REPRODUCTIVE LIFE HISTORY AND HOST FISH SELECTIVITY OF IMPERILED FRESHWATER MUSSELS IN THE GUADALUPE RIVER, TEXAS

A Thesis

by

JACK FORREST DUDDING

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Roel R. Lopez
Co-Chair of Committee,	Charles R. Randklev
Committee Members,	Delbert M. Gatlin, III
	Nova J. Silvy
Head of Department,	David J. Caldwell

May 2019

Major Subject: Wildlife and Fisheries Sciences

Copyright 2019 Jack Forrest Dudding

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 threats and various conservation and management strategies. In Texas, located within the southwestern United States, 11 species are pending review for listing under the Endangered Species Act, and information on their reproductive life history and age/ growth is not available. To address this knowledge gap, I examined these aspects for three species (*Cyclonaias pustulosa* (Pimpleback), *Cyclonaias necki* (Guadalupe Orb), and *Fusconaia mitchelli* (False Spike) from a site in the lower Guadalupe River, located in central Texas. The resulting information was then compared with existing life-history information for closely related congeners. I observed peak gamete production in late January and early February for all three species, indicating that spawning occurred during this time. Brooding was observed in all species between March and June, and brooding behavior and glochidia morphology were similar to those of congeners. Accumulated degree days were important in regulating the timing of gametogenesis and potentially the duration of brooding. Fecundity estimates for *C. necki* and *F. mitchelli* were much lower than the values reported for congeners and increased with age and length, although length was a better predictor than age. Trematode infestation rates were high (~30%) in *C. pustulosa* and *C. necki*, and sex ratios were skewed toward males, indicating that females may be disproportionately affected. The age and growth estimates for *C. necki* and *F. mitchelli* closely mirror those of related congeners, although the maximum observed age for *C. petrina* did not meet theoretical expectations based on the estimated growth (*K*) rate for this species, indicating reduced longevity. Taken together, my findings suggest that *C. petrina* and *F. mitchelli* are

experiencing impacts to reproduction that may have negative consequences on long-term population maintenance and persistence for these species in the Guadalupe River.

I also conducted laboratory trials that tested host suitability of 12 fish species (4 families, and 11 genera) for *C. necki* and 8 species (4 families and 7 genera) for *F. mitchelli*. For *C. necki*, four host species, *Ictalurus punctatus* (Channel Catfish), *Pylodictus olivarus* (Flathead Catfish), *Noturus gyrinus* (Tadpole Madtom), and *Ameirus natalis* (Yellow Bullhead) were identified. The transformation period was 11 to 22 days for *I. punctatus* (peak metamorphosis at 15 days), 16 days for *P. olivaris*, and *A. natalis*, and 10 days for *N. gyrinus*. Two host species, *Cyprinella lutrensis* (Red Shiner) and *Cyprinella venusta* (Blacktail Shiner), were identified for *F. mitchelli*. The transformation period was 18 days for both *C. lutrensis* and *C. venusta*. The hosts identified in this study combined with current information on their status within the Guadalupe River indicate that imperilment of *C. necki* and *F. mitchelli* may be partially related to the status of their host fish. These results also provided critical information for informing recovery activities such as translocation and captive propagation, if deemed necessary for both species.

ACKNOWLEDGEMENTS

I would like to express eternal and sincere gratitude to both my committee chair, Dr. Roel Lopez, and my co-chair and advisor, Dr. Charles Randklev, for their limitless patience and effort they put forth to enlighten and share their wisdom with me. Words cannot adequately convey their support and guidance, both academically and professionally. I also would like to thank my committee members Drs. Nova Silvy and Delbert Gatlin for their invaluable expertise and support throughout the course of my graduate program.

To the many colleagues and friends that contributed to the fieldwork, lab work, and company along the way, I extend a multitude of thanks. Special thanks to Jennifer Khan for her friendship, support and considerable help with my study. I am also thankful for the invaluable guidance and technical knowledge provided by Michael Hart whose friendship, advise, and support has made this possible. I'm especially thankful to Clint Robertson (Texas Parks and Wildlife) who provided funding for my study and helped with fish collection in the Guadalupe River. I want to thank Dr. Kevin Conway, who provided specimens for host-trials. Many thanks to the following field and laboratory technicians, whose hard work helped make data collection possible for my study: Madeline Freiburger, Megan Hess, Kelsey Marshall, Bianca Perez, Anna Pieri, J. T. Sparks, and Geoff Szafranski.

Finally, I would like to extend my thanks to my family for their loving support through grad school. To my parents and brother (Carlton, Whitney, and Gates) for their continuous love, encouragement, and support. To my aunts (Patricia, Connie, and Nonie) who were always there to lend their support and continued interest in my life. And thank you to everyone else, for your support and companionship.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supported by a thesis committee consisting of Dr. Lopez (chair), Dr. Randklev (co-chair), and Dr. Silvy of the Department of Wildlife and Fisheries Science and Dr. Gatlin of the Intercollegiate Faculty of Nutrition.

All other work conducted for the thesis was completed by the student independently.

Funding Sources

Graduate study was supported by funding from the United States Fish and Wildlife Service (Grant TX T-130-1), Texas A&M University, and Texas A&M Natural Resource Institute.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Freshwater mussels (Bivalvia: Unionidae) are long-lived, sedentary benthic invertebrates represented by approximately a thousand species worldwide (Haag 2012). North America boasts the greatest diversity of freshwater mussel species, with ~300 recognized taxa (Williams et al. 2017), but habitat degradation and fragmentation has reduced this number. As a result, unionid mussels are considered one of the most sensitive and rapidly declining faunal groups in North America, with 29 species (10%) already considered extinct and another 195 (65%) having a state or federal conservation designation (Williams et al. 1993, Neves 1999, Haag 2009, Haag and Williams 2014). These declines will likely have long-term negative impacts to freshwater ecosystems because of the important services they provide, such as nutrient cycling and providing habitat for other aquatic taxa (Vaughn and Hakenkamp 2001, Vaughn et al. 2008, Spooner et al. 2012).

Unionid mussels have a unique mode of reproduction compared to other freshwater bivalves in which their larvae (glochidia) are obligate parasites on the gills or fins of fishes (Haag and Stanton 2003). Like other bivalves, unionid mussels broadcast spawn, in which males release sperm into the water column, which are then filtered out by females to fertilize the eggs (McMahon and Bogan 2001). Fertilized eggs and glochidia are brooded in the interbranchial chambers of the gills (marsupia) until they are mature (Kat 1984, Richard et al. 1991). The timing of these events can vary by species, and species can be generalized into short-term (tachytictic) vs. long-term (bradytictic) brooders, although there are exceptions to both (see Watters 1998). In general, short-term brooders spawn in the late winter and early spring, with females brooding for a short period (2-8 weeks) after fertilization. Long-term brooders spawn in

the late summer and fall, with females brooding through the winter until spring. Following brooding, mussels release their larvae either passively or actively through the use of lures or conglutinates, which mimic prey items for fish (Haag 2012). The larvae then attach to the fins or gill filaments of their host fish and then undergo transformation into a free-living juvenile after a period of several weeks (Barnhart et al. 2008). The timing and duration of both spawning and brooding is influenced, in part, by seasonal variations in flow, water temperature, water quality, and food availability (Roe and Lydeard 1998, Galbraith and Vaughn 2009, Gascho-Landis et al. 2012), although few studies have specifically tested these associations (but see Haggerty et al. 1995, Haggerty and Garner 2000, Galbraith and Vaughn 2009). In Texas, such information is either unknown or based on anecdotal evidence (Howells et al. 1996, 1997, Ford and Oliver 2015), which will likely hinder conservation assessments, particularly those that focus on modeling population demography in response to future threats, such as climate change or different water management strategies (Mims and Olden 2012).

The nature of mussel-host fish relationships can be general (multiple fish host species for a single mussel species) or specific (a single host fish species for a single mussel species). To date, hosts are only positively known for approximately 130 of the 300 species of mussels endemic to the United States and Canada. Texas boasts the greatest diversity of freshwater mussels in the southwestern United States; however, 13 of the 52 species that occur in the state have no known or confirmed hosts at this time. (Haag 2012, Ford and Oliver 2015). Knowledge of host fish associations is important for conservation efforts because this information can be used to determine whether a species' imperilment is related to loss of its host fish (Kelner and Sietman 2000), which in turn can help focus recovery activities. For example, Kelner and Sietman (2000) found that decline of *Reginaia ebena* (ebonyshell) in the Upper Mississippi

River was due to the extirpation of its host-fish, skipjack herring (*Alosa chysochloris*). Similarly, declines in *Elliptoideus sloattianus* (purple bankclimber) are thought to coincide with decline of the Gulf Sturgeon (*Acipenser oxyrinchus desotoi*) in the Apalachicola-Chattahoochee-Flint basin in southeastern North America (Georgia, Alabama, Florida; Fritts et al. 2012).

Given the importance of reproductive life history and host-fish associations to mussel conservation planning and recovery efforts, the purpose of this thesis was to examine both for three rare freshwater mussel species pending listing under the U.S. Endangered Species Act: *Cyclonaias pustulosa* (Pimpleback), *Cyclonaias necki* (Guadalupe Orb), and *Fusconaia mitchelli* (False Spike). The specific objectives of my study were to : (1) assess seasonal gamete production throughout the year to determine the phenology of spawning and brooding; (2) use water temperature, discharge, and photoperiod data from the Guadalupe River to determine what environmental cues may signal reproduction; (3) conduct host-fish trials to determine primary and marginal hosts; and (4) discuss how reproductive life history characteristics for these species may relate to their current distribution and status.

CHAPTER II

REPRODUCTIVE LIFE HISTORY OF TWO IMPERILED AND ONE WIDELY DISTRIBUTED FRESHWATER MUSSEL SPECIES FROM THE SOUTHWESTERN UNITED STATES: *FUSCONAIA MITCHELLI* (FALSE SPIKE), *CYCLONAIAS NECKI* (GUADALUPE ORB), AND *CYCLONAIAS PUSTULOSA* (PIMPLEBACK)

Introduction

Freshwater mussels (Bivalvia: Unionidae; hereafter mussels) are currently one of the most imperiled groups of organisms in North America (Master et al. 2000), with 29 (10%) of the recognized taxa considered extinct and 195 (65%) species listed as endangered, threatened, or of special concern (Williams et al. 1993, Neves 1999, Haag 2009). 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, as filter feeders, mussels can influence primary and secondary production (Allen et al. 2012, Spooner et al. 2012, Atkinson et al. 2013). Additionally, their presence within stream bottoms can help stabilize substrates, and along with their shells, provide habitat for other benthic organisms (Vaughn and Hakenkamp 2001, Vaughn and Spooner 2006).

Unionid mussels possess a unique reproductive life history involving larval (hereafter glochidia) parasitism of primarily fish (Haag 2012). Like other bivalves, unionid mussels broadcast spawn, in which males release sperm into the water column, which are then filtered out by females to fertilize the eggs (McMahon and Bogan 2001). Fertilized eggs and glochidia are brooded in the interbranchial chambers of the gills (marsupia) until they are mature (Kat 1984,

Richard et al. 1991). The timing of these events can vary by species, and species can be generalized into short-term (tachytictic) vs. long-term (bradytictic) brooders, although there are exceptions to both (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 weeks) after fertilization. Long-term brooders spawn in the late summer and fall, with females brooding through the winter until spring. Following brooding, mussels release their larvae either passively or actively through the use of lures or conglutinates, which mimic prey items for fish (Haag 2012). The larvae then attach to the fins or gill filaments of their host fish and then undergo transformation into a free-living juvenile after a period of several weeks (Barnhart et al. 2008).

The environmental determinants of spawning and brooding are likely dependent on adequate flow, water quality (e.g., temperature) and food availability (Roe et al. 1997, Galbraith and Vaughn 2009), although few studies have specifically tested these associations (but see Haggerty et al. 1995, Haggerty and Garner 2000, Galbraith and Vaughn 2009). Moreover, all of the studies have been conducted in the midwestern or southeastern United States, where flow and water temperatures differ from those in arid and semi-arid regions, such as Texas, which is located in the southwestern United States. Therefore, the transferability of early mussel reproductive life history and age and growth data from these previous studies to different species or different populations of the same species in arid and semi-arid regions is unknown. This will likely be an issue, as information on mussel reproduction,and age/ growth forms the basis for understanding life-history evolution and population dynamics, both of which inform conservation and recovery efforts (Haag and Staton 2003, Berg et al. 2008).

Texas boasts the greatest diversity of freshwater mussels in the southwestern United States, with approximately 52 species. Human-induced impacts have resulted in significant

population declines, particularly for many Texas endemics, leaving rivers with fewer and smaller populations and species with reduced ranges (e.g., Burlakova et al. 2011, Randklev et al. 2013*a*, *b*, 2018). As a result, the Texas Parks and Wildlife Department has listed 15 species as state threatened (TPWD 2010), of which 11 are under review for listing under the Endangered Species Act (USFWS 2011), and one has already been listed (USFWS 2018). Currently, 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). This will likely hinder conservation assessments, particularly those that focus on modeling population demography in response to future threats, such as climate change or different water management strategies (Mims and Olden 2012).

Given the importance of understanding the reproductive life history of species for their management and conservation, and the lack of such information for most of the imperiled and many of the common mussel species in Texas, the objectives of this study were to (1) evaluate the timing of gamete production, spawning, brooding, and potential environmental cues in *Cyclonaias necki* (Guadalupe Orb), *Cyclonaias pustulosa* (Pimpleback), and *Fusconaia mitchelli* (False Spike), (2) assess the fecundity, age at maturation, age and growth, and (3) compare the resulting information with existing life-history information for mussels and discuss the implications for management and conservation.

Methods

Study Site

This study was conducted in the mainstem of the lower Guadalupe River within central Texas, USA, 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 considered 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. 1994, Perkin and Bonner 2011). The flow regime in the lower Guadalupe River is modified by seven mainstem impoundments, including Canyon Lake reservoir, a deep storage bottom release reservoir (Perkin and Bonner 2011). My study site was located in the lower Guadalupe River near the town of Hochheim, Texas (Figure 2.1), and was chosen based on prior freshwater mussel surveys in this river (Tsakiris and Randklev 2016), which identified this reach as containing stronghold populations of my focal species.

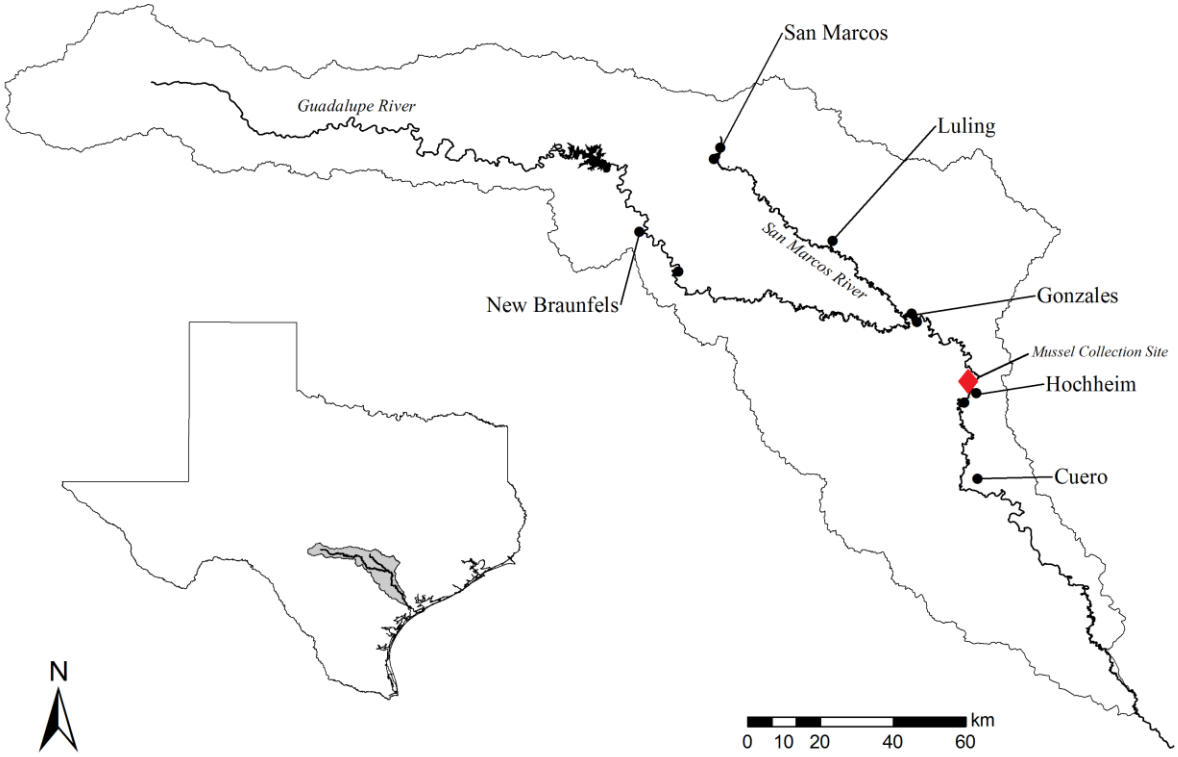

Figure 2.1. Map showing location of study sites within the lower Guadalupe River in central Texas.

Test Organisms

I examined the early reproductive life history of *C. necki* (Guadalupe Orb), *C. pustulosa* (Pimpleback), and *F. mitchelli* (False Spike). *Cyclonaias necki* occurs in the Guadalupe drainage (Randklev et al. 2017, Johnson et al. 2019), though Burlakova et al. (2018) erroneously reported it from the San Antonio drainage. Recent collections of live individuals demonstrate that it currently persists in the upper and lower Guadalupe and San Marcos rivers (Guadalupe drainage) (Howells 2010*c*, Randklev et al. 2017). *Cyclonaias pustulosa* is considered a common and widely distributed species that ranges from eastern reaches of the Great Lakes Basin throughout much of the Mississippi Basin, including East and Central Texas (Williams et al. 2008, Johnson et al. 2019). Within the Guadalupe River Basin, this species is known from the San Antonio and Guadalupe rivers and adjacent tributaries (Howells 2010*b*, Randklev et al. 2017) and is currently known to occur in the lower Guadalupe, San Marcos, San Antonio, and Medina rivers and Cibolo Creek (Howells 2010*b*, Randklev et al. 2017). *Fusconaia mitchelli*, once believed to be extinct until its recent rediscovery in the Guadalupe River Basin (Randklev et al. 2013*b*), is thought 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 (Brazos drainage) and Little (Brazos drainage) rivers (Howells 2010*a*, Randklev et al. 2013*b*, Randklev et al. 2017).

Gamete Sampling

From 14 November 2016, to1 December 2017, mussels were collected via visual and tactile searches using scuba or snorkeling on a bimonthly to monthly schedule (~2 to 4 week intervals). A total of 20 *C. necki*, 30 *C. pustulosa*, and 20 *F. mitchelli* was collected during each

sampling period. None of these species are known to be sexually dimorphic, and the sampled individuals were chosen at random. I 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, approximately positioned at the midline of the shell and halfway into the visceral mass. Approximately ~0.1 to 0.5 mL of gonadal fluid was extracted from each individual, evident by its milky coloration or red/pink color in the case of *F. mitchelli*. The samples were fixed in 0.5 mL of 10% buffered formalin solution stained with methylene blue and transported on ice back to the laboratory for analysis. Following the gonadal fluid sampling, each individual was marked on both valves with vinyl tags (Hallprint Tags, Holden Hills, Australia), and the maximum shell length (mm) was measured using calipers. The mussels were tagged to prevent resampling because the syringe technique, although non-lethal, can damage the gonads and thus could bias the subsequent quantification of gametes. All collected mussels were placed back into the river substrate.

In the laboratory, sperm concentration was quantified using a hemocytometer and a compound microscope (400X; Galbraith and Vaughn 2009). Egg concentration was quantified by gently agitating the sample, pipetting a 10-µL subsample onto a glass slide, and then counting the number of eggs using a compound microscope (40X–100X). Mean egg diameter was then estimated by measuring 50 randomly selected eggs using a compound microscope fitted with an ocular micrometer (Tsakiris et al. 2016).

Gravidity and Fecundity

To assess gravidity at the time of sampling, I collected up to 15 individuals per sampling period. Collected individuals were gently pried open using a nasal speculum, and then the gills were inspected for signs of inflation and discoloration, indicating the presence of fertilized eggs

or glochidia. Gravid females were then immediately placed into individual plastic bags containing river water and stored in insulated coolers with ice for transport back to the laboratory. In the laboratory, the gravid females were placed into individual perforated containers in flow-through aquariums with reconstituted water similar to that at their collection site. Within 24–120 hours, all individuals had released their gill contents, which were removed from the perforated containers, washed into a 55-µm mesh filter and then suspended in 100 mL of water. In cases where the gill contents were clumped together, a solution of 5% NaOH was used over a period of 5 minutes to dissolve the clumps (Haag and Staton 2003). Total fecundity was then estimated by extrapolating the counts of the gill contents from ten, 200 -µL aliquots (2 mL total; Haag and Staton 2003); shell length was measured following the fecundity estimation.

Age and Growth

Thin sectioning was used to estimate the age and growth of sacrificed females with known fecundity. A single valve of each collected individual was coated in epoxy (EpoxiCure 2 Epoxy Resin and Hardener) and dried overnight. The epoxied shells were then cut into 1.0–1.5-mm thin sections along an axis running from the umbo to the dorsal margin using a Buehler Isomet 1000 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 using Crystalbond 509 clear mounting adhesive (SPI Supplies) and sanded using Dia-Sharp® Bench Stones (Diamond Machining Technology) at progressively finer grit sizes to increase the visibility of the annual growth lines (hereafter annuli). Using light microscopy, the annuli were identified 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. A von Bertalanffy growth curve was fit

to the resulting age and length estimates for each species to determine the following: L_t, which is the length (mm) at a given time (age in years); L_∞, which is the predicted mean maximal length (mm) for the population; K, which is the Brody growth constant; and t_0, which is the theoretical time at which L = 0 (Ricker 1975). To account for the younger age classes, I back-calculated the external annuli using a subsample of the larger-sized individuals for which age had been previously estimated.

Environmental data

Water temperature (°C) and depth (m) were recorded at 15-minute intervals using HOBO level loggers (Onset, Bourne, MA) deployed at the study site for the entire duration of the study. Flow data were obtained from a nearby USGS gaging station (#08174700; 1.6 km downstream of the study site) by relating the water depth recorded at my site to the corresponding water depth and discharge measured at the gaging station. Photoperiod per day was based on estimates of day length obtained from the U.S. Naval Observatory Astronomical Application Department. Accumulated degree days from the start of the study were calculated using the University of California Statewide Integrated Pest Management Program online degree day calculator (Baskerville and Emin 1969, UC IPM 2018). To calculate degree days, I followed Galbraith and Vaughn (2009), whereby limits of growth were set to occur between 10 and 30 °C based on metabolic rate data for *Cyclonaias pustulosa*, a close congener of both my focal *Cyclonaias* species (Spooner and Vaughn 2008). Maximum and minimum daily temperatures were determined from logger data, and a single sine method was employed to calculate the number of degree days since the start of the study (Galbraith and Vaughn 2009).

Statistical Analysis

The timing of gamete reproduction for each species was determined by plotting the mean sperm concentration in males and oocyte diameter against the sample date. A sharp decline in gamete concentration or gamete size signified that spawning had occurred. I used a general additive model (GAM) approach to evaluate which environmental parameters (e.g., mean daily temperature, accumulated degree days, photoperiod, or mean daily flow) were associated with gamete production. I chose this approach because GAMs are robust to assumptions regarding independence and multicollinearity. The resulting models were ranked based on Akaike's Information Criterion (AIC_c) adjusted for sample size. AIC_c weights (w), which range from 0 to 1, were calculated, and the model with the highest weight was considered to be the best-approximating model (Anderson and Burnham 2002). I considered models to be plausible if their $AIC_c \leq 2$. To assess the departure from a 1:1 sex ratio, a chi-squared goodness-of-fit test was performed in R, and only results in which $P < 0.05$ were considered significant. Finally, a von Bertalanffy growth equation was fit for both species using the fishmethods package, and GAMs were implemented using the MGCV package in the R program (version 3.4.3; R Project for Statistical Computing, Vienna, Austria).

Results

In total, gonadal fluid was extracted from 843 individuals: 247 *C. necki*, 368 *C. pustulosa,* and 228 *F. mitchelli.* The sex ratio of *C. necki* differed significantly from 1:1 (χ^2 goodness-of-fit test, $P < 0.0001$), with males representing 62.1% of the sampled individuals. The sex ratio of *C. pustulosa* significantly differed from 1:1 (χ^2 goodness-of-fit test, $P < 0.001$), with males representing 73.9% of sampled individuals. The sex ratio of *F. mitchelli* did not differ significantly from 1:1 (χ^2 goodness-of-fit test, $P > 0.05$), with males representing 58.7% of the

sampled individuals. All sampled individuals were dioecious, and no hermaphroditism was observed. The shell length of the sampled individuals ranged from 29 to 59 mm (mean = 45.15 mm ± 0.34 SE) for *C. necki*, 27 to 61 mm (mean = 47.07 ± 0.32 SE) for *C. pustulosa*, and 30 to 65 mm (mean = 48.48 mm ± 0.38 SE) for *F. mitchelli*. Independent samples t-test with unequal variance revealed shell length differed significantly between males and females in both *C. pustulosa* and *C. necki* ($P < 0.05$), but not in *F. michelli* ($P > 0.05$). In *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 for *C. necki* and from late January to early February for *F. mitchelli*. In contrast, the mean sperm concentration of *C. pustulosa* peaked from late January to early March (Figure 2.2). Sharp declines in sperm concentrations following these events suggest that spawning had occurred. However, the sperm concentration remained at higher levels for *C. necki* and *C. pustulosa* than for *F. mitchelli*. The mean egg diameter peaked from late winter to early summer in all three species. Individuals brooding mature glochidia were observed shortly after spawning until between March and June, with peak brooding occurring for all species in early April. However, a single individual of *C. necki* was observed with fully mature glochidia in September.

Females in all three species were tetragenous, brooding glochidia in both their inner and outer gills. However, for *C. necki* and *C. pustulosa*, glochidia were held only in the central portion of the gills. Embryos of *C. necki* and *C. pustulosa* were white in color and remained so throughout maturation. Embryos of *F. mitchelli* were deep red to pink, growing increasingly

lighter in color with maturity. Glochidia of *C. necki* and *C. pustulosa* were brooded in lanceolate-shaped conglutinates in the marsupia, but the conglutinates did not maintain their structure after the glochidia were released from the gills. *Fusconaia mitchelli* also brooded glochidia in lanceolate-shaped conglutinates, but in contrast to *C. necki* and *C. pustulosa*, they maintained their structure following release. Earliest onset of reproduction for *C. necki* was observed at 3 years of age (shell length of 36 mm). For *F. mitchelli*, onset of reproduction was observed at 5 years of age (shell length of 42 mm). Onset of reproduction in *C. pustulosa* was observed at a shell length of 33 mm.

The glochidia of *C. necki* were semi-elliptical in shape, hookless, and 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). The glochidia of *C. pustulosa* were semi-elliptical in shape, hookless, and 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). The glochidia of *F. mitchelli* were semi-elliptical in shape, hookless, and 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).

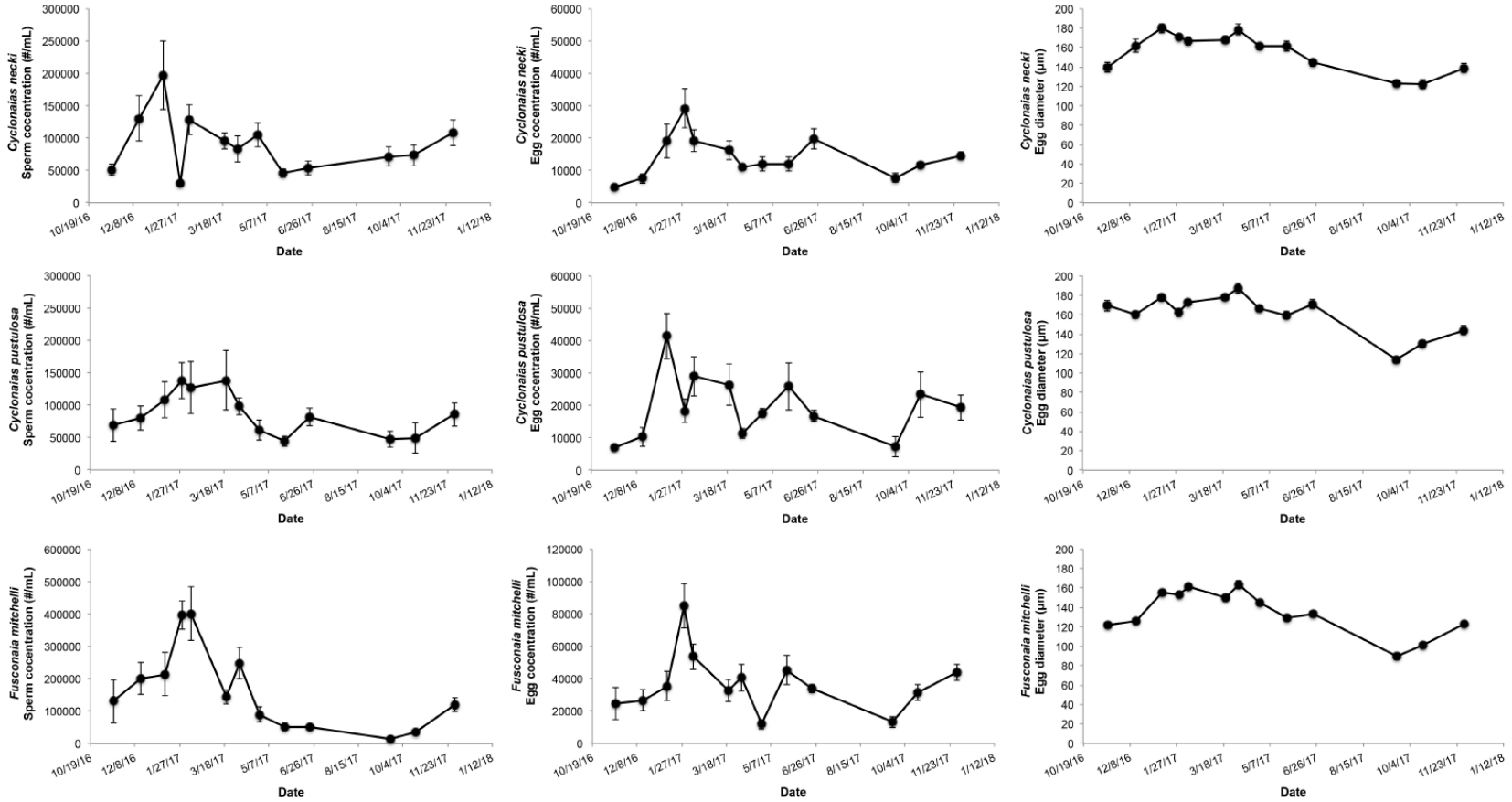

Figure 2.2. Mean sperm concentration (#/mL), mean egg concentration (#/mL), and mean egg diameter (µm) observed in *Cyclonaias necki* (Guadalupe Orb), *Cyclonaias pustulosa* (Pimpleback) and *Fusconaia mitchelli* (False Spike) from November 2016 to December 2017. Error bars denote ± 1 SE

Spawning/Brooding-Environmental Relationships

Models relating the accumulated degree days to the log of the sperm concentration and mean egg diameter generally performed the best (AICc selection; Table 2.1), and all GAMs were characterized by significant smoothing functions (Table 2.1). For *C. necki*, *C. pustulosa*, and *F. mitchelli*, the relationship between the mean egg diameter and accumulated degree days was well supported (i.e., R^2 ranging from 0.51 to 0.63; Table 2.2), and the shape of best-fit line was sinusoidal, peaking in late winter and early spring, declining from late spring to fall, and then increasing again, which mirrors my results relating the mean egg diameter to the calendar date (Figure 2.3). In contrast, for *C. necki* and *C. pustulosa*, the relationship between the log of the sperm concentration and the accumulated degree days was not well supported (i.e., R^2 ranging from 0.07 to 0.10; Table 2.2) despite being significant. The shape of the best-fit line was generally flat, indicating that, on average, the sperm concentration was the same throughout the year (Figure 2.3). Additionally, for *C. necki*, the relationship between water temperature and the log of the sperm concentration also was well supported (Table 2.1). For both water temperature and accumulated degree days, the ΔAIC_c was less than 2, and the AIC weights (w_i) were generally similar, indicating that either model could be the most parsimonious (Table 2.1). For *F. mitchelli*, the relationship between the log of the sperm concentration and the accumulated degree days was well supported ($R^2 = 0.56$), and the best-fit line between the two mirrored that of the relationship between the mean egg diameter and the accumulated degree days (Figure 2.3).

Fecundity

The fecundity of *C. necki* and *F. mitchelli* was estimated based on 34 and 31 individuals, respectively. Individuals of *C. pustulosa* were not included because samples could not be obtained due to high flows. For *C. necki*, the fecundity averaged 5,849 embryos (± 533 SE) and

ranged from 1,080 to 13,150 embryos. For *F. mitchelli*, the fecundity averaged 12,726 embryos (± 1,600 SE) and ranged from 2,340 to 32,250 embryos per individual. When evaluating the relationships among fecundity, age, and length, all GAMs were characterized by significant smoothing functions, except for the relationship between fecundity and length for *F. mitchelli*, which was moderately significant ($P = 0.09$; Table 2.3). The shape of the response across all species was positive, such that mean fecundity generally increased with age or length (Figure 2.4). However, length explained more variation in fecundity than age (Table 2.3; Figure 2.4). Older and younger individuals were uncommon in both species, and the fecundity for these individuals was similar to the average for each species.

Table 2.1. Summary of small-sample Akaike information criterion (AIC_c) selection of univariate general additive models (GAM) relating environmental factors with gametogenesis (i.e., egg diameter and sperm concentration) for *Cyclonaias necki* (Guadalupe Orb), *Cyclonaias pustulosa* (Pimpleback), and *Fusconaia mitchelli* (False Spike). ΔAIC_c = AIC_c of model relative to the lowest AIC_c, w_i = Akaike weight, and K = number of parameters in the model.

Species Model	AIC_c	ΔAIC_c	w_i	K
Cyclonaias necki – Male				
Log(Sperm Conc.) ~ Water temperature (°C)	354.55	0.00	0.65	4
Log(Sperm Conc.) ~ Accumulated Degree Days	355.93	1.37	0.33	6
Log(Sperm Conc.) ~ Discharge (m^3/s)	362.03	7.48	0.02	4
Log(Sperm Conc.) ~ Day length (h)	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 (m^3/s)	589.41	15.88	< 0.001	4
Egg diam. ~ Water temperature (°C)	603.67	30.13	< 0.001	3
Egg diam. ~ Day length (h)	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 (m^3/s)	638.33	6.33	0.04	5
Log(Sperm Conc.) ~ Water temperature (°C)	647.36	15.36	< 0.001	3
Log(Sperm Conc.) ~ Day length (h)	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 (m^3/s)	537.10	19.68	< 0.001	10
Egg diam. ~ Water temperature (°C)	544.08	26.66	< 0.001	10
Egg diam. ~ Day length (h)	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 (m^3/s)	373.01	10.23	0.01	10
Log(Sperm Conc.) ~ Water temperature (°C)	375.71	12.93	< 0.001	10
Log(Sperm Conc.) ~ Day length (h)	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 (m^3/s)	950.75	49.72	< 0.001	5
Egg diam. ~ Water temperature (°C)	974.04	73.02	< 0.001	3
Egg diam. ~ Day length (h)	987.23	86.20	< 0.001	3

Table 2.2. Generalized additive modeling (GAM) results for *Cyclonaias necki* (Guadalupe Orb), *Cyclonaias pustulosa* (Pimpleback), and *Fusconaia mitchelli* (False Spike) relating mean egg diameter (µm) or log sperm concentration (#/mL) with accumulated number of degree days.

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

Table 2.3. Generalized additive modeling (GAM) results for *Fusconaia mitchelli* (False Spike) and *Cyclonaias petrina* (Texas Pimpleback) relating fecundity to age (years) 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
Cyclonaias necki	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
Fusconaia mitchelli	Fecun. ~ Length	0.32	35.0	1.30	1.57	<0.0001

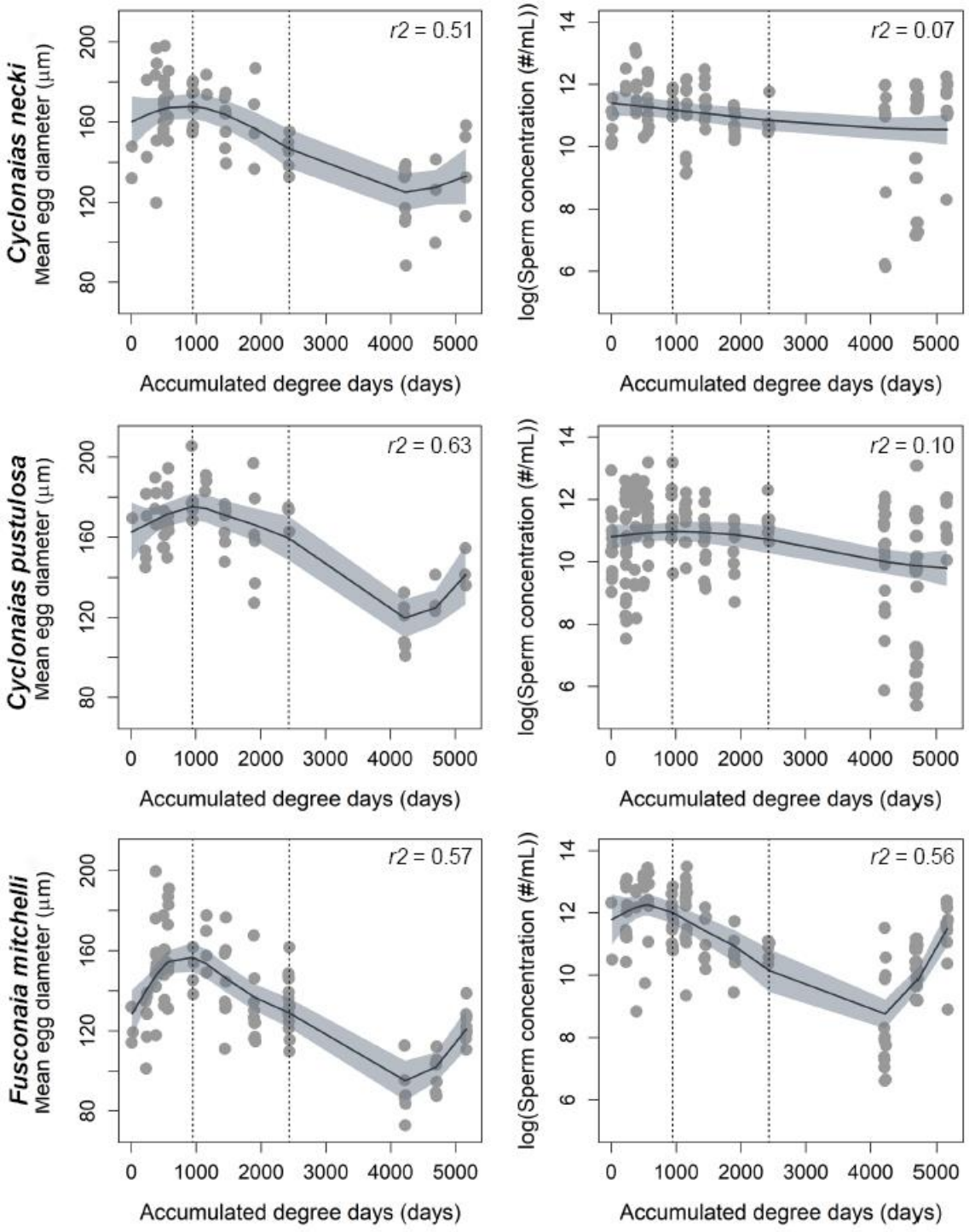

Figure 2.3. Plotted general additive models relating mean egg diameter (µm) and log sperm concentration (#/mL) with accumulated degree days for *Cyclonaias necki* (Guadalupe Orb), *Cyclonaias pustulosa* (Pimpleback), and *Fusconaia mitchelli* (False Spike). Shaded polygons denote 95% confidence intervals and dotted lines designate the brooding period. Coefficient of determination is given for each model.

Trematodes

Of the 247 *C. necki* sampled, 70 (28.3%) showed signs of digenean trematode parasitism via the observation of sporocyst and cercaria life phases. Similarly, of the 368 *C. necki* sampled, digenean trematodes were observed in the gonadal fluid of 130 (35.3%) individuals. Digenean trematodes were not observed in the gonadal fluid of *F. mitchelli*. Individuals infested with digenean trematodes could not be sexed because the gonadal fluid did not contain gametes and the individuals had been effectively castrated. Linear regressions between length and frequency of trematode infection was not significant for *C. necki* ($P > 0.05$; $R^2 = 0.024$), but it was for *C. pustulosa* ($P < 0.05$; $R^2 = 0.263$).

Growth

Growth significantly varied between *C. necki* and *F. mitchelli*, while the longevity was generally the same between the two species (Table 2.4; Figure 2.4). Specifically, *C. necki* had a low growth constant (K = 0.142; Table 2.4) compared to *F. mitchelli* (K = 0.231; Table 2.4), while the maximum ages of thin-sectioned individuals were similar, 13 vs. 15, respectively. Individuals of *C. pustulosa* were not included in this analysis because sufficient number of samples could not be obtained due to high flows.

Table 2.4. Population growth parameters for females of *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, and max age = maximum observed age. 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	55.491	47.525	89.361	0.142	0.05	0.246	-1.636	-4.368	-0.317	13
Fusconaia mitchelli	58	0.99	56.428	52.964	61.234	0.231	0.178	0.291	-0.112	-0.665	0.282	15

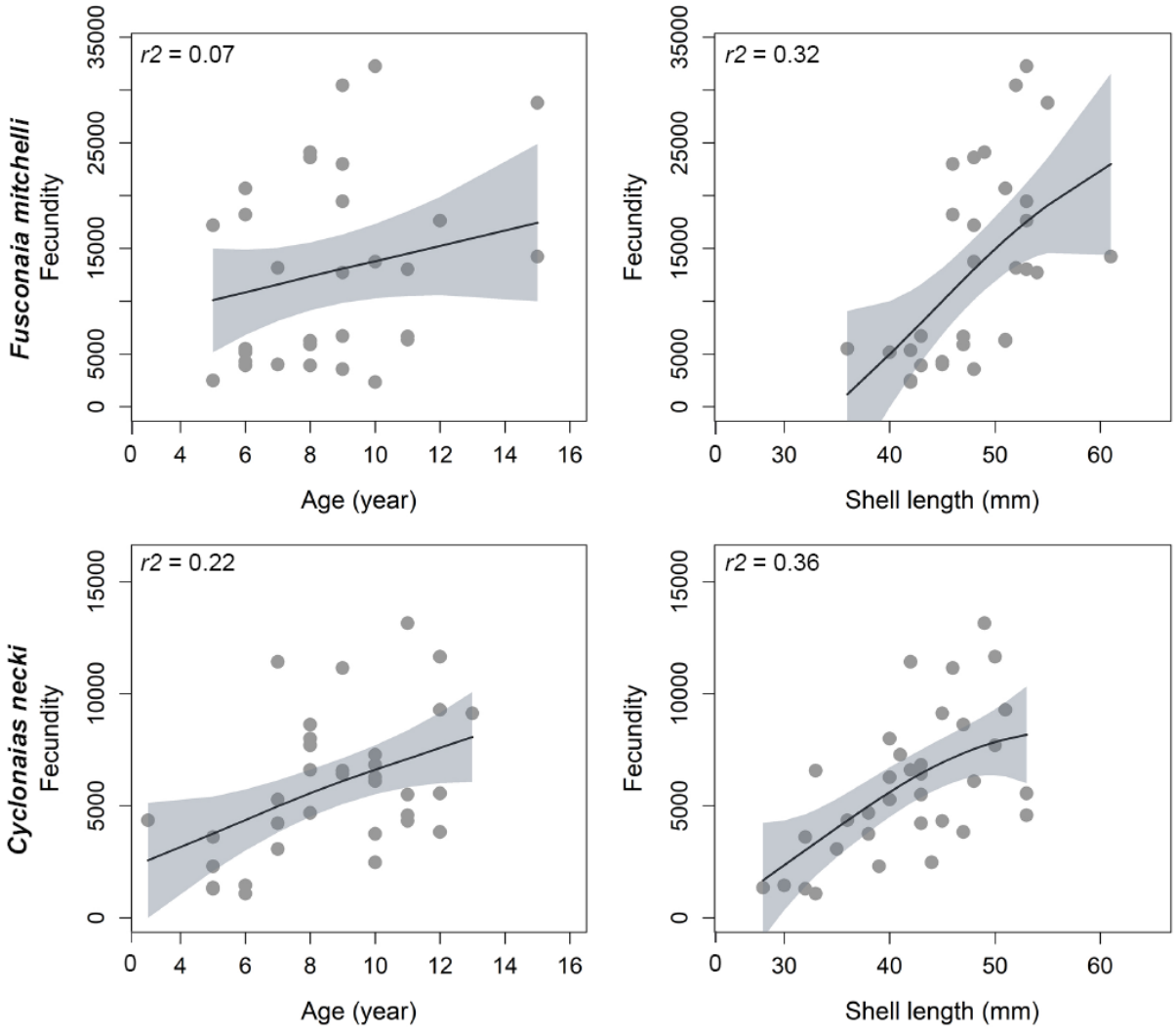

Figure 2.4. Plotted general additive models relating fecundity to age and length for *Cyclonaias necki* (Guadalupe Orb) and *Fusconaia mitchelli* (False Spike). Shaded polygons denote 95% confidence intervals.

Discussion

Spawning and Brooding

My results demonstrate that *C. necki, C. pustulosa* and *F. mitchelli* are short-term (i.e., tachytictic) brooders and have reproductive traits similar to those of closely related congeners within the Quadrulini and Pleurobemini tribes (Barnhart et al. 2008, Haag 2013). In general, gamete production peaked from late January to early March, although there was some variability depending on the species, followed by a decline in the gamete concentration in the gonads, indicating 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, which could indicate a protracted period of spawning, which also has been observed in congeners within Quadrulini (Haggety et al. 1995, Garner et al. 1999, Haag 2013). Glochidia maturation was relatively quick, lasting from March to July, which also is characteristic of short-term brooders and mirrors the pattern of brooding in several closely related congeners (Haag 2013). Despite these similarities, I found subtle differences in the timing of spawning and brooding for my focal species relative to closely related congeners outside of Texas. These shifts are not unexpected, as my study populations occur farther south than those in any prior quantitative study of mussel gametogenesis, and such shifts are therefore likely to correspond to latitudinal differences in temperature, which are known to affect mussel growth and reproduction (Haag 2012).

I also found *C. necki, C. pustulosa*, and *F. mitchelli* brood glochidia in both the inner and outer gills (i.e., they are tetragenous) and use host-infection strategies that mirror those of closely related congeners (Coker et al. 1921, Haag and Staton 2003). I observed that for most of the individuals sampled, regardless of species, the gills were often only partially charged, which has

been observed in populations of *C. pustulosa* outside of Texas and in congeners such as *Fusconaia cerina* (Gulf Pigtoe) (Haag and Staton 2003). This phenomenon has been attributed to poor recruitment (Haggerty et al. 1995), but Haag and Staton (2003) argue that this is a regular characteristic of mussel reproduction, at least for their focal species. My observations appear to support this hypothesis.

For both *Cyclonaias* species, I observed the release of white lanceolate-shaped conglutinates, which fell apart shortly after release. It is unknown whether these conglutinates are "functional" and able to infect host fish or are "puerile" and disintegrate as fertilized eggs develop into glochidia (Barnhart et al. 2008). The conglutinates within Quadrulini are usually puerile, although some species, and populations of *C. pustulosa* outside of Texas are known to be able to hold clumps of conglutinates (Barnhart et al. 2008). The release of glochidia or conglutinates within *Cyclonaias* species, such as *C. pustulosa*, occurs via an expansion of the mantle, termed the "mantle magazine", which serves as a temporary reservoir until small quantities are released following stimulation by touch, vibrations or shadows (Barnhart et al. 2008). I did not observe a mantle magazine in *C. necki* or *C. pustulosa*, but I may have overlooked it, which is not unexpected because the mantle magazine of species within the *Pustulosa* lineage (see Johnson et al. 2019), in which both species belong, is often reduced (Barnhart et al. 2008, Sietman et al. 2012). For *F. mitchelli,* I observed the release of pink, leaf-like conglutinates, with unfertilized eggs acting as structural elements, which 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 items for cyprinid minnows (Barnhart et al. 2008). White et al. (2008) observed similar behaviors in *Fusconaia burkei*, a closely related congener that uses

conglutinates to infect its host fish. In that study, *F. burkei* was reported to release pink, sub-cylindrical, conglutinates and to use *Cyprinella venusta* as a host which mirrors my findings for *F. mitchelli* (see Dudding et al. 2019 for mussel-host information for this species). Similar behaviors also have been observed in *F. cerina*, another closely related congener, with sub-cylindrical conglutinates, structural eggs, and cyprinid hosts (Haag and Staton 2003, Haag and Warren 2003).

Spawning/Brooding-Environmental Relationships

My results indicate that accumulated degree days, a measure of the total heat to which an organism has been subjected over time, is a good predictor of gamete production, explaining 51–63% of the variation in gametogenesis, although there were exceptions. For *C. necki* and *C. pustulosa*, the relationship between sperm production and accumulated degree days accounted for less than 10% of the variation in reproductive status, although this was the most parsimonious model. The likely reason for this result is the males of both species had a high incidence of sterilizing trematodes (see discussion below), which negatively affected my estimates of sperm concentration and, as a consequence, my ability to model the determinants of mussel gametogenesis. However, this weak association also may be characteristic of the male reproductive status of these species. For example, studies of *C. pustulosa* outside of Texas also have shown the relationship between the log of the sperm concentration and accumulated degree days is subtle, accounting for approximately 15% of the total variation in gametogenesis for males (Galbraith and Vaughn 2009). A potential explanation for this pattern is protracted, asynchronous spawning, which is not unexpected given that river systems in this part of central Texas are warm water systems and as such experience a greater number of accumulated degree days. Because metabolic and physiological processes of mussels are governed by temperature

and constrained within specific predictable thermal ranges, greater degree days likely means a wider reproductive window, as shown with my 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 a mean fecundity of 28,369 embryos per female in a population of *C. pustulosa* from the Sipsey River, AL, although their estimates per individual varied, ranging from 49 to 50,625. In my study, the mean fecundity of *C. necki* averaged 5,849 embryos (± 533 SE) and ranged from 1,080 to 13,150 embryos. The fecundity of *C. pustulosa* was not estimated due to a series of high-flow events that precluded the collection of gravid females. In that same study, Haag and Staton (2003) estimated mean fecundity of *Fusconaia cerina* to be 23,890 per female, ranging from 8,750 to 55,422. For *F. mitchelli*, I estimated the mean fecundity to be 12,726 embryos (± 1600 SE), ranging from 2,340 to 32,250 embryos per individual. The low fecundity of my focal species relative to closely related congeners outside of Texas raises questions about whether my estimates are representative of both species or a byproduct of a poor reproductive year due to trematode parasitism and/or human-induced impacts to flow and water temperature. However, the lower fecundity, particularly for *C. necki* and presumably *C. pustulosa*, could be due to protracted brooding for these species, which seems plausible based on my observations of asynchrony in spawning and gravidity in these species.

Size was a good predictor of fecundity for both *C. necki* and *F. mitchelli*, with fecundity increasing with shell length; age also was predictive, but less so compared to length. Similar relationships have been reported for other mussel species, which suggests 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 play an important role in population maintenance (Haag and Staton 2003), but only up to a point, as my fecundity-length and -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 suggested that this was evidence of reproductive senescence.

Age and Growth

The age at sexual maturity was similar between *C. necki* (3 years of age) and *F. mitchelli* (5 years of age), but younger classes were not examined for either species because sampling these age classes is difficult and tends to be destructive. Because of this the true age at maturity for both species may be much younger than reported here. However, when comparing these results to those of other studies, my estimates mirror those findings (Haag and Staton 2003). For example, Haag and Staton (2003) found that for *C. pustulosa* only 37% of 3-year-old individuals were sexually mature, but this percentage gradually increased, although 100% maturity was not achieved until age 7. For *F. cerina*, sexual maturity was delayed and was estimated to occur at approximately 5 years of age (Haag 2012), although it is unknown whether this represents 100% maturity or just the youngest age at which sexual maturity was detected. My estimates for *C. necki* and *F. mitchelli* reflect the latter, so it is likely that 100% maturity is not achieved until much later, presumably approximating the reported ages for *C. pustulosa* and *F. cerina*.

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* (K = 0.23 vs. 0.14 for *C. necki*). Typically, *K* and longevity are inversely related such that increases in *K* correspond to decreases in longevity and vice versa, and the proposed explanation

for this relationship is related to oxidative stress and other cellular damage during growth (Haag and Rypel 2011, Haag 2012). However, in my study, the maximum age of both species was generally the same, 13 years for *C. necki* and 15 for *F. mitchelli*, which could indicate that *C. necki* may not be realizing its maximum longevity in the Guadalupe. Comparing my growth estimates with populations and congeners outside of Texas underscores this point. For example, *C. pustulosa* from the Licking River, KY, was reported to have a *K* value of 0.14, which is notably similar to my estimate for *C. necki*, and the maximum observed age for that population was 39 years, which is three times greater than what I observed for *C. necki* (Haag and Rypel 2011). In contrast, *F. cerina* from the Sipsey River, Alabama, was reported to have a *K* value of 0.17, which is similar to my estimate for *F. mitchelli*, and the maximum age for that population was 15 years, which mirrors what I 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 in the Guadalupe it is unknown which of these is impacting longevity for *C. necki*.

Trematodes

Trematode infestations are thought to be rare in most mussel populations, typically affecting fewer than 5% of individuals (Haag 2012). In comparison, I observed a high incidence of sterilizing trematodes in both *C. necki* and *C. pustulosa* such that almost 30% of the individuals sampled could not be diagnosed as male or female. 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). Taken together, these findings suggest that generalizations regarding the rarity of trematode

infestations may not be correct, at least in Texas, and that such infestations appear to be widespread and not just restricted to *Cyclonaias* species.

I also found the sex ratios of both *C. necki* and *C. pustulosa* were skewed toward males, indicating that females may be disproportionately impacted. Galbraith and Vaughn (2011) evaluated the population performance of mussels downstream of large-scale impoundments and observed similar patterns for *C. pustulosa*, although the degree to which this occurred varied by population such that only one of three sites showed a strong skew towards males. However, the rates of infestation were low, less than 4% across all sites, which perhaps means the trematode infestation rates for their study populations had not increased significantly enough to begin impacting the sex ratios. Similarly, Seagroves (2017), evaluating the reproductive biology of *L. bracteata*, found that only females were affected by trematodes and the sex ratio was skewed toward males in infested populations. It is unclear why female mussels are disproportionately affected, although this observation may be related to the fact that female gonads provide a greater energetic return than those of males. For example, Taskinen and Valtonen (1995) found that female *Anodonta piscinalis* demonstrated higher trematode infection rates than males. Similarly, Müller et al. (2015) found that trematode infection rates for *Anodonta anatine* were significantly higher in females than males. Unfortunately, no such studies have been performed on closely related taxa in North America aside from observations of trematode presence.

Trematode infestation can have severe consequences on the persistence of mussel populations by reducing the number of reproducing individuals and 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*, whereby a reduced number of males and females are participating in reproduction; sterilizing trematodes would then likely further reduce this number (Haag and

Staton 2003, Galbraith and Vaughn 2010, Haag 2012). The cause of mussel trematode infestations is unknown, but is hypothesized to be associated with degraded body conditions stemming from disturbed habitats, isolated populations and river impoundment (Heard 1975, Gangloff et al. 2008, Galbraith and Vaughn 2011, Haag 2012).

Conclusions

This study was successful in determining the timing of spawning and brooding, glochidia morphology, fecundity, age and growth of two imperiled freshwater mussel species endemic to central Texas and one common and widespread species: *C. necki*, *F. mitchelli*, and *C. pustulosa*. I found the patterns of spawning and brooding closely mirrored findings for congeners outside of Texas, but there were subtle differences in timing, which were likely the result of latitudinal variation in stream temperatures. I also determined that accumulated degree days is a significant environmental cue for mussel gametogenesis, confirming the results of previous studies on mussel reproduction that have shown the importance of water temperature in regulating the timing of mussel reproduction. This finding is important because it highlights the importance of natural flows and thermal regimes to mussel population viability and the urgent need for ecologically sustainable water management in the Guadalupe basin. The latter is particularly important because I show the fecundity of both *C. necki* and *F. mitchelli* is low relative to that of congeners and the longevity of *C. necki* appears to be reduced. I also observed trematodes in *C. necki* and *C. pustulosa* at rates above what is considered typical. Taken together, my findings suggest the populations of at least *C. necki* and *F. mitchelli* are experiencing impacts to reproduction that may have negative consequences to population maintenance and persistence, although the causal mechanisms remain unknown. Finally, the interspecific differences in spawning, brooding, fecundity, age at maturity, and age and growth across *C. necki*, *C. pustulosa*

and *F. mitchelli* indicate different life-history strategies, which to date remain poorly known for most mussel species in Texas. This is unfortunate, as such information is important for a better understanding of evolutionary and ecological relationships as well as predicting how different species will respond to future environmental challenges and associated conservation and management strategies.

CHAPTER III

HOST FISH ASSOCIATION OF TWO HIGHLY IMPERILED MUSSEL SPECIES FROM THE SOUTHWESTERN UNITED STATES: *CYCLONAIAS NECKI* (GUADALUPE ORB) AND *FUSCONAIA MITCHELLI* (FALSE SPIKE)*

Introduction

North America boasts the greatest diversity of freshwater mussels (hereafter, mussels) with approximately 300 species (Haag 2012, Williams et al. 2017), but over the course of the last century, anthropogenic impacts have resulted in widespread declines, making mussels among the most imperiled group of organisms in North America (Master et al. 2000). Freshwater mussels provide a range of ecosystem services, including nutrient cycling (Vaughn et al. 2008), filtering suspended sediments (Spooner and Vaughn 2008), stabilizing substrates (Vaughn and Hakenkamp 2001), and providing microhabitats for aquatic macroinvertebrates (Vaughn and Spooner 2006). Thus, their decline will likely have long-term negative consequences for the ecological function of riverine systems.

Freshwater mussels have a unique reproductive life history in which they require a fish (except in the case of the salamander mussel, *Simpsonaias ambigua*) to briefly host their parasitic larvae (glochidia) to successfully reproduce (Watters and O'Dee 1998). Male mussels release sperm into the water column, which are then filtered from the water column by females where fertilization occurs internally (Haag 2012). The fertilized eggs are brooded from

---

*Reprinted with permission from "Host fish association of two highly imperiled mussel species from the southwestern United States: *Cyclonaias necki* (Golden Orb) and *Fusconaia mitchelli* (False Spike)" by Jack Dudding, Michael Hart, Jennifer Morton, Clinton R. Robertson, Roel Lopez, and Charles R. Randklev, 2019. *Freshwater Mollusk Biology and Conservation*, Volume 22, Issue Number 1, Copyright 2019 by Freshwater Mollusk Conservation Society.

fertilization to mature larvae (viable glochidia) within modified gills (marsupia) of the female mussels. Upon maturation of the larvae, female mussels can attract their host(s) by using modified mantle tissue lures, disguising their larvae in packages (i.e., conglutinates) that resemble food items, or passively releasing their glochidia into the water column (Barnhart et al. 2008, Sietman et al. 2012). This entire process can last several months, and success is strongly dependent on adequate flows, water quality (e.g., temperature), food availability, and fish host availability (Roe et al. 1997, Galbraith and Vaughn 2009).

The nature of mussel-host fish relationships varies by species and can be general (multiple fish host species for a single mussel species) or specific (a single host fish species for a single mussel species). To date, hosts are only positively known for approximately 130 of the 300 species of mussels endemic to the United States and Canada. Texas boasts the greatest diversity of freshwater mussels in the southwestern United States; however, 13 of the 52 species which occur in the state have no known or confirmed hosts at this time (Haag 2012, Ford and Oliver 2015). *Cyclonaias necki* (Guadalupe Orb; Burlakova et al. 2018) and *Fusconaia mitchelli* (False Spike; Dall 1896) are two of these unstudied species, and many questions regarding their reproductive biology and host fish associations remain unanswered (Howells et al. 1996, 1997, Ford and Oliver 2015). This lack of information is problematic because both species are being reviewed for listing under the U.S. Endangered Species Act (USFWS 2011).

Knowledge of host fish associations is important for conservation efforts because this information can be used to determine whether a species' imperilment is related to loss of its host fish (Kelner and Sietman 2000), which in turn can help focus recovery activities. For species that do become listed and/or are the focus of restoration programs, knowledge of host associations can guide captive propagation techniques for population augmentation and

reintroduction (Jones et al. 2004). Finally, knowledge of mussel-host fish relationships can be helpful in the development of a more complete understanding of how host fish abundance and dispersal impact freshwater mussel population and community ecology, which is unknown for the vast majority of mussel species (Schwalb et al. 2015, FMCS 2016).

Given the role that host fish information plays in conservation and management of rare mussel species and the potential listing of *C. necki* and *F. mitchelli*, the objectives of this study were to: (1) identify primary and marginal hosts of *C. necki* and *F. mitchelli*; and (2) use the resulting information to discuss management and conservation implications of my findings and potential future research opportunities.

Methods

Species

The focal species of this study are *C. necki* and *F. mitchelli*, which are endemic to central Texas and considered imperiled (USFWS 2011). The historical range of *C. necki* is believed to include only the Guadalupe drainage (Randklev et al. 2017, Johnson et al. 2019), although recent studies have mistakenly described it as occurring in the San Antonio River drainage (see Burlakova et al. 2018). Current live collections of this species are known from the Cypress, Blanco, San Marcos and Guadalupe rivers (Randklev et al. 2017, Johnson et al. 2019). *Fusconaia mitchelli* historically ranged from the Brazos, Colorado, and Guadalupe drainages in Texas (Strecker 1931, Stansbery 1971, Pfeiffer et al. 2016). To date, live collections of *F. mitchelli* have been made in the lower Guadalupe, lower San Saba, Llano and San Gabriel rivers, Brushy Creek and the Little River (Howells 2010, Randklev et al. 2013*b*, Randklev et al. 2017).

Study Site

The present study was conducted in the Guadalupe River drainage of central Texas. The Guadalupe basin is located in the Floodplains and Low Terraces of the Western Gulf Coastal Plain ecoregion (Griffith et al. 2004). This basin is characterized by underlying karst geology, with limestone bedrock in the upper reaches and alluvial sediments in the lower reaches (Blum et al. 1994, Scanlon et al. 2004). Flow within the Guadalupe basin is derived from groundwater and spring inputs and impoundment release, primarily from Canyon Lake reservoir (Young et al. 1972, Perkin and Bonner 2011). The Guadalupe River has seven mainstem impoundments, which were constructed between 1928 and 1962; the largest impoundment is a bottom release dam forming Canyon Lake reservoir and the rest are run-of-the river reservoirs (Young et al. 1972). The region is susceptible to hydrologic extremes, ranging from intense precipitation and flooding events to severe droughts (Scanlon et al. 2004). Gravid female *C. necki* and *F. mitchelli* were collected from the Guadalupe River between Gonzales and Cuero, TX, and potential host fish were collected from sites on the Guadalupe, San Marcos, and Blanco rivers, all of which are part of the Guadalupe drainage (Figure 3.1).

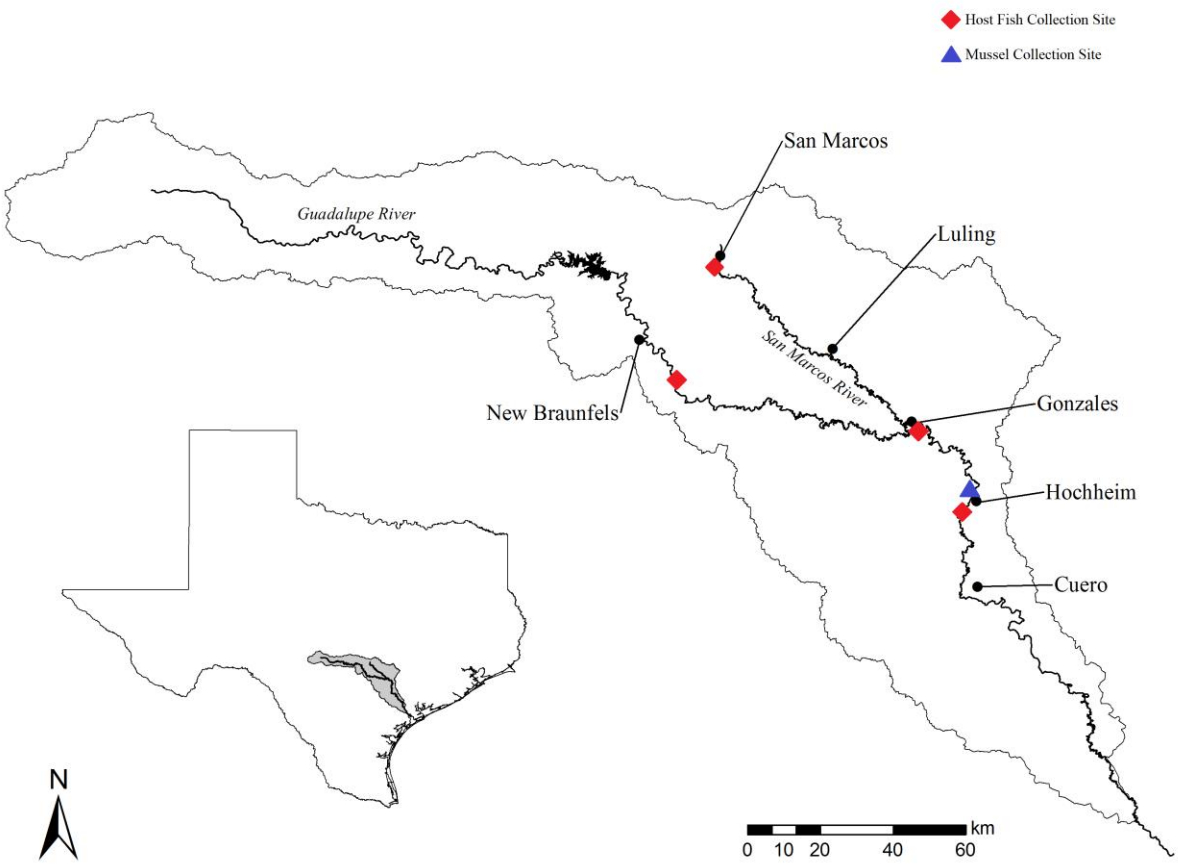

Figure 3.1. Map of the Guadalupe River basin of Texas showing the collection site for gravid *Cyclonaias necki* (Guadalupe Orb) and *Fusconaia mitchelli* (False Spike) and host fish collection sites. *Noturus gyrinus* (Tadpole Madtom) were collected from a single site on the Brazos River, Texas, which is not shown on this map.

Collection

Gravid individuals of my focal species were collected during the spring (mid-March through late April 2017). Because neither of my species were sexually dimorphic, females were identified based on visual inspection for the presence of inflated and discolored gills, which was characteristic of gravid females. Handling of gravid mussels for some species, particularly those belonging to the tribes Pleurobemini and Quadrulini, can induce brood abortion; therefore, collected individuals were placed into individual plastic bags filled with river water to retain aborted gill contents (Bruenderman and Neves 1993, Yeager and Neves 1986). Following collection, mussels were transported in insulated coolers to the Texas A&M AgriLife Extension and Research Center in Dallas, Texas. In the laboratory, I visually inspected the contents of each plastic bag for aborted gill contents (i.e., glochidia, conglutinates, or undeveloped embryos). Gravid females were placed into 55-µm mesh-lined containers in recirculating flow-through systems, with temperature (21–25 °C) and water chemistry matching those of the Guadalupe River.

Potential host fish were collected from sites where mussels were not present and at least 30 days prior to the observed brooding period to minimize the chance of using fish with prior glochidia infestation or acquired immunity to glochidia (Zale and Neves 1982, Rogers and Dimock 2003). Fish were collected via seine and electrofishing to obtain at least five individuals of each species (see below for experimental design). All fish were visually inspected to ensure no current infestation from glochidia. Following collection, fish were transported to the laboratory in covered stock tanks under aeration with water from the collection site, which was treated with NaCl to maintain a 3–5 ppt salinity to reduce handling stress and disease outbreak. Upon arrival, fish were separated by species into recirculating holding systems with water temperature and

chemistry matching the collection site. I held potential host fishes for a 30-day quarantine period to allow any encysted glochidia to drop off.

Experimental Design - Host-Testing

I conducted laboratory host suitability trials using standard methods (Zale and Neves 1982), in which I induced glochidial infections in potential host fishes and monitored for rejection of glochidia or metamorphosis of juvenile mussels. Specifically, released glochidia were flushed from containers holding gravid females and suspended in 100 mL of water. Ten, 200-µL subsamples were removed with a pipette while vigorously stirring with a large rubber-bulb syringe to ensure glochidia were evenly distributed in the container. Subsamples were evaluated under a dissecting microscope to ensure that glochidia were mature (i.e., developed valves and presence of an adductor muscle) and viable by introducing a saturated NaCl solution to observe the closure of valves (Zale and Neves 1982, ASTM 2006). Viability was enumerated as:

$$\frac{(\#\text{ open initially}) - (\#\text{ open after exposure})}{\text{total }\#\text{ of glochidia}}$$

Broods with viability ≥70% were used to infect fish, which is lower than ≥90% viability (which is a good indicator of infectivity; Fritts et al. 2014). In my study, most of the gravid females collected rarely had glochidia viability greater than this amount, instead averaging around ~70%. Glochidia from one or multiple females were used to infect fish depending on the amount of available glochidia.

Fishes were infected with glochidia by placing them into a bath containing ~4,000 glochidia L^{-1}. The bath was aerated and vigorously stirred with a rubber-bulb syringe to keep glochidia suspended. Fish were exposed in the bath for 15 minutes then transferred to individual 2.75-L tanks using dips nets. I monitored transformation success of glochidia on individual fish

in a recirculating (AHAB) system. Each trial consisted of five replicate tanks, containing a single infected fish. For some species tested, there were not enough individuals for five replicates so less replicates were used (2–4). Each of the replicate tanks were self-cleaning such that water exited each from the bottom rather than the top, which ensured the glochidia and/or juveniles were removed from the tank without disturbing the fish. The water from each tank passed through a 55-µm mesh filter cup, which was examined every other day for sloughed glochidia or juvenile mussels. Each tank also was flushed with an increased flow rate for 15 minutes to remove any glochidia or juveniles that may not have made it into the filter cup at standard flows. Water temperature was maintained at 23°C, matching average water temperatures of the Guadalupe River during the period of glochidia release. Fish were fed blood worms and brine shrimp daily. Trials were continued until no further glochidia were found in filter cups for four consecutive monitoring events.

Analyses

I empirically determined host suitability through visual observation and by calculating metamorphosis rate by species. Specifically, successful glochidial metamorphosis was defined by the presence of juveniles, which showed valve growth beyond the original glochidial valve, the presence of a fully formed and active foot, and paired adductor muscles. The metamorphosis rate (M%) was calculated as follows for each individual fish:

$$\frac{\#\ \text{juveniles}}{(\#\ \text{juveniles}) + (\#\ \text{sloughed glochidia})} \times 100$$

Results

Cyclonaias necki

I collected 29 gravid female of *C. necki* for use in host fish trials. Water temperature at the time of collection ranged from 21.1 to 31.6°C (mean = 25.8°C). Of those individuals, only 11 released mature glochidia that could be used for host fish trials (i.e., viability ≥70%). Most gravid females (~60%) aborted immature embryos; however, I was unable to verify if fertilization had occurred. A total of 12 species was used in host trials, however juvenile metamorphosis was observed in only four species, all of which were ictalurids: *Ictalurus punctatus* (Channel Catfish), *Pylodictis olivaris* (Flathead Catfish), *Ameiurus natalis* (Yellow Bullhead), and *Noturus gyrinus* (Tadpole Madtom) (Table 3.1). *Ictalurus punctatus* (*n* = 2) produced 183 juveniles with a metamorphosis rate of 38.2% (± 8.99 SE); followed by *P. olivaris* (*n* = 2) producing 130 juveniles with an average metamorphosis rate of 34.1% (± 2.09 SE); and *N. gyrinus* (*n* = 3) producing 194 juveniles with an average metamorphosis rate of 27.6% (± 2.88SE). Only 8 juveniles were recovered from *A. natalis* (*n* = 3) yielding a metamorphosis rate of 2.5% (± 0.52 SE). The period for juvenile metamorphosis was 11 to 22 days for *I. punctatus* (peak metamorphosis at 15 days), 10 days for *N. gyrinus*, 15 days for *A. natalis*, and 16 days for *P. olivaris*.

Table 3.1. Results of the host trials for *Cyclonaias necki* (Guadalupe Orb), including fish species tested, number of replicates (Rep), total number of juvenile mussels collected (Juv), total number of glochidia attached (#Glch), days to juvenile mussel transformation (Trans), and mean metamorphosis rate (%M) with standard error (± 1 SE) in parentheses.

Species	Rep	Juv	#Gloch	Trans	% M (SE)
Ameiurus natalis (Yellow Bullhead)	3	8	378	15	2.51 (0.52)
Ictalurus punctatus (Channel Catfish)	2	183	459	11-22	38.24 (8.99)
Pylodictis olivaris (Flathead Catfish)	2	130	388	16	34.08 (2.09)
Noturus gyrinus (Tadpole Madtom)	3	194	697	10	27.56 (2.88)
Cyprinella lutrensis (Red Shiner)	5	0	7	0	0
Cyprinella venusta (Blacktail Shiner)	5	0	14	0	0
Lepomis macrochirus (Bluegill)	5	0	29	0	0
Micropterus treculii (Guadalupe Bass)	5	0	225	0	0
Macrhybopsis marconis (Burrhead Chub)	5	0	29	0	0
Campostoma anomalum (Central Stoneroller)	5	0	35	0	0
Etheostoma spectabile (Orangethroat Darter)	5	0	36	0	0
Pimephales vigilax (Bullhead Minnow)	5	0	0	0	0

Fusconaia mitchelli

I collected 34 gravid females for use in host fish trials. Water temperature at the time of collection ranged from 21.1 to 31.6°C (mean = 25.8°C). Of the individuals collected, only 10 released mature glochidia that could be used for host fish trials (i.e., viability ≥70%). Most gravid females (~60%) aborted immature embryos and for those individuals, I was unable to verify if fertilization had occurred. Of the eight species evaluated, two cyprinid species, *Cyprinella lutrensis* (Red Shiner) and *Cyprinella venusta* (Blacktail Shiner) successfully transformed glochidia (Table 3.2), yielding a total of 48 juveniles. *Cyprinella lutrensis* (*n* = 3) produced 75% (*n* = 36) of metamorphosed juveniles while the remaining 25% (*n* = 12) were produced by *C. venusta* (*n* = 3). The average metamorphosis rate for *C. lutrensis* was 32.5% (± 9.11 SE), and transformation was observed in three of the five trials; the average metamorphosis rate for *C. venusta* was 34.5% (± 3.51 SE) and transformation also was observed in three of the five trials. The transformation period for *F. mitchelli* was 18 days for both *C. lutrensis* and *C. venusta*.

Table 3.2. Results of the host trials for *Fusconaia mitchelli* (False Spike) including the list of fish species tested, number of replicates (Rep), total number of juvenile mussels collected (Juv), total number of glochidia attached (#Glch), days to juvenile mussel transformation (Trans), and mean metamorphosis rate (%M) with standard errors (± 1 SE) in parentheses.

Species	Rep	#Juv	#Glch	Trans	% M (SE)
Ameiurus natalis (Yellow Bullhead)	5	0	45	0	0
Cyprinella lutrensis (Red Shiner)	3	36	156	18	32.51 (9.11)
Cyprinella venusta (Blacktail Shiner)	3	12	54	18	34.49 (3.51)
Lepomis macrochirus (Bluegill)	5	0	12	0	0
Gambusia affinis (Western Mosquitofish)	5	0	20	0	0
Pimephales vigilax (Bullhead Minnow)	5	0	22	0	0
Campostoma anomalum (Central Stoneroller)	5	0	5	0	0

Discussion

I found that *C. necki* uses *I. punctatus, P. olivaris, N. gyrinus,* and *A. natalis* as hosts. However, high transformation rates on *I. punctatus, P. olivaris,* and *N. gyrinus* suggests these fish species are likely the primary hosts while low transformation rates on *A. natalis* suggests that this species is likely a marginal host. Additionally, *C. lutrensis,* and *C. venusta* were identified as host fish for *F. mitchelli.* My results also indicate that *C. necki* and *F. mitchelli* are specialists, with host use restricted to a single family or genus of fishes, which matches similar findings of laboratory host trials of closely related congeners. For example, mussel-host fish associations within *Cyclonaias* and *Quadrula* use ictalurid fishes (Haggerty et al. 1995, Hove et al. 2011, Hove et al. 2012, Harriger et al. 2015), and my findings for *C. necki* provide additional support for this inference. This means for other threatened Texas mussel species belonging to the genus *Cyclonaias* whose host fish are unknown, such as *Cyclonaias pustulosa* (Pimpleback) or *Cyclonaias houstonensis* (Smooth Pimpleback), phylogeny can serve as a means to predict host use (Haag and Warren 2003). Similarly, cyprinid fishes have been identified as primary hosts for the genus *Fusconaia* (Neves 1991, Bruenderman and Neves 1993, White et al. 2008), which my results further corroborate.

Freshwater mussels are sessile (Allen and Vaughn 2009, Gough et al. 2012) and as a result, host fish are the primary means of dispersal, which can affect mussel population and community structure (Mansur and da Silva 1999, Barnhart et al. 2008, Horký et al. 2014). Generally, smaller freshwater fishes (e.g., darters and sculpin) have reduced home ranges compared to larger fishes (e.g., ictalurids) (Funk 1957, Freeman 1995, Minns 1995, Rodriguez 2002, Petty and Grossman 2004) and such information may provide insight into the conservation status of a given mussel species. Similarly, fish size influences upstream and downstream

movement, with smaller fish moving less than larger fish; which is likely tied to reproduction and larval dispersal (Gerking 1950, Hall 1972, Minns 1995). In this study, I found that *C. necki* uses ictalurids as hosts, which exhibit potamodromous migratory behavior (Pellet et al. 1998), suggesting greater dispersal capacity and perhaps resiliency to human impacts. This could explain why *Cyclonaias* and *Quadrulid* mussel species in Texas appear to be more broadly distributed with multiple stronghold populations spread throughout their range (Randklev et al. 2017). However, for *C. necki, N. gyrinus* also was identified as a host. This species of fish is diminutive, maintains a small home range (often a single riffle) and is rare within the Guadalupe drainage (Perkin and Bonner 2011, GBRA and TPWD 2014). If *N. gyrinus* proves to be the primary ecological host (see below) for *C. necki*, then my findings would suggest that this species' decline could be associated with the conservation status of its host fish. If this turns out not to be the case, ongoing declines in ictalurid fishes within Texas rivers (Anderson et al. 1995) may still be evidence that its decline is related, in part, to it host fish. For *F. mitchelli*, I found that it uses cyprinids as hosts, which typically have a small home range and dispersal capacity and are generally sensitive to anthropogenic impacts (Irmscher and Vaughn 2015). Thus, the patchy distribution of *F. mitchelli* within its presumptive range and the fact that stronghold populations are aggregated in reaches away from human impacts could be the result of its host-fish (Brittain and Eikeland 1988, Watters 1992, McLain and Ross 2005). However, *C. lutrensis* is known to be tolerant of poor water quality and habitat, which could mean the imperilment of *F. mitchelli* is unrelated to its host fish. However, in this study I was only able to test four of the 10 minnow species known to occur in the lower Guadalupe River due to the fact the remaining six species have become increasingly rare (e.g., *Notropis buchanani*, Ghost Shiner; Perkin and

Bonner 2011). Thus, because I did not test these other species, conservationists and managers should not assume that *F. mitchelli's* imperilment is unrelated to the status of its host fish.

Host specificity for species like *C. necki* and *F. mitchelli* is important because it minimizes competition for host fish (Bauer 2001, Rashleigh and DeAngelis 2007) and potentially increases reproduction success via host attraction and successful metamorphosis (Barnhart et al. 2008). However, high host specificity comes with a cost in human-dominated landscapes, as it ties the fate of the mussel species with the fish, such that extirpation of the host fish results in reproductive and recruitment failure for the mussel (McNichols et al. 2011). Habitat fragmentation and impoundments inhibit host fish dispersal, alter fish assemblages and community structure, and displace or extirpate host fish necessary for persistence of mussel populations (Watters 1996, Vaughn and Taylor 1999). The consequence of these impacts to mussels was diminished gene flow and reduced colonization, which overtime can lead to extirpation or extinction (Watters 1996, Bogan 2008, Newton et al. 2008). For example, declines in *Reginaia ebenus* (Ebonyshell) in the Upper Mississippi River have been attributed to extirpation of its host fish, *Alosa chrysochloris* (Skipjack Herring), caused by river impoundment (Kelner and Sietman, 2000, Hart et al. 2018). Similarly, declines in *Elliptoideus sloatianus* (Purple Bankclimber) are thought to coincide with decline of the *Acipenser oxyrinchus desotoi* (Gulf Sturgeon) in the Apalachicola-Chattahoochee-Flint basin in southeastern North America (Georgia, Alabama, Florida; Fritts et al. 2012). For *C. necki* and *F. mitchelli*, it is unknown whether their declines are associated with impoundments, either directly through habitat loss or indirectly by loss of host fish. However, impoundments cannot be ruled out because the Guadalupe River is highly managed with seven mainstem impoundments, including Canyon Lake reservoir, a deep storage reservoir that significantly alters mainstem discharge (Young et al.

1972, Edwards 1978). Recent studies of fish assemblage structure within the Guadalupe River has demonstrated significant shifts in fish assemblages following mainstem impoundment (Perkin and Bonner 2011).

Although I was successful in identifying hosts for *C. necki* and *F. mitchelli* there were shortcomings to the methods herein. First, low fecundity and difficulty recovering viable glochidia limited my capacity to test a broader range of fish species. However, this is a common issue for most host-fish studies, especially those focused on rare species. That said, additional host testing may yield further insights into host suitability and better determination of primary and marginal hosts. For example, the association between *C. necki* and *N. gyrinus* should be further evaluated given that *N. gyrinus* was collected from the Brazos drainage. Laboratory host studies have shown that mussels tested with fish species from the same river system have higher metamorphosis success than laboratory trials that use fish from a different basin from where mussels are collected (Haag 2012). Thus, it is likely that *N. gyrinus* collected from the Guadalupe River would have had higher metamorphosis success and juvenile production rates than what I observed in this study. Second, pipetting glochidia directly onto the host gills instead of using glochidia baths would likely be a better approach for cyprinids, especially if large-scale production of juveniles is desired. This is because *Fusconaia* species use conglutinates to attract and elicit sight-feeding minnows to ingest glochidia, thereby breaking the conglutinate membrane and freeing glochidia in close proximity to the gills (Barnhart et al. 2008). Thus, if I had pipetted glochidia onto the gills, I would have likely seen greater attachment and transformation success. For example, Bertram et al. (2017) examining host-suitability of *Fusconaia askewi* (Texas Pigtoe), a closely related congener of *F. mitchelli*, via molecular identification of newly transformed juveniles from naturally infested fish from the wild,

confirmed that *C. lutrensis* and *C. venusta* were hosts for this species. Metamorphosis success in their study was 29.4% for *C. lutrensis* and 46.3% for *C. venusta*, which is similar to what I observed during my laboratory trials (32.51% and 34.49%, respectively) for these same fish species with *F. mitchelli*. However, juvenile production differed with a greater number of individuals produced from naturally infested fish. Third, my study entailed identifying hosts though laboratory infections (termed physiological hosts), which may not be the same in natural settings (termed ecological hosts; Levine et al. 2012). Thus, future host studies for my focal species should reconcile this knowledge gap by identifying ecological hosts and then comparing those to the results presented in this study. A "DNA barcoding" approach could be a way to do this, particularly in river systems with more than one species, and entails collecting naturally infested fish from the wild, chosen, in part, based on information from laboratory trials like my study. Collected individuals are transported back to the laboratory and held in an AHAB system or aquaria until glochidia or juveniles are released from the fish. Glochidia and juveniles are then identified using a molecular approach (e.g., Boyer et al. 2011).

Despite these limitations, this study was successful in identifying hosts for two highly imperiled species from the southwestern United States. The fish hosts I identified will enable captive propagation programs to begin recovery reintroduction efforts, although comprehensive genetic management plans should be developed before captive-raised animals are released into the wild (McMurray and Roe 2017). Future management and conservation efforts regarding *C. necki* and *F. mitchelli* should take into consideration host fish abundance, habitat, and population connectivity, now that this information is known. In addition, other metrics, such as mussel demography and abundance, should be considered when assessing population viability and developing recovery goals.

CHAPTER IV

CONCLUSIONS

The results of my study indicate that *Cyclonaias pustulosa* (Pimpleback), *Cyclonaias necki* (Guadalupe Orb), and *Fusconaia mitchelli* (False Spike) are short-term brooders (tachytictic), spawning in late January and early February, followed shortly by a brooding period between March and July. Fecundity for these species was relatively low in comparison with other unionid mussel species. Age and growth estimates from shell thin sectioning indicate that *C. necki* and *F. mitchelli* have reduced longevity relative to congeners in other drainages. The presence of digenean trematodes within the gonads of the *Cyclonaias* species in this study suggests reproduction for populations of *C. pustulosa* and *C. necki* is likely impaired, and as such the long-term persistence of both species in this basin may be in jeopardy. I also found that gamete production is likely driven by accumulated degree days and discharge, corroborating early studies on this topic which has important implications for water management practices in this basin.

The results of host trials for *F. mitchelli* and *C. petrina* indicate they are host specialists, with hosts restricted to a single family or genus. Two cyprinid species (one primary and one marginal) were identified as hosts for *F. mitchelli,* and four ictalurid species (three primary and one marginal) were identified as hosts for *C. petrina*. The hosts identified in this study, combined with current information on their status within the Guadalupe River, indicate that imperilment of *C. necki* and *F. mitchelli* may be partially related to the status of their host fish. This information also will likely be useful for guiding captive propagation programs, if deemed necessary, for population recovery and augmentation.

LITERATURE CITED

Allen, D. C., and C. C. Vaughn. 2009. Burrowing behavior of freshwater mussels in experimentally manipulated communities. Journal of the North American Benthological Society 28:93–100.

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.

Anderson, A. A., C. Hubbs, K. O. Winemiller, and R. J. Edwards. 1995. Texas freshwater fish assemblages following three decades of environmental change. The Southwestern Naturalist 40:341–321.

ASTM (American Society of Testing Materials). 2006. Standard guide for conducting laboratory toxicity tests with freshwater mussels. E2455-06. Pages 1393–1444 *in* Annual Book of ASTM Standards, Volume. 11.06. American Society of Testing Materials International, West Conshohocken, Pennsylvania.

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.

Bauer, G. 2001. Factors affecting naiad occurrence and abundance. Pages 155–162 *in* G. Bauer and K. Wachtler, editors. Ecology and evolution of the freshwater mussels Unionoida. Ecological Studies (Analysis and Synthesis), vol 145. Springer, Berlin, Heidelberg.

Berg, D. J., T. D. Levine, J. A. Stoeckel, and B. K. Lang. 2008. A conceptual model linking demography and population genetics of freshwater mussels. Journal of the North American Benthological Society 27:395–408.

Bertram, E. P., J.S. Placyk, M. G. Williams, and L. R. Williams. 2017. Verification of two cyprinid host fishes for the Texas Pigtoe, *Fusconaia askewi*. Freshwater Mollusk Biology and Conservation 20:65–70.

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.

Bogan, A. E. 2008. Global diversity of freshwater mussels (Mollusca, Bivalvia) in freshwater. Hydrobiologia 595:139–147.

Boyer, S. L., A. A. Howe, N. W. Juergens, and M. C. Hove. 2011. A DNA-barcoding approach to identifying juvenile freshwater mussels (Bivalvia: Unionidae) recovered from naturally infested fishes. Journal of the North American Benthological Society 30:182–194.

Brittain, J. E., and T. J. Eikeland. 1988. Invertebrate drift–a review. Hydrobiologia 166:77–93.

Bruenderman, S. A., and R. J. Neves. 1993. Life history of the endangered fine-rayed pigtoe *Fusconaia cuneolus* (Bivalvia: Unionidae) in the Clinch River, Virginia. American Malacological Bulletin 10:83–91.

Burlakova, L. E., and A. V. Karatayev. 2011. State-Wide assessment of unionid diversity in Texas. Final Performance Report to State Wildlife Grants Program. Federal Aid Grant T-43.

Burlakova, L., A. Karatayev, E. Froufe, A. E. Bogan, and M. Lopes-Lima. 2018. A new freshwater bivalve species of the genus *Cyclonaias* from Texas (Unionidae: Ambleminae: Quadrulini). The Nautilus 132:45–50.

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

Dudding, J. F. 2019. Reproductive life history and host fish selectivity of imperiled freshwater mussels in the Guadalupe River, Texas. Master's thesis, Texas A&M University, College Station, Texas.

Dudding, J. F., M. Hart, J. Morton, C. R. Robertson, R. Lopez, and C. R. Randklev. 2019. Host fish association of two highly imperiled mussel species from the southwestern United States: *Cyclonaias necki* (Golden Orb) and *Fusconaia mitchelli* (False Spike). Freshwater Mollusk Biology and Conservation 22: *In press.*

Edwards, R. J. 1978. The Effect of Hypolimnion Reservoir Releases on Fish Distribution and Species Diversity. Transactions of the American Fisheries Society 107:71–77.

FMCS (Freshwater Mollusk Conservation Society). 2016. A national strategy for the conservation of native freshwater mollusks. Freshwater Mollusk Biology and Conservation 19:1–21.

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.

Freeman, M. C. 1995. Movements by two small fishes in a large stream. Copeia 1995:361–367.

Fritts, A. K., M. W. Fritts II, D. L. Peterson, D. A. Fox, and R. B. Bringolf. 2012. Critical linkage of imperiled species: Gulf Sturgeon as host for Purple Bankclimber mussels. Freshwater Science 31:1223–1232.

Fritts, A. K., M. C. Barnhart, M. Bradley, N. Liu, W. G. Cope, E. Hammer, and R. B. Bringolf. 2014. Assessment of toxicity test endpoints for freshwater mussel larvae (glochidia). Environmental Toxicology and Chemistry 33:199–207.

Funk, J. L. 1957. Movement of stream fishes in Missouri. Transactions of the American Fisheries Society 85:39–57.

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.

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.

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.

GBRA (Guadalupe-Blanco River Authority), and TPWD (Texas Parks and Wildlife Department). 2014. Supplemental Biological Data Collection, Lower Guadalupe River Priority Instream Flow Study. Texas Water Development Board Research and Planning Fund Contract No. 1248311360, Austin, Texas.

Gerking, S. D. 1950. Stability of a stream fish population. The Journal of Wildlife Management 14:193–202.

Gough, H. M., A. M. Gascho Landis, and J. A. Stoeckel. 2012. Behaviour and physiology are linked in the responses of freshwater mussels to drought. Freshwater Biology 57:2356–2366.

Griffith, G. E., S. A. Bryce, J. M. Omernik, and A. Rogers. 2004. Ecoregions of Texas. U.S. Geological Survey, Reston, Virginia.

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. 2009. Extreme longevity in freshwater mussels revisited: sources of bias in age estimates derived from mark-recapture experiments. Freshwater Biology 54:1474–1486.

Haag, W. R. 2012. North American freshwater mussels: natural history, ecology, and conservation. Cambridge University Press 2012:1–535.

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.

Haag, W. R., and J. D. Williams. 2014. Biodiversity on the brink: an assessment of conservation strategies for North American freshwater mussels. Hydrobiologia 735:45–60.

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.

Hall, C. A. 1972. Migration and metabolism in a temperate stream ecosystem. Ecology, 53:585–604.

Harriger, K. M., J. R. Knight, and M. G. Wegener. 2015. Host-fish identifications for two Gulf Coast-drainage freshwater mussels, *Lampsilis straminea* (Southern Fatmucket) and *Quadrula succissa* (Purple Pigtoe). Southeastern Naturalist, 14:243–253.

Hart, M. A., W. R. Haag, R. B. Bringolf, and J. A. Stoeckel. 2018. Novel technique to identify large river host fish for freshwater mussel propagation and conservation. Aquaculture Reports 9:10–17.

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

Horký, P., K. Douda, M. Maciak, L. Závorka, and O. Slavík. 2014. Parasite-induced alterations of host behaviour in a riverine fish: the effects of glochidia on host dispersal. Freshwater Biology 59:1452–1461.

Hove, M. C., B. E. Sietman, J. E. Bakelaar, J. A. Bury, D. J. Heath, V. E. Pepi, J. E. Kurth, J. M. Davis, D. J. Hornbach, and A. R. Kapuscinski. 2011. Early life history and distribution of Pistolgrip (*Tritogonia verrucosa* (Rafinesque, 1820)) in Minnesota and Wisconsin. The American Midland Naturalist 165:338–354.

Hove, M. C., M. T. Steingraeber, T. J. Newton, D. J. Heath, C. L. Nelson, J. A. Bury, J. E. Kurth, M. R. Bartsch, W. S. Thorpe, M. R. McGill, and D. J. Hornbach. 2012. Early life history of the Winged Mapleleaf mussel (*Quadrula fragosa*). American Malacological Bulletin 30:47–57.

Howells, R. G. 2010. Rare mussels: summary of selected biological and ecological data for Texas. BioStudies, Kerrville, Texas.

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

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

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

Howells, R. G., R. W. Neck, and H. D. Murray. 1996. Freshwater mussels of Texas. Texas Parks and Wildlife Press, 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 a UMRCC Symposium, Rock Island, Illinois.

Irmscher, P., and C. C. Vaughn. 2015. Limited movement of freshwater mussel fish hosts in a southern US river. Hydrobiologia 757:223–233.

Johnson, N. A., C. H. Smith, J. M. Pfeiffer, C. R. Randklev, J. D. Williams, and J. D. Austin. 2019. Integrative taxonomy reveals both cryptic and overestimated species level diversity in the freshwater mussel genus *Cyclonaias*. Manuscript submitted for publication.

Jones, J. W., R. J. Neves, S. A. Ahlstedt, and R. A. Mair. 2004. Life history and propagation of the endangered dromedary pearlymussel (*Dromus dromas*) (Bivalvia: Unionidae). Journal of the North American Benthological Society 23:515–525.

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

Kelner, D. E., and B. E. Sietman. 2000. Relic populations of the ebony shell, *Fusconaia ebena* (Bivalvia: Unionidae), in the upper Mississippi River drainage. Journal of Freshwater Ecology 15:371–377.

Levine, T. D., B. K. Lang, and D. J. Berg. 2012. Physiological and ecological hosts of *Popenaias popeii* (Bivalvia: Unionidae): laboratory studies identify more hosts than field studies. Freshwater Biology 57:1854–1864.

Mansur, M. C. D., and M. da Silva. 1999. Description of glochidia of five species of freshwater mussels (Hyriidae: Unionoidea) from South America. Malacologia 41:475–483.

Master, L. L., B. A. Stein, L. S. Kutner, and G. A. Hammerson. 2000. Vanishing assets: Conservation status of U.S. 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.

McLain, D. C., and M. R. Ross. 2005. Reproduction based on local patch size of *Alasmidonta heterodon* and dispersal by its darter host in the Mill River, Massachusetts, USA. Journal of the North American Benthological Society 24:139–147.

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.

McMurray, S. E., and K. J. Roe. 2017. Perspectives on the controlled propagation, augmentation, and reintroduction of freshwater mussels (Mollusca: Bivalvia: Unionoida). Freshwater Mollusk Biology and Conservation 20:1–12.

McNichols, K. A., G. L. Mackie, and J. D. Ackerman. 2011. Host fish quality may explain the status of endangered *Epioblasma torulosa rangiana* and *Lampsilis fasciola* (Bivalvia:Unionidae) in Canada. Journal of the North American Benthological Society 30:60–70.

Mims, M. C., and J. D. Olden. 2012. Life history theory predicts fish assemblage response to hydrologic regimes. Ecology 93:35–45.

Minns, C. K. 1995. Allometry of home range size in lake and river fishes. Canadian Journal of Fisheries and Aquatic Sciences, 52:1499–1508.

Müller, T., M. Czanoleski, A. M. Labecka, A. Cichy, K. Zajac, D. Dragosz-Kluska. 2015. Factors affecting trematode infection rates in freshwater mussels. Hydrobiologia 742:59–70.

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

Newton, T. J., D. A. Woolnough, and D. L. Strayer. 2008. Using landscape ecology to understand and manage freshwater mussel populations. Journal of the North American Benthological Society 27:424–439.

Pellett, T. D., G. J. Van Dyck, and J. V. Adams. 1998. Seasonal migration and homing of Channel Catfish in the lower Wisconsin River, Wisconsin. North American Journal of Fisheries Management 18:85–95.

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.

Petty, J. T., and G. D. Grossman. 2004. Restricted movement by mottled sculpin (Pisces: Cottidae) in a southern Appalachian stream. Freshwater Biology 49:631–645.

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. 2013*a*. 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., 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. 2013*b*. Distribution of extant populations of *Quadrula mitchelli* (False Spike). Elipsaria 15:18–21.

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

Rashleigh, B., and D. L. DeAngelis. 2007. Conditions for coexistence of freshwater mussel species via partitioning of fish host resources. Ecological Modelling 201:171–178.

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. Fisheries Research Board of Canada Bulletin 191:1-382.

Rodríguez, M. A. 2002. Restricted movement in stream fish: the paradigm is incomplete, not lost. Ecology 83:1–13.

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.

Rogers, C. L., and R. V. Dimock, Jr. 2003. Acquired resistance of bluegill sunfish *Lepomis macrochirus* to glochidia larvae of the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) after multiple infections. Journal of Parasitology 89:51–56.

Scanlon, B. R., M. Musgrove, A. Sansom, H. Xie, B. Wilcox, J. Sharp, C. Alexander, B. Vieux, R. Jackson, S. Archer, K. Keese, and R. Reedy. 2004. Unique aspects of proposed San Antonio/Guadalupe Hydrologic Observatory in Texas. American Geophysical Union, Fall Meeting 2004, San Francisco, California.

Schwalb, A. N., T. J. Morris, and K. Cottenie. 2015. Dispersal abilities of riverine freshwater mussels influence metacommunity structure. Freshwater Biology 60:911–921.

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

Sietman, B. E., J. M. Davis, and M. C. Hove. 2012. Mantle display and glochidia release behaviors of five quadruline freshwater mussel species (Bivalvia: Unionidae). American Malacological Bulletin 30:39–46.

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.

Shiver, M. 2002. Reproduction and propagation of the Neosho mucket, *Lampsilis rafinesqueana*. Master’s Thesis, Southwest Missouri State University, Springfield, Missouri.

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.

Stansbery, D. H. 1971. Rare and endangered freshwater mollusks in eastern United States. Pages 5–18 *in* S. E. Jorgensen and R. E. Sharp, editors. Proceedings of a Symposium on Rare and Endangered Mollusks (Naiads) of the U.S.. U.S. Fish and Wildlife Service, Twin Cities, Minnesota.

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

Taskinen, J. and E. T. Valtonen. 1995. Age-, size-, and sex-specific infection of *Anodonta piscinalis* (Bivalvia: Unionidae) with *Ryipidocotyle fennica* (Digenea: Bucephalidae) and its influence on host reproduction. Canadian Journal of Zoology 73:887–897.

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.

Tsakiris, E. T., and C. R. Randklev. 2016. Structural changes in freshwater mussel (Bivalvia: Unionidae) assemblages downstream of Lake Somerville, Texas. The American Midland Naturalist 175:120–127.

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, Oakland, California.

USFWS (U.S. 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 (U.S. Fish and Wildlife Service). 2018. Endangered and threatened wildlife and plants; endangered species status for Texas hornshell. Federal Register 83:5720–5735.

Vaughn, C. C., and C. C. Hakenkamp. 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology 46:1431–1446.

Vaughn, C. C., S. J. Nichols, and D. E. Spooner. 2008. Community and foodweb ecology of freshwater mussels. Journal of the North American Benthological Society 27:409–423.

Vaughn, C. C., and D. E. Spooner. 2006. Unionid mussels influence macroinvertebrate assemblage structure in streams. Journal of the North American Benthological Society 25:691–700.

Vaughn, C. C., and C. M. Taylor. 1999. Impoundments and the decline of freshwater mussels: a case study of an extinction gradient.. Conservation Biology 13:912–920.

Watters, G. T. 1992. Unionids, fishes, and the species-area curve. Journal of Biogeography 19:481–490.

Watters, G. T. 1996. Small dams as barriers to freshwater mussels (Bivalvia, Unionoida) and their hosts. Biological Conservation 75:79–85.

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.

White, M. P., H. N. Blalock-Herod, and P. M. Stewart. 2008. Life history and host fish identification for *Fusconaia burkei* and *Pleurobema strodeanum* (Bivalvia: Unionidae). American Malacological Bulletin 24:121–125.

Williams, J. D., A. E. Bogan, R. S. Butler, K. S. Cummings, J. T. Garner, J. L. Harris, N. A. Johnson, and G. T. Watters. 2017. A revised list of the freshwater mussels (Mollusca: Bivalvia: Unionida) of the United States and Canada. Freshwater Mollusk Biology and Conservation 20:33–58.

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.

Yeager, B. L., and R. J. Neves. 1986. Reproductive cycle and fish hosts of the rabbit's foot mussel, *Quadrula cylindrica strigillata* (Mollusca: Unionidae) in the upper Tennessee River drainage. American Midland Naturalist 116:329–340.

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.

Zale, A. V., and R. J. Neves. 1982. Reproductive biology of four freshwater mussel species (Mollusca: Unionidae) in Virginia. Freshwater Invertebrate Biology 1:17–28.