Reproductive life history of 2 imperiled and 1 widely-distributed freshwater mussel species from the southwestern United States

Jack F. Dudding[1,3], Michael Hart[1,4], Jennifer M. Khan[1,5], Clinton R. Robertson[2,6], Roel Lopez[1,7], and Charles R. Randklev[1,8]

[1]Texas A&M Natural Resources Institute, Texas A&M AgriLife Research Center at Dallas, 17360 Coit Road, Dallas, Texas 75252 USA
[2]Texas Parks and Wildlife Department, Inland Fisheries Division, 505 Staples Road Building 1, San Marcos, Texas 78666 USA

Abstract: Information on mussel reproductive life history, age, and growth is important for understanding evolutionary and ecological relationships and predicting how species will respond to conservation and management strategies intended to mitigate threats. In Texas, located within the southwestern United States, 11 species are pending review for listing under the Endangered Species Act, and information on mussel reproductive life history, age, and growth is lacking for most of these species. To address this knowledge gap, we examined life-history traits for 2 imperiled mussel species (*Cyclonaias necki*, Guadalupe Orb, and *Fusconaia mitchelli*, False Spike) and 1 common, widely-distributed species (*C. pustulosa*, Pimpleback) from a site in the lower Guadalupe River, located in Central Texas. The resulting information was then compared with existing life-history information for mussels. We observed peak sperm production between late January to early March and peak mean egg diameter from late winter to early summer in all 3 species. Brooding was observed in all species, usually between March and June, and brooding behavior and glochidia morphology were similar to those of congeners studied in other locations. Accumulated degree days was important in regulating the timing of gametogenesis and potentially the duration of brooding for all 3 species. Fecundity estimates for *C. necki* and *F. mitchelli* were much lower than the values reported for congeners in other locations. Fecundity was associated with both mussel age and shell length, although length was a better predictor than age. Trematode infestation rates were high (~30%) in *C. necki* and *C. pustulosa*, and sex ratios were skewed toward males, which could mean that females are disproportionately affected. The age distribution and individual growth rate for *C. necki* and *F. mitchelli* closely mirror those of related congeners, although the maximum observed age for *C. necki* did not meet theoretical expectations based on the estimated growth rate for this species. It is unknown why fecundity is reduced for *C. necki* and *F. mitchelli* or why *C. necki* may have reduced longevity, but these differences could be the result of environmental change.

Key words: life history, reproduction, unionids, Texas, endangered species, *Cyclonaias necki*, *Cyclonaias pustulosa*, *Fusconaia mitchelli*

Freshwater mussels (Bivalvia:Unionidae) are one of the most imperiled groups of organisms in North America (Master et al. 2000). Twenty-nine (10%) of these taxa are considered extinct and 195 (65%) are listed as endangered, threatened, or of special concern (Williams et al. 1993, Neves 1999, Haag 2012). Within the next century, up to 50% of these imperiled taxa are projected to go extinct in the absence of intense conservation actions (Ricciardi and Rasmussen 1999). These declines will likely have long-term negative consequences for freshwater ecosystems because mussels are filter feeders that can strongly influence primary and secondary production (Allen et al. 2012, Spooner et al. 2012, Atkinson et al. 2013).

Unionid mussels have a unique reproductive life history, which makes them an ideal model for understanding how human-mediated impacts affect freshwater ecosystems and to identify actions that help guide conservation and recovery efforts. Unionid mussels have a complex reproductive life

E-mail addresses: [3]jack.dudding@gmail.com; [4]michael.hart@ag.tamu.edu; [5]jennifer.morton@ag.tamu.edu; [6]clint.robertson@tpwd.texas.gov; [7]roel.lopez@ag.tamu.edu; [8]To whom correspondence should be addressed: crandklev@ag.tamu.edu

DOI: 10.1086/707774. Received 6 December 2018; Accepted 28 August 2019; Published online 30 January 2020.
Freshwater Science. 2020. 39(1):000–000.

cycle that involves a parasitic larval stage (glochidia), which typically utilizes various species of fish (Haag 2012). Males release spermatozeugmata (aggregates of individual sperm) into the water column, which females then take in to fertilize the eggs (McMahon and Bogan 2001). Females brood the fertilized eggs, and then glochidia, in the interbranchial chambers of their gills (marsupia) until the glochidia are mature (Kat 1984, Richard et al. 1991). The timing of spawning and brooding can vary by species and can be broadly categorized as either short-term (tachytictic) or long-term (bradytictic) (see Watters and O'Dee 2000). In general, short-term brooders spawn in the late winter and early spring, with females brooding for a short period (2–8 wk) after fertilization. Long-term brooders spawn in the late summer and autumn, with females brooding through the winter until spring. Following brooding, unionid mussels release their larvae. This release can either occur passively into the water column or actively through the use of lures or conglutinates, which mimic fish prey items (Haag 2012). In either case, the released larvae typically attach to the fins or gill filaments of their host fish and undergo transformation into free-living juveniles after several weeks (Barnhart et al. 2008).

The environmental determinants of spawning and brooding phenology are considered to be regulated by adequate flow, water quality (e.g., temperature), and food availability (Roe et al. 1997, Galbraith and Vaughn 2009), but few studies have specifically tested these associations (but see Haggerty et al. 1995, Haggerty and Garner 2000, Watters et al. 2001, Galbraith and Vaughn 2009). For studies that have examined the environmental determinants of mussel reproduction most have taken place in the midwestern or southeastern United States. Therefore, these studies may not be representative of early mussel reproductive life history, age, and growth data to different species or different populations of the same species in arid and semi-arid regions, such as Texas, located in the southwestern United States.

Texas boasts the greatest diversity of freshwater mussels in the southwestern United States with ~52 species. However, human-induced changes have resulted in significant population declines, particularly for many Texas endemics (e.g., Randklev et al. 2013a, b, 2018). As a result, the Texas Parks and Wildlife Department has listed 15 species as state threatened (TPWD 2010). Eleven of these species are under review for listing under the Endangered Species Act (USFWS 2011), and 1 has already been listed (USFWS 2018). Information on the early reproductive life history of many of these species is either unknown or based on anecdotal evidence (Howells et al. 1996, 1997, Ford and Oliver 2015), which will likely hinder conservation efforts for these species. Our objectives were to: 1) evaluate the timing of gamete production, spawning, brooding, and potential environmental cues for 2 imperiled mussel species (*Cyclonaias necki*, Guadalupe Orb, and *Fusconaia mitchelli*, False Spike) and 1 common, widely-distributed species (*C. pustulosa*, Pimpleback) from a site in the lower Guadalupe River, located in Central Texas, 2) assess their fecundity, age at maturation, longevity, and growth, and 3) compare the resulting information with existing life-history information for congeners and discuss the implications for their management and conservation.

METHODS

Study site

Our study was conducted in the mainstem of the lower Guadalupe River within Central Texas, USA (Fig. 1), which runs through the floodplains and low terraces of the Western Gulf Coastal Plain ecoregion (Griffith et al. 2007). The geology of the lower Guadalupe River is characterized by alluvial sediments, and land use is primarily ranching and agriculture (Sharif et al. 2010). The climate in the region is subtropical–subhumid and is susceptible to hydrologic extremes ranging from intense precipitation and flooding events to severe droughts (Blum et al. 1994). Baseflows are sourced from a combination of spring-fed tributaries, local groundwater inputs, upstream dam releases, and surface runoff (Young et al. 1972, Perkin and Bonner 2011). The flow regime in the lower Guadalupe River is modified by 7 mainstem impoundments, including Canyon Lake, a deep storage bottom-release reservoir (Perkin and Bonner 2011). Our study site was located in the lower Guadalupe River near the town of Hochheim, Texas (Fig. 1). We chose this site because prior freshwater mussel surveys in this river (Tsakiris et al. 2016, 2017) identified this reach as containing stronghold populations of our focal species.

Test organisms

We examined the early reproductive life history of *C. necki* (Guadalupe Orb), *C. pustulosa* (Pimpleback), and *F. mitchelli* (False Spike). *Cyclonaias necki* is endemic to the Guadalupe drainage (Randklev et al. 2017, Johnson et al. 2018). Recent collections of live individuals demonstrate that *C. necki* currently persists in the upper and lower Guadalupe and San Marcos rivers (Guadalupe drainage; Howells 2010c, Randklev et al. 2017, Johnson et al. 2018). *Cyclonaias pustulosa* is a common and widely-distributed species that occurs in the Great Lakes Basin and much of the Mississippi Basin, including East and Central Texas (Williams et al. 2008, Johnson et al. 2018). Within the Guadalupe River basin, *C. pustulosa* occurs in the San Antonio and Guadalupe rivers and adjacent tributaries (Howells 2010b, Randklev et al. 2017), the lower Guadalupe, San Marcos, San Antonio, and Medina rivers, and Cibolo Creek (Howells 2010b, Randklev et al. 2017). *Fusconaia mitchelli* was believed to be extinct until its recent rediscovery in the Guadalupe River basin (Randklev et al. 2013b). This species is known to have ranged across the Brazos, Colorado, and Guadalupe drainages of Central Texas (Strecker 1931, Howells et al. 1996, Pfeiffer et al. 2016). Live individuals of this species have recently been collected from the lower Guadalupe (Guadalupe drainage), San Saba (Colorado drainage), Llano (Colorado drainage), San Gabriel

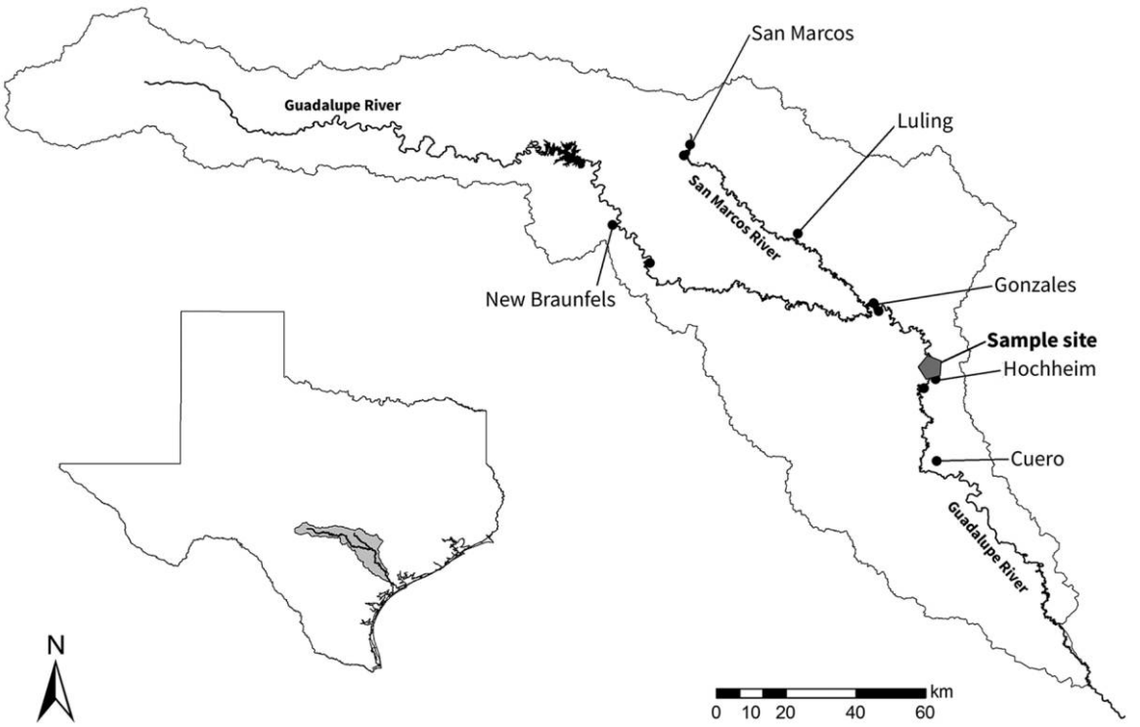

Figure 1. Map showing location of the study site within the lower Guadalupe River in Central Texas.

(Brazos drainage), and Little (Brazos drainage) rivers (Howells 2010a, Randklev et al. 2013b, 2017).

Gamete sampling

We used scuba and snorkeling to collect mussels via visual and tactile searches, depending on water level, approximately every 2 to 4 wk from 14 November 2016 to 1 December 2017. We collected a total of 20 *C. necki*, 30 *C. pustulosa*, and 20 *F. mitchelli* during each sampling period. None of these species are known to be sexually dimorphic so the sampled individuals were chosen at random. We used the syringe technique (Galbraith and Vaughn 2009, Tsakiris et al. 2016) to sample the gonadal fluid of each individual by inserting a 20-gauge hypodermic needle through the foot, at approximately the midline of the shell, halfway into the visceral mass. We extracted ~0.1 to 0.5 mL of gonadal fluid from each individual. In *C. necki* and *C. pustulosa*, gonadal fluid is a milky color, whereas in *F. mitchelli* it is a red or pink color. We fixed the samples in 0.5 mL of 10% buffered formalin solution stained with methylene blue and transported them on ice to the laboratory for analysis. Following the gonadal fluid sampling, we marked both valves on each individual with vinyl tags (Hallprint Fish Tags, Holden Hills, Australia) and measured the maximum shell length (mm) with calipers. The mussels were tagged to prevent resampling because the syringe technique may damage the gonads, so resampling could have biased the subsequent quantification of gametes. All tagged mussels were placed back into the river substrate.

The timing of gamete production for each species was determined by quantifying collected gamete samples in the laboratory. We quantified individual sperm concentration with a hemocytometer and a compound microscope (400×; Galbraith and Vaughn 2009). We quantified egg concentration by gently agitating the sample, pipetting a 10-μL subsample onto a glass slide, and counting the number of eggs with a compound microscope (40–100×). Mean egg diameter was then estimated by measuring 50 randomly-selected eggs with a compound microscope fitted with an ocular micrometer (Tsakiris et al. 2016).

Gravidity and fecundity

To assess gravidity, we collected up to 15 additional individuals/sampling period. We gently pried these individuals open with a nasal speculum and inspected their gills for signs of inflation and discoloration, which indicate the presence of fertilized eggs or glochidia. We took note of where within the gills glochidia were brooded. Gravid females were immediately placed into individual plastic bags containing river water and stored in insulated coolers with ice for transport back to the laboratory.

We assessed the fecundity of the gravid females in the laboratory by placing them into individual perforated containers in flow-through aquaria filled with reconstituted water similar to the water at their collection site. Hardness, alkalinity, and salinity were matched following the 5-salt recipe of Smith et al. (1997). All individuals released their gill contents within 24 to 120 h. We then removed the gill contents from the perforated containers, washed them into a 55-μm-mesh filter, and suspended them in 100 mL of water. We took note of whether conglutinates were produced

and their structure. We used a solution of 5% NaOH to dissolve clumps or conglutinates over a period of 5 min (Haag and Staton 2003). We then estimated total fecundity by extrapolating the counts of the gill contents from ten 200-μL aliquots (2 mL total) (Haag and Staton 2003). After we estimated fecundity, we measured the shell length of each individual to see if fecundity varied by size or age. We also used a microscope to record the overall morphology of glochidia of *C. necki*, *C. pustulosa*, and *F. mitchelli*. Measurements of total length, height, and hinge length were taken following Hoggarth (1999). Individuals of *C. pustulosa* were not included in the fecundity analysis because high flows prevented us from sampling a sufficient number of gravid females.

Age and growth

To estimate age and growth, we took thin sections of shells from 54 sacrificed male and female individuals of *C. necki* and 54 individuals of *F. mitchelli*. A single valve was coated in epoxy (EpoxiCure™ 2 Epoxy Resin and Hardener; Buehler, Lake Bluff, Illinois) and cured overnight. The epoxied shells were then cut into thin sections (1.0–1.5 mm) along an axis running from the umbo to the dorsal margin with a Buehler IsoMet 1000 Precision Cutter low-speed saw equipped with a diamond wafering blade (12.7 mm) (Haag and Commens-Carson 2008). The resulting thin sections were mounted on standard, unfrosted microscope slides with Crystalbond 509 clear mounting adhesive (SPI® Supplies, West Chester, Pennsylvania) and sanded with DiaSharp® Bench Stones (DMT®, Marlborough, Massachusetts) at progressively-finer grit sizes to ~300 μm to increase the visibility of the annual growth lines (annuli). We used light microscopy to identify the annuli as internal lines within the shell matrix that extended from the umbo and crossed the periostracum without interruption (Haag and Rypel 2011). A second counter was used to validate the annulus counts, and discrepancies between the 2 readers were either reconciled or individual specimens with incongruent counts were removed from the dataset. We then fit a von Bertalanffy growth curve to the age and length estimates for each species to determine the length (L_t, mm) at a given time (t, age in y); L_∞, the predicted mean maximal length (mm) for individuals in the population that depicts the asymptotic length at which growth is 0; K, the Brody growth constant that depicts the rate at which an individual approaches L_∞ (mm/y); and t_0, the theoretical time at which $L = 0$ (Ricker 1975). To account for the younger age classes that were not sampled, we back-calculated the external annuli with a subsample of the large individuals for which age had been previously estimated. Individuals of *C. pustulosa* were not included in this analysis because high flows prevented us from sampling a sufficient number of individuals.

Environmental data

To assess potential environmental determinants of spawning and brooding, we measured water temperature and depth, discharge, and accumulated degree days (a measure of the total heat an organism has experienced over time). Water temperature (°C) and depth (m) were recorded at 15-min intervals with HOBO® Water Level Loggers (Onset®, Bourne, Massachusetts) deployed at the study site for the duration of the study. We obtained flow data from a nearby United States Geographical Survey gaging station (#08174700; 1.6 km downstream of the study site) by relating the water depth recorded at the study site to the corresponding water depth and discharge measured at the gaging station. Photoperiods were based on estimates of day length obtained from the US Naval Observatory Astronomical Application Department (https://www.esrl.noaa.gov/gmd/grad/solcalc/sunrise.html). We calculated accumulated degree days from the start of the study with the University of California Statewide Integrated Pest Management Program online degree day calculator (Baskerville and Emin 1969, UC IPM 2018). We followed Galbraith and Vaughn (2009) and defined the limits of growth between 10 and 30°C based on metabolic rate data for *C. pustulosa*, 1 of our focal species and a close congener of *C. necki* (Spooner and Vaughn 2008). We then determined the maximum and minimum daily temperatures from logger data and used a single sine method to calculate the number of degree days accumulated since the start of the study (Galbraith and Vaughn 2009).

Statistical analyses

The timing of gamete production for each species was determined by plotting sperm or egg concentration and oocyte diameter against the sample date. We expected to see an increase in gamete concentration or oocyte diameter as reproduction progressed and a peak where all 3 were highest, signifying that spawning had occurred.

We used a general additive model (GAM) approach to assess the relationship between the measured environmental parameters (e.g., mean daily temperature, accumulated degree days, photoperiod, and mean daily flow) and number and timing of gametes produced. We modeled each variable individually. We chose GAMs because they use smoothers that are robust to assumptions about independence and multicollinearity and can capture non-linear relationships among variables. Significance of the smoothing function is used to evaluate change in the response variable, which in this study was an increase or decrease in gametes produced over time. We used the *mgcv* package to implement GAMs in R (version 3.4.3; R Project for Statistical Computing, Vienna, Austria). To identify which parameter best predicted gamete production, we ranked the resulting GAM models based on Akaike's Information Criterion adjusted for sample size (AIC_c). We then calculated AIC_c weights (w) for each model and considered the model with the highest weight to be the best-supported model (Anderson and Burnham 2002). However, we considered all models with $\Delta AIC_c \leq 2$ to be plausible.

To assess the departure from a 1:1 sex ratio, we did an χ^2 goodness-of-fit test in R and considered results with $p \leq 0.05$ to be significant.

To assess whether occurrence of trematodes differed by size, we used ordinary least squares regression to assess the relationship between mean shell length and frequency of trematode infection (presence of sporocysts and cercaria life stages) across sample dates in R. We considered results with $p \leq 0.05$ to be significant.

We used an independent samples *t*-test with unequal variance in R to assess if shell length differed between males and females of each species. We considered results with $p \leq 0.05$ to be significant.

We fit the von Bertalanffy growth equation for both species with the *fishmethods* package in R to estimate age and growth. We then used GAMs to relate fecundity with age and length estimates and to determine onset of sexual maturity. In this analysis, a significant smoothing function indicates significant change in fecundity with increases in either age or length.

RESULTS

In total, we extracted gonadal fluid from 843 individuals: 247 *C. necki*, 368 *C. pustulosa*, and 228 *F. mitchelli*. All sampled individuals were dioecious, and no hermaphroditism was observed. The sex ratio of *C. necki* differed significantly from 1:1 (χ^2 goodness-of-fit test, $p < 0.0001$), and males comprised 62.1% of the sampled individuals. The sex ratio of *C. pustulosa* also differed significantly from 1:1 (χ^2 goodness-of-fit test, $p < 0.001$), and males comprised 73.9% of sampled individuals. The sex ratio of *F. mitchelli* did not differ significantly from 1:1 (χ^2 goodness-of-fit test, $p > 0.05$), and males comprised 58.7% of the sampled individuals.

The shell length of the sampled individuals ranged from 29 to 59 mm (mean SE = 45.15 ± 0.34) for *C. necki*, 27 to 61 mm (47.07 ± 0.32) for *C. pustulosa*, and 30 to 65 mm (48.48 ± 0.38) for *F. mitchelli*. The independent samples *t*-test with unequal variance revealed that shell length differed significantly between males and females in both *C. necki* and *C. pustulosa* ($p < 0.05$), but not in *F. mitchelli* ($p > 0.05$). In both *C. necki* and *C. pustulosa*, mean shell length was larger for males than females (mean male shell length: 45.93 mm for *C. necki* and 47.94 mm for *C. pustulosa*; mean female shell length: 44.10 mm for *C. necki* and 43.24 mm for *C. pustulosa*).

Spawning and brooding

The mean sperm concentration peaked in late January in *C. necki* (Fig. 2A), from late January to early March in

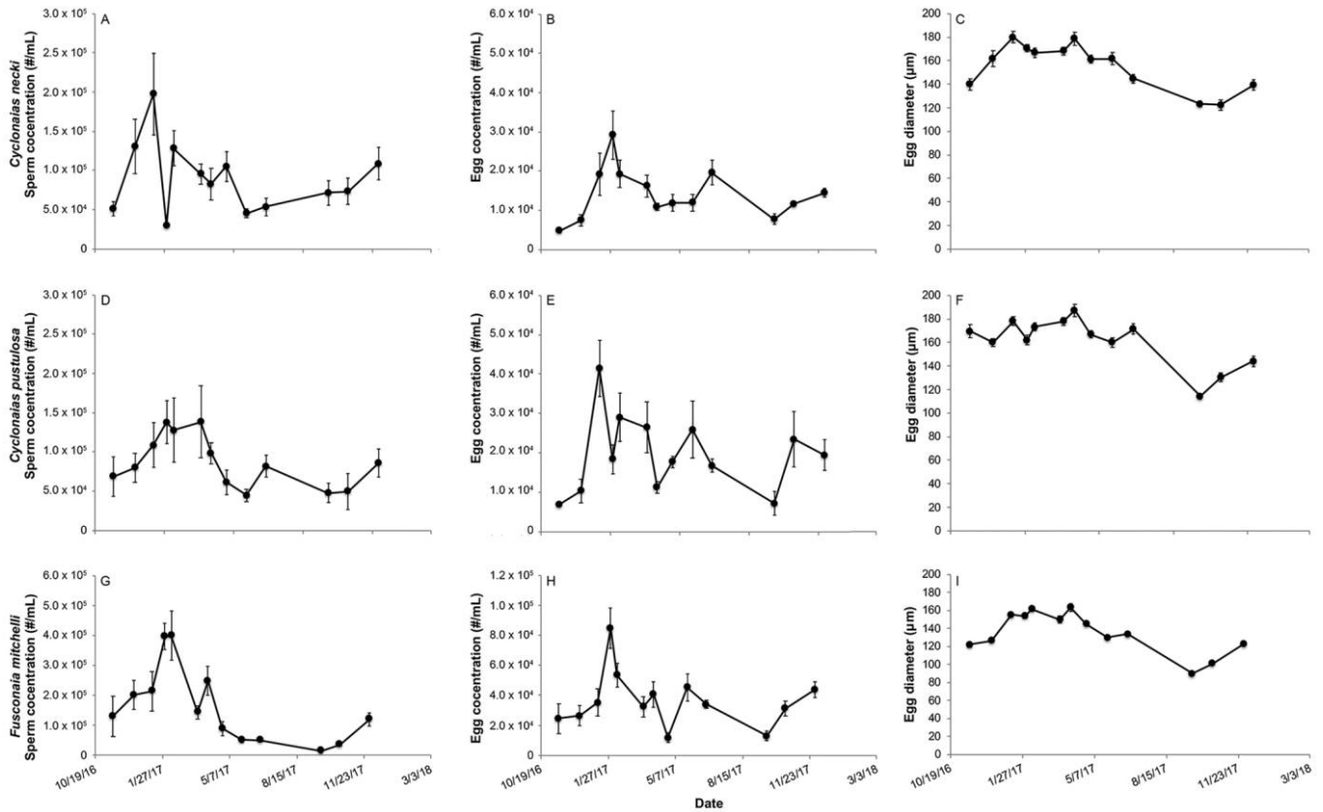

Figure 2. Mean sperm concentration (#/mL), mean egg concentration (#/mL), and mean egg diameter (µm) observed in *Cyclonaias necki* (Guadalupe Orb; panels A–C), *C. pustulosa* (Pimpleback; panels D–F), and *Fusconaia mitchelli* (False Spike; panels G–I) from November 2016 to December 2017. Error bars denote ±1 standard error.

C. pustulosa (Fig. 2D), and from late January to early February in *F. mitchelli* (Fig. 2G). Sharp declines in sperm concentrations following these events suggest that spawning had occurred. However, the sperm concentration remained elevated following peaked concentrations for *C. necki* and *C. pustulosa* compared to *F. mitchelli* (Fig. 2A, D, G). The mean egg concentration peaked during the same period as mean sperm concentration (Fig. 2B, E, H), which further indicates that spawning had likely occurred following these events. Mean egg diameters were largest from late winter to early summer in all 3 species (Fig. 2C, F, I). We observed individuals brooding mature glochidia shortly after spawning, between March and June, and peak brooding for all species occurred in early April. However, a single individual of *C. necki* with fully mature glochidia was observed in September.

Females of all 3 species were tetragenous, brooding glochidia in both their inner and outer gills. However, the position along the gills where these species brooded glochidia differed. For *C. necki* and *C. pustulosa*, glochidia were held primarily in the central portion of the marsupia, whereas for *F. mitchelli* brooding was distributed throughout the marsupia. *Cyclonaias necki* and *C. pustulosa* embryos were white and remained so throughout maturation. *Fusconaia mitchelli* embryos were deep red to pink but grew increasingly lighter in color with maturity. *Cyclonaias necki* and *C. pustulosa* brooded glochidia in lanceolate-shaped conglutinates in the marsupia, but the conglutinates did not maintain their structure after the glochidia were released. *Fusconaia mitchelli* also brooded glochidia in lanceolate-shaped conglutinates, but the conglutinates maintained their structure after they were released.

All 3 species had semi-elliptical and hookless glochidia. *Cyclonaias necki* glochidia had the following mean valve dimensions: length = 264 μm (± 2.098 SE); width = 324 μm (± 2.098 SE); and hinge length = 129 μm (± 2.627 SE). *Cyclonaias pustulosa* glochidia had the following mean dimensions: length = 215 μm (± 1.581 SE); width = 262.5 μm (± 1.904 SE); and hinge length = 87.5 μm (± 1.273 SE). *Fusconaia mitchelli* glochidia had the following mean valve dimensions: length = 172 μm (± 1.897 SE); width = 159 μm (± 1.703 SE); and hinge length = 168 μm (± 1.265 SE).

Spawning/brooding-environment relationships

Models that related the accumulated degree days to mean egg diameter and log of the sperm concentration for all 3 species generally were the most parsimonious (AIC_c selection; Table 1). All GAMs had significant smoothing functions, indicating that mean egg diameter and log of the sperm concentration varied with accumulated degree days (Table 2, Fig. 3A–F). For mean egg diameter, all 3 species peaked during low number of accumulated degree days (Fig. 3A, C, E), which coincides with late winter and early spring and is the same pattern we observed in relating egg diameter to calendar date (Fig. 2A–I). For the log of sperm concentration, *C. necki* and *C. pustulosa* showed peak concentration during low number of accumulated degree days, which then gradually decreased (Fig. 3B, D). For *F. mitchelli*, log of the sperm also peaked during lower number of accumulated degree days but then dramatically decreased (Fig. 3F). These patterns mirror our results relating sperm concentration to calendar date for all 3 species (Fig. 2A–I).

Fecundity

We estimated fecundity of *C. necki* and *F. mitchelli* based on 34 and 31 individuals, respectively. We did not estimate fecundity of *C. pustulosa* because high-flow conditions prevented sample collection. For *C. necki*, fecundity averaged 5849 embryos/individual (± 533 SE; range: 1080–13,150). For *F. mitchelli*, fecundity averaged 12,726 embryos/individual (±1600 SE; range: 2340–32,250). Most GAMs that evaluated the relationship between fecundity and age or length had significant smoothing functions, but the relationship between fecundity and age for *F. mitchelli* was only marginally significant ($p = 0.09$; Table 3). The shape of the response across both species and relationships was positive, such that mean fecundity generally increased with age or length (Fig. 4A–D). Shell length explained more variation in fecundity than did age (Table 3, Fig. 4A–D).

Trematodes

Of the 247 *C. necki* sampled, 70 (28.3%) showed signs of parasitism by digenean trematodes based on the presence of sporocyst and cercaria life phases in their gonadal fluid. Similarly, of the 368 *C. pustulosa* sampled, digenean trematodes were observed in the gonadal fluid of 130 (35.3%) individuals. However, no digenean trematodes were observed in the gonadal fluid of *F. mitchelli*. Individuals infested with digenean trematodes could not be sexed since the individuals had been castrated and the gonadal fluid did not contain gametes. The linear regression of frequency of trematode infection on shell length was significant for *C. pustulosa* ($p < 0.05$, $r^2 = 0.26$), but the regression for *C. necki* was not ($p > 0.05$, $r^2 = 0.02$).

Age and growth

The age range for samples of *C. necki* was 1 to 13 y ($n = 54$) and of *F. mitchelli* was 1 to 15 y ($n = 58$). The earliest age of reproduction for *C. necki* was 3 y (shell length of 36 mm), whereas for *F. mitchelli* the earliest was 5 y (shell length of 42 mm). Onset of reproduction in *C. pustulosa* was observed at a shell length of 33 mm.

Individual growth varied between *C. necki* and *F. mitchelli*, whereas longevity was similar between the 2 species (Table 4, Fig. S1). Specifically, *C. necki* had a low growth constant ($K = 0.142$; Table 4) compared to *F. mitchelli* ($K = 0.231$), whereas the maximum age of thin-sectioned individuals was 13 y for *C. necki* and 15 y for *F. mitchelli* (Table 4).

DISCUSSION

Spawning and brooding

Our results demonstrate that *C. necki, C. pustulosa*, and *F. mitchelli* are short-term brooders that have reproductive traits similar to those of closely-related congeners within

Table 1. Summary of small-sample Akaike Information Criterion (AIC_c) selection of univariate general additive models relating environmental factors to gametogenesis (i.e., egg diameter and sperm concentration) for *Cyclonaias necki* (Guadalupe Orb), *C. pustulosa* (Pimpleback), and *Fusconaia mitchelli* (False Spike). Measurements: discharge (m^3/s), water temperature (°C), day length (h). ΔAIC_c = AIC_c of model relative to the lowest AIC_c, w_i = Akaike weight, K = number of parameters in the model, conc = concentration, diam = diameter.

Species and model	AIC_c	$DAIC_c$	w_i	K
Cyclonaias necki – Male				
Log(Sperm conc) ~ Accumulated degree days	355.93	1.37	0.33	6
Log(Sperm conc) ~ Discharge	362.03	7.48	0.02	4
Log(Sperm conc) ~ Water temperature	354.55	0.00	0.65	4
Log(Sperm conc) ~ Day length	364.30	9.75	<0.001	3
Cyclonaias necki – Female				
Egg diam ~ Accumulated degree days	573.54	0.00	0.99	6
Egg diam ~ Discharge	589.41	15.88	<0.001	4
Egg diam ~ Water temperature	603.67	30.13	<0.001	3
Egg diam ~ Day length	620.43	46.89	<0.001	3
Cyclonaias pustulosa – Male				
Log(Sperm conc) ~ Accumulated degree days	632.00	0.00	0.95	4
Log(Sperm conc) ~ Discharge	638.33	6.33	0.04	5
Log(Sperm conc) ~ Water temperature	647.36	15.36	<0.001	3
Log(Sperm conc) ~ Day length	650.02	18.03	<0.001	5
Cyclonaias pustulosa – Female				
Egg diam ~ Accumulated Degree Days	517.42	0.00	0.99	7
Egg diam ~ Discharge	537.10	19.68	<0.001	10
Egg diam ~ Water temperature	544.08	26.66	<0.001	10
Egg diam ~ Day length	575.76	58.33	<0.001	3
Fusconaia mitchelli – Male				
Log(Sperm conc) ~ Accumulated degree days	362.77	0.00	0.99	7
Log(Sperm conc) ~ Discharge	373.01	10.23	0.01	10
Log(Sperm conc) ~ Water temperature	375.71	12.93	<0.001	10
Log(Sperm conc) ~ Day length	431.85	69.08	<0.001	7
Fusconaia mitchelli – Female				
Egg diam ~ Accumulated degree days	901.03	0.00	0.99	7
Egg diam ~ Discharge	950.75	49.72	<0.001	5
Egg diam ~ Water temperature	974.04	73.02	<0.001	3
Egg diam ~ Day length	987.23	86.20	<0.001	3

the Quadrulini and Pleurobemini tribes (Barnhart et al. 2008, Haag 2013). In general, sperm and egg production peaked from late January to early March, although there was some variability depending on the species, which indicates that spawning likely takes place during this time. However, for both *Cyclonaias* species, particularly *C. pustulosa*, sperm concentrations remained elevated until late spring/beginning of summer. This long-term elevation in gamete concentrations could indicate a protracted period of spawning, which has also been observed in congeners within Quadrulini (Haggerty et al. 1995, Garner et al. 1999, Haag 2013). Glochidia maturation was relatively quick in all species and lasted from March to July, which is characteristic of short-term brooders and mirrors the pattern of brooding in several closely-related congeners (Haag 2013).

Cyclonaias necki, *C. pustulosa*, and *F. mitchelli* brood glochidia in both the inner and outer gills (i.e., they are tetragenous), which is similar to closely-related congeners (Coker et al. 1921, Haag and Staton 2003). Most of the individuals we sampled, regardless of species, had partially-charged gills, which has been observed in populations of *C. pustulosa* outside of Texas and in congeners such as *F. cerina* (Gulf Pigtoe) (Haag and Staton 2003). Partially-charged gills have been attributed to poor recruitment (Haggerty et al. 1995), but Haag and Staton (2003) argue that this is a regular characteristic of mussel reproduction.

Both *Cyclonaias* species released white lanceolate-shaped conglutinates, which contained glochidia, that fell apart shortly after release. It is unknown whether these conglutinates are functional and able to infect host fish or are puerile

Table 2. Generalized additive modeling (GAM) results for *Cyclonaias necki* (Guadalupe Orb), *C. pustulosa* (Pimpleback), and *Fusconaia mitchelli* (False Spike) relating mean egg diameter (μm) or log sperm concentration (#/mL) to the accumulated number of degree days.

Species	Sex	Adjusted r^2	Deviance explained	Estimated df	*F*-value	*p*-value
Cyclonaias necki	Male	0.07	8.2	1.3	0.92	0.004
	Female	0.51	53.1	3.33	7.53	<0.0001
C. pustulosa	Male	0.10	10.6	2.1	2.06	<0.0001
	Female	0.63	66.0	4.3	11.72	<0.0001
Fusconaia mitchelli	Male	0.56	57.8	4.7	17.19	<0.0001
	Female	0.57	59.2	4.6	15.13	<0.0001

and disintegrate as fertilized eggs develop into glochidia (Barnhart et al. 2008, Watters 2008). Most species within Quadrulini have puerile conglutinates, although individuals of some species hold clumps of conglutinates, including individuals in populations of *C. pustulosa* outside of Texas (Barnhart et al. 2008). For *F. mitchelli,* we observed the release of pink, leaf-like conglutinates, with unfertilized eggs acting as structural elements. This characteristic is unique to the Pleurobemini tribe (Haag and Staton 2003, Haag and Warren 2003, Barnhart et al. 2008). Unlike in *C. necki* and *C. pustulosa*, these conglutinates are likely functional and probably facilitate host infection by resembling food

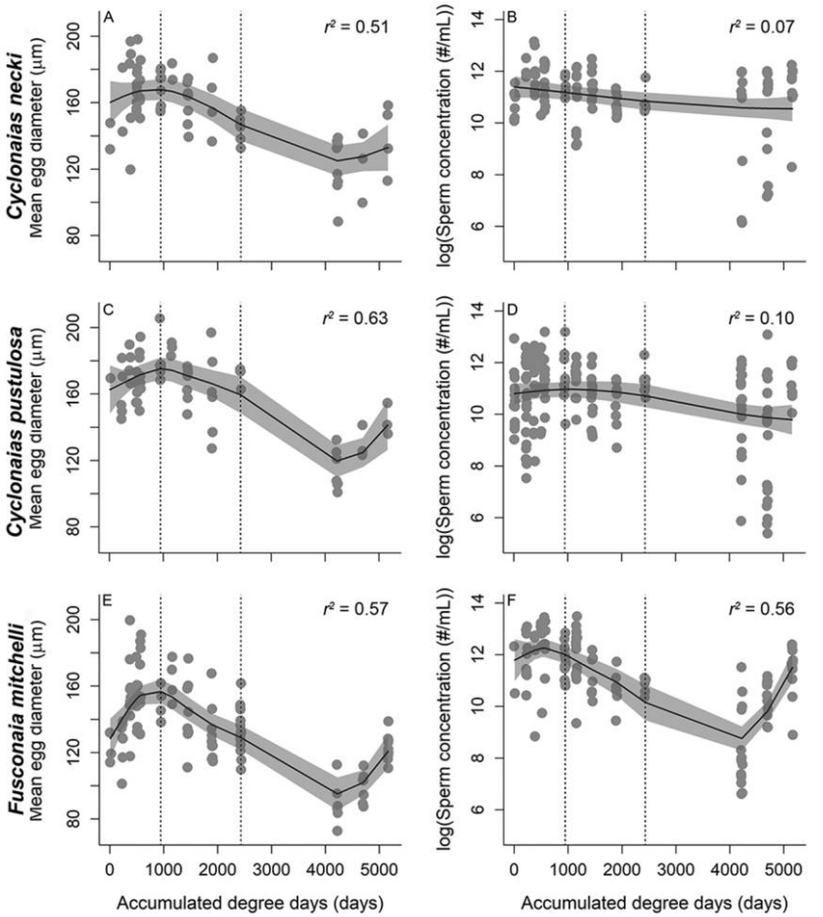

Figure 3. Relationship between mean egg diameter (μm) and log sperm concentration (#/mL) with accumulated degree days for *Cyclonaias necki* (Guadalupe Orb; panels A and B), *C. pustulosa* (Pimpleback; panels C and D), and *Fusconaia mitchelli* (False Spike; panels E and F). The solid line is the best-fit line for each relationship. Shaded polygons denote 95% confidence intervals, and dotted lines bracket the brooding period. The coefficient of determination (r^2) is given for each model.

Table 3. Generalized additive modeling results for *Fusconaia mitchelli* (False Spike) and *Cyclonaias petrina* (Texas Pimpleback) relating fecundity (Fecun) to age (y) and maximum shell length (mm).

Species	Model	Adjusted r^2	Deviance explained	Estimated df	*F*-value	*p*-value
Cyclonaias necki	Fecun ~ Age	0.22	24.4	1.10	1.02	0.003
	Fecun ~ Length	0.36	38.9	1.69	2.03	<0.0001
Fusconaia mitchelli	Fecun ~ Age	0.07	9.08	0.76	0.26	0.09
	Fecun ~ Length	0.32	35.0	1.30	1.57	<0.0001

items for cyprinid minnows (Barnhart et al. 2008, Dudding et al. 2019).

Spawning/brooding-environment relationships

Our results indicate that accumulated degree days, which are a measure of the total heat an organism has experienced over time, is a good determinant of gamete production. For all 3 species, egg and sperm concentration was maximized during low number of degree days and brooding followed during intermediate number of accumulated degree days. This pattern makes sense given that mussels are ectotherms and gametogenesis and glochidia development are presumably regulated by water temperature (Haag 2012). The occurrence of spawning during late winter and brooding during spring and early summer are likely adaptations to ensure mussel reproduction is completed before water temperatures exceed thermal optima for not only glochidia and juveniles but also adults. This reproductive window may also minimize impacts to energy allocation for juveniles and adults during summer and late autumn when growth and maintenance are likely maximized (Haag 2012).

We also observed exceptions to the overall pattern in gamete production across all 3 species. For example, *C. necki* and *C. pustulosa* sperm production remained somewhat elevated following peak spawning, which is likely characteristic of the male reproductive status of these species. For example, studies of *C. pustulosa* outside of Texas have also shown that sperm concentration remains elevated post-peak spawning (Galbraith and Vaughn 2009). The reason for this is not well understood but could be indicative of asynchronous spawning, wherein most individuals spawn during a specific period in time but some otherwise reproductively-active members of a population do not. Some of these other individuals do eventually reproduce but at a different time when conditions are favorable. This strategy may be a form of bet-hedging to ensure some individuals

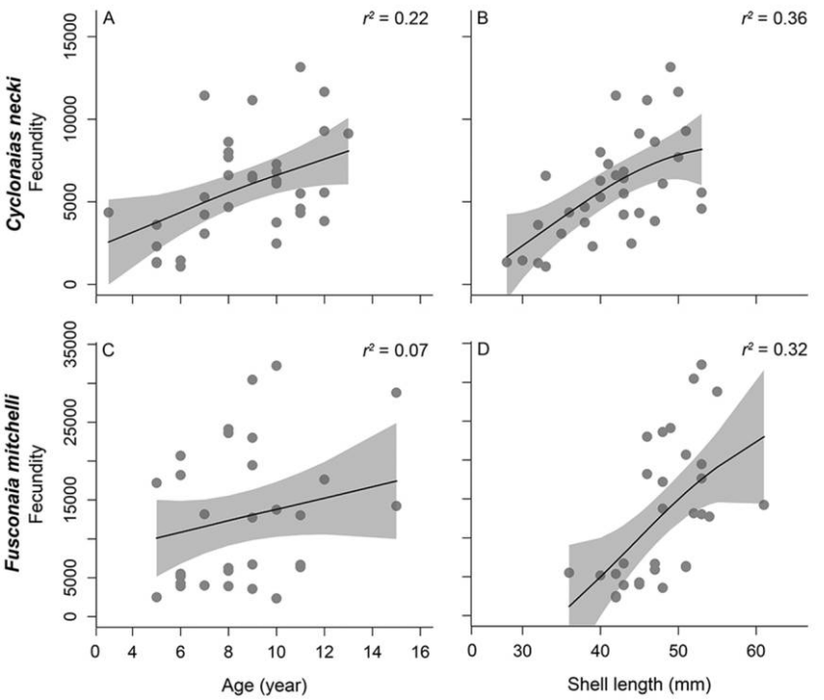

Figure 4. Relationship between fecundity and age or length for *Cyclonaias necki* (Guadalupe Orb; panels A and B) and *Fusconaia mitchelli* (False Spike; panels C and D). The solid line indicates the best-fit line for each relationship. Shaded polygons denote 95% confidence intervals.

Table 4. Population growth parameters for *Cyclonaias necki* (Guadalupe Orb) and *Fusconaia mitchelli* (False Spike) derived from fitted von Bertalanffy growth curves for each species. Pseudo-r^2 = coefficient of determination, L_∞ = the predicted mean maximal length (mm) for the population, K = the Brody growth constant, t_0 = is the theoretical time at which $L = 0$, max age = maximum observed age, n = number observed, Cl-lower = lower confidence limit, Cl-upper = upper confidence limit. 95% confidence intervals are provided for each parameter estimate.

Species	n	Pseudo-r^2	L_∞	Cl-lower	Cl-upper	K	Cl-lower	Cl-upper	t_0	Cl-lower	Cl-upper	Max age
Cyclonaias necki	54	0.99	55.49	47.63	89.49	0.14	0.05	0.24	−1.64	−4.30	−0.36	13
Fusconaia mitchelli	58	0.99	56.43	53.01	61.34	0.23	0.18	0.29	−0.12	−0.67	0.28	15

of a population reproduce in a changing environment. In our study area, such a strategy could be advantageous as the Guadalupe River is subject to frequent droughts and floods. Asynchronous spawning has also been observed in fish species experiencing similar environmental conditions in Texas (Durham and Wilde 2008, Robertson et al. 2016). Also, hydrologic extremes combined with a warmwater system like the Guadalupe River should affect the magnitude and variation in accumulated degree days, which means a wider reproductive window, as shown with our results, for species inhabiting these systems.

Fecundity

Fecundity of *C. necki* and *F. mitchelli* was low in comparison to that of other unionid species (Haag and Staton 2003, Haag 2013). For example, Haag and Staton (2003) estimated that female *C. pustulosa* from the Sipsey River in Alabama had a mean fecundity of 28,369 embryos/female (range = 49–50,625) and that female *F. cerina* had a mean fecundity of 23,890 embryos/female (range = 8750–55,422). In our study, the mean fecundity of *C. necki* was 5849 embryos (±533 SE) and ranged from 1080 to 13,150 embryos. We estimated the mean fecundity of *F. mitchelli* to be 12,726 embryos (±1600 SE), ranging from 2340 to 32,250 embryos/female. Thus, our focal species had low fecundity relative to closely-related congeners outside of Texas. This finding raises questions about whether our estimates are a byproduct of a poor reproductive year because of trematode parasitism, human-induced impacts to flow and water temperature, or both. However, the lower fecundity, particularly for *C. necki* and presumably *C. pustulosa*, could also be a result of protracted brooding for these species, which seems plausible based on our observations of potential asynchrony in spawning and gravidity in these species.

Fecundity increased with shell length for both *C. necki* and *F. mitchelli.* Age was also correlated with fecundity, but to a lesser extent than length. Similar relationships have been reported for other mussel species, indicating that this is a common characteristic of mussels (Haag and Staton 2003, Haag 2012). The increase in fecundity with length and age also indicates that large, older individuals may play an important role in population maintenance (Haag and Staton 2003). However, our fecundity–length and fecundity–age relationships show a potential decline in very large or old individuals. Haag and Staton (2003) made similar observations in their study of the early life history of mussels from the Sipsey and Tallahatchie rivers of Alabama and Mississippi and also suggested that this was evidence of reproductive senescence.

Age and growth

The age at sexual maturity was similar between *C. necki* (3 y of age) and *F. mitchelli* (5 y of age), but we did not examine younger classes of either species because sampling these age classes is difficult and tends to be destructive. Thus, the true age at maturity for both species may be younger than reported here. However, our results are similar to those of other studies (Haag and Staton 2003). For example, Haag and Staton (2003) found that 37% of 3-y-old *C. pustulosa* individuals were sexually mature and reached 100% maturity at age 7. For *F. cerina*, sexual maturity is estimated to occur at ~5 y of age (Haag 2012), although it is unknown whether this represents full maturity or just the youngest age at which sexual maturity was detected. In this study, estimates for *C. necki* and *F. mitchelli* reflect the age at which we detected sexual maturity, and full maturity is probably not achieved until much later.

Growth and longevity differed between *C. necki* and *F. mitchelli* such that K, the rate at which a species approaches its growth asymptote (Haag 2012), was 60% higher in *F. mitchelli* than in *C. necki* (K = 0.23 vs 0.14, respectively). K and longevity are typically inversely related, such that increases in K correspond to decreases in longevity and vice versa, possibly because of increased oxidative stress and other cellular damage during faster growth (Haag and Rypel 2011, Haag 2012). However, the species in our study had similar maximum ages (13 y for *C. necki* and 15 y for *F. mitchelli*). This result could indicate that *C. necki* may not be realizing its maximum longevity in the Guadalupe River. For example, *C. pustulosa* from the Licking River, Kentucky, USA, had a K value of 0.14, which is similar to our estimate for *C. necki*, but a maximum observed age of 39 y, which is 3× greater than what we observed for *C. necki* (Haag and Rypel 2011). In contrast, *F. cerina* from the Sipsey River, Alabama, USA, had a K value of 0.17, which is similar to our estimate for *F. mitchelli*, and the maximum age for that population was 15 y, mirroring what we observed for *F. mitchelli* (Haag and Rypel 2011). Environmental factors, such as water

temperature, stream flow, and eutrophication, can cause variation in age and growth (Haag 2012), but the cause of reduced longevity for *C. necki* remains unknown.

Trematodes

Trematode infestations are probably rare in most mussel populations and typically affect <5% of individuals, although infection rate may vary by species (Gentner and Hopkins 1966, Pekkarinen 1993, Haag 2012). In our study, sterilization by trematodes was common in both *C. necki* and *C. pustulosa*, such that almost 30% of the individuals sampled could not be sexed. Similar infestation rates have been found in populations of *C. petrina* (Texas Pimpleback) and *Lampsilis bracteata* (Texas Fatmucket) from the San Saba River, a tributary of the Colorado River (Tsakiris et al. 2016, Seagroves 2017). Thus, trematode infestations may be more common than initially thought, at least in Texas, which could be an issue for rare mussel species. Trematode infestations can have severe consequences on the persistence of mussel populations by potentially reducing the number of reproducing individuals, thereby lowering the effective population size. This can be especially problematic if mussel densities are already low, such as in the case of *C. necki* (Haag and Staton 2003, Galbraith and Vaughn 2011, Haag 2012). The cause of trematode infestations in mussels is unknown but may be associated with degraded body condition stemming from disturbed habitats, isolated populations, and river impoundment (Heard 1975, Gangloff et al. 2008, Galbraith and Vaughn 2011, Haag 2012).

ACKNOWLEDGEMENTS

Author contributions: JFD performed the research, assisted in analyzing the data, and prepared the manuscript. MH and JMK assisted with the field and laboratory work and offered written content and constructive suggestions on various drafts of the manuscript. CRR provided funding, helped with field work, and assisted with writing of the manuscript. RL provided input and guidance on study design and editorial assistance with the manuscript. CRR contributed to study design, conducted the data analysis, and helped frame, write, and edit the manuscript.

We thank Megan Hess, Kelsey Marshall, Geoffrey Szafranski, and Eric Tsakiris for assistance in the field and laboratory. Special thanks are extended to Editor Chuck Hawkins, Associate Editor Carla Atkinson, Technical Editor Katie Sirianni, and 2 anonymous referees for helpful comments on the manuscript. Financial support for this study was provided by United States Fish and Wildlife Service Grant Texas T-130-1.

LITERATURE CITED

Allen, D. C., C. C. Vaughn, J. F. Kelly, J. T. Cooper, and M. H. Engel. 2012. Bottom-up biodiversity effects increase resource subsidy flux between ecosystems. Ecology 93:2165–2174.

Anderson, D. R., and K. P. Burnham. 2002. Avoiding pitfalls when using information-theoretic methods. The Journal of Wildlife Management 66:912–918.

Atkinson, C. L., C. C. Vaughn, K. J. Forshay, and J. T. Cooper. 2013. Aggregated filter-feeding consumers alter nutrient limitation: Consequences for ecosystem and community dynamics. Ecology 94:1359–1369.

Barnhart, M. C., W. R. Haag, and W. N. Roston. 2008. Adaptations to host infection and larval parasitism in Unionoida. Journal of the North American Benthological Society 27:370–394.

Baskerville, G. L., and P. Emin. 1969. Rapid estimation of heat accumulation from maximum and minimum temperatures. Ecology 50:514–517.

Blum, M. D., R. S. Toomey, and S. Valastro. 1994. Fluvial response to Late Quaternary climatic and environmental change, Edwards Plateau, Texas. Palaeogeography, Palaeoclimatology, Palaeoecology 108:1–21.

Coker, R. E., A. F. Shira, H. W. Clark, and A. D. Howard. 1921. Natural history and propagation of fresh-water mussels. US Bureau of Fisheries Bulletin 37:75–181.

Dudding, J., M. Hart, J. Khan, C. R. Robertson, R. Lopez, and C. R. Randklev. 2019. Host fish associations for two highly imperiled mussel species from the southwestern United States: *Cyclonaias necki* (Guadalupe Orb) and *Fusconaia mitchelli* (False Spike). Freshwater Mollusk Biology and Conservation 22:12–19.

Durham, B. W., and G. R. Wilde. 2008. Asynchronous and synchronous spawning by Smalleye Shiner *Notropis buccula* from the Brazos River, Texas. Ecology of Freshwater Fish 17:528–541.

Ford, D. F., and A. M. Oliver. 2015. The known and potential hosts of Texas mussels: Implications for future research and conservation efforts. Freshwater Mollusk Biology and Conservation 18:1–14.

Galbraith, H. S., and C. C. Vaughn. 2009. Temperature and food interact to influence gamete development in freshwater mussels. Hydrobiologia 636:35–47.

Galbraith, H. S., and C. C. Vaughn. 2011. Effects of reservoir management on abundance, condition, parasitism, and reproductive traits of downstream mussels. River Research and Application 27:193–201.

Gangloff, M. M., K. K. Lenertz, and J. W. Feminella. 2008. Parasitic mite and trematode abundance are associated with reduced reproductive output and physiological condition of freshwater mussels. Hydrobiologia 610:25–31.

Garner, J. T., T. M. Haggerty, and R. F. Modlin. 1999. Reproductive cycle of *Quadrula metanevra* (Bivalvia: Unionidae) in the Pickwick Dam tailwater of the Tennessee River. The American Midland Naturalist 141:277–283.

Gentner, H. W., and S. H. Hopkins. 1966. Changes in the trematode fauna of clams in the Little Brazos River, Texas. Journal of Parasitology 52:458–461.

Griffith, G. E., S. A. Bryce, J. M. Omernik, and A. Rogers. 2007. Ecoregions of Texas. Texas Commission on Environmental Quality, Austin, Texas.

Haag, W. R. 2012. North American freshwater mussels: Natural history, ecology, and conservation. Cambridge University Press, Cambridge, United Kingdom.

Haag, W. R. 2013. The role of fecundity and reproductive effort in defining life-history strategies of North American freshwater mussels. Biological Reviews 88:745–766.

Haag, W. R., and A. M. Commens-Carson. 2008. Testing the assumption of annual shell ring deposition in freshwater mussels. Canadian Journal of Fisheries and Aquatic Sciences 65:493–508.

Haag, W. R., and A. L. Rypel. 2011. Growth and longevity in freshwater mussels: Evolutionary and conservation implications. Biological Reviews 86:225–247.

Haag, W. R., and L. J. Staton. 2003. Variation in fecundity and other reproductive traits in freshwater mussels. Freshwater Biology 48:2118–2130.

Haag, W. R., and M. L. Warren. 2003. Host fishes and infection strategies of freshwater mussels in large mobile basin streams, USA. Journal of the North American Benthological Society 22:78–91.

Haggerty, T. M., and J. T. Garner. 2000. Seasonal timing of gametogenesis, spawning, brooding and glochidia discharge in *Potamilus alatus* (Bivalvia: Unionidae) in the Wheeler Reservoir, Tennessee River, Alabama, USA. Invertebrate Reproduction and Development 38:35–41.

Haggerty, T. M., J. T. Garner, G. H. Patterson, and L. C. Jones Jr. 1995. A quantitative assessment of the reproductive biology of *Cyclonaias tuberculata* (Bivalvia: Unionidae). Canadian Journal of Zoology 73:83–88.

Heard, W. H. 1975. Sexuality and other aspects of reproduction in *Anodonta* (Pelecypoda: Unionidae). Malacologia 15:81–103.

Hoggarth, M. A. 1999. Descriptions of some of the glochidia of the Unionidae (Mollusca: Bivalvia). Malacologia 41:1–88.

Howells, R. G. 2010a. False spike (*Quadrula mitchelli*): Summary of selected biological and ecological data for Texas. BioStudies, Kerrville, Texas. Report on file with Save Our Springs Alliance, Austin, Texas.

Howells, R. G. 2010b. Golden Orb (*Quadrula aurea*): Summary of selected biological and ecological data for Texas. BioStudies, Kerrville, Texas. Report on file with Save Our Springs Alliance, Austin, Texas.

Howells, R. G. 2010c. Texas Pimpleback (*Quadrula petrina*): Summary of selected biological and ecological data for Texas. BioStudies, Kerrville, Texas. Report on file with Save Our Springs Alliance, Austin, Texas.

Howells, R. G., C. Mather, and J. Bergmann. 1997. Conservation status of selected freshwater mussels in Texas. Pages 117–128 *in* K. S. Cummings, A. C. Buchanan, C. A. Mayer, and T. J. Naimo (editors). Conservation and Management of Freshwater Mussels II: Proceedings of an Upper Mississippi River Conservation Committee Symposium, Rock Island, Illinois.

Howells, R. G., R. W. Neck, and H. D. Murray. 1996. Freshwater mussels of Texas. Texas Parks and Wildlife Press, Austin, Texas.

Johnson, N. A., C. H. Smith, J. M. Pfeiffer, C. R. Randklev, J. D. Williams, and J. D. Austin. 2018. Integrative taxonomy reveals both cryptic and overestimated species level diversity in the freshwater mussel genus *Cyclonaias*. Scientific Reports 8: 15892.

Kat, P. W. 1984. Parasitism and the Unionacea (Bivalvia). Biological Reviews 59:189–207.

Master, L. L., B. A. Stein, L. S. Kutner, and G. A. Hammerson. 2000. Vanishing assets: Conservation status of US species. Pages 93–118 *in* B. A. Stein, L. S. Kutner, and J. S. Adams (editors). Precious heritage: The status of biodiversity in the United States. Oxford University Press, Oxford, United Kingdom.

McMahon, R. F., and A. E. Bogan. 2001. Mollusca: Bivalvia. Pages 331–430 *in* J. H. Thorp and A. P. Covich (editors). Ecology and Classification of North American Freshwater Invertebrates. Academic Press, San Diego, California.

Neves, R. J. 1999. Conservation and commerce: Management of freshwater mussel (Bivalvia: Unionoidae) resources in the United States. Malacologia 41:461–474.

Pekkarinen, M. 1993. Reproduction and condition of unionid mussels in the Vantaa River, South Finland. Archiv fur Hydrobiologie 127:357–375.

Perkin, J. S., and T. H. Bonner. 2011. Long-term changes in flow regime and fish assemblage composition in the Guadalupe and San Marcos Rivers of Texas. River Research and Applications 27:566–579.

Pfeiffer III, J. M., N. A. Johnson, C. R. Randklev, R. G. Howells, and J. D. Williams. 2016. Generic reclassification and species boundaries in the rediscovered freshwater mussel '*Quadrula*' *mitchelli* (Simpson in Dall, 1896). Conservation genetics 17: 279–292.

Randklev, C. R., M. S. Johnson, E. T. Tsakiris, J. Groce, and N. Wilkins. 2013a. Status of the freshwater mussel (Unionidae) communities of the mainstem of the Leon River, Texas. Aquatic Conservation: Marine and Freshwater Ecosystems 23:390–404.

Randklev, C. R., N. A. Johnson, T. Miller, J. M. Morton, J. Dudding, K. Skow, B. Bosman, M. Hart, E. T. Tsakiris, K. Inoue, and R. R. Lopez. 2017. Freshwater mussels (Unionidae): Central and West Texas final report. Texas A&M Institute of Renewable Natural Resources, College Station, Texas.

Randklev, C. R., T. Miller, M. Hart, J. Morton, N. A. Johnson, K. Skow, K. Inoue, E. T. Tsakiris, S. Oetker, R. Smith, C. Robertson, and R. Lopez. 2018. A semi-arid river in distress: Contributing factors and recovery solutions for three imperiled freshwater mussels (Family Unionidae) endemic to the Rio Grande basin in North America. Science of the Total Environment 631–632:733–744.

Randklev, C. R., E. T. Tsakiris, R. G. Howells, J. E. Groce, M. S. Johnson, J. A. M. Bergmann, C. Robertson, A. Blair, B. Littrel, and N. A. Johnson. 2013b. Distribution of Extant Populations of *Quadrula mitchelli* (False Spike). Elipsaria 15:18–21.

Ricciardi, A., and J. B. Rasmussen. 1999. Extinction rates of North American freshwater fauna. Conservation Biology 13:1220–1222.

Richard, P. E., T. H. Dietz, and H. Silverman. 1991. Structure of the gill during reproduction in the unionids *Anodonta grandis*, *Ligumia subrostrata*, and *Carunculina parva texasensis*. Canadian Journal of Zoology 69:1744–1754.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Bulletin 191. Bulletin of the Fisheries Research Board of Canada, Ottawa, Canada.

Robertson, S. M., T. H. Bonner, and J. N. Fries. 2016. Effects of habitat utilization on the life histories of two imperiled, sympatric Dionda (Cyprinidae) in the Rio Grande basin, Texas. The American Midland Naturalist 175:222–232.

Roe, K. J., A. M. Simons, and P. Hartfield. 1997. Identification of a fish host of the Inflated Heelsplitter *Potamilus inflatus* (Bivalvia: Unionidae) with a description of its glochidium. American Midland Naturalist 138:48–54.

Seagroves, A. 2017. Reproductive ecology of *Lampsilis bracteata* (Unionoidea: Bivalvia). MS thesis. Texas State University, San Marcos, Texas.

Sharif, H. O., A. A. Hassan, S. Bin-Shafique, H. Xie, and J. Zeitler. 2010. Hydrologic modeling of an extreme flood

in the Guadalupe River in Texas. Journal of the American Water Resources Association 46:881–891.

Smith, M. E., J. M. Lazorchak, L. E. Herrin, S. Brewer-Swartz, and W. T. Thoeny. 1997. A reformulated, reconstituted water for testing the freshwater amphipod, *Hyalell azteca*. Environmental Toxicology and Chemistry 30:1229–1233.

Spooner, D. E., and C. C. Vaughn. 2008. A trait-based approach to species' roles in stream ecosystems: Climate change, community structure, and material cycling. Oecologia 158:307–317.

Spooner, D. E., M. A. Xenopoulos, C. Schneider, and D. A. Woolnough. 2012. Coextirpation of host-affiliate relationships in rivers: The role of climate change, water withdrawal, and host-specificity. Global Change Biology 17:1720–1732.

Strecker, J. K. 1931. The naiades or pearly fresh-water mussels of Texas. Baylor University Museum Special Bulletin 2:1–71.

TPWD (Texas Parks and Wildlife Department). 2010. Threatened and endangered nongame species. Texas Register 35. Chapter 65. Wildlife subchapter G. 31 TAC §65.175. Adopted rules: 249–251. Texas Secretary of State, Austin, Texas. (Available from: https://texreg.sos.state.tx.us/public/readtac$ext.TacPage?sl=T&app=9&p_dir=P&p_rloc=171974&p_tloc=&p_ploc=1&pg=44&p_tac=&ti=31&pt=2&ch=65&rl=261)

Tsakiris, E. T., C. R. Randklev, A. Blair, M. Fisher, and K. W. Conway. 2017. Effects of translocation on survival and growth of freshwater mussels within a West Gulf Coastal Plain river system. Aquatic Conservation: Marine and Freshwater Ecosystems 17:1240–1250.

Tsakiris, E. T., C. R. Randklev, and K. W. Conway. 2016. Effectiveness of a nonlethal method to quantify gamete production in freshwater mussels. Freshwater Science 35:958–973.

UC IPM (University of California Statewide Integrated Pest Management Program). 2018. UC IPM Online: Statewide Integrated Pest Management Program. The Regents of the University of California. (Available from: http://ipm.ucanr.edu/)

USFWS (United States Fish and Wildlife Service). 2011. Endangered and threatened wildlife and plants: 90-day finding on petitions to list nine species of mussels from Texas as threatened or endangered with critical habitat. Federal Register 74:66260–66271.

USFWS (United States Fish and Wildlife Service). 2018. Endangered and threatened wildlife and plants: Endangered species status for Texas Hornshell. Federal Register 83:5720–5735.

Watters, G. T. 2008. The morphology of conglutinates and conglutinate-like structures in North American freshwater mussels: A scanning-electron microscopy study. Novapex 9(6):1–20.

Watters, G. T., and S. H. O'Dee. 2000. Glochidial release as a function of water temperature: Beyond bradyticty and tachyticty. Proceedings of the Conservation, Captive Care, and Propagation of Freshwater Mussels Symposium 1998:135–140.

Watters, G. T., S. H. O'Dee, and S. Chordas III. 2001. Patterns of vertical migration in freshwater mussels. Journal of Freshwater Ecology 16:541–549.

Williams, J. D., A. E. Bogan, and J. T. Garner. 2008. The freshwater mussels of Alabama and the Mobile Basin of Georgia, Mississippi, and Tennessee. University of Alabama Press, Tuscaloosa, Alabama.

Williams, J. D., M. L. Warren Jr, K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18:6–22.

Young, W. C., H. H. Hannan, and J. W. Tatum. 1972. The physiochemical limnology of a stretch of the Guadalupe River, Texas, with five main-stream impoundments. Hydrobiologia 40:297–319.