Host Fish Associations for Two Highly Imperiled Mussel Species from the Southwestern United States: Cyclonaias necki (Guadalupe Orb) and Fusconaia mitchelli (False Spike)

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REGULAR ARTICLE

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

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ABSTRACT

Most freshwater mussels (Unionidae) require a specific host fish to advance their life cycle. Currently, hosts are known for only one-third of the mussel species endemic to the United States and Canada. Texas boasts the greatest diversity of freshwater mussels in the southwestern United States. However, information on mussel-host relationships for ~52 species known to occur within the state is either lacking or incomplete, including two species, *Cyclonaias necki* (Guadalupe Orb) and *Fusconaia mitchelli* (False Spike), currently under review for listing under the U.S. Endangered Species Act. To address this deficiency, we conducted laboratory trials that tested 12 fish species (four families and 11 genera) for *C. necki* and eight species (four families and seven genera) for *F. mitchelli*. For *C. necki*, we identified four host species, *Ictalurus punctatus* (Channel Catfish), *Pylodictis olivarus* (Flathead Catfish), *Noturus gyrinus* (Tadpole Madtom), and *Ameiurus natalis* (Yellow Bullhead). The transformation period was 11 to 22 d for *I. punctatus* (peak metamorphosis at 15 d), 16 d for *P. olivaris* and *A. natalis*, and 10 d for *N. gyrinus*. For *F. mitchelli*, we identified two host species, *Cyprinella lutrensis* (Red Shiner) and *Cyprinella venusta* (Blacktail Shiner); for both, the transformation period was 18 d. Current information on the status of these six host species within the Guadalupe River suggests that imperilment of *C. necki* and *F. mitchelli* may be partly related to the status of their host fishes. Our results also provide critical information for informing recovery activities, such as translocation and captive propagation, if deemed necessary for one or both mussel species.

KEY WORDS: Unionidae, host fish, glochidia, juveniles, freshwater mussels, Guadalupe River

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 cycling nutrients (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). Accordingly, their decline will likely have long-term negative consequences for the ecological function of riverine systems.

Freshwater mussels have a unique life history in that, to successfully reproduce, most require a fish to briefly host their parasitic larvae (glochidia) (Watters and O'Dee 1998). Male mussels release sperm into the water column, and it is filtered from the water by females; fertilization is internal (Haag 2012). The fertilized eggs are brooded to mature larvae (glochidia) within the modified gills (marsupia) of the female

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mussels. After the glochidia mature, female mussels may attract their host(s) by using modified mantle tissue lures, disguising their larvae in packages (i.e., conglutinates) that resemble food items, or passively release their glochidia into the water column (Barnhart et al. 2008; Sietman et al. 2012). This entire process can last several months, and its success depends 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, host use is known reasonably well for ~130 North American mussel species, but it remains poorly described for the remaining two-thirds of native species (Haag 2012). Texas boasts the greatest diversity of freshwater mussels in the southwestern United States; unfortunately, for 13 of the 52 species that occur in the state, researchers do not know or have not confirmed host fishes (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; Howells 1997; Ford and Oliver 2015). This lack of information is problematic because both species are currently 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 the 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, a knowledge of mussel-host fish relationships can help us develop a more complete understanding of how host fish abundance and dispersal impact freshwater mussel population and community ecology, information unknown for the vast majority of mussel species (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*, our objectives 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 and to identify potential future research opportunities.

METHODS

Mussel Species

The focal species of this study were *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 River basin (Randklev et al. 2017; Johnson et al. 2018), although recent studies have mistakenly described it as occurring in the San Antonio River basin (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. 2018). *Fusconaia mitchelli* historically ranged throughout the Brazos, Colorado, and Guadalupe river basins 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, San Gabriel, and Little rivers as well as in Brushy Creek (Howells 2010; Randklev et al. 2013, 2017).

Study Site

Our study was conducted in the Guadalupe River basin of central Texas. Located in the floodplains and low terraces of the Western Gulf Coastal Plain ecoregion (Griffith et al. 2007), the Guadalupe 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). Flow within the 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. Gravid female *C. necki* and *F. mitchelli* were collected from the Guadalupe River between Gonzales and Cuero, Texas, and potential host fish were predominantly collected from sites on the Guadalupe, San Marcos, and Blanco rivers, all of which are part of the Guadalupe basin (Fig. 1), with the exception of *Noturus gyrinus* (Tadpole Madtom), which was collected from a single site on the Brazos River.

Collection

We collected gravid individuals of the two focal species during the spring (mid-March through late April 2017). Because neither species is sexually dimorphic, females were identified based on visual inspection for the presence of inflated and discolored gills, which is characteristic of gravid females. Because the handling of gravid mussels for some species, particularly those belonging to the tribes Pleurobemini and Quadrulini, can induce brood abortion, we placed collected individuals into individual plastic bags filled with river water to retain aborted gill contents (Yeager and Neves 1986; Bruenderman and Neves 1993). Following collection, we transported mussels in insulated coolers to the Texas A&M AgriLife Research & Extension Center in Dallas, Texas. In the laboratory, we visually inspected the contents of each plastic

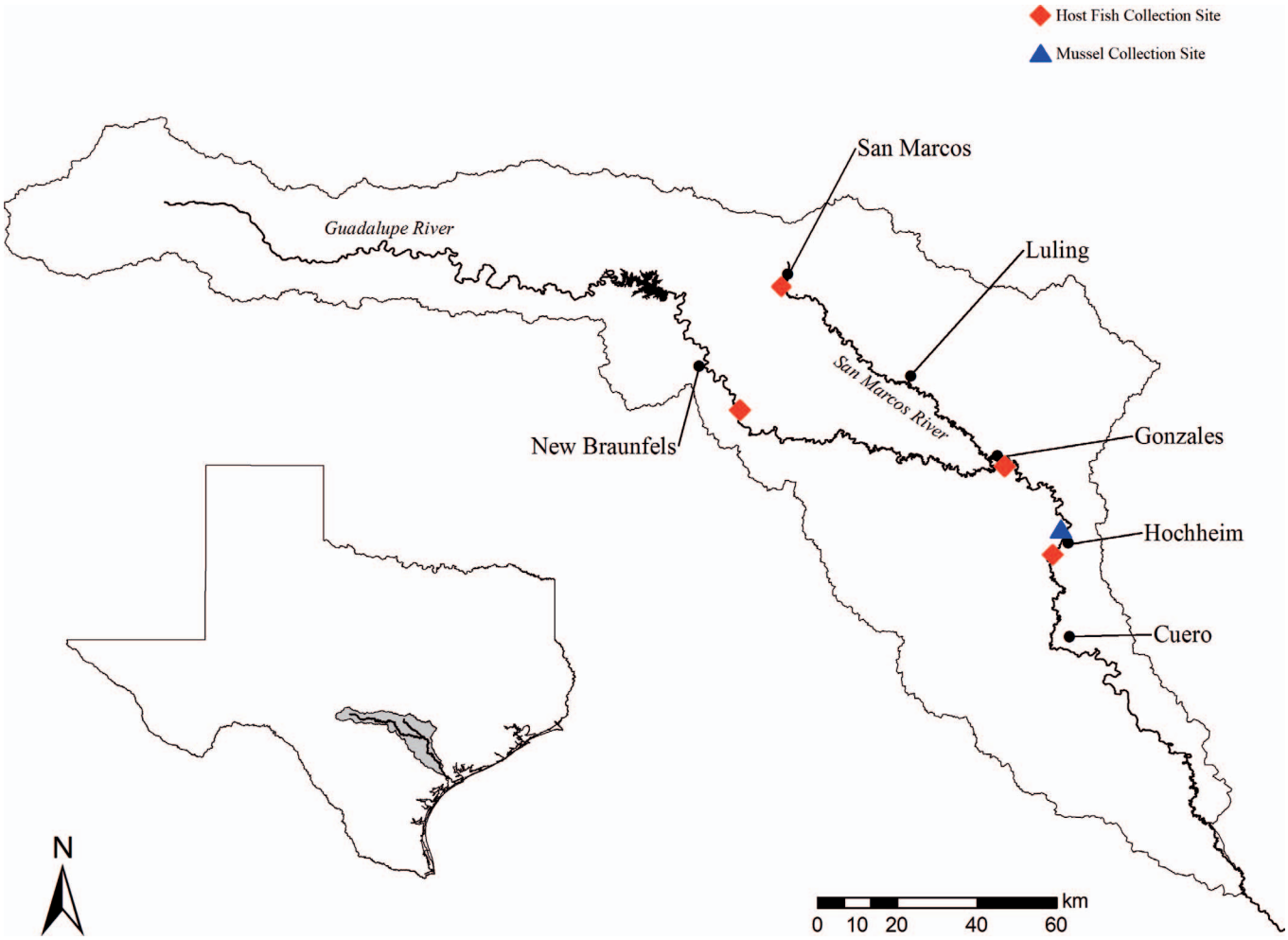

Figure 1. Map of the Guadalupe River basin of Texas showing the collection site for gravid *Cyclonaias necki* (Guadalupe Orb) and *Fusconaia mitchelli* (False Spike) and host fish collection sites. *Noturus gyrinus* (Tadpole Madtoms) were collected from a single site on the Brazos River, Texas, which is not shown on this map.

bag for aborted gill contents (i.e., glochidia, conglutinates, or undeveloped embryos). We placed gravid females 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 fishes were collected from sites where mussels were not present and at least 30 d 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). We attempted to collect as many species of fish as possible while also making sure to collect suspected hosts. Fishes were collected via seine and electrofishing with a goal of collecting at least five individuals of each species (see below for experimental design). We visually inspected all fish to ensure no current infestation from glochidia. Following collection, we transported fish 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, we separated fish by species into recirculating holding systems with water temperature and chemistry matching the collection site at time of sampling. We held potential host fishes for a 30-d quarantine period to allow any encysted glochidia to drop off.

Experimental Design: Host Testing

We conducted laboratory host suitability trials using standard methods (Zale and Neves 1982), inducing glochidial infections in potential host fishes and monitoring for rejection of glochidia or metamorphosis of juvenile mussels. Specifically, we flushed released glochidia from containers holding gravid females and suspended them in 100 mL of water. While vigorously stirring with a large rubber-bulb syringe to ensure glochidia were evenly distributed in the container, we used a pipette to remove ten 200-µL subsamples. We evaluated these subsamples under a dissecting microscope to ensure that glochidia were mature (i.e., developed valves and presence of an adductor muscle) and viable (ascertained by introducing a

Table 1. Results of the host trials for *Cyclonaias necki* (Guadalupe Orb) including the list of fish species tested (Trial), number of replicates (No. Rep: number of tested individuals for a given species of fish; number in parentheses denotes number of individuals that produced juveniles), total number of juvenile mussels collected (No. Juv), total number of glochidia attached (No. Glch), days to juvenile mussel transformation (Trans), and mean metamorphosis rate (% M) with standard errors (±1 SE) in parentheses.

Species Trial	No. Rep	No. Juv	No. Glch	Trans (d)	% M (SE)
Ameiurus natalis (Yellow Bullhead)	3 (3)	8	378	15	2.51 (0.52)
Ictalurus punctatus (Channel Catfish)	2 (2)	183	459	11–22	38.24 (8.99)
Pylodictis olivaris (Flathead Catfish)	2 (2)	130	388	16	34.08 (2.09)
Noturus gyrinus (Tadpole Madtom)	3 (3)	194	697	10	27.56 (2.88)
Cyprinella lutrensis (Red Shiner)	5 (0)	0	7	0	0
Cyprinella venusta (Blacktail Shiner)	5 (0)	0	14	0	0
Lepomis macrochirus (Bluegill)	5 (0)	0	29	0	0
Micropterus treculii (Guadalupe Bass)	5 (0)	0	225	0	0
Macrhybopsis marconis (Burrhead Chub)	5 (0)	0	29	0	0
Campostoma anomalum (Central Stoneroller)	5 (0)	0	35	0	0
Etheostoma spectabile (Orangethroat Darter)	5 (0)	0	36	0	0
Pimephales vigilax (Bullhead Minnow)	5 (0)	0	0	0	0

saturated NaCl solution to observe the closure of valves) (Zale and Neves 1982; ASTM 2006). Viability was enumerated as

$$\frac{(\text{No. open initially}) - (\text{No. open after exposure})}{\text{Total no. of glochidia}}.$$

Broods with viability of $\geq$ 70% were used to infect fish. Depending on the number of available glochidia, we used glochidia from one or multiple females to infect fish.

To infect fishes with glochidia, we placed them into a bath containing ~4,000 glochidia L^{-1}. The bath was aerated and vigorously stirred with a turkey baster to keep glochidia suspended. Fishes were exposed in the bath for 15 min and then transferred to individual 2.75 L tanks using dip nets. We monitored transformation success of glochidia on individual fish in a recirculating (AHAB) system. Each trial consisted of five replicate tanks, each containing a single infected fish. For some of the species tested, we did not have enough individuals for five replicates, so we used two to four replicates. Each of the replicate tanks was self-cleaning; water exited from the bottom rather than the top, ensuring that the glochidia and/or juveniles were removed from the tank. The water from each tank passed through a 55 μm mesh filter cup, which we examined every other day for sloughed glochidia or juvenile mussels. We also flushed each tank with an increased flow rate for 15 min prior to monitoring the filter cups 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. Fishes were fed bloodworms and brine shrimp daily. We continued trials until no further glochidia were found in filter cups for four consecutive monitoring events.

Analyses

We 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. We calculated the metamorphosis rate (% M) as follows for each individual fish:

$$\frac{\text{No. juveniles}}{(\text{No. juveniles}) + (\text{No. sloughed glochidia})} \times 100.$$

RESULTS

Cyclonaias necki

We collected 29 gravid females (marsupium appeared inflated and had a grainy appearance relative to noninflated individuals) 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, and for those individuals we were unable to quantify viability. We used a total of 12 fish species in host trials, but 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 1). *Ictalurus punctatus* (n = 2) produced 183 juveniles with a metamorphosis rate of 38.24% (± 8.99 SE), followed by *P. olivaris* (n = 2), which produced 130 juveniles, an average metamorphosis rate of 34.08% (± 2.09 SE). *Noturus gyrinus* (n = 3) produced 194 juveniles with an average metamorphosis rate of 27.56% (±2.88 SE). We recovered only eight juveniles from *A. natalis* (n = 3), a metamorphosis rate of 2.51% (±0.52 SE). The period for juvenile metamorphosis was 11 to 22 d for *I. punctatus* (peak

Table 2. Results of the host trials for *Fusconaia mitchelli* (False Spike) including the list of fish species tested (Trial), number of replicates (No. Rep: number of tested individuals for a given species of fish; number in parentheses denotes number of individuals that produced juveniles), total number of juvenile mussels collected (No. Juv), total number of glochidia attached (No. Glch), days to juvenile mussel transformation (Trans), and mean metamorphosis rate (% M) with standard errors (±1 SE) in parentheses.

Species Trial	No. Rep	No. Juv	No. Glch	Trans (d)	% M (SE)
Ameiurus natalis (Yellow Bullhead)	5 (0)	0	45	0	0
Cyprinella lutrensis (Red Shiner)	3 (3)	36	156	18	32.51 (9.11)
Cyprinella venusta (Blacktail Shiner)	3 (3)	12	54	18	34.49 (3.51)
Lepomis macrochirus (Bluegill)	5 (0)	0	12	0	0
Gambusia affinis (Western Mosquitofish)	5 (0)	0	20	0	0
Pimephales vigilax (Bullhead Minnow)	5 (0)	0	22	0	0
Campostoma anomalum (Central Stoneroller)	5 (0)	0	5	0	0

metamorphosis at 15 d), 10 d for *N. gyrinus*, 15 d for *A. natalis*, and 16 d for *P. olivaris*.

Fusconaia mitchelli

We 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 we were unable to quantify viability. Of the eight species evaluated, two cyprinid species, *Cyprinella lutrensis* (Red Shiner) and *Cyprinella venusta* (Blacktail Shiner), successfully transformed glochidia (Table 2), yielding a total of 48 juveniles. *Cyprinella lutrensis* ($n = 3$) produced 75% ($n = 36$) of metamorphosed juveniles, while *C. venusta* ($n = 3$) produced the remaining 25% ($n = 12$). The average metamorphosis rate for *C. lutrensis* was 32.51% (±9.11 SE), while the average metamorphosis rate for *C. venusta* was 34.49% (±3.51 SE). For both *C. lutrensis* and *C. venusta*, transformation was observed in three of the five trials, and the transformation period for *F. mitchelli* was 18 d.

DISCUSSION

Our results show that *C. necki* and *F. mitchelli* are likely 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 (see Supplemental Table 1). Specifically, for *C. necki*, we found that it uses *I. punctatus*, *P. olivaris*, *N. gyrinus*, and *A. natalis* as hosts. However, high transformation rates on *I. punctatus*, *P. olivaris*, and *N. gyrinus* suggest that these fish species are likely the primary hosts, while low transformation rates on *A. natalis* suggest that this species is likely a marginal host. Other *Cyclonaias* and *Quadrula* species also have been shown to use ictalurids as hosts (Haggerty et al. 1995; Hove et al. 2011, 2012; Harriger et al. 2015), and our findings for *C. necki* provide additional support for this inference. For *F. mitchelli*, we found that it uses *C. lutrensis* and *C. venusta* as hosts, corroborating previous studies identifying Cyprinid fishes as primary hosts for the genus *Fusconaia* (Neves 1991; Bruenderman and Neves 1993; White et al. 2008). Taken together, our findings provide further evidence that phylogeny may be used to predict host use for other threatened species for which the host is unknown (Haag and Warren 2003).

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, a characteristic likely tied to their reproduction and larval dispersal (Gerking 1950; Hall 1972; Minns 1995). The ictalurids we found to serve as hosts for *C. necki* exhibit potamodromous migratory behavior (Pellet et al. 1998), suggesting greater dispersal capacity and perhaps resiliency to human impacts. That behavior might explain why *Cyclonaias* and *Quadrula* mussel species in Texas appear to be more broadly distributed with multiple stronghold populations spread throughout their range (Randklev et al. 2017). However, we also identified *N. gyrinus* as a host for *C. necki*. This species of fish is diminutive, maintains a small home range (often a single riffle), and is rare within the Guadalupe basin (Perkin and Bonner 2011; GBRA and TPWD 2014). If *N. gyrinus* proves to be the primary ecological host (see below) for *C. necki*, then our 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 *C. necki's* decline is related, in part, to its host fish. For *F. mitchelli*, we 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, its host fish relationship may explain *F. mitchelli's* patchy distribution within its presumptive range and the fact that stronghold populations are aggregated in reaches away from human impacts (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 that the imperilment of *F. mitchelli* is unrelated to its host fish. In this study, we were able to test only four of the 10 minnow species known to occur in the lower Guadalupe due to the fact that the remaining six species have become increasingly rare (e.g., *Notropis buchanani*, Ghost Shiner; Perkin and Bonner 2011). Because we 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 may minimize competition for host fish (Bauer 2001; Rashleigh and DeAngelis 2007) and potentially increase 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 that of the fish, such that extirpation of the host fish results in 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 the host fish necessary for mussel populations to persist (Watters 1996; Vaughn and Taylor 1999). The consequence of these impacts to mussels are diminished gene flow and reduced colonization, which over time 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 the 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. Impoundments cannot be ruled out because the Guadalupe River is highly managed with seven mainstem impoundments, including Canyon Lake reservoir, which is a deep storage reservoir that significantly alters mainstem discharge and water temperatures via hypolimnetic releases (Young et al. 1972; Edwards 1978). Recent studies of fish assemblage structure within the Guadalupe River have demonstrated significant shifts in fish assemblages following mainstem impoundment (Perkin and Bonner 2011).

We were successful in identifying a suite of hosts for two mussel species of high conservation concern (*C. necki* and *F. mitchelli*) from the southwestern United States, which to our knowledge is novel. Based on these results we provide the following recommendations for future host studies for these and other mussel species from this region. First, low fecundity and difficulty collecting viable glochidia limited our capacity to test a broader range of fish species, 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 basin. 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 than where mussels are collected (Haag 2012). Thus, it is possible that *N. gyrinus* collected from the Guadalupe would have had higher metamorphosis success and juvenile production rates than what we observed in this study. Second, if large-scale production of juveniles is desired, pipetting glochidia directly onto the host gills instead of using glochidia baths might use glochidia more effectively. In our study, cyprinids were more diminutive compared to other fishes we tested, meaning their gills had a smaller surface area for glochidia attachment compared to other tested species. Thus, if we had pipetted glochidia onto the gills, we possibly would have seen greater attachment success. However, it is important to note that this method is unlikely to change which hosts are primary versus marginal. Third, our study entailed identifying hosts through 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 our 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; it entails collecting naturally infested fish from the wild, chosen, in part, based on information from laboratory trials like our 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).

The fish hosts we identified in this study 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). When assessing population viability and developing recovery goals, future management and conservation efforts regarding *C. necki* and *F. mitchelli* should take into consideration host fish abundance and habitat and population connectivity (now that this information is known) in addition to other metrics, such as mussel demography and abundance.

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