fishes. Our objective was to characterize the reproductive biology of Pecos Bluntnose Shiner at the population level. Specifically, we used temporal patterns in gonadosomatic index (GSI), ovarian condition, and egg count and maturity to determine the timing and length of the spawning season in the Pecos River, New Mexico.

METHODS

Study Area

Pecos Bluntnose Shiner occurs in the Pecos River from its confluence with Taiban Creek to Brantley Reservoir, New Mexico (Fig. 1), with the core population occurring in Chaves County (Hoagstrom et al. 2008). We collected fish for laboratory examination from Willow Creek to Scout Camp, an approximately 78.4-km length of stream. This segment of the Pecos River is predominately a wide (>15 m), shallow (<0.5 m), sand-bottom stream (Hoagstrom and Brooks 2005, Hoagstrom et al. 2008). We chose 7 sites in the core population segment for histological examination (Fig. 1). Although the study sites were not randomly selected, their fish communities are similar to those at random sampling locations (Archdeacon and Davenport 2013), and we believe the sampling sites adequately represent the Pecos Bluntnose Shiner population in the river.

Stream flow data were obtained from the United States Geological Survey surface water monitoring station at Near Acme Gage, New Mexico (USGS Station 08386000) and Below Sumner Gage (USGS Station 08384500) to compare the monthly flow regime to monthly GSI values. Reservoir releases from Sumner Reservoir for water management are apparent, as the discharge at the Below Sumner Gage is typically zero; flows above zero are due only to reservoir releases and not rainfall, whereas the Near Acme Gage reflects both reservoir releases and increased discharge due to summer rainfall. The Near Acme Gage is located downstream of U.S. Highway 70 and the Below Sumner Gage is located downstream of Sumner Reservoir (Fig. 1).

Fish Collections

We collected Pecos Bluntnose Shiner during 25 monthly sampling trips from May 2009 to October 2011. No samples were collected in September 2011 due to time constraints. We used a 3.0 × 1.0-m seine (3 mm mesh) to collect Pecos Bluntnose Shiner. Because of its threatened status, we retained only specimens of Pecos Bluntnose Shiner from sites with high catch rates. Therefore, we retained only specimens from 1–6 sites ($\bar{x}$ = 3) per month. Between 13 and 120 ($\bar{x}$ = 36) Pecos Bluntnose Shiner were preserved in 10% formalin during each trip (Table 1). After 3–7 d, we transferred all preserved fish to 70% ethanol before laboratory examination.

Laboratory Examination

We used digital calipers to measure standard length (SL; ±0.01 mm). We determined fish mass (±0.01 g) before removal of the digestive tract, liver, and gonads. Then, both eviscerated fish mass (±0.01 g) and gonad mass (±0.001 g) were recorded. Prior to determination of mass, fish were blotted with paper towels to remove excess surface liquids. We used a dissecting microscope to determine sex, classify stage of reproductive development, and count oocytes. We deposited all retained specimens at the University of New Mexico's Museum of Southwestern Biology under access code MSB ACC2006-V:23.

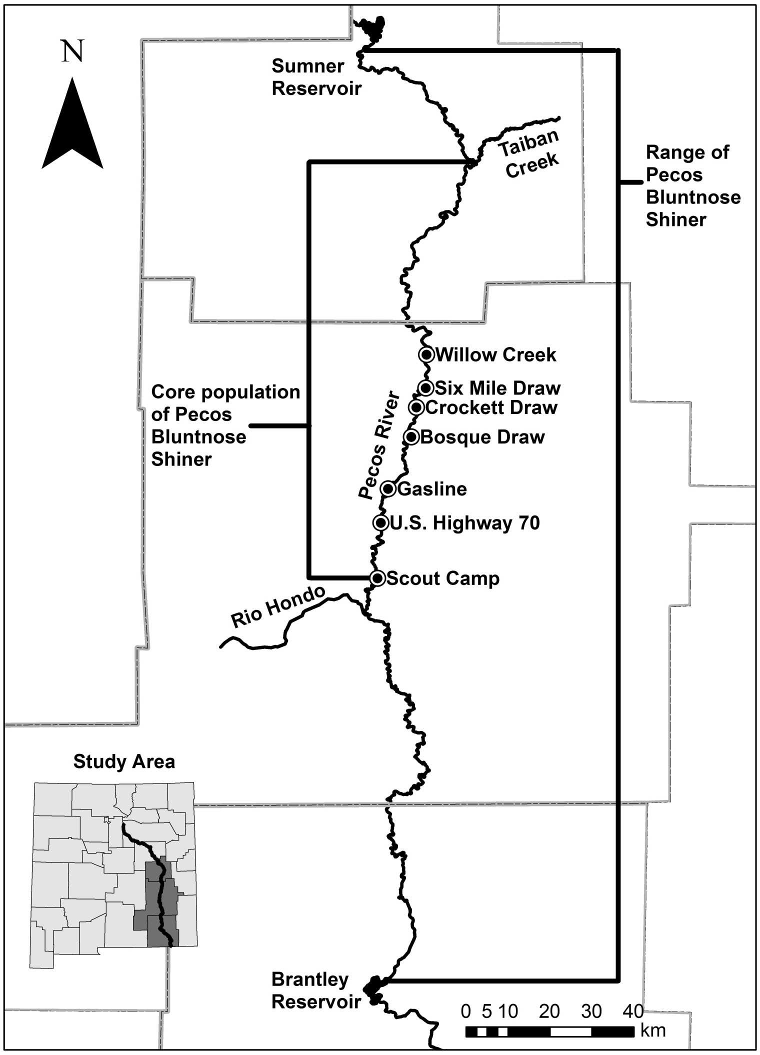

Fig. 1. Map of the Pecos River region in southeastern New Mexico. Fish sampling locations are represented by dots with circles.

Ovarian Stages and GSI

We visually classified gross ovarian and oocyte condition based on a modification of the reproductive development scheme provided by Heins and Rabito (1986). Each specimen was classified twice. If there was disagreement in classification between the first and second classifications, we examined the gonads a third time to make a final determination. If there was no agreement, the fish was dropped from the analysis. We classified ovaries as one of 4 developmental stages: latent (LA), maturing (EM), mature (MA), and ripe (RI) (Heins and Rabito 1986, Heins and Baker 1993, Phillip 1993). Flaccid ovaries or ovaries that were very small and thin, slightly translucent, or with atresia were classified as latent. Ovaries that were small to medium in size and opaque and white in color were classified as early maturing. Ovaries that were greatly enlarged and filling the body cavity and opaque and cream to white in color were classified as mature. Ovaries varying in size with ripe, ovulated ova concentrated in the posterior portion were classified as ripe. Male gonads were classified as latent (small strands of clear tissue), mature (opaque and well visible), or

TABLE 1. The number of male and female Pecos Bluntnose Shiner collected by month in the Pecos River, New Mexico. Numerals in parentheses indicate the number of specimens excluded from gonadosomatic index (GSI) calculations.

Date	Males	Females	Total
May 2009	59 (9)	61 (12)	120 (21)
Jun 2009	6	12	18
July 2009	22	21	43
August 2009	18 (1)	24	42 (1)
September 2009	11	11	22
October 2009	8 (2)	12	20 (2)
November 2009	17	26	42
December 2009	7	6	13
February 2010	20 (2)	30	50 (2)
March 2010	3	10	13
April 2010	10	34 (1)	44 (1)
May 2010	14 (1)	19	33 (1)
June 2010	19 (1)	27 (1)	46 (2)
July 2010	16	14	30
August 2010	6 (1)	13	19 (1)
September 2010	10	16	26
October 2010	10 (3)	17	27 (3)
February 2011	30 (3)	39 (2)	69 (5)
March 2011	11 (3)	14	25 (2)
April 2011	2	12	14
May 2011	13 (1)	16 (1)	29 (2)
June 2011	20	12	32
July 2011	18	9	27
August 2011	17	28 (1)	45 (1)
October 2011	7 (1)	8	15 (1)
TOTAL	374 (28)	491 (18)	865 (46)

ripe (opaque and large) based on size and color of the testes. We calculated the proportion of females in each stage for all samples. We calculated GSI as (mass of gonad/mass of eviscerated fish) · 100.

Reproductive Condition over Time and Fish Length

We separated reproductive condition into 2 categories, reproductive (ripe and mature females) and nonreproductive (latent and early maturing females). We constructed 5 logistic regression models in R 3.0.3 (R Core Team 2014) to predict the proportion of reproductive females (Crawley 2007). We built a simple, null model with no explanatory variables for proportion of reproductive females. We constructed 2 models, each with a single variable predicting proportion of reproductive females, one with SL and one with month. Finally, we built 2 models with both SL and month predicting the proportion of reproductive females, one with main effects only and one with the interaction effect. We ranked the models with Akaike's information criterion (AIC) and computed the AIC weight for each candidate model (Burnham and Anderson 2002).

Egg Counts

We counted eggs from 71 mature females collected during the spawning season (May–September) to determine clutch size. We separated oocytes from ovarian tissue using forceps, and then all mature ova in both ovaries were counted. Because we could not determine whether females had spawned previously, we report only the counts of eggs per month and the counts of eggs per size class of female as measures of reproductive activity, rather than attempting to estimate size-specific fecundity.

RESULTS

Between May 2009 and October 2011, we retained 760 fish for laboratory examination during 25 monthly sampling trips (Table 1). In all 3 years, median female GSI increased in April or May (2009), peaked in June, and then gradually declined, returning to prespawning levels by September (Fig. 2). Mean monthly GSI for females and males during the study ranged from 1.2%–23.6% and 0.13%–0.75%, respectively.

In all 3 years, we observed a high proportion of EM females in April. MA females were

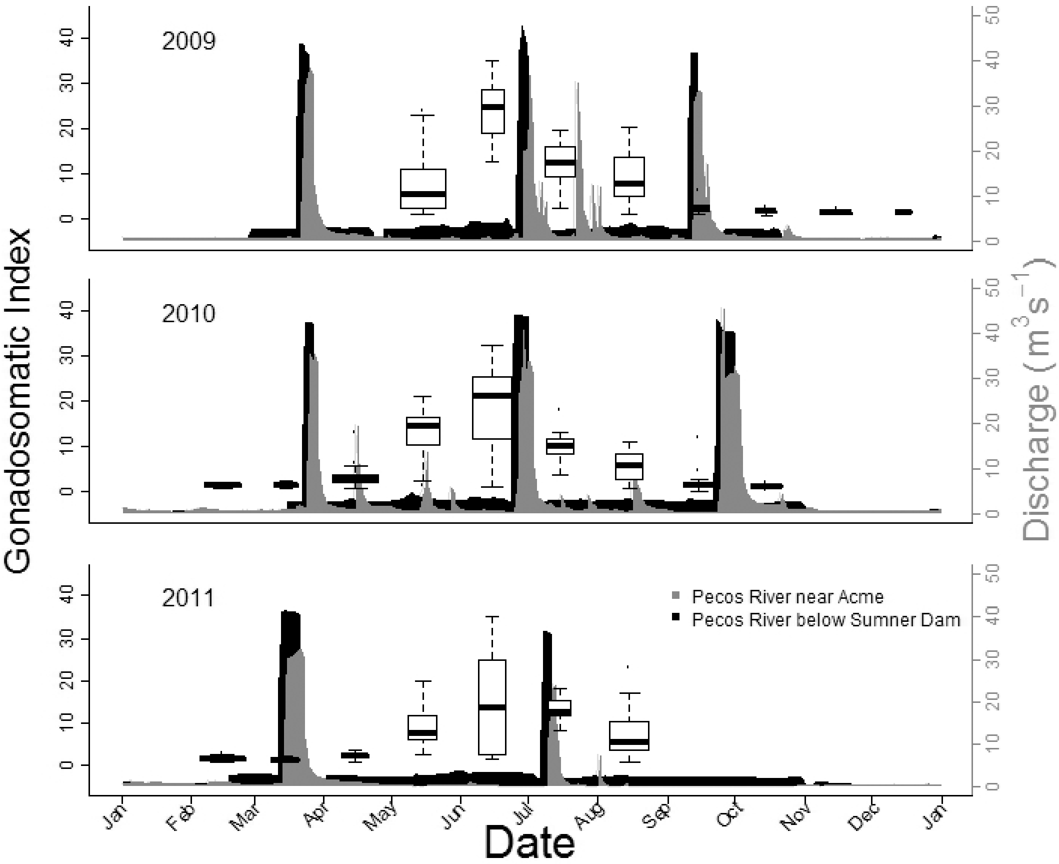

Fig. 2. Gonadosomatic index (GSI) for female Pecos Bluntnose Shiners collected monthly, 2009–2011, in the Pecos River, New Mexico, (left *y*-axis), and mean daily discharge at 2 gaging stations in the Pecos River (right *y*-axis). Box-and-whisker plots represent the median, interquartile range, and minimum or maximum excluding outliers. Outliers are a distance ≥1.5 times the interquartile range from the median. Outliers are represented as dots. Box width is proportional to sample size.

predominant in May–August (Fig. 3). In 2009, we observed the highest proportion of RI females in July. In 2010 and 2011, we observed the highest proportion of RI females in June. By September, most females were latent, except 3 fish containing ripe eggs. All females collected in October–December were reproductively latent.

We found strong evidence that reproductive condition varied by month and SL, as no other model had significant AIC weight (Table 2). In April, there was little relation between SL and reproductive activity, while in May about 50% of females were reproductive by the time they were approximately 31 mm SL (Fig. 4). In June, 50% of females were reproductive by approximately 26 mm SL (Fig. 4). By July, there was no relation between SL and reproductive condition, as nearly every female was reproductive; conversely in August there was no relation between SL and reproductive condition, as many female ovaries were latent (Fig. 4). The smallest female with mature ovaries was 25.8 mm SL, whereas the smallest male with mature testes was 26.8 mm SL.

The number of mature ova per female ranged from 48 to 1498. The mean number of mature ova in each length class ranged from 71 to 1182 (Table 3). The most mature ova (1498) were found in a female measuring 59.5 mm SL in June 2009. Mean number of ova per mature female peaked in June, and then decreased from July to August (Fig. 5).

Discharge was variable among years. Peak flows (>10 $m^3 \cdot s^{-1}$) during the study period were created by sustained water releases from

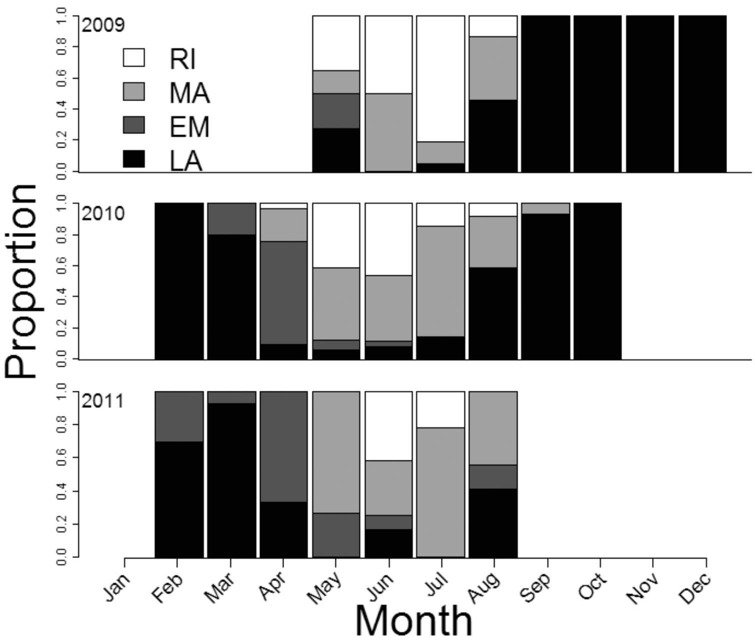

Fig. 3. Spine plot of ovarian stages in Pecos Bluntnose Shiners collected monthly, 2009–2011, in the Pecos River, New Mexico. The ovarian stages are RI (ripe), MA (mature), EM (early maturing), and LA (latent).

TABLE 2. Average (mean), minimum (min.), and maximum (max.) number of mature ova in standard length classes, May–August 2009–2011 in the Pecos River, New Mexico. *N* = number of fish sampled.

SL Class (mm)	Mean SL (mm)	*N*	Min.	Max.	Mean
25.0–29.9	27.6	7	48	113	71
30.0–34.9	32.3	21	48	328	149
35.0–39.9	37.4	15	69	379	161
40.0–44.9	42.4	12	123	478	290
45.0–49.9	47.4	11	74	633	401
50.0–54.0	51.9	2	317	482	400
55.0–59.9	57.4	3	713	1498	1182

Sumner Reservoir in combination with rain events. There were 3 Sumner Reservoir releases between June and September 2009, with discharges of approximately 33–38 $m^3 \cdot s^{-1}$. There were also 3 sustained water releases in 2010. In 2011, a sustained water release occurred in April and July; flows remained relatively low throughout the year. While most 2009–2011 rainfall events resulted in stream flow peaks $<20\ m^3 \cdot s^{-1}$, a monsoonal event on 22 July 2009 produced flows $>35\ m^3 \cdot s^{-1}$. Reservoir releases created higher volume and longer duration flows (up to 14 d) than rainfall events. Each year, one sustained reservoir release coincided with Pecos Bluntnose Shiner peak reproductive activity.

DISCUSSION

Mean monthly GSI and the proportion of females in each reproductive stage indicate that Pecos Bluntnose Shiner had a single reproductive season mainly extending from May through August, with a peak from June to July. We also found some evidence of limited reproduction as early as April and as late as September. Average gonadosomatic index, reproductive stage, and number of eggs per individual all decline beginning in August, reaching levels indicative of reproductive quiescence in October. Quiescence appeared to extend through March of the following year. The presence of mature ovaries in April–September corroborates

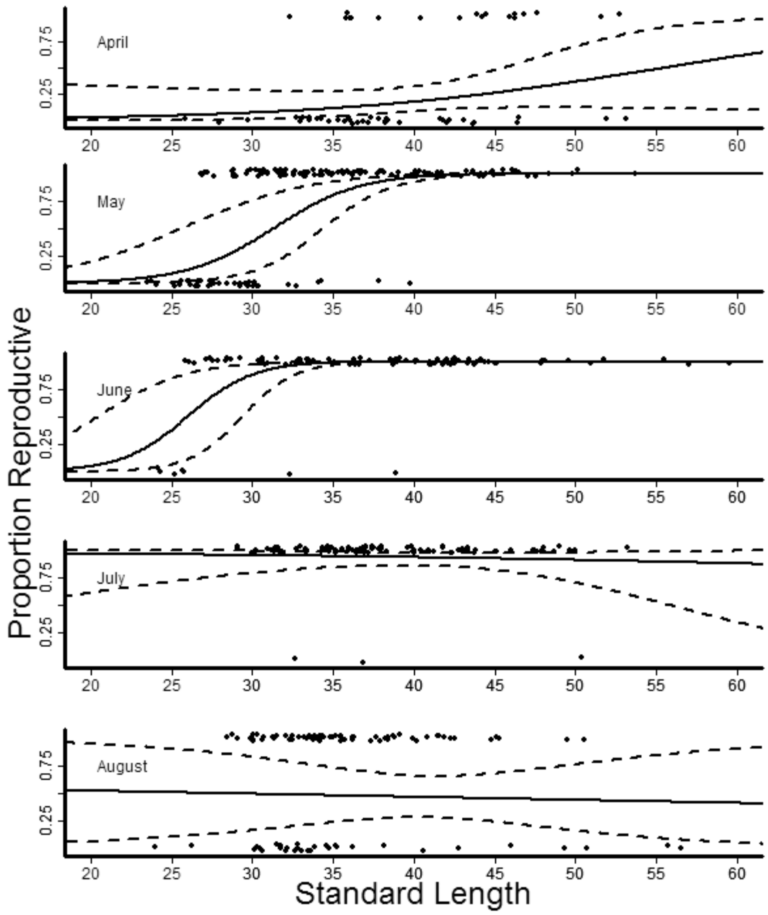

Fig. 4. Relation between ovary condition (nonreproductive = latent and early maturing, reproductive = mature and ripe) and standard length, by month, for female Pecos Bluntnose Shiners. Dashed lines represent the 95% prediction band.

TABLE 3. ΔAIC and AIC weights of candidate models predicting the proportion of reproductive female Pecos Bluntnose Shiner in the Pecos River, New Mexico 2009–2011.

Model parameters	ΔAIC	AIC weight
SL * Month	275.13	0.9996
SL + Month	290.77	0.0004
Month	321.21	0.0000
SL	588.80	0.0000
Intercept-only	591.73	0.0000

Hatch et al. (1985), who reported that Pecos Bluntnose Shiner has a protracted spawning season based on the observation of late-season larvae. Hatch et al. (1985) also observed few females with mature oocytes in September, and all fish sampled in October–November were reproductively latent, similar to our study.

Female Pecos Bluntnose Shiner in our study were sexually mature at a much smaller size than previously reported (Hatch et al. 1985, Bestgen and Platania 1990). Time of year has a much stronger influence on female reproductive condition than size alone. In May and June, about 50% of females between 25 and 30 mm SL were mature. By July, almost all female Pecos Bluntnose Shiner were mature. Hatch et al. (1985) and Bestgen and Platania (1990) examined fish collected in September and later; neither study examined fish collected March through August. Hoagstrom et al. (2008) assumed that fish >40 mm SL were adults, based on those previous studies. By examining fish over the entire reproductive season, we have demonstrated that fish were mature and reproductive at a much smaller size.

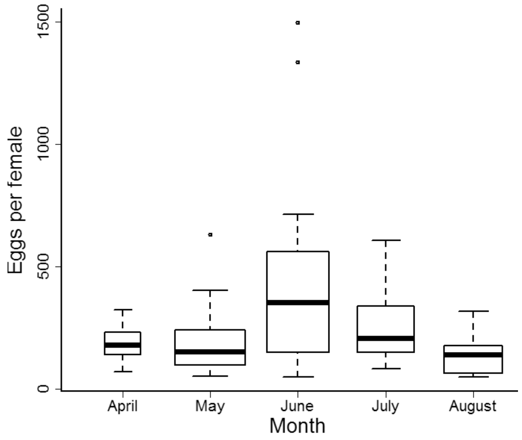

Fig. 5. Number of mature eggs by month per female Pecos Bluntnose Shiner collected between April and August, 2009–2011, in the Pecos River, New Mexico. Box-and-whisker plots represent the median, interquartile range, and minimum or maximum excluding outliers. Outliers are a distance ≥1.5 times the interquartile range from the median. Outliers are represented as dots. Box width is proportional to sample size.

Ovary size and number of ova increase with size and age in some *Notropis* species (Heins and Clemmer 1976, Kerns and Bonneau 2002, Roberts et al. 2006, Perkin et al. 2012). Our findings were no different; the number of mature ova produced by a female Pecos Bluntnose Shiner increased with SL, and the variance in number of ova produced also increased with SL, though we could not estimate total annual fecundity based on size. The mean number of mature eggs per female was highly variable; however, there was a clear peak in June, corresponding with mean female GSI. Our data do not allow us to determine a clutch-size or a size-specific fecundity because we cannot determine whether a single individual has spawned prior to collection. However, our results do suggest that larger females produce more eggs annually than smaller females, but females become mature at a relatively small size.

Recently, collection of both population- and individual-scale reproductive data clarified complex spawning ecology of similar species of *Notropis* in western Texas (Durham and Wilde 2014). Due to study design constraints, we were unable to determine whether Pecos Bluntnose Shiner spawns multiple times within the season; this would require precise oocyte size frequency and multiple collections per month during the reproductive season. However, the presence of mature ova in all sizes over an extended period suggests that individual Pecos Bluntnose Shiners do spawn multiple times per season. Group-synchronous spawning fish can shed their whole clutch over a short period of a few days or weeks (i.e., single spawners; Holden and Raitt 1974, Rinchard and Kestemont 1996) or multiple times within an entire year (Dieterman et al. 2006). During the reproductive season, these species undergo synchronous spawning based on environmental cues, such as changes in stream flow (Bye 1984, Heins and Rabito 1986, Durham and Wilde 2008). We observed a single oocyte size in the ovaries of Pecos Bluntnose Shiner, suggesting that a single group of maturing eggs is shed in a short spawning period. This is similar to the research of Hatch et al. (1985) who noted uniform egg sizes in mature female laboratory specimens. Additional studies incorporating histological analysis of oocytes would further clarify *Notropis* reproductive biology (Heins and Rabito 1986, Durham and Wilde 2008, 2014).

Reservoir releases from Sumner Reservoir are a regular part of water management in the Pecos River. Depending on water availability, up to 3 releases occur each year. Water is stored in upstream reservoirs to limit evaporative loss, and is transferred downstream as needed (Robertson 1997). In each of the 3 years of our study, a release from Sumner Reservoir to downstream irrigation districts coincided with peak spawning activity. High-magnitude, long-duration releases may have a negative impact on reproduction for certain reproductive guilds of Great Plains fishes (Dudley and Platania 2007).

Pecos Bluntnose Shiner has reproductive strategies analogous to other *Notropis* species. Other Pecos River fish, including the nonnative Arkansas River Shiner (*Notropis girardi*) and native Sand Shiner (*Notropis stramineus*), spawn during peak summertime flow events, although Sand Shiner does not have semibuoyant eggs (Moore 1944, Summerfelt and Minckley 1969, Platania and Altenbach 1998). The Plains Minnow (*Hybognathus placitus*) is a nonnative and abundant multiple-batch spawning fish in the Pecos River with a reproductive season that also peaks in June, typically during periods of increased flows, and also produces semibuoyant, nonadhesive eggs

(Lehtinen and Layzer 1988, Taylor and Miller 1990). The July release from Sumner Reservoir is a sustained, high-volume release which is both longer in duration and greater in volume than rainfall events. While fish may be encouraged to spawn during these high-flow events, (Platania and Altenbach 1998), other studies have shown that reproductive propagules (i.e., eggs and larvae) are transported downstream much farther than during rain events (Dudley and Platania 2007). While Pecos Bluntnose Shiner has persisted in the face of reservoir releases, many eggs and larvae are likely transported to Brantley Reservoir during sustained July releases, as the releases coincide with the peak in reproductive effort in the female population. Hoagstrom et al. (2008) speculated that mortality of recently hatched larval fish is high once the larvae are transported into Brantley Reservoir. Timing reservoir releases to avoid peak spawning activity of Pecos Bluntnose Shiner may help retain eggs and larvae in areas near spawning activities. However, changing the timing of reservoir releases may increase evaporation of water.

Water management devised to maintain adequate in-stream flow is important in the conservation efforts of North American Plains cyprinids. Water management agencies can benefit wild stocks of protected fish by maintaining perennial flows in habitat designated as critical or habitat within core reproductive populations (Hoagstrom et al. 2008). Perennial flows would provide favorable spawning conditions and aid in successful recruitment of young-of-year fishes (Durham and Wilde 2008, Munz and Higgins 2013). The current flow regime on the Pecos River is supplemented with stored water from a fish conservation pool, forbearance agreements with irrigation districts, and groundwater pumped into the river channel. However, during irrigation season, surface flow intermittence on the Pecos River remains of highest conservation concern (U.S. Fish and Wildlife Service 2006). Despite downstream transport concerns, reservoir releases have been timed to augment flows during critical low-flow periods, typically June and July, when river drying is imminent. While these managed flows may displace some reproductive effort downstream into unfavorable habitat, the managed release functions to prolong surface flow, and, it is speculated, increase survival of all life stages of fish that would otherwise perish in a dry river bed. Ultimately, the information provided here will be helpful to management agencies tasked with decisions on how and when to release water to aid conservation of Pecos Bluntnose Shiner.

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