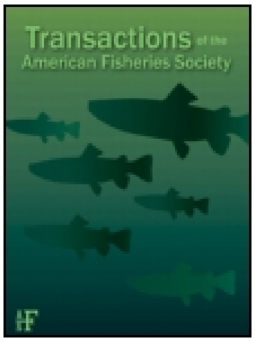

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Anthony W. Rodger, Kevin B. Mayes & Kirk O. Winemiller

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NOTE

Preliminary Findings for a Relationship between Instream Flow and Shoal Chub Recruitment in the Lower Brazos River, Texas

Anthony W. Rodger*
Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas 77843-2258, USA

Kevin B. Mayes
Texas Parks and Wildlife Department, Inland Fisheries, River Studies, Post Office Box 1685, San Marcos, Texas 78667, USA

Kirk O. Winemiller
Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, Texas 77843-2258, USA

Abstract
Seasonal flow pulses in rivers facilitate spawning, dispersal, and early life stage survival of many fish species. To evaluate the effectiveness of current flow standards to sustain threatened fish populations, we investigated the relationship between hydrology and recruitment of the Shoal Chub *Macrhybopsis hyostoma*, a broadcast-spawning minnow in the Brazos River, Texas. From March 2013 to March 2014, we collected metalarval and juvenile Shoal Chub bimonthly at night using arrays of stationary drift nets. Otoliths were examined to estimate age, and the relationship between hatch date and discharge was analyzed. Shoal Chub recruited under both base-flow and pulse-flow conditions, including intervals of increasing, decreasing, and stable discharge. However, hatch dates of surviving fish indicated greater levels of recruitment during flow pulses, particularly on the rising limb. Greatest recruitment occurred during flow pulses of a magnitude defined as two per season according to the method of hydrological analysis adopted by the state's environmental flow program. Our findings imply that the state's current environmental flow standards for the lower Brazos River may be insufficient to sustain Shoal Chub populations and additional research on this issue is warranted.

Flow regimes of rivers throughout the continental USA have been altered by human actions (Benke 1990). According to Cooper (2013), there are over 19,000 barriers fragmenting stream networks in the U.S. Great Plains region, which have caused major alterations to natural flow regimes (Costigan and Daniels 2012). Dam impacts are greatest for fishes with life history strategies sensitive to variation in magnitude, duration, and timing of flow pulses (Bunn and Arthington 2002; Lytle and Poff 2004). Dams in the Great Plains region appear to have resulted in extirpation of populations of numerous species, especially minnows (Perkin et al. 2015a, 2015b).

Multiple species within several genera of North American cyprinids belong to a reproductive guild that broadcast nonadhesive semibuoyant ova in rivers of the Great Plains (Bottrell et al. 1964; Lehtinen and Layzer 1988; Platania and Altenbach 1998; Hoagstrom and Turner 2013). Spawning during high-flow pulses facilitates passive dispersal of fertilized eggs and prevents eggs and larvae from settling onto the river bottom and being covered by fine sediments (Platania and Altenbach 1998; Wilde and Urbanczyk 2014). Drifting eggs and larvae eventually may be deposited or actively swim into backwaters and other low-velocity habitats along river margins, where conditions are favorable for survival, feeding, and growth of early life stages (Taylor and Miller 1990; Dudley and Platania 1999; Hoagstrom 2014). Asynchronous spawning by minnows of this reproductive guild has been

*Corresponding author: anthony.rodger@odwc.ok.gov
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observed over a range of flow conditions, however, flow pulses may synchronize spawning (Durham and Wilde 2008b, 2014). Several species (e.g., Arkansas River Shiner *Notropis girardi* and Peppered Chub *Macrhybopsis tetranema*) in the upper Canadian River, New Mexico and Texas, were observed to spawn within isolated pools; however, this resulted in recruitment failure (Bonner 2000; Durham and Wilde 2006). Frequent spawning events over an extended reproductive period are believed to increase odds of successful reproduction and recruitment in highly variable lotic habitats (Durham and Wilde 2009a). Rapid larval development facilitates recruitment under favorable conditions associated with flow pulses (Bottrell et al. 1964; Platania and Altenbach 1998; Hoagstrom and Turner 2013).

Shoal Chub *Macrhybopsis hyostoma* is a species within the Speckled Chub *M. aestivalis* complex (Eisenhour 1999, 2004) of benthic minnows that inhabit rivers in the central USA. Other species in the *M. aestivalis* complex have been identified as members of the pelagic broadcast-spawning reproductive guild (Bottrell et al. 1964; Platania and Altenbach 1998; Hoagstrom and Turner 2013). Given similarities in morphology and close phylogenetic relationship, Shoal Chub are hypothesized to belong to this reproductive guild (Perkin and Gido 2011), and observations of captive spawning support this classification (Aaron Urbancyzk, Texas Tech University, personal communication). Shoal Chub are benthic insectivores and habitat specialists that prefer unregulated stretches of rivers with moderately strong currents over clean sand or gravel substrates (Luttrell et al. 2002; Williams 2011; Pierce et al. 2014). Life spans of *Macrhybopsis* spp. seldom exceed 2 years (Wilde and Durham 2008; Williams 2011; Perkin et al. 2013). Consequently, 1 or 2 years of failed or poor recruitment could threaten local populations. Recent declines in Shoal Chub abundance and geographic distribution have been attributed to fragmentation and hydrologic alteration caused by dams and reservoir operations (Luttrell et al. 1999). The objective of this study was to evaluate the effectiveness of current flow standards to sustain populations of the Shoal Chub in the flow-regulated Brazos River, Texas. We investigated the relationship between hydrology and juvenile recruitment within a major river segment without dams, where the population appears relatively stable (Bonner and Runyan 2007).

METHODS

Study site.—Field collections were made at a site on the lower Brazos River near State Highway 105 near Navasota, Brazos County, Texas (30°21′45″N, 96°09′16.8″W;

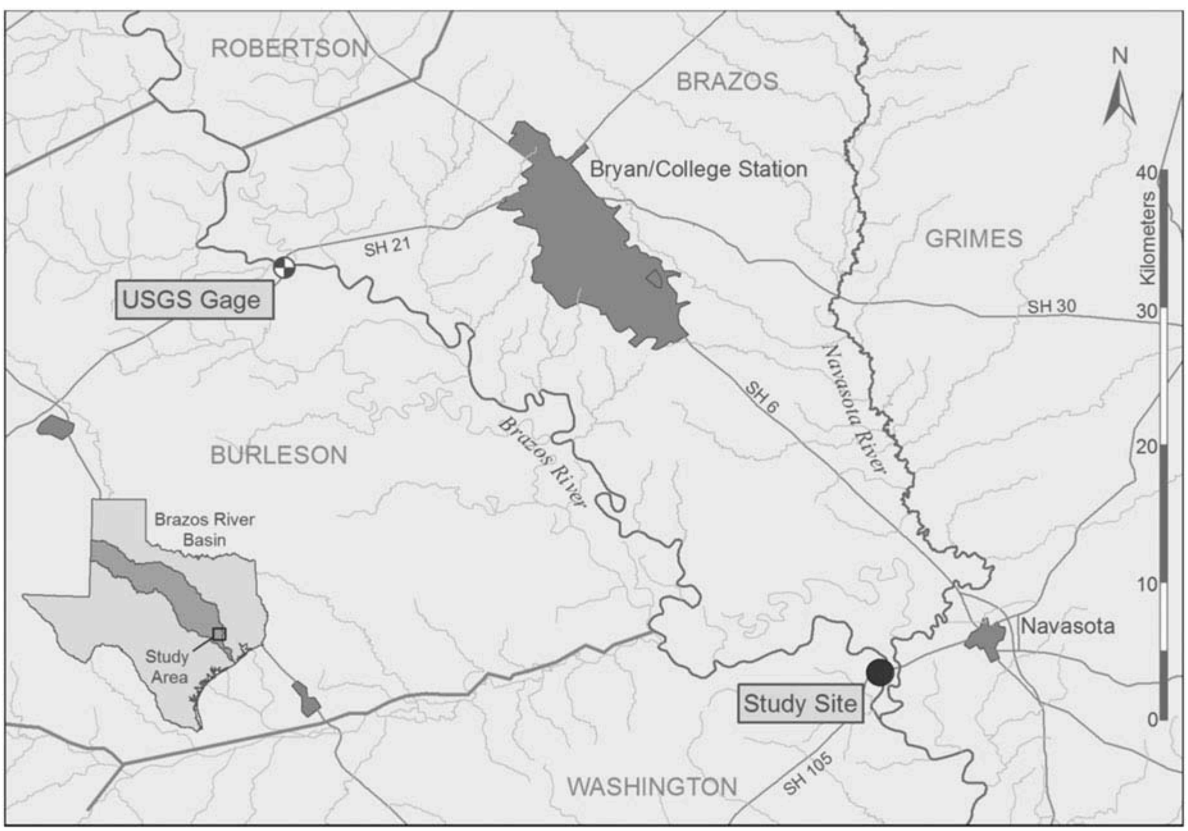

FIGURE 1. Study area, including the location of the sampling site and U.S. Geological Survey gauge 08108700 on the lower Brazos River, Texas.

Figure 1). Originating in New Mexico, the Brazos River flows approximately 2,736 km southeast and discharges into the Gulf of Mexico at Freeport, Texas (Brazos Bay and Basin Expert Science Team [Brazos BBEST] 2012). The upper and middle Brazos River main stem and tributaries have been altered by construction of 16 major reservoirs for flood control and water supply (Brazos BBEST 2012). Although the lower Brazos River remains unimpounded, flows are regulated by these upstream reservoirs. The lower Brazos River extends 330 km from Waco, Texas, to the Gulf of Mexico. Historical discharge records indicate highest flows generally occur during winter and spring (Zeug and Winemiller 2008); however, unpredictable summer rainstorms can generate high flow pulses and overbanking flows. The lower Brazos River fish assemblage has maintained moderate integrity, notwithstanding several noticeable disappearances and population declines of fluvial specialists, including Sharpnose Shiner *Notropis oxyrhynchus*, Smalleye Shiner *N. buccula*, Chub Shiner *N. potteri*, and Plains Minnow *Hybognathus placitus* (Bonner and Runyan 2007).

Field surveys.—From March 2013 to March 2014 larval fish surveys were generally conducted every other week from March 2013 through October 2013 during the Shoal Chub reproductive season (Williams 2011) and once per month from November 2013 to February 2014. Drift nets with a rectangular opening 0.45 m wide by 0.30 m tall and length of 1 m with a mesh size of 500 µm were set at wadeable depths within current on gently sloping sandbars. Nine drift nets were set in three arrays perpendicular to the shoreline. The three nets in each array were positioned in a straight line extending from shore towards the middle of the channel. Water depth (cm) and current velocity (m/s) were measured at the center of each drift net opening at the beginning and end of each sampling interval using a meter stick and Marsh–McBirney current velocity meter (Flo-Mate Model 2000, Frederick, Maryland), respectively. Drift-net samples were collected every 2 h, the first sample being at 2000 hours and the last sample at 0200 hours, yielding a total of 36 individual drift-net samples on each date. From November to February, sampling effort was reduced; the last collections were initiated at 2200 hours, yielding a total of 18 individual drift-net samples. After each 2-h interval, samples from each drift net were washed into individual Whirl-Pac bags containing 95% ethanol and transported to the laboratory at Texas A&M for processing. In addition to drift nets, sampling with seine nets was done concurrently to augment sample sizes of metalarvae and juvenile Shoal Chub. Unfortunately, seining failed to capture any Shoal Chub (≤20 mm; preliminary work demonstrated unacceptable age discrepancies between readers aging individuals >20 mm).

Discharge data.—Daily discharge data were obtained from the nearest upstream U.S. Geological Survey (USGS) streamflow gauge, USGS 08108700 Brazos Rv at SH21 near Bryan, Texas (Figure 2). This gauge is located approximately 92 river kilometers (rkm) upstream from our study site.

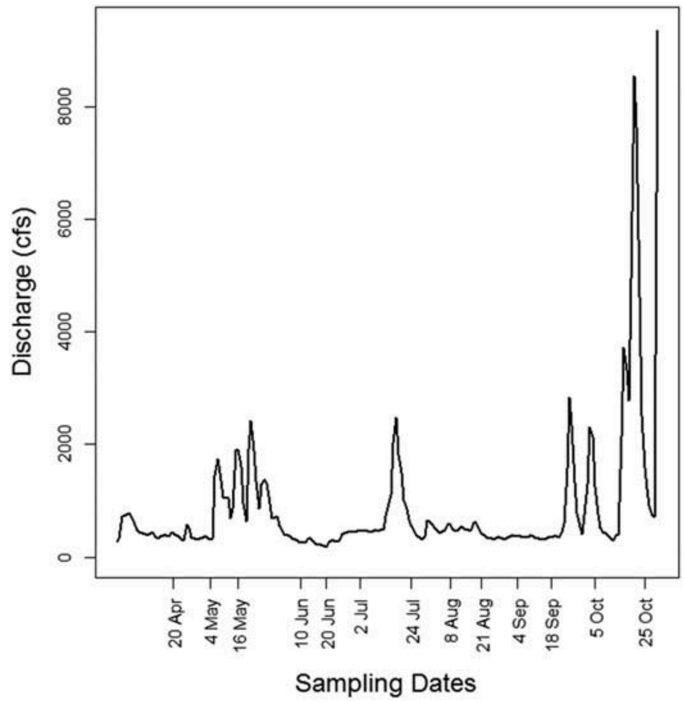

FIGURE 2. Daily maximum discharge from U.S. Geological Survey gauge 08108700 Brazos River at SH21 near Bryan, Texas, during the 2013 reproductive season (April–October).

Laboratory methods.—Larval fish were separated from other contents of the Whirl-Pacs and then counted and stored in labeled vials in 70% ethanol. Larval fish were sorted by developmental stage prior to taxonomic identification. Identifications proceeded from the juvenile stage and progressed through successively earlier stages. Developmental stages were categorized as protolarvae, mesolarvae, metalarvae, or juveniles based on characteristics reported by Snyder and Muth (2004). Given lack of published keys for early stage larvae (e.g., protolarvae and mesolarvae) of cyprinids of the southern USA, we were only able to positively identify Shoal Chub metalarvae and juveniles.

Daily growth increments during otolith formation in young-of-the-year cyprinids of the Brazos River have been validated as a reliable means to estimate hatch dates (Durham and Wilde 2008a). Total length (mm) and standard length (mm) were recorded for each specimen prior to otolith examination. Asteriscus otoliths, the largest otoliths in Cyprinidae (Secor et al. 1992), were removed using a dissecting microscope with two polarizing filters, one mounted between the light source and otolith, and one mounted between the objective lens and otolith. Each otolith was fixed to a glass slide using thermoplastic cement. A drop of immersion oil was placed on the slide, and daily growth rings were counted with the aid of a compound microscope at 40× magnification. Daily growth rings were counted independently by two readers. Age estimates from the two readers that were within 10% agreement were accepted as valid and retained for analysis. The daily age estimate was recorded as the mean of the two observer estimates (Durham and Wilde 2006, 2009a). To estimate fertilization date (i.e., spawning date) based on estimated age, 1 d was added to final daily growth ring counts based on finding that eggs of *Macrhybopsis* spp. hatch ≤28 h after fertilization (Bottrell et al. 1964; Urbancyzk, personal communication).

Statistical analysis.—Linear and quadratic regressions were used to investigate the potential relationship between the number of surviving Shoal Chub (surviving to metalarvae and juvenile stages) that resulted from spawning on a given date and the magnitude of discharge on that date. A chi-square test was used to determine if there was an association between discharge categories (subsistence, base, high flow pulse, or overbanking) on dates of spawning and the number of Shoal Chub that survived to metalarvae and juvenile stages. This test measures discrepancies between an observed frequency distribution and a distribution expected under a null hypothesis of random distribution of observations among categories. Expected frequencies for the null hypothesis were calculated by counting the number of days the river was classified into various flow categories, dividing that value by the total number of days in the reproductive season (April to October), and then multiplying by the total number of Shoal Chub specimens. Very few cyprinid protolarvae were captured in March; thus, we considered April the start of the 2013 reproductive season. A chi-square test also was used to compare surviving Shoal Chub hatch dates in relation to intervals categorized as having increasing, decreasing, or stable flows.

A flow separation procedure (see Opdyke et al. 2014) was used to separate daily streamflow data from USGS 08108700 into components of the flow regime: subsistence, base, high-flow pulses, and overbank flows. Daily streamflow was classified as subsistence if flow was <299 cfs (8.475 m^3/s). The minimum flow for classification as a high-flow pulse was 833 cfs (23.59 m^3/s), and the maximum flow for base flows was 5,370 cfs (152.06 m^3/s). For daily streamflows between minimum and maximum values for base and pulse flows, the percent change determined the flow component. If the previous day was a base flow and there was a discharge increase greater than 25%, the daily flow was classified as a pulse. If the previous day was a pulse and discharge decrease was less than 5%, the daily flow was classified as base flow. Daily flow exceeding 41,200 cfs (1,167 m^3/s) was classified as overbanking flow. Maximum daily discharge values were used in place of mean daily discharge values because maximum values better characterize the nature of the highly variable flow regime. Tables 1 and 2 illustrate the flow separation process using an abbreviated period from the 2013 spawning season.

For the chi-square test analyzing flow trends, a flow was categorized as increasing if the percent change was greater than +5% from the previous day's flow. The flow was considered decreasing if the percent change was less than –5%. Stable flows were determined to be flows where the percent change was between –5% and +5% compared with the previous day's flow. All statistical analyses were conducted using R (R Core Team 2015).

RESULTS

Sixty-eight out of 70 total metalarvae and juvenile fish identified as Shoal Chub yielded valid otolith growth estimates; mean length was 10.1 mm SL (range: 7.1–18.8 mm) and mean age was 11 d (range: 7–34 d). To support daily age estimates, linear regression was used to determine relationships between otolith diameter, daily growth ring counts and fish standard length. Linear regressions were highly significant for estimated age versus otolith diameter (R^2 = 0.82, P < 0.0001), age versus standard length (R^2 = 0.87, P < 0.0001), and standard length versus otolith diameter (R^2 = 0.94, P < 0.0001). Strong relationships among these

TABLE 1. Parameters used by the Brazos Bay and Basin Expert Science Team as indicators of hydrologic alteration for flow separation (Adapted from Brazos BBEST 2012).

				Parameters that apply between minimum and maximum (%)		
Environmental flow location	Subsistence flow limit (cfs)	Minimum flow for pulse flow (cfs)	Maximum flow for base flows (cfs)	If increase is greater, go from base to pulse	If decrease is less, go from pulse to base	Overbank flow limit (cfs)
Brazos at SH 21 near Bryan	299	833	5,370	25	5	41,200

TABLE 2. Example flow data illustrating classification of maximum daily flows during the 2013 spawning season.

Date in March 2013	Discharge	Rate of change	Flow classification
4	279	–11	Subsistence
5	281	1	Subsistence
6	314	12	Base
7	320	2	Base
8	672	110	Base
9	739	10	Base
10	758	3	Base
11	788	4	Base
12	2,730	246	Pulse
13	2,670	–2	Base

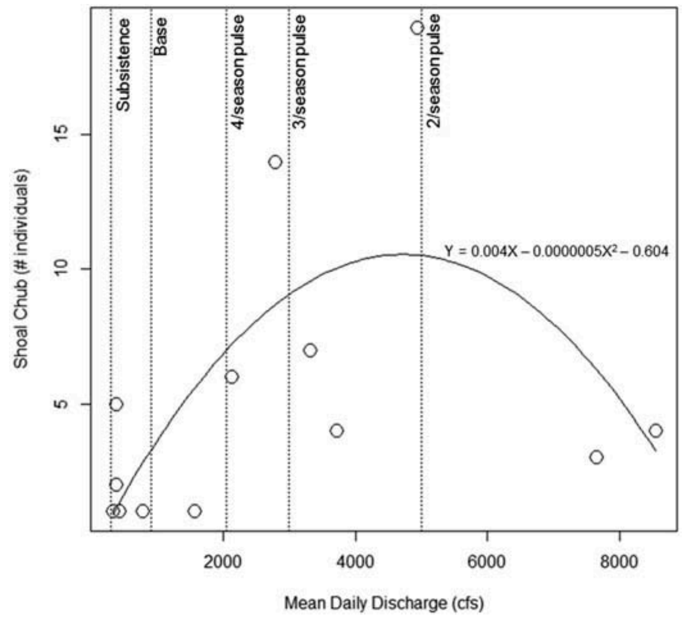

FIGURE 3. Polynomial regression between discharge and number of Shoal Chub hatched. Nonlinear relationship was significant (R^2 = 0.46, P = 0.048). Vertical dotted lines represent separate summer (June–October) flow tiers calculated with discharge data from U.S. Geological Survey gauge near Bryan, Texas (Brazos BBEST 2012). During the study period, there were no one/season flow pulses or overbank events.

variables indicate consistent patterns of otolith formation and supports use of daily growth rings as reliable indicators of age (Miller and Storck 1982; Durham and Wilde 2006). Simple linear regression between number of surviving Shoal Chub and maximum daily discharge on the date of fertilization was nonsignificant, with discharge explaining little variation in the number of surviving individuals (R^2 = 0.09, P = 0.33). However, quadratic regression between number of surviving Shoal Chub and maximum daily discharge on the estimated date when fertilization occurred was significant (R^2 = 0.46, P = 0.048; Figure 3). Recruitment was greatest between 2,000 and 5,000 cfs, which correspond to four/season, three/season, and two/season flow pulses during summer based on the environmental flow regime recommended by the Brazos BBEST (2012). Highest recruitment was observed at discharge magnitudes corresponding to two/season flow pulses (Figure 3). October 16 and 17 yielded the highest number of Shoal Chub recruits. Both dates were categorized as high-flow pulses, and maximum daily discharges were 2,790 cfs and 4,940 cfs, respectively.

During the 2013 spawning season (214 d), 145 d were classified as base flows, 43 d as high flow pulses, and 26 d were subsistence flows. There were no one/season flow pulses or overbank flows between April and October. Estimated fertilization dates of surviving Shoal Chub were significantly associated with flow levels (χ^2 = 180.4, df = 2, P < 0.0001; Figure 4). Four times as many Shoal Chub were spawned during flow pulses than expected based on the null hypothesis. Fewer surviving Shoal Chub were spawned during base and subsistence flow conditions than expected by chance. During the 2013 reproductive season, increasing flows occurred on 57 d, decreasing flows on 90 d, and stable flows on 67 d. Surviving Shoal Chub produced from spawning on dates with increasing flows were twice as abundant as expected (χ^2 = 180.4, df = 2, P < 0.0001), and fewer surviving Shoal Chub than expected were produced from spawning on dates with decreasing or constant flow (χ^2 = 28.9, df = 2, P < 0.0001; Figure 4).

DISCUSSION

Studies of reproductive characteristics in broadcast-spawning minnows have documented asynchronous spawning throughout long reproductive seasons, but synchronized spawning is associated with high-flow pulses (Durham and Wilde 2008b, 2009a, 2014). For example, flow pulses in the upper Brazos River coincided with greater numbers of drifting eggs and larvae of Smalleye Shiner (Durham and Wilde 2008b). Due to our inability to identify the earliest larval stages to species level, the direct relationship between Shoal Chub protolarvae abundance and flows in the lower Brazos River could not be analyzed. However, a more relevant issue for population dynamics than the number of eggs fertilized is the number of fertilized eggs that ultimately survive and recruit into the breeding population. Therefore, our analysis focused on the relationship between early life-stage survival and flow variation. In the lower Brazos River, surviving Shoal Chub resulted from spawning that took place during base and pulse flow conditions (none had been produced during subsistence flows), including intervals of increasing, decreasing, and consistent discharge. However, significantly more surviving Shoal Chub were produced during the ascending limb of flow pulses.

Enhanced recruitment of broadcast-spawning minnows during periods of elevated discharge probably occurs for several reasons. Flow pulses may increase availability and quality of habitat for early life stages (Cross and Moss 1987; Hoagstrom

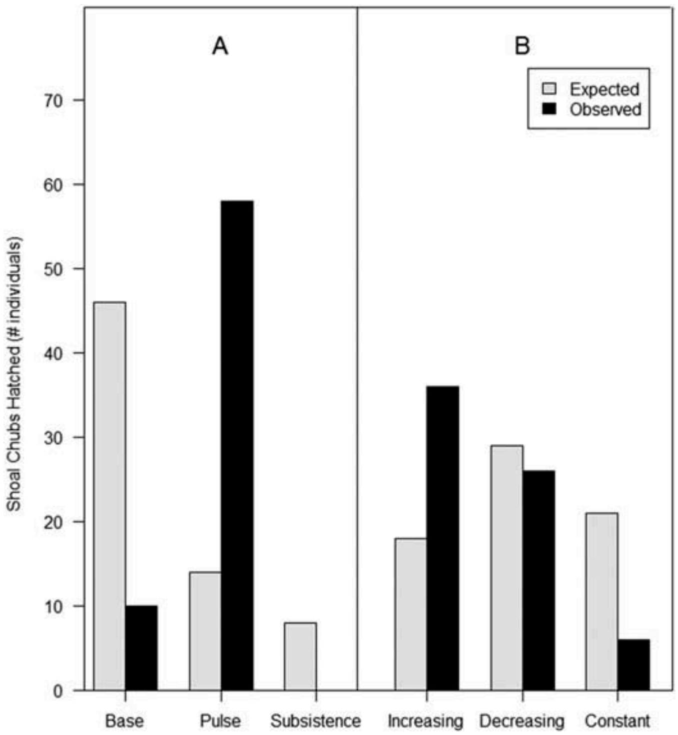

FIGURE 4. Frequency distribution of surviving Shoal Chub and flow categories for estimated spawning dates that produced them. Light bars represent expected frequencies under a null model of random occurrence, and black bars represent observed frequencies. Result were highly significant for both **(A)** flow levels (χ^2 = 180.4, df = 2, P < 0.0001) and **(B)** flow trends (χ^2 = 28.9, df = 2, P < 0.0001) .

and Turner 2013). Pulse events also keep ova and larvae suspended in the water column, thereby avoiding abrasion from contact with substrates and siltation (Cross and Moss 1987). During flow-pulse attenuation, many larvae are retained within low-velocity habitats that function as nurseries (Medley et al. 2007; Widmer et al. 2012; Hoagstrom and Turner 2013), and habitat expansion during flow pulses could reduce predator density and competition (Economou 1991; Cushing and Horwood 1994; Bertrand et al. 2004). During flow pulses, predation mortality may be further reduced by increases in sediment suspension that reduces detection by visually orienting predators (Bonner and Wilde 2002; Hoagstrom and Turner 2013).

To maintain local populations along the longitudinal river continuum, pelagic broadcast-spawning cyprinids either need to limit downstream transport of eggs and larvae, or undergo upstream movements to counter downstream drift (Hoagstrom and Turner 2013). The costs associated with upstream migration would be reduced if spawning occurs during short-lived flow pulses of moderate magnitude that facilitate retention of drifting propagules in nursery habitats during pulse subsidence (Medley et al. 2007; Widmer et al. 2012). We detected a significant nonlinear relationship between number of surviving Shoal Chub and flow magnitude on their estimated dates of fertilization (i.e., parental spawning event). This provides evidence, albeit based on a limited sample size, that Shoal Chub recruitment is positively associated with discharge, but only to a certain threshold (Figure 3). Larger flow pulses may be detrimental to survival of fertilized eggs or larvae. The discharge associated with highest Shoal Chub recruitment matched the recommendation of a science team appointed by the state (Brazos BBEST 2012) for environmental flows at the USGS gauge upstream from our field site (two/season pulses of 5,000 cfs each).

Dams and water diversions have greatly altered natural flow regimes of North American rivers (Lytle and Poff 2004; Lehner et al. 2011; Costigan and Daniels 2012); consequently, pelagic broadcast-spawning minnows have been extirpated or undergone range reductions (Winston et al. 1991; Luttrell et al. 1999; Worthington et al. 2014; Perkin et al. 2014). Population dynamics of these minnows have been shown to be influenced by seasonal and interannual variation in discharge (Durham and Wilde 2009a, 2009b, 2014; Wilde and Durham 2008). Flow regimes must maintain discharge variability that synchronizes spawning and enhances recruitment. We found preliminary evidence for a threshold in the magnitude of flow pulses for the Shoal Chub, but other broadcast-spawning cyprinids may have different pulse requirements (e.g., species differ in egg buoyancy and larval development times; Perkin and Gido 2011). For the river reach of our study site, two flow pulses of 5,000 cfs (volume averaging 38,100 acre-feet with an 8-d duration) during summer were recommended for protection by the Brazos BBEST (2012), which is similar to the two pulse-flow magnitudes (2,790 cfs and 4,940 cfs) that yielded greatest Shoal Chub recruitment in our study. The state adopted lower flow standards that protect three pulses of 2,060 cfs during years with average precipitation, two pulses of 2,990 cfs during wet years, and one pulse of 2,060 during dry years (TCEQ 2014). Our preliminary findings imply that these adopted environmental flow standards for high-flow pulses may not provide sufficient protection for persistence of Shoal Chub in the lower Brazos River.

More research is urgently needed to improve understanding of flow–ecology relationships in the Brazos and other rivers for validation of environmental flow standards. Species that are particularly sensitive to flow variation, such as broadcast-spawning fishes, provide a useful ecological metric for evaluating environmental flows. Our 1-year study of Shoal Chub in the lower Brazos River revealed a curvilinear relationship between recruitment and flow, suggesting intermediate flow pulses during the reproductive season may be most important for population persistence. To refine estimates of environmental flows that yield highest fish recruitment, future studies should analyze age and abundance data from multiple years encompassing a broad range of flow conditions.

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ORCID

Anthony W. Rodger http://orcid.org/0000-0002-1258-7043

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