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REGULAR ARTICLE

SEASONALITY OF GAMETE PRODUCTION OF CYCLONAIAS SPECIES IN CENTRAL TEXAS

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ABSTRACT

Reproductive traits, which can impact population health, are important life-history characteristics for freshwater mussels. Little research has been done on the reproductive ecology of mussels, and crucial information is missing for many threatened and endangered species, especially in Texas. The objective of this study was to examine gamete production, parasitic infection rates, and sex ratios of two freshwater mussel species (*Cyclonaias petrina* and *Cyclonaias pustulosa*) in the Llano and San Saba rivers in central Texas. Gamete densities and egg diameters of *C. petrina* in the Llano River varied seasonally, with peak gamete densities occurring in December and February 2017 and being significantly lower from June through September 2017, while the relative abundance of the largest size classes of eggs was highest in February 2018. Few to no differences were detected in gamete production and egg diameter sizes for *C. petrina* between rivers. *Cyclonaias pustulosa* had significantly higher sperm densities and smaller egg diameters compared to *C. petrina* but exhibited similar egg densities in the San Saba River. There were no significant differences in gamete densities between rivers and no significant correlations between shell length and gamete density. Infection rates of parasitic trematodes varied from <1% to 14%, with the highest infection rates occurring in *C. petrina* in the San Saba River. Sex ratio of *C. petrina* was slightly skewed toward females in the Llano River and toward males in the San Saba River, with *C. pustulosa* having a 1:1 sex ratio in the San Saba River. The high percentage of samples without gametes suggests that the reproductive outputs of *Cyclonaias* appear to be more limited in the San Saba River due to several potential stressors. Further research will need to investigate the relative importance of the various stressors that affect the reproductive ecology of mussels and their persistence.

KEY WORDS: Unionidae, gonadal fluid, reproduction, conservation, parasite, trematodes

INTRODUCTION

Traits such as reproductive timing and sex ratio are important life-history characteristics because they affect population dynamics and can be used to inform future conservation efforts. Yet, life-history data are still lacking for many unionid mussels (Haag 2012). While significant efforts have been put towards host-fish identification and the development of propagation techniques for freshwater mussels, less research has been done on the reproductive ecology of mussels, and crucial information is missing for many threatened and endangered species. Most information available on the reproductive ecology of freshwater mussels (family Unionidae) is from species within the tribes Amblemini and

Lampsilini of the subfamily Ambleminae, and the tribe Anodontini of the subfamily Unioninae (e.g., Heard 1975; Haag and Staton 2003; Moles and Layzer 2008; Haag 2012). Less research has been conducted on species within the tribe Quadrulini of the subfamily Ambleminae (but see, e.g., Jirka and Neves 1992; Woody and Holland-Bartels 1993; Haag and Staton 2003).

The tribe Quadrulini contains six genera: *Cyclonaias*, *Megalonaias*, *Quadrula*, *Theliderma*, *Tritogonia*, and *Unio* (Williams et al. 2017). Species in the tribe are classified as equilibrium strategists (Haag 2012) and, in general, can be characterized as being relatively long-lived (i.e., life span over 25 yr) with relatively low growth rates and late maturity. They primarily use either mucoid conglutinates or mantle magazines to attract host fish (Haag and Staton 2003; Barnhart et al. 2008; Sietman et al. 2012). *Cyclonaias*,

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Quadrula, and *Tritogonia* species are catfish specialists and *Theliderma* species are minnow specialists, whereas *Megaloniaias* is classified as a host generalist (Haag 2012). Fish hosts of *Unio* are currently unknown. Despite a general knowledge of life-history strategies and host-fish use of several species, there is still a lack of knowledge on the reproductive ecology (e.g., reproductive effort and timing) for most species within the tribe.

Reproductive effort and timing of mussels plays a critical role in determining the reproductive health and viability of populations. Since fecundity is often strongly related to body size, smaller *Cyclonaias*, *Quadrula*, and *Theliderma* species often have much lower fecundity rates (5–63-fold lower) compared to larger species such as *Megaloniaias nervosa* and *Tritogonia verrusco* (Haag 2013). Species in *Quadrulini* are short-term brooders (tachytictic) and, in general, gamete production increases in the fall (September–November) and reaches peak concentrations in the winter or early spring before spawning occurs in May or June (although monthly variability can exist between species and populations). The lowest gamete concentrations occur during the late summer for most species within the tribe for which data are available (Yeager and Neves 1986; Jirka and Neves 1992; Garner et al. 1999; Culp et al. 2011; Hove et al. 2011; Tsakiris et al. 2016; Dudding et al. 2020). Exceptions to this reproductive pattern have been documented for *Megaloniaias nervosa* (Woody and Holland-Bartels 1993; Haggerty et al. 2005) and a northern population of *Quadrula fragosa* (Hove et al. 2012), in which brooding and spawning occurred relatively late (summer–fall) compared to other *Quadrulini* species. Information on the seasonality of gamete production is still lacking for many species of *Quadrulini*, as is the degree of variation between populations of the same species. A better understanding of the seasonality of gamete production would improve the timing of collection of brooding mussels and host fishes and ultimately make propagation methods more predictable and efficient.

In addition to reproductive timing, sex ratio and the number of reproducing individuals is crucial in determining the reproductive potential of mussel populations (Haag and Staton 2003; Berg et al. 2008; Haag 2013). Most *Quadrulini* were found to have a relatively equal sex ratio; where variation in sex ratio has been reported, most populations showed an increased proportion of males (summarized in Berg et al. 2008 and Haag 2012). However, female-dominated populations have been documented for *M. nervosa*, *Theliderma metanerva*, and *Theliderma cylindrica* (Woody and Holland-Bartels 1993; Garner et al. 1999; Galbraith and Vaughn 2009). The exact causes of skewed sex ratios are usually not well understood in freshwater mussel populations (Haag 2012), but a strongly skewed sex ratio could lead to decreased reproductive health within a mussel population. For example, parasitic trematodes have been shown to alter sex ratios and limit the reproductive output of freshwater mussels; however, the impacts of these parasites on most freshwater mussel species are not well understood (e.g., Taskinen and Valtonen 1995; Müller et al. 2015).

Texas has approximately 50 species of freshwater mussels (Howells 2014). Like other populations throughout North America, Texas mussels have experienced significant population declines (Howells et al. 1996; Burlakova et al. 2011a; Ford and Oliver 2015; Mitchell et al. 2019). In order to better understand and inform management strategies, it is imperative to examine basic life-history traits of these species. Only three studies have investigated gamete production of mussels within Texas (Tsakiris et al. 2016; Seagroves et al. 2019; Dudding et al. 2020). Hence, the objective of this study was to evaluate the reproductive timing of two congeneric species of mussels from central Texas. *Cyclonaias petrina*, endemic to this region, is a candidate for federal listing; *Cyclonaias pustulosa* is widespread throughout the Mississippi River drainage and some coastal drainages of the Gulf of Mexico. Central Texas populations of the latter were previously named *Cyclonaias houstonensis*, but that name was recently synonymized with *C. pustulosa* (Johnson et al. 2018; Lopes-Lima et al. 2019). The reproductive ecology of *C. pustulosa* has been studied relatively well compared to other species in the tribe but little research has been conducted in subtropical rivers located in semiarid regions such as central Texas (but see Tsakiris et al. 2016; Dudding et al. 2020). Almost no information on the reproductive ecology of *C. petrina* is known, although a recent study examined monthly gamete production and gamete parasitism by digenetic trematodes (Tsakiris et al. 2016). Since *C. petrina* and *C. pustulosa* were found to be genetically different in central Texas (Johnson et al. 2018), it is important to understand how they may differ in their reproductive ecology. Our objectives were to (1) describe seasonal variation in gamete production (egg and sperm densities and egg diameters) for *C. petrina* in the Llano River from February 2017 to February 2018 and for both species in the San Saba River for the periods June–November 2017 and February 2018; (2) compare gamete production between (a) populations of *C. petrina* located in different rivers and (b) two species within the San Saba River; (3) examine the relationship between shell length and gamete density; (4) quantify rates of infection with parasitic trematodes; and (5) determine sex ratios of each species.

METHODS

We used three study sites in two rivers located in central Texas: one site in the upper Llano River and two sites in the lower San Saba River (Fig. 1). Both rivers are spring-fed tributaries of the Colorado River. At the San Saba sites, the habitat consisted of riffles with a mix of sand, gravel, cobble, and boulder substrate while the Llano River site was a run with substrate consisting of mostly bedrock with patches of boulder, cobble, and silt. We chose these sites because both species occurred in high local abundances and site access was easy.

The Llano and San Saba rivers are in the Edwards Plateau ecoregion, which is generally characterized by grasslands and juniper/oak/mesquite woodlands in shallow soils underlain by

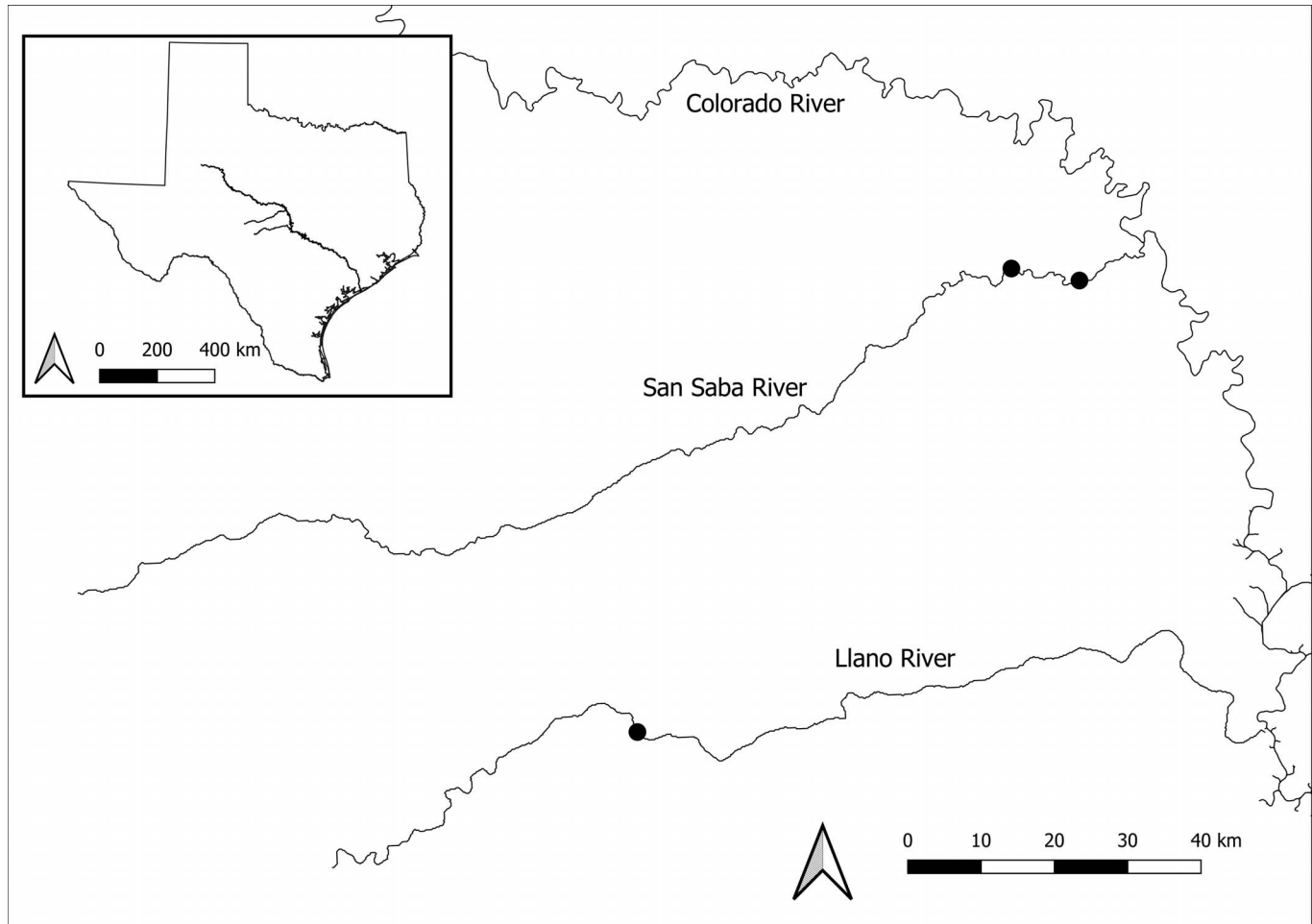


Figure 1. Site map for gamete collections in the Llano and San Saba rivers, Texas.

limestone (Griffith et al. 2007; tpwd.texas.gov). The Llano River (~169 km long) is the most urbanized tributary of the middle Colorado River but still has substantial semiarid ranchland and farmland (TPWD 1974; Heitmuller and Hudson 2009). Landcover in the San Saba River (~225 km long) is characterized by semiarid ranchland in the middle and upper stretches but is dominated by pecan orchards and row crops in the lower stretch (TPWD 1974; Griffith et al. 2007; RPS Espey 2013). Historically, the Llano River contained approximately 14 species of freshwater mussels, but it has suffered great losses in terms of species diversity over the past several decades (Strecker 1931, Howells et al 1996; Burlakova et al. 2011b; Burlakova and Karatayev 2012, Mitchell et al. 2019). It contains two candidate species for federal listing (*C. petrina* and *Lampsilis bracteata*). The loss of species richness has been less severe in the San Saba River, compared to other tributaries of the Colorado River. It still harbors around 13 of an original 16 species (Strecker 1931, Howells et al 1996; Burlakova et al. 2011b; Burlakova and Karatayev 2012; Mitchell et al. 2019), and four extant species are candidates for federal listing (*C. petrina*, *L. bracteata*, *Fusconaia mitchelli*, *Truncilla macrodon*).

Using visual and tactile methods, we collected 10 new individuals of *C. petrina* monthly in the Llano River from February 2017 to February 2018, except for October 2017 ($n=9$). The survey period was shorter in the San Saba River, where we collected individuals of *C. petrina* and *C. pustulosa* monthly from June 2017 to February 2018. We collected 10 individuals of *C. petrina* during each sampling event, but we were not able to find 10 individuals on all sampling dates for *C. pustulosa* so that sample size was six in July and August and one in September. In order to prevent unnecessary mortality as a result of mussels potentially not being able to rebury themselves in the sediment at colder temperatures (Block et al. 2013), we did not collect samples in December 2017 and January 2018 in the San Saba River because water temperatures were below 10°C at the time of sampling. All sampled mussels were uniquely marked during each survey period with shell tags to prevent resampling of gonadal fluid during future collection periods. After collecting consistently low numbers of nontagged *C. pustulosa* during the summer, we started to collect individuals at an alternate site that had similar environmental conditions and community assemblage, located approximately 9 km from our original site. A Mantel

correlogram analysis based upon data from continuous surveys completed within the lower San Saba River identified mussels from these two sites as belonging to a “single mussel patch” (Mitchell 2020). *Cyclonaias petrina* was collected from the same site in the San Saba River throughout the study.

Gonadal fluid was sampled from all individuals using a nonlethal syringe (BD 5-mL syringe Luer-Lok™ with BD PrecisionGlide™ needle; BD, Franklin Lakes, New Jersey, USA) technique (see Tsakiris et al. 2016). Gamete samples were fixed with 10% formalin, dyed with 0.01% methylene blue, and transported to the laboratory for analysis. Since our study species are not sexually dimorphic, mussels were sexed from collected gamete samples (Saha and Layzer 2008). Sperm were quantified in 10- μ L subsamples (transferred with micropipette; Fisherbrand Elite Fisher Scientific Co., Pittsburgh, Pennsylvania, USA) with a compound microscope (400 \times) and improved Neubauer hemocytometer (INCYTO DHC- N01-5; INCYTO, Covington, Georgia, USA). Sperm concentration (n /mL of gonadal fluid) was extrapolated from subsamples using Equations 1 and 2:

$$\text{Number/mL} = \# \text{ sperm in 5 small center squares} * 5 \\ * \text{dilution factor} * 10^4 \quad [1]$$

Dilution factor =

$$\frac{\text{Total volume (containing formalin and methyl blue)}}{\text{Initial sample volume (gonadal fluid)}} \quad [2]$$

We estimated egg concentration and diameter by counting and measuring the number of eggs in a 10- μ L subsample at 100 \times magnification on a glass slide and extrapolating the number of eggs to 1 mL of gonadal fluid after accounting for dilution, similar to sperm estimates. The presence or absence of trematodes within each gamete sample was recorded.

We used one-way analysis of variance (ANOVA) to examine differences in mean gamete densities and egg diameter sizes sampled in different months. We did a separate analysis for each species, at both sites and for each sex. Two-way ANOVA was used to determine whether gamete densities (i.e., egg or sperm densities) and egg diameters differed significantly between (1) rivers (Llano vs. San Saba) and sampling period (month) for *C. petrina*, and (2) between species (*C. petrina* and *C. pustulosa*) and sampling period within the San Saba River. Two-way ANOVA tests only compared months in which data were available for both rivers, both species, or both sexes. Multiple comparisons for ANOVA tests were examined using a Tukey honestly significant difference (HSD) post hoc analysis. Pearson product-moment correlations were used to estimate the association between gamete density and mussel-body size (shell length measured anterior to posterior) for each sampling period for both species (males and females separately) and rivers. A two-way ANOVA was also used to determine whether length of mussels differed significantly between mussels with and without gametes and between sampling periods. We employed Student's *t*-tests to compare the body sizes of mussels that

were infected with trematodes to those that were not. We used chi-square goodness-of-fit tests to assess whether sex ratios were significantly different from a male to female ratio of 1:1. Assumptions of normality and homogeneity of variance were tested using the Shapiro-Wilk and Levene's tests, respectively. Densities were log₁₀(x) transformed to better meet the assumptions for all analyses.

RESULTS

We collected gamete samples from 252 mussels, which included 199 samples from *C. petrina* (129 from Llano River, 70 from San Saba River), and 53 samples from *C. pustulosa* in the San Saba River. Length (mean \pm SD) of collected *C. petrina* was 49.1 \pm 7.7 mm and 59.8 \pm 10.7 mm for the Llano and San Saba rivers, respectively. Length of *C. pustulosa* was 65.7 \pm 6.1 mm in the San Saba River. Gametes were found in 76% ($n = 191$) of samples from both species. Most samples without gametes (42 of 61 samples) were collected from the San Saba River, where 43% of *C. petrina* and 23% of *C. pustulosa* were found without gametes. Samples in which no gametes were found were collected from mussels with a wide range of body sizes (37–72 mm). Size did not differ significantly between mussels with and without gametes for all sampling periods ($F_{[1,232]} = 2.48$, $P = 0.12$). In both rivers, no significant correlations between shell length and gamete density were found for either species of each sex during each sampling period ($r = 0.16$ – 0.78 ; $P > 0.05$ in all cases).

In the Llano River, differences in gamete densities between months were statistically significant for both sperm ($F_{[12,27]} = 7.39$, $P < 0.001$) and eggs ($F_{[12,57]} = 19.3$, $P < 0.001$). Sperm densities of *C. petrina* were highest in December 2017 and lowest (three orders of magnitude lower) in the summer and early fall (June–September 2017; Fig. 2A). Egg densities of *C. petrina* were highest in February and March 2017 and tended to decline during warmer months (one order of magnitude lower) before slightly increasing the following fall (October–December 2017; Fig. 2B). In the San Saba River, gamete densities were not different between months for *C. petrina* (sperm: $F_{[6,33]} = 1.07$, $P = 0.4$; eggs: $F_{[5,8]} = 3.32$, $P = 0.06$) or *C. pustulosa* (sperm: $F_{[6,34]} = 0.33$, $P = 0.91$; eggs: $F_{[5,13]} = 1.45$, $P = 0.27$; Fig. 2C, D). There was no statistically significant difference in sperm and egg densities (Tukey HSD: $P > 0.05$) of *C. petrina* between rivers when months in which data available for both rivers were compared, except for higher sperm densities in July 2017 in the San Saba River. For all months combined, *C. pustulosa* had significantly higher sperm densities (1.5-fold higher) compared to *C. petrina* ($F_{[1,34]} = 8.57$, $P < 0.01$) in the San Saba River (Fig. 2C), but exhibited similar egg densities ($F_{[1,21]} = 0.04$, $P = 0.85$; Fig. 2D).

Egg diameters varied between 30 and 435 μ m and differently sized eggs were present throughout the year (Figs. 3–5). Mean egg diameter size varied significantly between months for both *C. petrina* (Llano River: $F_{[12,1861]} = 5.58$, $P < 0.001$; San Saba River: $F_{[4,183]} = 7.01$, $P < 0.001$) and *C.*

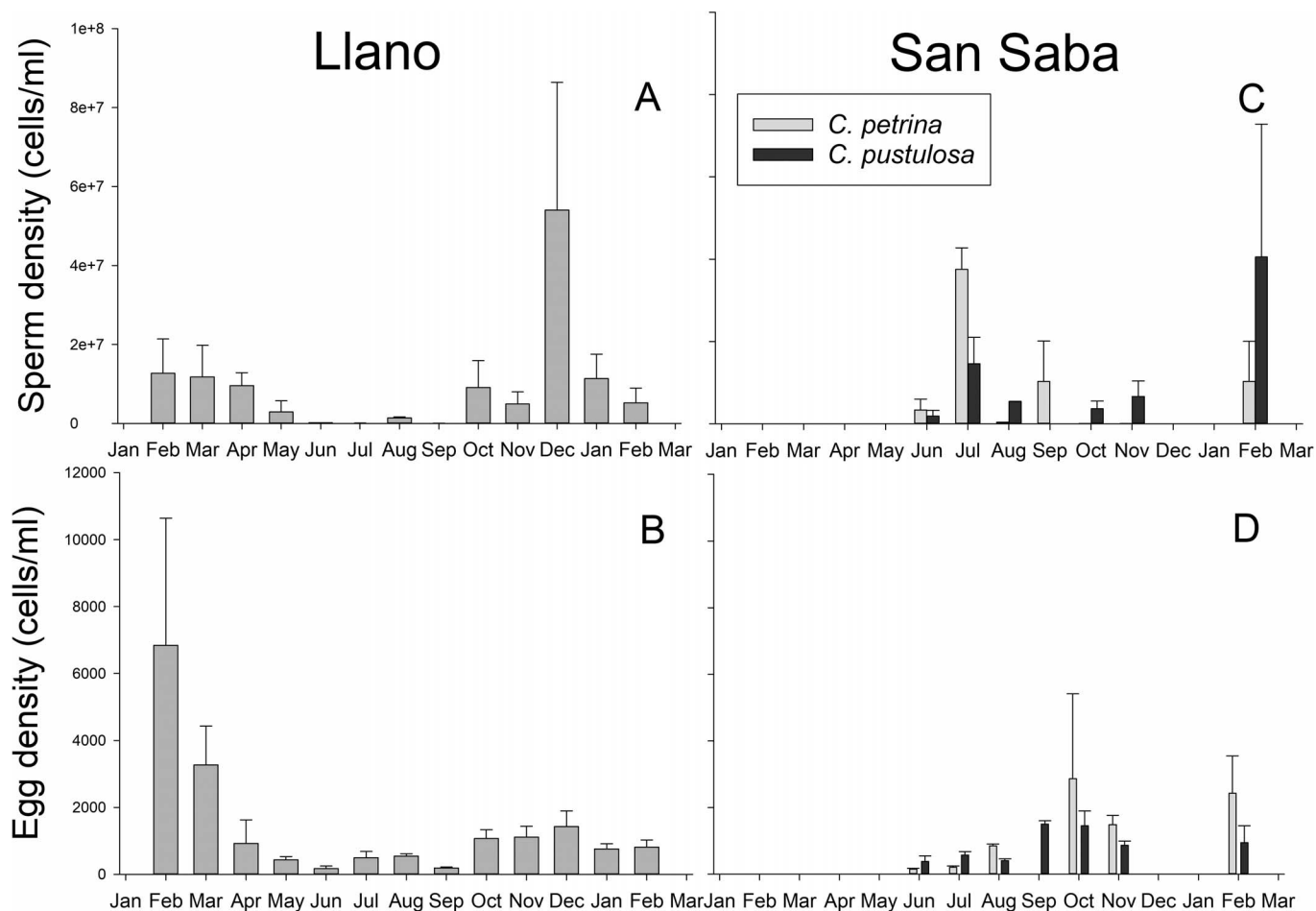


Figure 2. Monthly gamete production (mean \pm SD) for two candidate species of mussels in the Llano and San Saba rivers, Texas. Gray bars denote *Cyclonaias petrina* and black bars denote *Cyclonaias pustulosa*.

pustulosa (San Saba River: $F_{[6,360]} = 8.69$, $P < 0.001$). The relative frequency of the largest size classes of eggs for both species was highest in February 2018 in both rivers and lowest in August 2017 in the San Saba River (Figs. 4, 5). In the Llano River it was lowest for *C. petrina* in February, June, and August 2017 (Fig. 3). There were no significant differences in mean egg diameters of *C. petrina* between the Llano River (182.9 μm) and the San Saba River (195.4 μm) when comparing months in which data was available for both sites (Tukey HSD: $P > 0.05$). Overall, *C. pustulosa* had a significantly smaller mean egg diameter (mean: 158.2 μm) compared to *C. petrina* in the San Saba River (all months combined; Tukey HSD: $P < 0.001$), but both species showed similar monthly trends (Figs. 4, 5).

Trematodes were found in 5% ($n = 13$) of all collected gamete samples, primarily from June to November, and almost exclusively (12 of 13 samples) from the San Saba River. Parasite incidence for *C. petrina* and *C. pustulosa* in the San Saba River was 14% ($n = 10$) and 4% ($n = 2$), respectively, and no differences in body size were found between mussels that were infected with trematodes and those that were not ($t =$

-0.53 , $df = 250$, $P > 0.05$). No gametes were found in samples that contained trematodes.

The sex ratio for *C. petrina* in the Llano River was skewed toward females (0.6 males per female, $\chi^2_1 = 7.13$, $P < 0.01$, $n = 110$), whereas in the San Saba River *C. petrina* was skewed toward males (2.2 males per female; $\chi^2_1 = 5.16$, $P < 0.05$, $n = 40$). The sex ratio for *C. pustulosa* in the San Saba River did not significantly differ from 1:1 ($\chi^2_1 = 0.02$, $P > 0.05$, $n = 41$).

DISCUSSION

We found that gamete density of *C. petrina* was lower during the summer and early fall months (June–September) and highest during the winter and early spring months (December–March, depending upon sex), which is in accordance with previous studies on *Cyclonaias* species (Jirka and Neves 1992; Haag and Staton 2003; Galbraith and Vaughn 2009; Tsakiris et al. 2016; Dudding et al. 2020). In addition, the higher percentage of samples without gametes in the San Saba River compared to the Llano River suggests that the reproductive potential of *C. petrina* may be more limited in

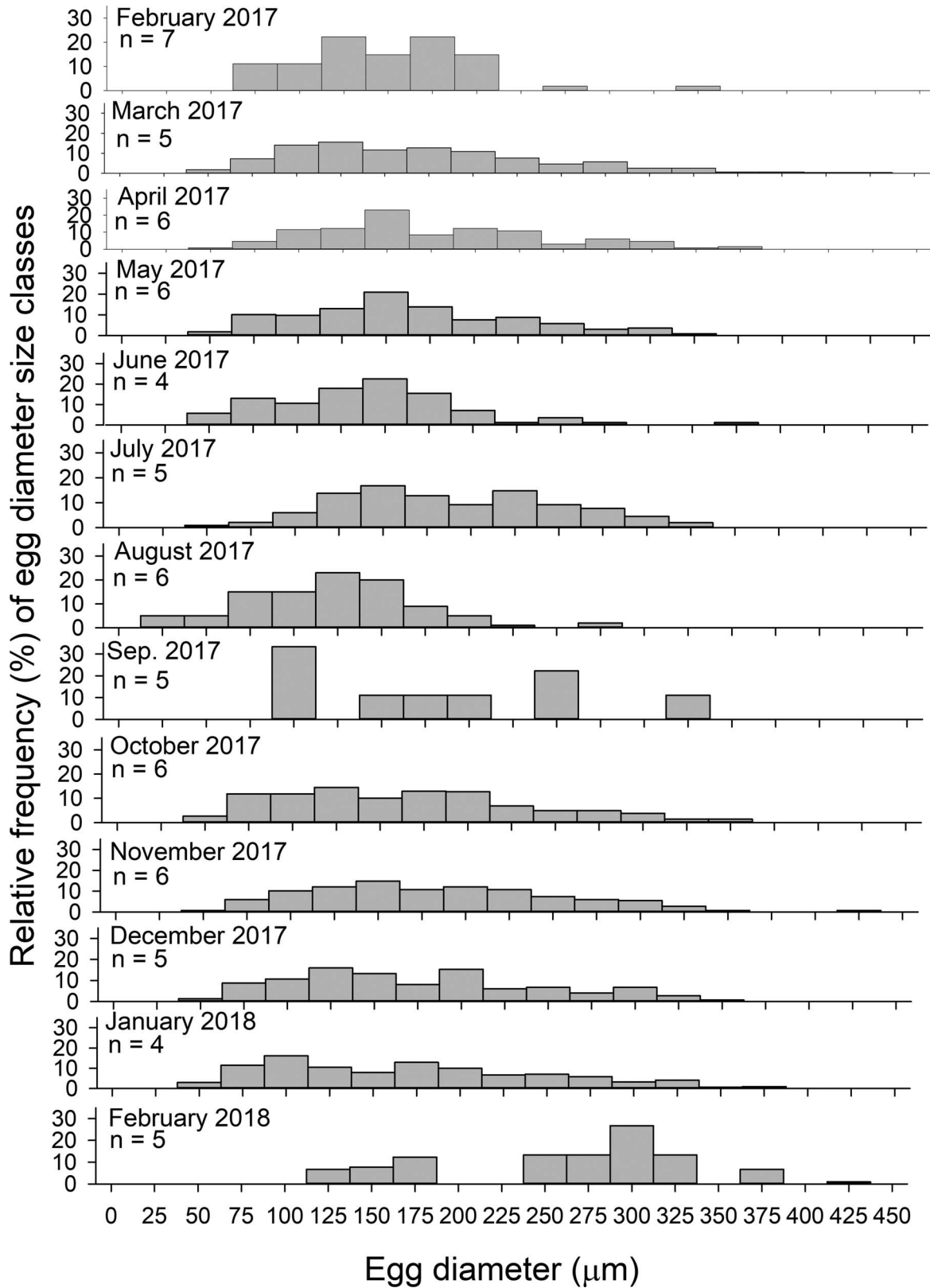


Figure 3. Egg size distribution of *Cyclonaias petrina* from the Llano River during monthly sampling events from February 2017 through February 2018.

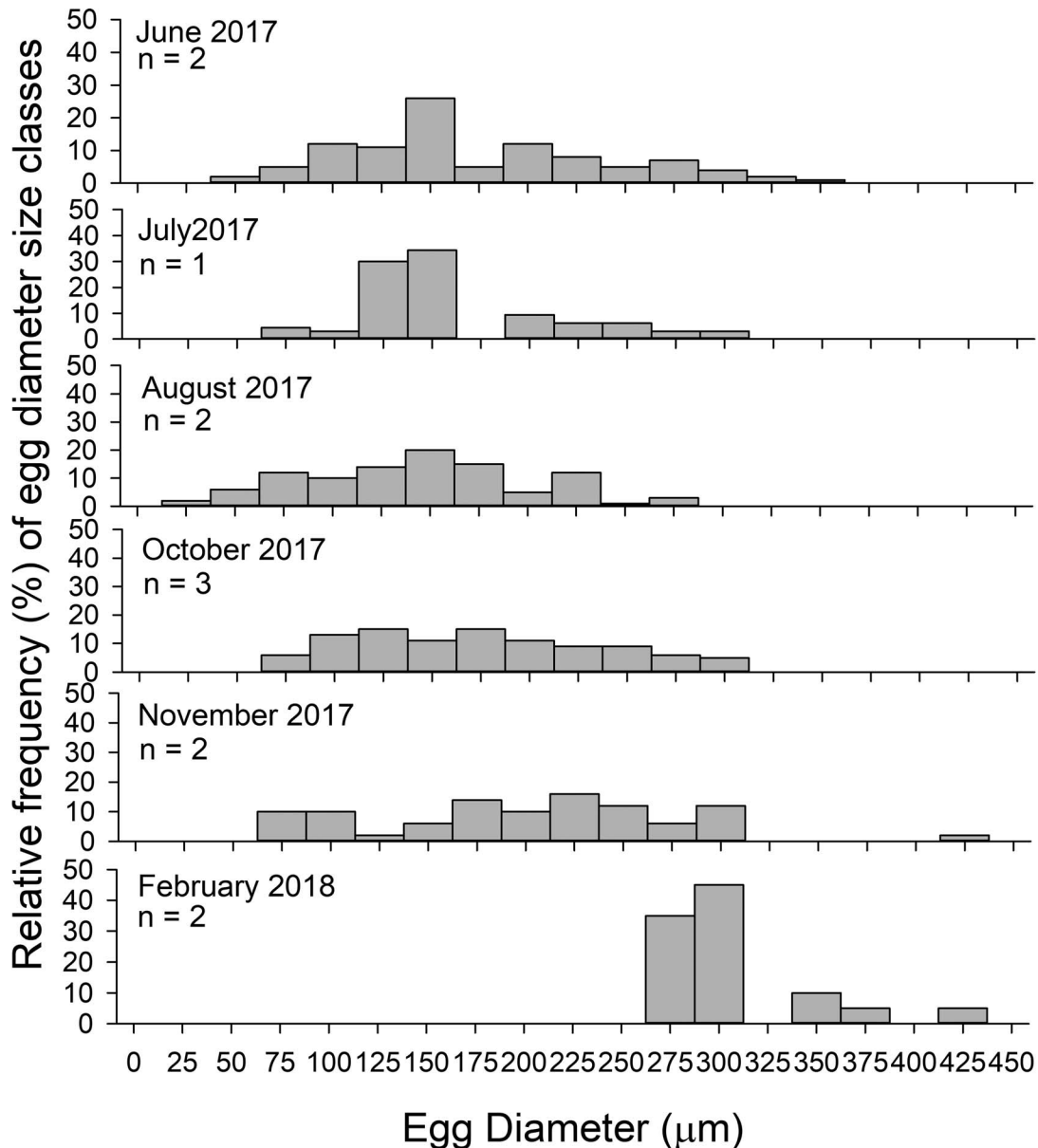


Figure 4. Egg size distribution of *Cyclonaias petrina* from the San Saba River during monthly sampling events from June 2017 through February 2018.

the former, which could be due to several potential stressors (see below).

We observed gamete densities increasing in the fall (October–November) and peaking in winter and early spring (December–March). However, seasonal variation in gamete densities represents variation in gamete production of mussels only if it is assumed that gamete fluid volume does not vary seasonally (Seagroves et al. 2019). Garner et al. (1999) suggested that mussels may benefit from starting gamete production in the fall to utilize increased nutrients within the river. Similar to our study, Tsakiris et al. (2016) reported the lowest gamete concentration of *Cyclonaias* during summer and early fall (June–September); however, they recorded peak gamete production 1–3 mo later than in our study. Low levels of active gametogenesis were found throughout the year in

Cyclonaias tuberculata with a pulse of mature gametes being produced and held during fall and early spring months (Jirka and Neves 1992), similar to our results. There seems to be little difference in gamete production between the two *Cyclonaias* species in the San Saba River, which is consistent with previous findings (Tsakiris et al. 2016).

Surprisingly, few studies on the reproductive ecology of Quadrulini mussels reported monthly changes in egg size (but see Yeager and Neves 1986; Garner et al. 1999; Haggerty et al. 2005; Tsakiris et al. 2016). Such changes tend to closely follow changes in gamete densities, with egg size being positively correlated with egg density (Yeager and Neves 1986; Garner et al. 1999; Haggerty et al. 2005; Tsakiris et al. 2016). Similar to our results, most short-term brooders that spawn in the spring and early summer (i.e., not including *M.*

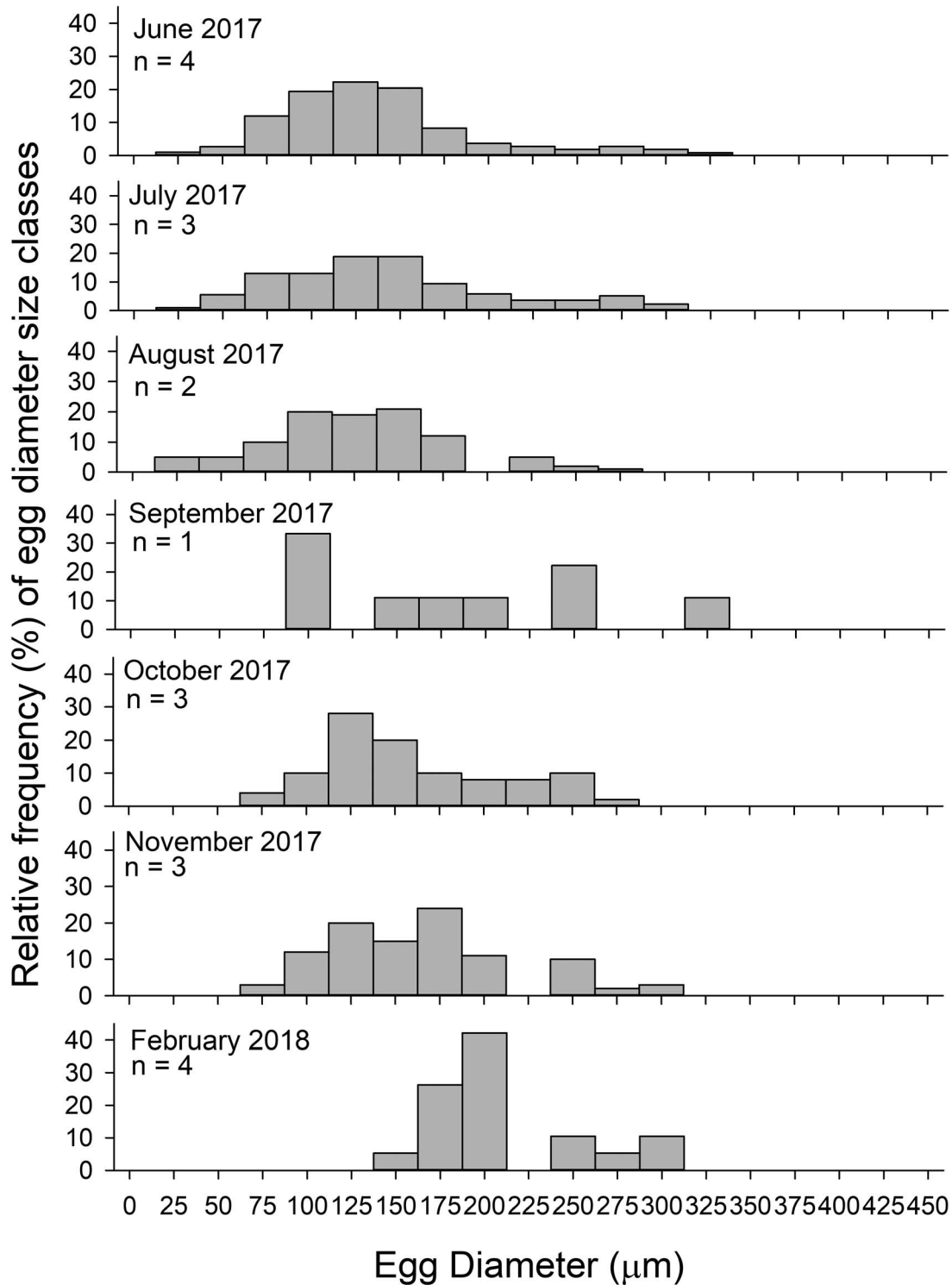


Figure 5. Egg size distribution of *Cyclonaias pustulosa* from the San Saba River during monthly sampling events from June 2017 through February 2018.

nervosa) have increases in egg diameters throughout the fall and reach maximum size from late winter to spring (February–April), before decreasing in size during the late summer (August–September; Yeager and Neves 1986; Garner et al. 1999; Tsakiris et al. 2016). Decreases in egg size within the gonads has been interpreted as an indicator of spawning, with

mature eggs presumably moving into the suprabranchial chamber to be fertilized (Garner et al. 1999). Substantial decreases in egg density for Llano River females in April 2017 (a pattern not seen in the San Saba River) also suggest that eggs had been released by the females from the gonads. Even though no brooding females were found in our study,

decreased egg densities and the estimated brooding time for tachytictic species (2–6 wk; Yokley 1972; Weaver et al. 1991; Garner et al. 1999) suggests that glochidia release occurred between April and June, similar to other species of *Quadrulini* (Yeager and Neves 1986; Jirka and Neves 1992; Haggerty et al. 1995; Garner et al. 1999; Haag and Staton 2003; Dudding et al. 2019).

Researchers have shown that gametogenesis can occur year round in many tachytictic species (Holland-Bartels and Kammer 1989; Jirka and Neves 1992; Garner et al. 1999; Tsakiris et al. 2016), which was observed in *C. petrina* in the Llano River, but not in the San Saba River. Similar to findings from another study investigating *Lampsilis bracteata* in central Texas (Seagroves et al. 2019), reproduction appeared to be more limited at sites in the San Saba River compared to the Llano River as suggested by a larger number of samples without gametes found in the San Saba River compared to the Llano River (43% vs. 11% when comparing months in which both rivers were sampled). Detrimental environmental conditions in the San Saba River may play a role, either by causing reproductive senescence in mussels as exhibited in populations of other taxa (Nussey et al. 2013) or by making mussels more susceptible to diseases and parasites (see below). Lower water quality in the San Saba River has been documented by the Texas Commission on Environmental Quality (TCEQ), with the lower San Saba River classified as impaired since 2008 due to elevated levels of *Escherichia coli*, likely resulting from nonpoint sources of pollution such as agriculture runoff and improper or lack of sewage treatment (TCEQ 2019).

As suggested by other studies, some mussels may experience decreased reproductive output due to factors other than water quality. Reproductive senescence caused by old age has been suggested (Bauer 1987; Downing et al. 1993; Haag and Staton 2003) but not supported for the vast majority of freshwater mussel species (Haag 2012). Based on age and growth data of *Cyclonaias* spp. in our study area, reproductive senescence due to old age likely did not play a role in explaining the lack of gametes within the majority of our samples because most of our mussels were less than 15 yr of age (Hayes 2020).

The higher incidence of trematodes in the San Saba River versus the Llano River may influence reproductive output in the former, potentially explaining the lack of gametes within our samples. Digenetic trematodes have been known to feed on gonadal tissue, lower reproductive output, negatively impact physiological condition, and limit growth of freshwater mussels (e.g., Taskinen 1998; Gangloff et al. 2008; Müller et al. 2015). Prevalence of parasitic trematodes in freshwater mussels has been shown to be related to season, mussel body size, age, and sex, with higher infection rates being found in larger and older females during warmer months (Huehner 1984; Taskinen et al. 1994; Taskinen and Valtonen 1995; Müller et al. 2015; Seagroves et al. 2019). Our study found increased prevalence of trematodes during the summer and fall months (June–November), but no relationship with mussel body size. Our data did not allow us to test for relationships

between parasite prevalence and mussel sex or age. Parasitic infection rates in mussels are usually low (<6%; Haag and Staton 2003), but some studies have shown relatively high (20–36%) infection rates in multiple species (Zale and Neves 1982; Tsakiris et al. 2016; Dudding et al. 2020). In the San Saba River, during congruent sampling trips, bucephalid trematodes (Bucephalidae) and unidentified parasites were found in gamete samples of *Lampsilis bracteata* (6% infection rate for all individuals) consistently and in low abundance from February 2017 to February 2018 but were not found in April and September 2017 (Seagroves et al. 2019). Conversely, parasites in our study were found mostly during the summer and fall months (June–November) in both *Cyclonaias* species. The differences in trematode infection patterns between our study and Seagroves et al. (2019) might be explained by differences in mussel or parasite life-history strategies or differential habitat use between mussels of different genera. Tsakiris et al. (2016) reported high infection levels (>20%) of digenetic trematodes in *C. petrina* and *C. pustulosa* within the San Saba River between July 2012 and July 2013 during an exceptional drought in Texas. Our lower infection rates within the San Saba River could be a product of different sampling sites, lower sample sizes, or differences in environmental conditions at the time of sampling.

Sex ratios for *C. petrina* were statistically different from 1:1 in both rivers, but *C. pustulosa* showed no difference from a 1:1 ratio. Reported sex ratios for other *Quadrulini* populations are close to equal or slightly male-biased (Yeager and Neves 1986; Jirka and Neves 1992; Haggerty et al. 1995; Garner et al. 1999; Haag and Staton 2003). Sex ratios less than 2:1 likely do not have any ecological significance (Haag 2012); however, in our study the ratio for *C. petrina* in the San Saba River slightly exceeded this. This deviation could be caused by differences in sex-specific stressors (e.g., increased female parasitism in which all gonads are removed; Kuris 1974; Müller et al. 2015) or low sample size (e.g., Ricklefs and Miller 2000; Haag 2012). In our study, the number of individuals without gametes present and those infected with trematodes was highest for *C. petrina* in the San Saba River. Possibly, the male-skewed sex ratio in the San Saba River could be the result of increased parasitism of female mussels. We cannot draw definitive conclusions because we were unable to determine the sex of *C. petrina* in the absence of gametes and because our small sample size ($n=40$) resulted in lower statistical power ($\beta=0.60$) for detecting differences in sex ratio. A sample size of about 100 individuals is needed to obtain accurate estimates of sex ratio within a mussel population (Haag 2012). Thus, more data would need to be collected in order to verify our results of a skewed sex ratio.

The results of this study have increased our insight into the reproductive ecology of *Cyclonaias* populations, which is important for their management and conservation, to predict and understand the impact of climate change and other human activities, and to improve the timing of collection of brooding mussels and host fish to facilitate propagation methods. Furthermore, our study highlights that the reproductive

potential of a species of freshwater mussel can vary substantially between tributaries within a single river basin. Further research is needed to investigate the relative importance of various stressors affecting the reproductive ecology of these mussels and the effects of reproductive variation on population persistence.

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