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Pages 1-17

Expanding the known ranges of the phreatic snails (Mollusca, Gastropoda, Cochliopidae) of Texas, USA

Dominique Alvear, Peter H. Diaz, J. Randy Gibson, Benjamin T. Hutchins, Benjamin Schwartz, and Kathryn E. Perez

Pages 18-24

Mesohabitat associations of the Devil Tryonia, *Tryonia diaboli* (Gastropoda: Truncatelloidea: Cochliopidae)

Peter H. Diaz, Dominique Alvear, and Kathryn E. Perez

Pages 25-35

Provenance and disposition of specimens appearing in color plates of the *Freshwater Mussels of Tennessee*

Gerald R. Dinkins

Pages 36-41

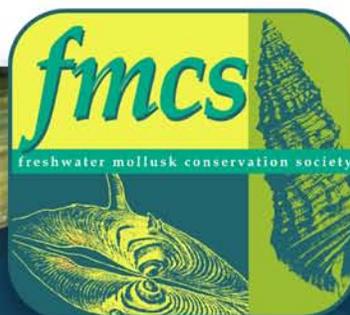
Freshwater mussel assemblage structure in a small Edwards Plateau impoundment with comments on conservation implications for Texas Fatmucket, *Lampsilis bracteata* (Gould 1855)

Kyle T. Sullivan and Bradley M. Littrell

Pages 42-54

Transgenerational effects of copper on a freshwater gastropod, *Planorbella pilsbryi*

Rebecca K. Osborne, Patricia L. Gillis, and Ryan S. Prosser



REGULAR ARTICLE

EXPANDING THE KNOWN RANGES OF THE PHREATIC SNAILS (MOLLUSCA, GASTROPODA, COCHLIOPIDAE) OF TEXAS, USA

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ABSTRACT

The Edwards-Trinity Aquifer System of Texas, USA, one of the world's most ecologically diverse groundwater systems, contains 14 species (across seven genera) of small, poorly studied freshwater snails. Their underground habitat and microscopic size make them difficult to study and identify. Most published records are from original species descriptions, and some have not been seen since they were described more than 100 years ago. Here we use ~150 new collections, including spring and hyporheic zone sampling from across the Texas portions of the Edwards-Trinity Aquifer System, to update the ranges of these species. Two species were very uncommon, if encountered at all; individuals that might be *Phreatodrobia imitata* were seen at one sampling location, and a single individual of *P. punctata* was encountered once. The most frequently encountered snail, *P. nugax*, can be highly abundant and is found across a wide range. Other notable findings include the rediscovery and greatly expanded range of *Stygopyrgus bartonensis*, the Barton Cavesnail, as well as range extensions of 100 km or more for several species.

KEY WORDS: cavesnails, Edwards-Trinity Aquifer System, conservation, hyporheic, Bou-Rouch pump

INTRODUCTION

The Edwards-Trinity Aquifer System of Texas, USA, is home to 14 poorly known, minute snail species (Table 1), all now placed in the Cochliopidae (Clark 2019). A few species were described >100 years ago (Pilsbry and Ferriss 1906), with the remainder mostly described in the last 35 years (Hershler and Longley 1986a, 1986b). Little is known about most of these species beyond their original descriptions. Their underground habitat, microscopic size, and difficulty of sampling make them challenging to study and identify.

The Edwards-Trinity Aquifer System includes three hydrologically interconnected aquifers: the Edwards, Trinity,

and Edwards-Trinity aquifers (Miller 2000). These aquifers are made up entirely of Cretaceous-aged carbonates, with the Edwards Formation lying stratigraphically above the formations that make up the Trinity Aquifer. Their degree of hydrogeologic connectivity is spatially heterogeneous, but in some regions the Trinity and Edwards aquifers are well connected and, for the purposes of hydrogeology, treated as a single aquifer. These aquifers underlie a sweeping arc through the north, central, and western parts of the state of Texas, with surface landscapes ranging from rolling hills to desert (Fig. 1). Most snail species discussed here occur in all three aquifers, so we do not discuss each aquifer separately but provide some background from the Edwards Aquifer, which is the best-studied. A prolific karst aquifer and a recognized global hotspot of stygobitic (groundwater faunal) biodiversity, the

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Table 1. List of Texas's endemic, phreatic snail species examined in this study. Conservation status ranks as assigned by Hutchins (2018) using Vulnerable for S3, Threatened for S2, and Endangered for S1.

Habitat	Texas Endemic	Species	Authority	AFS Common Name	Conservation Rank Status
Phreatic, hyporheic	Yes	<i>Balconorbis uvaldensis</i>	Hershler and Longley, 1986	Balcones Ghostsnail	Vulnerable
Phreatic, surface, hyporheic?	No?	<i>Cochliopina riograndensis</i>	(Pilsbry and Ferris, 1906)	Spiral Pebblesnail	Threatened*
Phreatic, hyporheic	No?	<i>Phreatoceras taylora</i>	(Hershler and Longley, 1986)	Nymph Trumpet	Vulnerable
Phreatic	Yes	<i>Phreatodrobia conica</i>	Hershler and Longley, 1986	Hueco Cavesnail	Threatened
Phreatic	Yes	<i>Phreatodrobia coronae</i>	Hershler, 1987	Crowned Cavesnail	Threatened
Phreatic	Yes	<i>Phreatodrobia imitata</i>	Hershler and Longley, 1986	Mimic Cavesnail	Endangered
Phreatic, hyporheic	Yes	<i>Phreatodrobia micra</i>	(Pilsbry and Ferriss, 1906)	Flattened Cavesnail	Threatened
Phreatic, hyporheic	Yes	<i>Phreatodrobia nugax</i>	(Pilsbry and Ferriss, 1906)	Domed Cavesnail	Vulnerable
Phreatic	Yes	<i>Phreatodrobia plana</i>	Hershler and Longley, 1986	Disc Cavesnail	Threatened
Phreatic, hyporheic	Yes	<i>Phreatodrobia punctata</i>	Hershler and Longley, 1986	High-hat Cavesnail	Endangered
Phreatic	Yes	<i>Phreatodrobia rotunda</i>	Hershler and Longley, 1986	Beaked Cavesnail	Threatened
Phreatic, hyporheic	Yes	<i>Stygopyrgus bartonensis</i>	Hershler and Longley, 1986	Barton Cavesnail	Endangered
Phreatic	Yes	<i>Texapyrgus longleyi</i>	Thompson and Hershler, 1991	Striated Hydrobe	Endangered
Phreatic surface	Yes	<i>Tryonia diaboli</i>	Pilsbry and Ferriss, 1906	Devils Tryonia	Endangered*

**Tryonia diaboli* and *Cochliopina riograndensis* were unranked in Hutchins, therefore the rankings from Johnson et al. (2013) are used for these species.

Edwards Aquifer is home to more than 50 described vertebrate and invertebrate species (Longley 1981; Culver and Sket 2000). Meteoric water recharges the aquifer along its northern and western margins, where Edwards limestones are exposed

at the surface. South and east, a series of *en échelon* faults mark the transition between the recharge zone and the confined zone, where Edwards limestones are confined below nonpermeable strata and the aquifer is under artesian pressure.

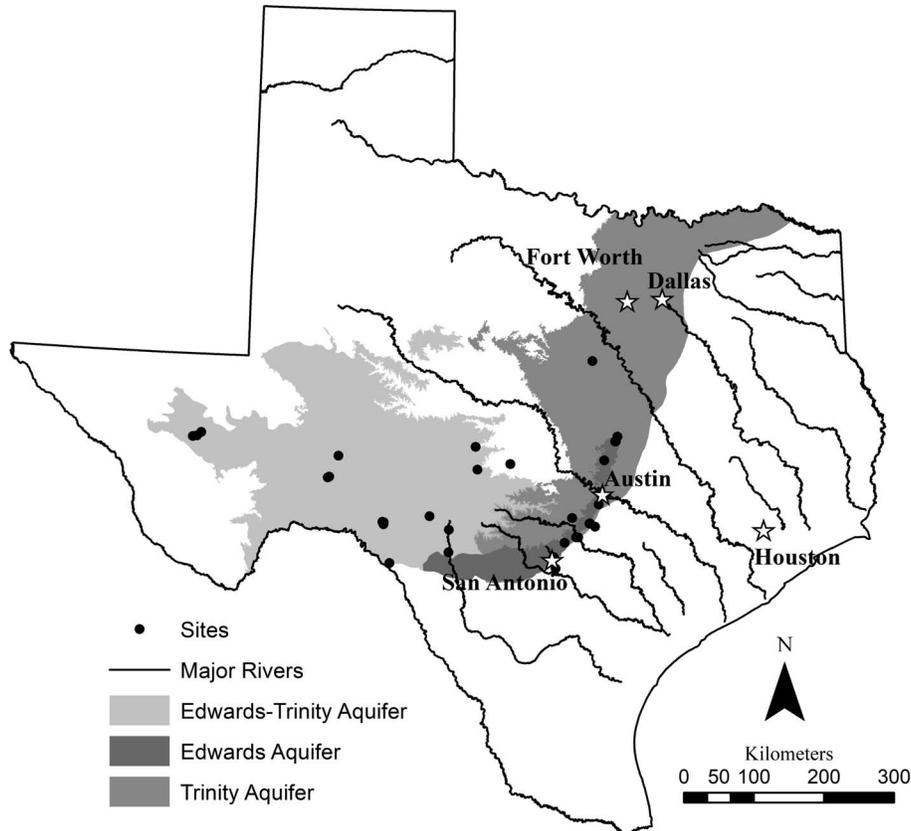


Figure 1. Map of Texas highlighting the three aquifers that comprise the Edwards-Trinity Aquifer System, major metropolitan areas, and streams. All localities drawn from taxonomic literature, and new sites are indicated.

Using stable isotope data, Hutchins et al. (2016) placed Edwards Aquifer *Phreatodrobia* spp. at the trophic level of primary consumer, presumably scraping and grazing on microbial mats and organic debris lining interconnected pores and conduits in the aquifer. In the phreatic (water-saturated zone, below the water table) portion of the Edwards Aquifer, invertebrate-dominated foodwebs are supported by (1) allochthonous (surface-derived photosynthetic) organic matter that enters the aquifer via surface connections in the recharge zone and (2) autochthonous (microbially derived chemolithoautotrophic) organic matter produced near a freshwater-saline water interface in the confined portion of the aquifer (Hutchins et al. 2016). Aquatic snails have been collected at both autochthonous- and allochthonous-dominated sites. They occur with a variety of other primary consumers (e.g., isopods and amphipods) as well as higher trophic level species that are potential predators (e.g., *Eurycea* salamanders, stygobitic catfish, cirolanid isopods, amphipods, and a flatworm *Sphalloplana* sp.). Species richness (and trophic complexity) decreases at sites where autochthonous organic matter is absent. Hyporheic zone (mixing zone of shallow groundwater and surface water along streams) ecology remains unstudied in Texas, but foodwebs are probably based on microbially processed particulates and dissolved organic carbon from downwelling surface waters (Vervier et al. 1992). As at phreatic aquifer sites, snails in hyporheic sites occur with a variety of potential predators, including flatworms, nematodes, salamanders, crustaceans, and aquatic insects (Hutchins et al. 2016), although none have been documented to make extensive use of snails as a food source. In some of the only available data, a gut-content analysis found 4% of the Salado salamander's (*Eurycea chisholmensis*) diet was *Phreatodrobia nugax* (Diaz and Warren 2018), but little other ecological information is known about these snails.

A recent conservation threat assessment (Hutchins 2018) ranked phreatic snails in Texas based on their reported range size and threats to their groundwater habitat. Researchers found that, due to a strong regulatory framework in place in much of the Edwards Aquifer, impacts of threats to species in the region are low. However, this was not the case in the western Edwards Plateau and Edwards-Trinity aquifers, where species face different anthropogenic stressors from industrial use rather than urban sprawl, and threats to many species were ranked medium. Hutchins (2018) and Johnson et al. (2013) assigned five of these phreatic snail taxa a conservation status of Endangered; six, Threatened; and three, Vulnerable. Several of these species were considered candidates for listing under the U.S. Endangered Species Act but were ultimately not listed due to insufficient information (USFWS 2009).

In this study, we address Issue no. 1 of the National Strategy for the Conservation of Freshwater Mollusks (FMCS 2016) by increasing the knowledge of the distribution of these species and providing information on collection methods. When possible, we also provide habitat information (Issue no. 4, FMCS 2016) for these poorly known, phreatic snail species.

METHODS

Sampling Methods

The new collections of snails examined in this study were from the hyporheic zone of spring-fed surface streams and from springs and other groundwater sites in the Edwards-Trinity Aquifer System (Fig. 1). Snail specimens were taken largely as a byproduct of sampling for other taxa, such as endangered salamanders or amphipods that occupy the same habitats, not as a result of randomized or directed sampling for snails. Sampling methods used in this study have been used in other studies of phreatic gastropods (Hershler and Longley 1986b). Depending on the type of site and access to the aquifer, a variety of sampling methods were used to collect specimens from springs, wells, streams, or the hyporheic zone. In streams, drift nets (0.45 by 0.30 m rectangular openings, 250 μm mesh) (Gibson et al. 2008) were deployed and checked periodically (Diaz and Warren 2018). Well samples were taken using (1) bottle traps (Nissen et al. 2018) that were baited with cotton substrate and pistachios or (2) drift nets placed on the outflow of flowing artesian wells. In some spring samples, the “mop head” technique (Hershler and Longley 1986b) was used. This method uses pieces of cotton mop heads placed in the spring orifice for several weeks, to allow bacteria and fungi to colonize, which in turn promotes colonization by snails. After the colonization period, the mop head is removed from the spring orifice and placed in a tray of water, allowing snails to be removed by hand. Additional detail on this sampling method can be found in Nissen et al. (2018). Stream, spring, and well samples were taken and preserved in 70–95% ethanol or isopropanol for identification. Hyporheic sites were sampled using a Bou-Rouch pump (Bou and Rouch 1967). The perforated interval of the pump spike was hammered into hyporheic sediments to a depth of 30–50 cm. A shallower interval was sampled only when tight sediments precluded reaching a depth of 30–50 cm. Approximately nine liters of hyporheic water was pumped from the hyporheic zone and filtered through a 200 μm -mesh net. Snails and sediments retained in the net were preserved in 95% ethanol in the field and sorted at 10 \times magnification in the lab.

In the records presented, we include new sample sites in the “new localities” category, as well as recollections from reported sites, because some large spring complexes have developed more detailed naming systems over time. For example, earlier collections from “Comal Springs” could be from several discrete spring orifices that are now individually named (e.g., Comal Springs Run 1). It is impossible to tell which spring orifices were sampled in those earlier collections, but it is important to distinguish between them as springs in a single complex often discharge water from different groundwater sources and have different faunal compositions. More generally, recollections confirm the continued presence of species in previously sampled sites—important information for conservation purposes.

Identifications

The source (literature, museum lot, or new collection) for each of these locality records, and a complete listing can be obtained from the corresponding author. New collections are vouchered at the Smithsonian Institution, USNM no. 1571271–1571310. To determine historic distributions, we included collection locality records ($n = 60$) and presented them on the distribution maps for each species, from the taxonomic literature describing these species (Pilsbry and Ferriss 1906; Hershler and Longley 1986a, 1986b; Thompson and Hershler 1991; Hershler 2001). Collection locality records for this study were not taken from unconfirmed museum lots, as identification in this group is difficult and other studies have shown misidentification rates of lots in museum collections to be a significant source of error in biodiversity studies (Shea et al. 2011; Goodwin et al. 2015). Below we include information on the diagnostic characteristics used to identify each species, but we also underwent an iterative training and identification process ourselves. For each locality record presented in this study that is from a new collection (hereafter “samples” or new collections, $n = 86$), the collector (coauthors: PD, RG, BH, or BS) assigned a preliminary identification, then the taxon experts (coauthors: KEP, DA) also assigned a preliminary identification. The taxon experts worked through the entire set of samples and assigned identifications based on the original species descriptions. At that point, we examined representatives of each species that had been identified by the species’ author, borrowed from the Smithsonian Institution, to refine our identifications; we then reidentified all samples. Identifications that were questionable required agreement from the two taxon experts (KEP, DA) for final identification. The final quality control step resulted in relatively few species reassignments (<1%), and all were juvenile *Phreatodrobia* spp. as detailed in the discussion below. Finally, 20 locality records are presented on the distribution maps from lots borrowed from the Smithsonian Institution, Zara Environmental Consulting, and the Texas Memorial Museum; as these are previously unpublished localities, they are presented here with the new collections.

RESULTS

We present 146 locality records of phreatic snails from the Edwards-Trinity Aquifer System. In the new collections presented in this study, we observed specimens matching the descriptions of 12 of the 14 described phreatic snails. We were unable to collect *Phreatodrobia imitata* specimens perfectly matching the species description, but we did find one population that was similar (additional details in species summary below). We refer to that population as *P. cf. imitata*. We collected a single individual attributable to *P. punctata* from near the type locality, but with some morphological differences from the species description and figures. We refer to that population as *P. cf. punctata*. Further work will be required to confirm the identification and full ranges of those two taxa. Below we present photographs of each species,

along with a map showing sites from the literature and new records from our sampling. We also include a summary of the key features used to distinguish each species from those that are similar and any notes about sampling or ecology that our data provide. If taxonomic concerns are apparent, comments are also included in each species summary. For drawings and definitions of snail morphological characters, we refer readers to Hershler and Ponder (1998) and Arnold (1965).

Cochliopidae

Balconorbis uvaldensis Hershler and Longley, 1986.—*Balconorbis uvaldensis* (Fig. 2) is distinguished from *Phreatodrobia* by having a pigmented shell, with a greater number of whorls, up to three, and pronounced growth lines on all whorls that follow the shape of the outer lip. At 0.90–1.22 mm width, *B. uvaldensis* is larger than *P. micra* at 1.00 mm or *P. plana* at 0.75–1.00 mm. *Balconorbis uvaldensis* can be distinguished from two *Phreatodrobia* (*P. nugax* and *P. micra*) species that have relatively circular aperture shapes and highly reflected and thickened lips by its elongate aperture (in the direction of the columellar axis) and only slightly reflected and thickened lip. This species is most likely to be confused with *P. plana*, as both have apertures that are elongate. However, the aperture in *P. plana* is enlarged to $\sim 1/3$ of the width of the shell, whereas the aperture of *B. uvaldensis* is $\sim 1/4$ of the width of the shell. If placed spire side down, *B. uvaldensis* is nearly flat, whereas *P. plana* is not, due to the enlargement of the aperture relative to the body whorl. The aperture shape also distinguishes *B. uvaldensis*; the outer edge of its aperture lip is slightly reflected and thickened, whereas the lip is not reflected or thickened in *P. plana*. *Balconorbis uvaldensis* has been observed in a relatively small region of the Edwards-Trinity Aquifer System; literature records were all from well sites, but hyporheic sampling added two sites on the Nueces River, and spring orifice sampling recovered this species from San Felipe Springs.

Cochliopina riograndensis (Pilsbry and Ferriss, 1906).—*Cochliopina riograndensis* (Fig. 3) is distinctive in shell morphology from the other phreatic and spring taxa described from the region; it is larger, average 2.23–2.80 mm width (Pilsbry and Ferriss 1906; Hershler 1985), globose, openly umbilicate, and usually with three to four brown pigmented bands around the body whorl. These pigmented bands usually mark distinctive spiral threads that extend the length of the shell. They are apparent in juveniles as well as adults, although they are not present in all individuals. Generally, the shell is slightly olive-colored, opaque, and shining; however, in some specimens collected from springs, the shell is nearly transparent, but it still retains faint pigment bands.

This species was described from river drift, with later workers describing it as epigeic; we also found it potentially occupying phreatic and hyporheic habitats. In Independence Creek (Terrell County), Leonard and Ho (1959) found this species alive “restricted to the edge of the stream, abundant

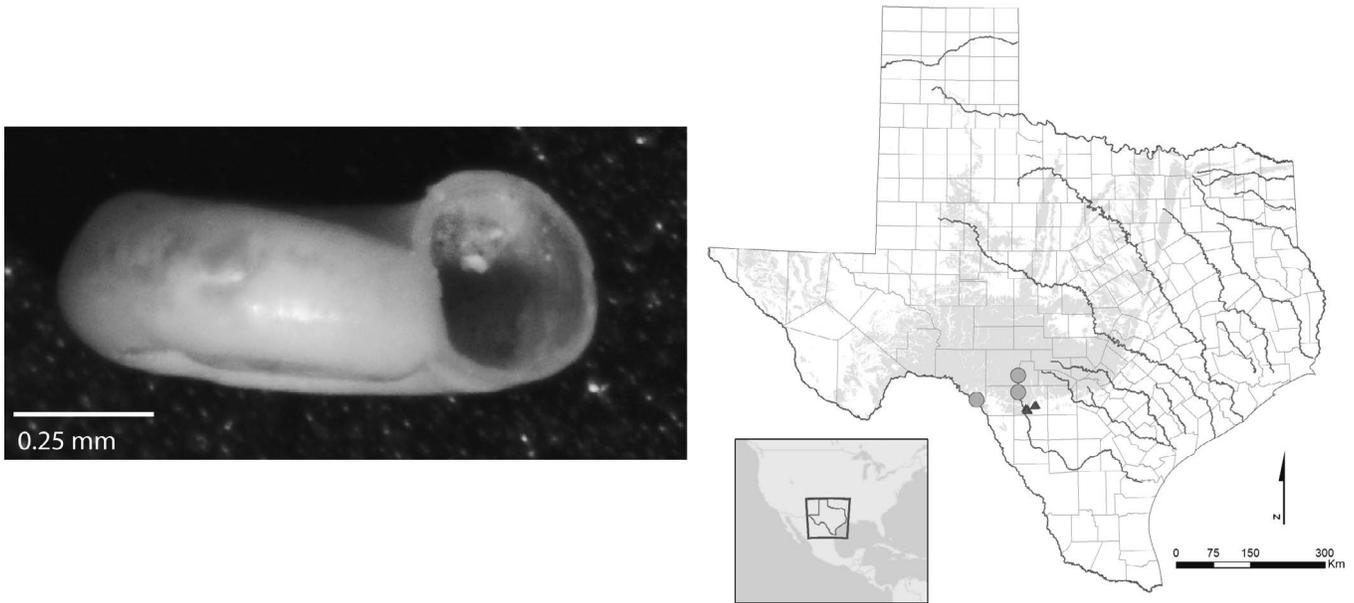
Balconorbis uvaldensis Hershler and Longley, 1986

Figure 2. *Balconorbis uvaldensis*, apertural view (0.95 mm width; Nueces at Barksdale, Edwards County, Texas). Map of Texas with Edwards-Trinity Aquifer System highlighted in gray. Counties and major river drainages are also illustrated. Literature records are indicated with triangles and new records with circles.

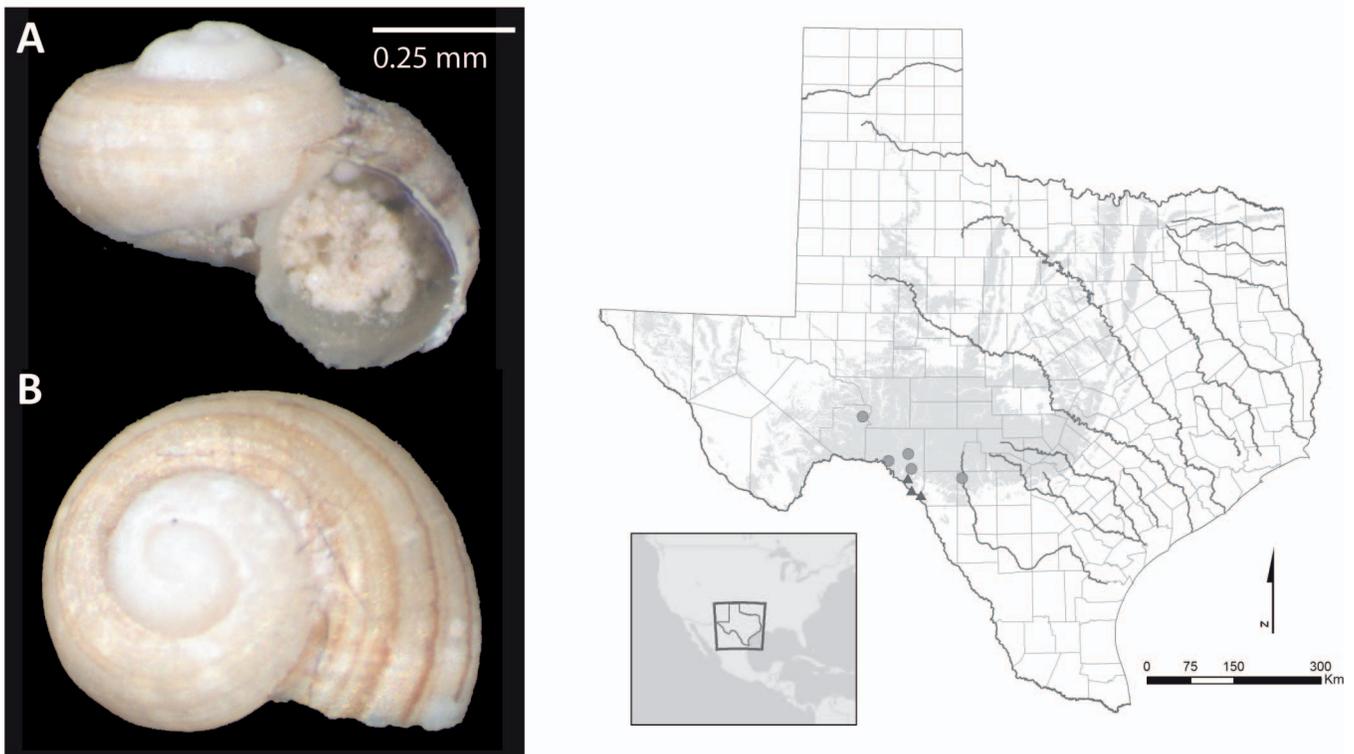
Cochliopina riograndensis Pilsbry and Ferriss, 1906

Figure 3. *Cochliopina riograndensis*, A: apertural view, B: spire view (1.31 mm wide; Caroline Springs, Independence Creek, Terrell County, Texas). Map of Texas with Edwards-Trinity Aquifer System highlighted in gray. Counties and major river drainages are also illustrated. Literature records are indicated with triangles and new records with circles.

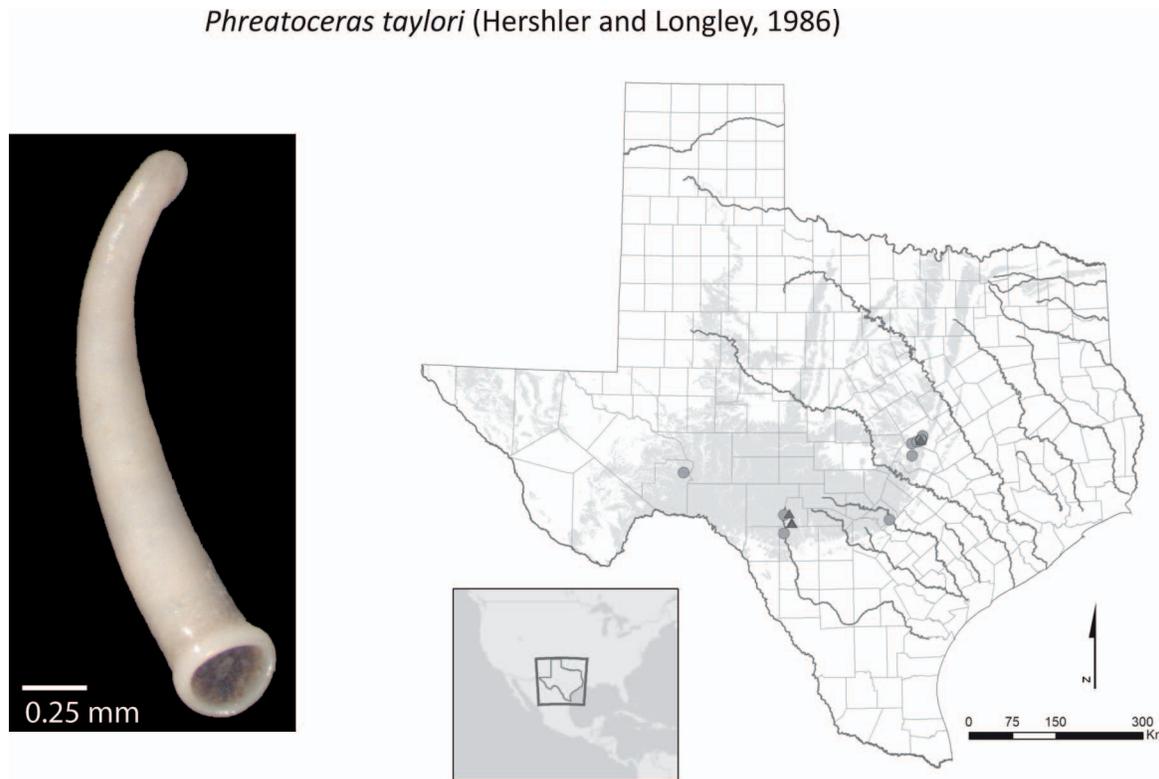


Figure 4. *Phreatoceras taylori* (2.18 mm length; Tahuaya Springs, Bell County, Texas). Map of Texas with Edwards-Trinity Aquifer System highlighted in gray. Counties and major river drainages are also illustrated. Literature records are indicated with triangles and new records with circles.

under cobbles, logs and in aquatic vegetation, and on fine mud on watercress.” In addition, sampling in Dolan and Finegan springs (Val Verde County) encountered live *C. riograndensis* ($N = 1,264$) in small numbers (2% of the individuals in the entire collection) from nets placed directly over water flowing from 19 spring orifices. These snails also were found in small numbers from riffles in Dolan Creek and the Devils River, but the greatest numbers by far were found in samples collected below the outflow of the orifice and in the transition zone between spring and spring run (89% of the individuals in the entire collection) (Diaz et al. 2018). Collections of live individuals from Snake Spring on Dolan Creek were found in nets placed over a spring orifice emerging directly from bedrock. All the shells of *C. riograndensis* from hyporheic sampling in our study were dead shells, so it is uncertain whether or not they were living in that habitat. We include this species here because, although it is found in spring runs, we hypothesize that it has a strong groundwater connection.

Cochliopina riograndensis also was reported from epigeal habitats on the surface of aquatic vegetation in a clear, cool, fast-flowing stream in the Cuatro Ciénegas basin in Coahuila, Mexico, and the Rio Salado de los Nadadores (20 km east of Cuatro Ciénegas), 290 km to the southwest of the nearest locality in Texas (Taylor 1966; Hershler 1985). It is worth noting, however, that there are morphological differences between shells figured from these locations, with the populations from Mexico having a more elevated, conical

shell, a solid pigmented band, and coarser striae (Hershler 1985).

Phreatoceras taylori (Hershler and Longley, 1986a).—*Phreatoceras taylori* (Fig. 4) is distinguished by its uncoiled trumpet-like shell. It has a round aperture and usually is slightly curved. This species was previously known only from the type locality and spring sites nearby, all in Real County (Hershler and Longley 1986a, 1986b). We expand its range to include spring and hyporheic samples as much as 240 km to the west at Independence Creek, Terrell County, and fill in sites within the documented range. A similar snail was reported from epigeal habitats in the Cuatro Ciénegas basin of Coahuila, Mexico, 380 km to the southwest (Hershler 1985) and fossils in other sites in Coahuila (Czaja et al. 2017); however, there are morphological differences between shells figured from these locations and those found in Texas.

Phreatodrobia conica Hershler and Longley, 1986.—*Phreatodrobia conica* (Fig. 5) is distinctive among phreatic hydrobiids of the Texas aquifers because it has a smooth, conical shell. It has a circular aperture with a thickened lip around the entire aperture. It can be distinguished from the surface-dwelling *Marstonia comalensis* by that species’ teardrop shaped aperture. It is distinguished from *P. punctata* and *P. imitata* by their characteristic shell sculpture and apertural lip. This species was previously known from well, cave, and spring samples in Bexar and Comal counties, and we report a

Phreatodrobia conica Hershler and Longley, 1986

Figure 5. *Phreatodrobia conica*, apertural view (0.75 mm width; Creek Springs, Robertson Springs, Bell County, TX). Map of Texas with Edwards-Trinity Aquifer System highlighted in gray. Counties and major river drainages are also illustrated. Literature records are indicated with triangles and new records with circles.

140-km range extension to the northeast at Tahuaya Springs, Bell County.

Phreatodrobia coronae Hershler, 1987.—*Phreatodrobia coronae* (Fig. 6) is similar in shape to *P. nugax* and *P. micra* in that it has a trochoid shape that is less (Fig. 6A) or more (Fig. 6B) depressed. This shape varies from nearly planar to trochoid in some individuals. However, the species is recognized by its distinctive uncoiled, hornlike protoconch. In other *Phreatodrobia* species, the protoconch is rounded and appressed to the other whorls, not free. In addition, *P. coronae* has unique sculpture in some populations, with distinct spiral lines on all whorls (except the protoconch) and large lamelliform costae in some individuals. The costae are highly variable with some individuals having just a hint of the structure (Fig. 6B) and others with costae extending nearly 1/3 the width of the whorl (Fig. 6A). The aperture is always attached to the body whorl in adult shells. We illustrated individuals with and without costae as well as more or less depressed for comparison. The range of this species is relatively restricted, with all known sites within 60 km of each other in Val Verde County, Texas. These additional samples expand the range to include further upstream in the Devils River at Snake, Dolan, and Finegan springs, and we confirm the population at San Felipe Springs is still extant. The Devils River groundwater region was considered by Hutchins (2018) as having high vulnerability due to proposed, unregulated groundwater extraction.

Phreatodrobia imitata Hershler and Longley, 1986.—*Phreatodrobia imitata* (Fig. 7) is distinguished by its tall, conical shape and distinctive shell sculpture. It has a smooth embryonic whorl followed by ribs that run in the direction of the apertural lip (collabral costae) and spiral lines on the remaining whorls. The aperture is round with a lip that is flared all around, with the greatest expansion in the low-outermost portion of the lip. This species has been recorded from Bexar County well sites in the literature, and we provide one potential new record of a population that might be *P. imitata*. *Phreatodrobia imitata* is described as variable in sculpture pattern with some individuals lacking the costae and with the lip sometimes not touching the body whorl. The individuals from Hidden Spring no. 2 in Bell County, Texas, may represent a large range extension and unusual sculpture pattern for *P. imitata*, or they may not be the same species. We figure them and refer to them as *P. cf. imitata*, as they are similar to *P. imitata*, but there is uncertainty in this identification. Soft tissues were not present in our sample, so we could not compare internal anatomical features to those described for *P. imitata*.

Phreatodrobia micra (Pilsbry and Ferriss, 1906).—*Phreatodrobia micra* (Fig. 8) has a very small, flattened (but not planispiral) shell. Other flat taxa, such as *P. plana* and *P. rotunda*, are distinguished by their distinctive aperture shapes while the aperture of *P. micra* is round. It is most similar to *P. nugax* and co-occurs with that species. *Phreatodrobia micra* is

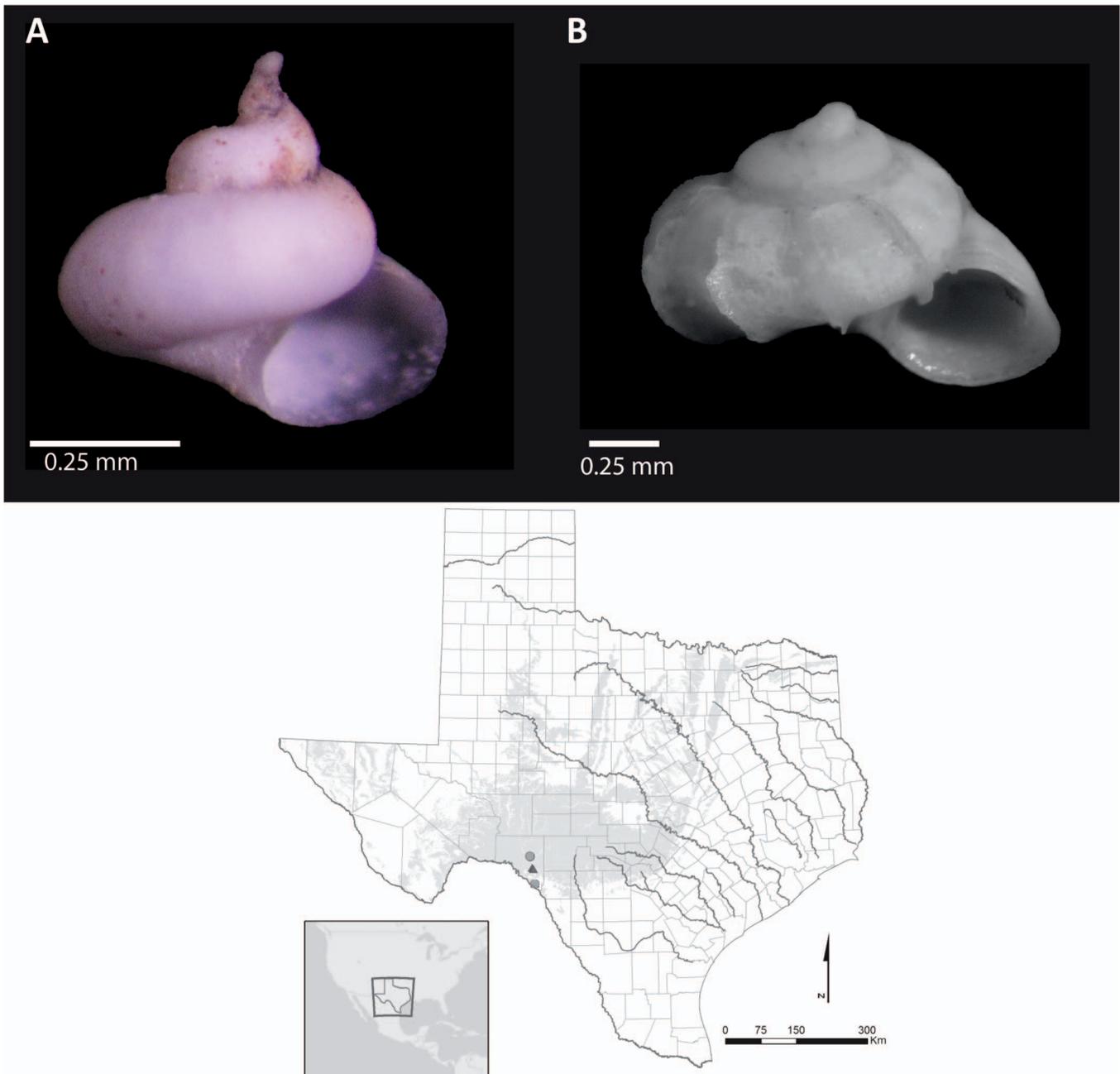
Phreatodrobia coronae Hershler 1987

Figure 6. A. *Phreatodrobia coronae*, apertural view (0.64 mm width; Blue Springs, Dolan Falls Preserve, Val Verde County, Texas); B. *Phreatodrobia coronae*, apertural view (1.51 mm width; Finegan Springs, Devils River, Val Verde County, Texas) lamellate individual. Map of Texas with Edwards-Trinity Aquifer System highlighted in gray. Counties and major river drainages are also illustrated. Literature records are indicated with triangles and new records with circles.

distinguished from *P. nugax* by a smaller adult size and even whorl expansion rate; moreover, the aperture is always attached to the body whorl. Additionally, in the redescription of *P. micra* and *P. nugax*, Hershler and Longley (1986b) included shells of *P. micra* with an average shell height of 0.41 mm and width of 0.95 mm for a h/w ratio of 0.43; shells of *P. nugax* had an average shell height of 1.02, width of 1.14, and a h/w ratio of 0.89.

Great care as well as observation of a size series is necessary to reliably distinguish *P. micra* morphologically from the juveniles of other flattened *Phreatodrobia* spp. Juvenile snails can be difficult or impossible to identify, and in the case of the flat *Phreatodrobia* species, they are positively misleading. In our final round of quality control, some individuals that were initially identified as *Phreatodrobia micra* were reidentified as juveniles of *P. plana*, *P. rotunda*,

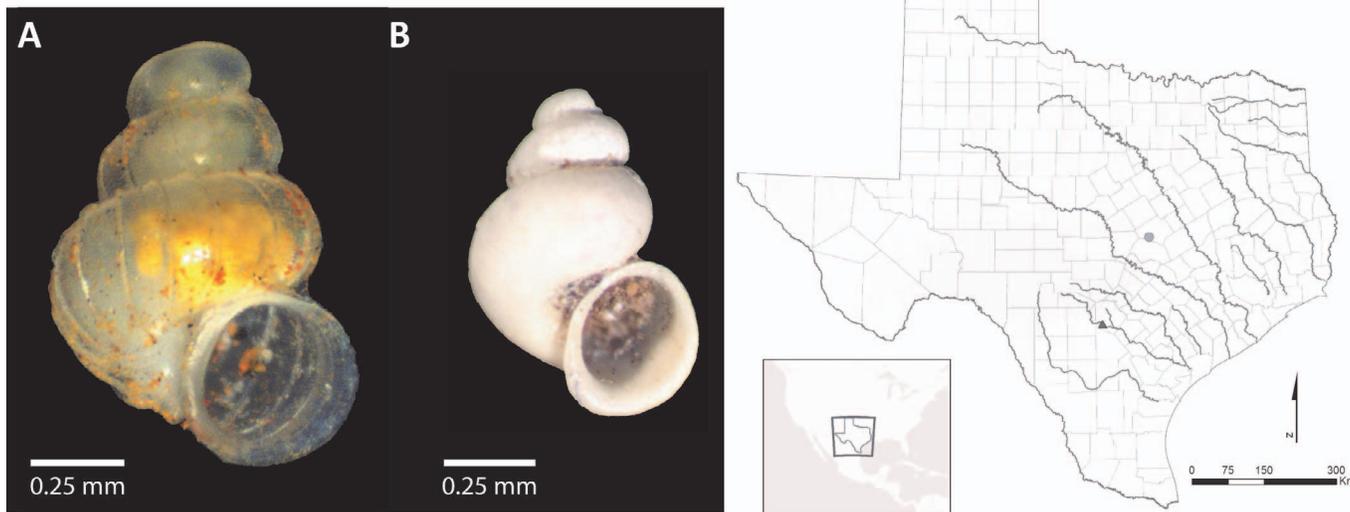
Phreatodrobia imitata Hershler and Longley, 1986

Figure 7. A: *Phreatodrobia imitata*, apertural view (1.13 mm length; Verstraeten well, Bexar County, Texas). B: *Phreatodrobia cf imitata*, apertural view (0.88 mm length; Hidden Spring no. 2, Bell County, Texas). Figured with relative sizes preserved to allow comparison. Map of Texas with Edwards-Trinity Aquifer System highlighted in gray. Counties and major river drainages are also illustrated. Literature records are indicated with triangles and new records with circles.

and *P. nugax*, all of which may co-occur at some sites. This reidentification occurred when the individuals were small and possessed just the embryonic whorl and half a whorl of additional growth. *Phreatodrobia plana*, *P. rotunda*, and *P. nugax* are similar in shape, sculpture, and size; all have

apertures that are detached from the body whorl as adults, but the aperture is attached during growth, making them look similar to *P. micra*. To distinguish juveniles of these other species from adult *P. micra*, we observed that if the shell near the aperture is very translucent, it is juvenile, and it becomes

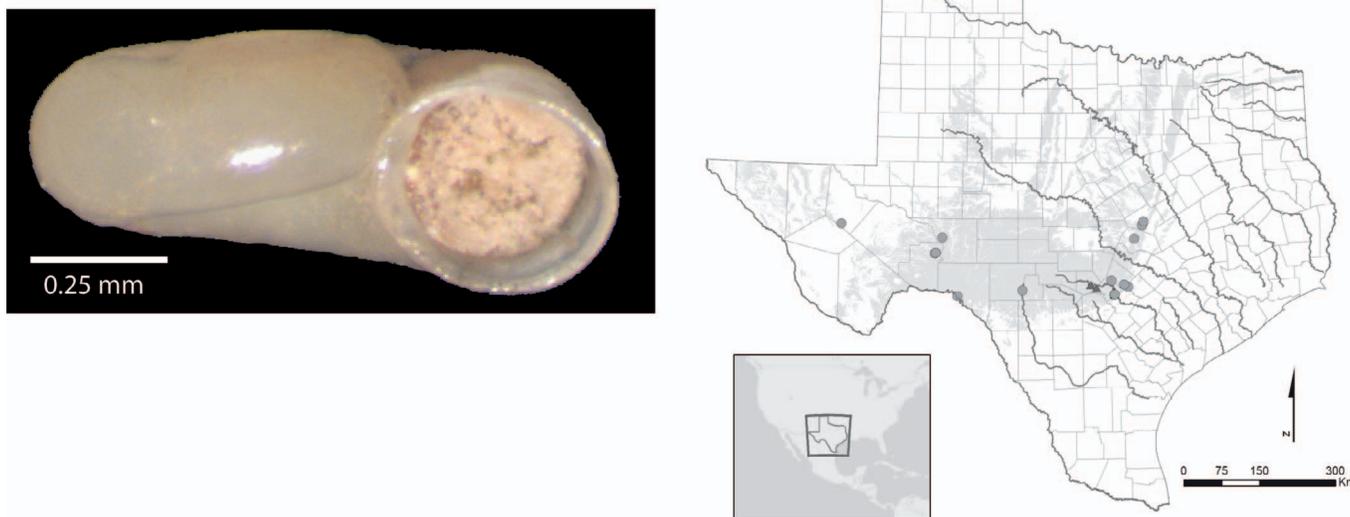
Phreatodrobia micra Hershler and Longley, 1986

Figure 8. *Phreatodrobia micra*, apertural view (1.08 mm width; Scull Rd. Crossing San Marcos River, Hays County, Texas). Map of Texas with Edwards-Trinity Aquifer System highlighted in gray. Counties and major river drainages are also illustrated. Literature records are indicated with triangles and new records with circles.

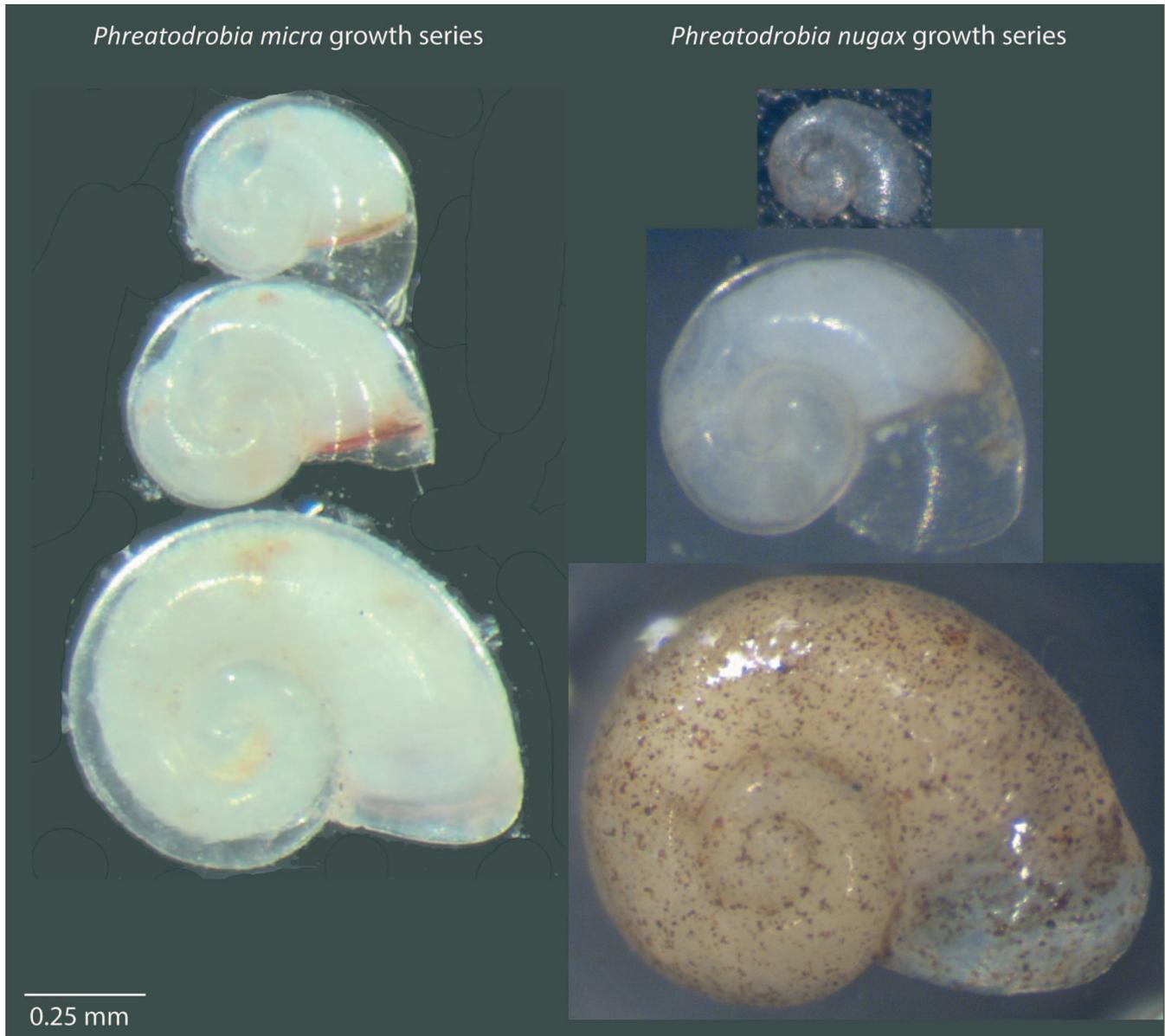


Figure 9. Comparison of a range of sizes of *P. micra* and *P. nugax* (adult *P. nugax* = 1.34 mm wide; San Antonio River, Bexar County, Texas). Relative sizes preserved to allow comparison.

less translucent with age (Fig. 9); a fully grown *P. micra* shell, while small, is not translucent and has a flared or thickened lip.

Phreatodrobia micra was known from cave, well, and spring sites from a restricted range in the central Edwards-Trinity Aquifer System (Comal, Kendall, and Hays counties). Our sampling expands the range 360 km to the west at San Solomon Springs, Reeves County, and 130 km to the northeast at Robertson Springs, Bell County, and it documents new occurrences from both hyporheic and spring samples.

Phreatodrobia nugax (Pilsbry and Ferriss, 1906).—*Phreatodrobia nugax* (Fig. 10), as currently understood, is highly variable in phenotype and includes shells that are variously flattened, elevated, conical, with and without ribbed sculpture, and with free or attached whorls. This species is characterized

by a rounded aperture that is often (not always) free from the previous whorl. The apertural lip is thickened and flared. The flare is most pronounced in adult individuals. It is most similar to *P. micra* but can be distinguished by its larger adult size (~1.75 mm adult width vs. 0.99 mm adult width), height/width ratio (detailed above in *P. micra* summary), and whorl expansion rate. In *P. micra*, when examining a view from the embryonic whorl, the whorl expansion rate is relatively even, so each whorl seems slightly wider than the previous whorl, whereas in *P. nugax* the whorl expansion rate is uneven, with the body whorl nearly twice as wide as the previous whorl. Finally, the aperture is always attached in *P. micra*, whereas in *P. nugax* adults it is often free. *Phreatodrobia nugax* is the most commonly encountered and broadly distributed phreatic

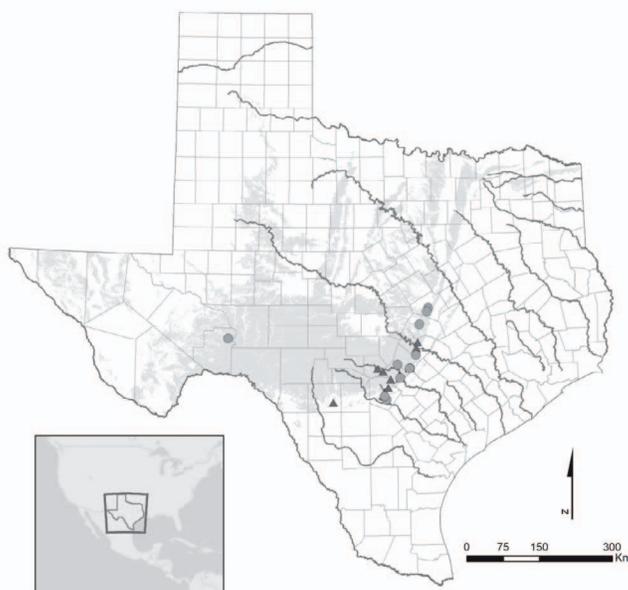
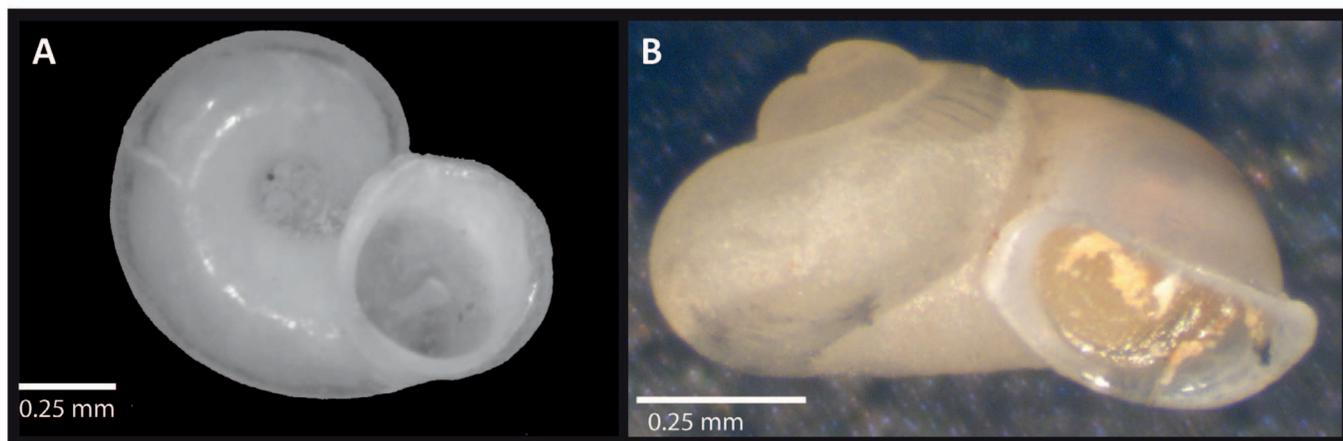
Phreatodrobia nugax Hershler and Longley, 1986

Figure 10. *Phreatodrobia nugax*, A: umbilical view, 1.09 mm width; and B: apertural view (1.02 mm width; San Marcos Artesian Well, Hays County, Texas). Map of Texas with Edwards-Trinity Aquifer System highlighted in gray. Counties and major river drainages are also illustrated. Literature records are indicated with triangles and new records with circles.

snail in the region and was known from well, drift, and spring samples in Uvalde, Kendall, Bexar, Comal, Hays, and Travis counties. This species's range, as currently understood, is extended by 280 km west (Independence Creek, Terrell County) and 80 km east (Tahuaya Springs, Bell County) and now includes the hyporheic zone.

Phreatodrobia plana Hershler and Longley, 1986b.—*Phreatodrobia plana* is recognized from the apertural view (Fig. 11). It has a flat shell aspect, depressed in both embryonic and umbilical aspects. The aperture is elongate, longest in width along the columellar axis. The aperture also extends above the spire where the lip is flared. In the flared portion, the apertural lip is thicker in larger (adult) individuals. This species has a very restricted range that was not extended by the few localities added in this study; all were within the

previously documented range and habitat in Hays and Comal counties.

Phreatodrobia punctata Hershler and Longley, 1986.—*Phreatodrobia punctata* has a rounded, conical shell, with a thick reflected aperture (Fig. 12). This species has similar morphology to the other relatively conical, minute, snail taxa such as *P. conica*, some *P. nugax*, and *M. comalensis*. It can be distinguished from *P. conica* by that species' round aperture and an apertural lip that is not reflected or flared. *Phreatodrobia punctata* can be distinguished from very conical individuals of *P. nugax* (e.g., Hershler and Longley 1986b; Fig. 4, individuals M and Y) by having an apertural lip that is appressed to the body whorl; the lip is usually not fused to the body whorl in *P. nugax*. It can be distinguished from *M. comalensis* by having a flared apertural lip, whereas the lip in *M. comalensis* is not flared or thickened.

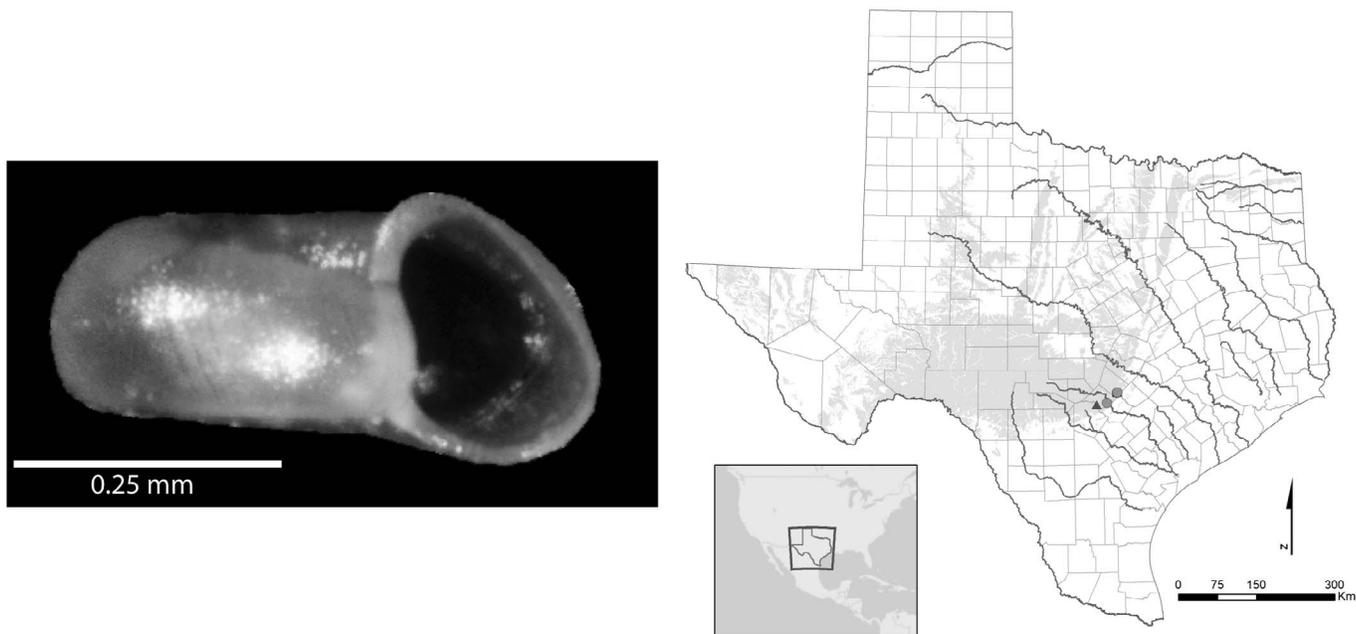
Phreatodrobia plana Hershler and Longley, 1986

Figure 11. *Phreatodrobia plana*, apertural view (0.51 mm width; Comal Springs Run 3, Comal County, Texas). Map of Texas with Edwards-Trinity Aquifer System highlighted in gray. Counties and major river drainages are also illustrated. Literature records are indicated with triangles and new records with circles.

The single individual of *P. cf. punctata* that we encountered was from a hyporheic sample in Sessom Creek, relatively close to the type locality (San Marcos Springs). The individual we encountered (Fig. 12) appears to be slightly juvenile, but it also differs from the species description by having a smoother shell, a pyriform (not rounded) lower apertural lip, and reflection where the lip meets the body whorl. This species' documented range is narrow, with all localities within 50 km of each other.

Phreatodrobia rotunda Hershler and Longley, 1986.—*Phreatodrobia rotunda* (Fig. 13) has a larger-sized (1.83–2.16 mm), planispiral shell. Individuals examined from Comal Springs have a more declined aperture (angled downward) than those from San Marcos Springs figured in the original description (Hershler and Longley 1986b). This species is diagnosed by the shape of the aperture, which is wide rather than elongate along the columellar axis (as found in *P. plana*) and which is always attached to the body whorl. The expansion rate of the whorls is relatively even, but in some individuals, the body whorl a quarter turn before the aperture has a pronounced “pinch” or dent where the whorl is slimmer for a fraction of the whorl. *Phreatodrobia rotunda* can be distinguished from *P. nugax* by the latter species' round aperture and flare on the outer lip only; in *P. rotunda* the aperture is wide and flares on both the inner and outer lip. This species has a narrow range, and we extended it by only 30 km to include Comal Springs and the San Marcos artesian well, near San Marcos Springs.

Stygopyrgus bartonensis Hershler and Longley, 1986.—*Stygopyrgus bartonensis* (Fig. 14) has an elongate shell, ~4× taller than it is wide with strong spiral lines throughout the

shell. *Stygopyrgus bartonensis* is most similar to *Texapyrgus longleyi* but differs by having the following characteristics: a slightly thickened lip that does not flare, more prominent and regular spiral lines and no collabral growth lines, fewer whorls (maximum of 4.6 vs. 5–5.5), sutures that are slightly impressed, whorls that are rounded, and a shell that is cylindrical. *Stygopyrgus bartonensis* was described from a few shells collected near the Barton Springs concession stand spring (Hershler and Longley 1986b). It was declined for listing consideration by USFWS due to insufficient information (USFWS 2009). We add two new localities (Mormon and Treadwell Springs) near the type locality and one from a 150 km distant hyporheic sample on the Llano River, greatly expanding the range occupied by this species.

Texapyrgus longleyi Thompson and Hershler, 1991.—*Texapyrgus longleyi* (Fig. 15) is distinguished from most of the other Texas phreatic species by being much (~4×) taller than it is wide and by usually having striations. The species most similar in shape are *Tryonia diaboli* and *Stygopyrgus bartonensis*. *Tryonia diaboli* is described as smooth-shelled, with rounded, deeply incised whorls and a lip that is barely touching the body whorl at the upper end. *Texapyrgus longleyi* usually has raised spiral and longitudinal lines resulting in a “crosshatched” sculpture pattern. *Stygopyrgus bartonensis* and *Te. longleyi* are similar in adult shell height (~1.3 mm); however, at adult height *Te. longleyi* has 5.5 whorls and *S. bartonensis* has 4.0–4.6 whorls. Both species have spiral striation, but they are weaker on the adapical shell of *S. bartonensis*. *Texapyrgus longleyi* has strong collabral striae that are not present in *S. bartonensis*. Finally, *Te. longleyi* has

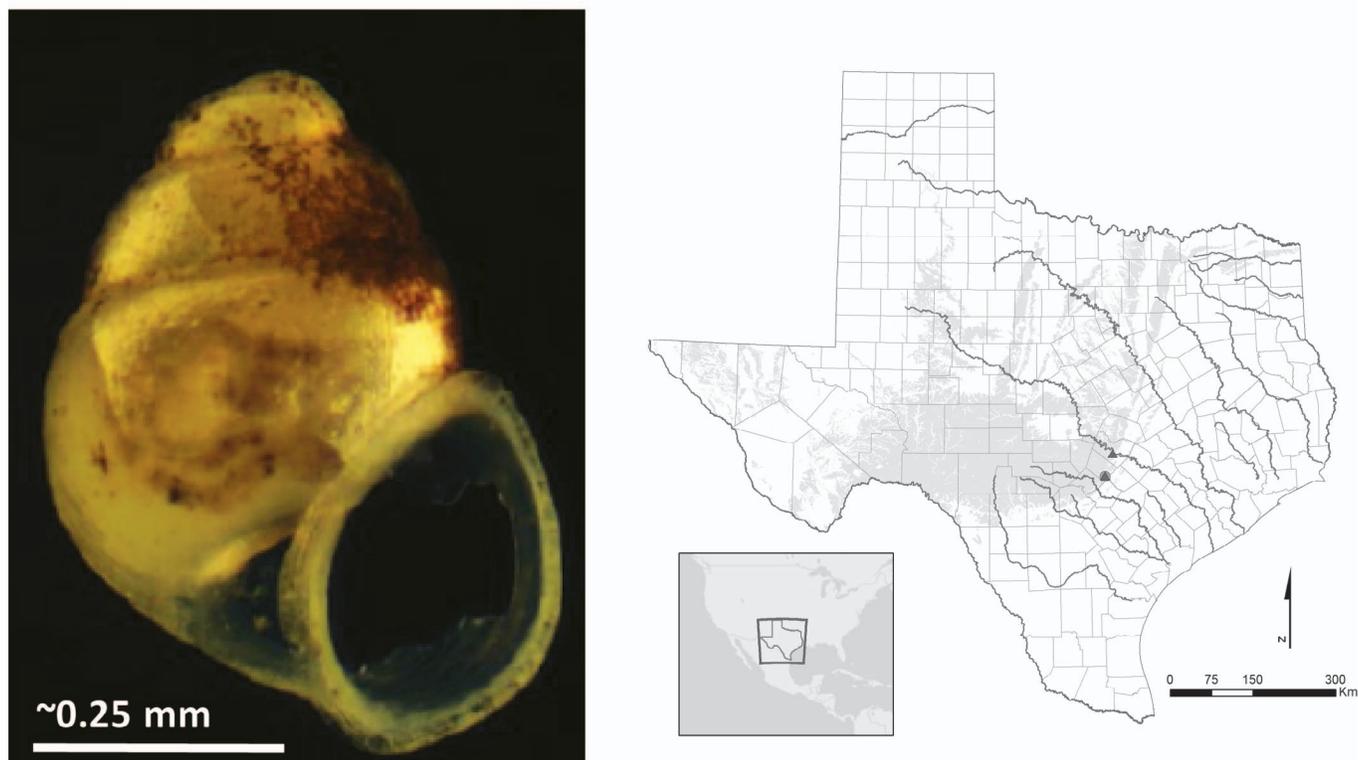
Phreatodrobia punctata Hershler and Longley, 1986

Figure 12. *Phreatodrobia punctata*, apertural view (average length of species 1.1 mm; Sessom Springs, Hays County, Texas). Map of Texas with Edwards-Trinity Aquifer System highlighted in gray. Counties and major river drainages are also illustrated. Literature records are indicated with triangles and new records with circles.

a prominent protoconch, which is less prominent in *S. bartonensis*. *Texapyrgus longleyi* was described from drift net sampling of the spring orifice feeding a small rheocrene just downflow from Slaughter Bend on the Devils River; we expand the known localities to include Dolan and Finegan springs, ~30 km upstream.

Tryonia diaboli Pilsbry and Ferriss, 1906.—*Tryonia diaboli* (Fig. 16) is most similar in overall shape to *Texapyrgus longleyi* and *Stygopyrgus bartonensis*. It is distinguished from *Te. longleyi* by the original description of a completely smooth shell, while *Te. longleyi* is highly sculptured. It is also distinguished from *S. bartonensis* by smooth sculpture, but that species is more columnar in shape while *T. diaboli* is distinctly tapered.

Tryonia diaboli was described from Devils River drift in 1906 (Pilsbry and Ferriss 1906), and we have not been able to find additional references to this species in the published literature, except for a ranking by Johnson et al. (2013). Along with *Te. longleyi* and *P. coronae*, it was encountered during a recent survey of the Devils River (Diaz et al. 2018).

DISCUSSION

The records presented here advance our understanding of the distributions of the phreatic snail fauna of the U.S. portion

of the Edwards-Trinity Aquifer System; however, there are still large regions of the aquifer that are not sampled or only incompletely. Our sampling, and that of prior investigators, has been primarily in the Balcones Fault region where there are concentrations of springs and artesian wells. The Edwards-Trinity Aquifer System extends 300 km north of our sampling, and there are large gaps in records throughout the northern and western portions of the aquifer. Sampling in those areas would likely extend the described species' ranges and reveal undescribed species.

An important consequence of this study was numerous new records for seven snail species from hyporheic samples in surface streams (indicated in Table 1). Previously, these snails had been recorded only from caves, springs and wells, and as river drift of uncertain provenance. Documentation of species from this new habitat has several implications for species conservation. First, surface streams can be an important target for conservation of groundwater species, particularly if hyporheic populations are a distinct ecological phenotype that could uniquely contribute to the species' evolutionary potential. Second, occurrence in the hyporheic zone potentially increases the area and connectivity of habitat that species occupy. Finally, the hyporheic zone is much more accessible and productive for research compared to efforts to study or sample fauna that can be accessed only via deep wells.

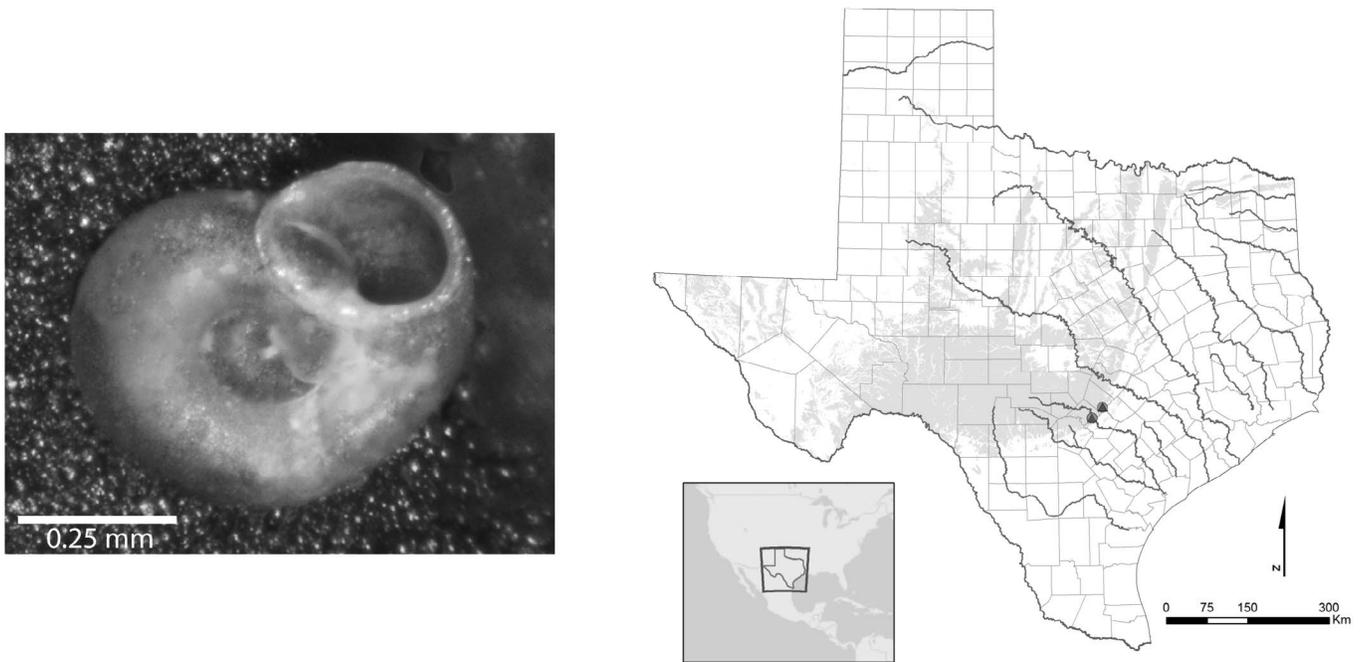
Phreatodrobia rotunda Hershler and Longley, 1986

Figure 13. *Phreatodrobia rotunda*, umbilical view (1.6 mm width; Comal Springs Run 3, Comal County, Texas). Map of Texas with Edwards-Trinity Aquifer System highlighted in gray. Counties and major river drainages are also illustrated. Literature records are indicated with triangles and new records with circles.

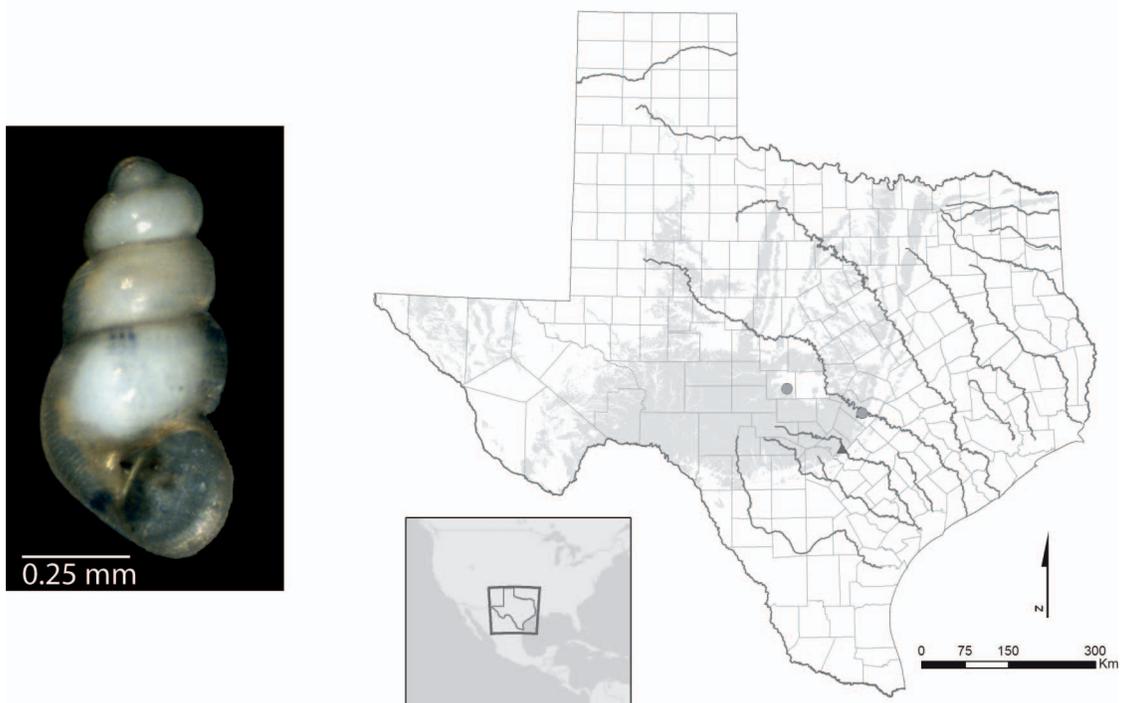
Stygopyrgus bartonensis Hershler and Longley, 1986

Figure 14. *Stygopyrgus bartonensis*, apertural view (1.08 mm length; Mormon Spring no. 3, Travis County, Texas). Map of Texas with Edwards-Trinity Aquifer System highlighted in gray. Counties and major river drainages are also illustrated. Literature records are indicated with triangles and new records with circles.

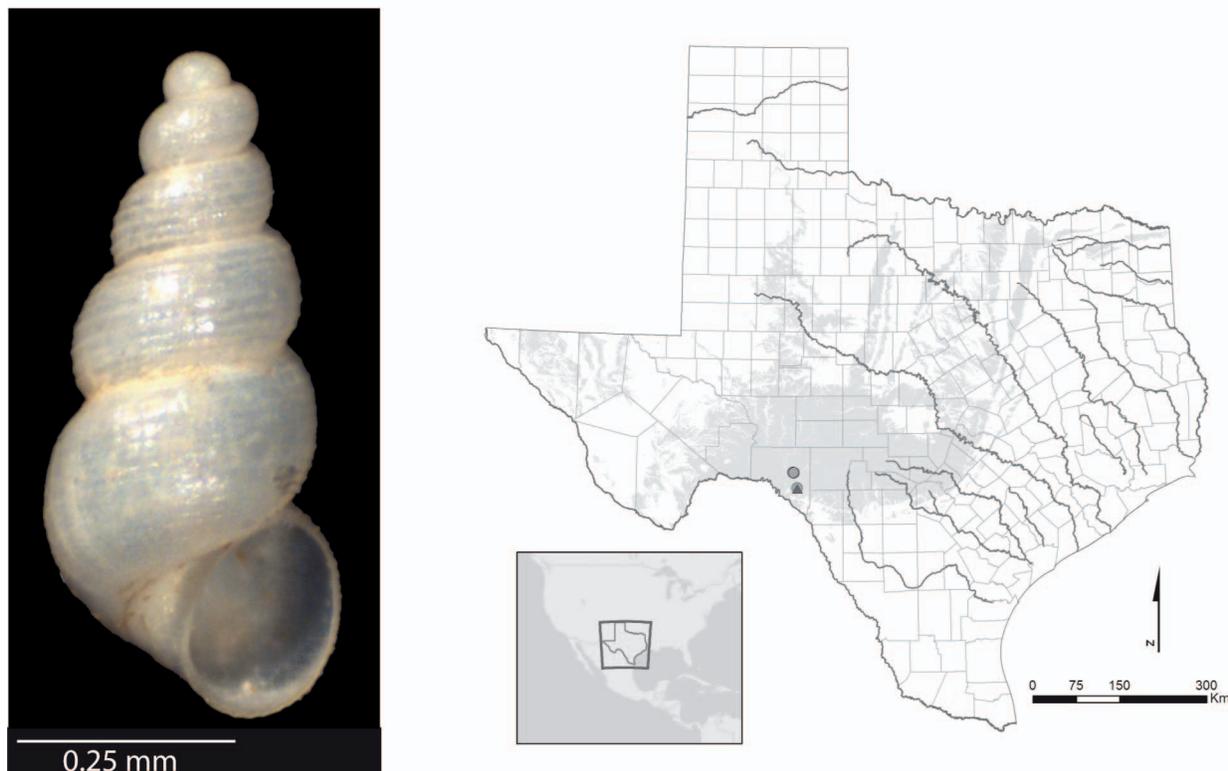
Texapyrgus longleyi Thompson and Hershler, 1991

Figure 15. *Texapyrgus longleyi*, apertural view (1.31 mm length; Finegan Springs, Devils River, Val Verde County, Texas). Map of Texas with Edwards-Trinity Aquifer System highlighted in gray. Counties and major river drainages are also illustrated. Literature records are indicated with triangles and new records with circles.

The hyporheic zone of rivers provides much larger cross sections of landscapes than is possible to sample with wells. However, it is worth considering that portions of deep aquifer habitats that are hydrologically isolated from surface recharge points might be less susceptible to environmental variability and point-source pollution if they are outside the recharge zone. Indeed, Hutchins et al. (2016) suggested that deep-aquifer habitats with foodwebs supported by *in-situ* primary production may serve as refugia for groundwater species during periods of increased aridification. Unfortunately, across much of Texas, hydraulic connectivity between the surface and subsurface is not well understood, and even confined aquifers are susceptible to contamination (Hutchins 2018).

Although it is possible for widespread species to be threatened, the phreatic snails of the greatest immediate conservation concern are those with narrow ranges and low abundances that occur in regions with great potential for modification and withdrawal of groundwater. *Phreatodrobia plana* and *P. rotunda* have very narrow ranges, but they are relatively frequent in samples from Comal and San Marcos Springs. Both spring systems have multiple federally protected endangered species and a local water regulatory framework in place for protection of the springs. Therefore, snails in these systems have a higher level of protection than some of the

other phreatic snail species. For example, *Tryonia diaboli*, *Texapyrgus longleyi*, and *Phreatodrobia coronae* are restricted to the Devils River region, described by Hutchins (2018) as having high vulnerability due to the lack of a regulatory framework for groundwater conservation and water extraction for oil and gas as well as human consumption. The species that are restricted to that region may face a much higher risk of extinction. In contrast to *P. plana* and *P. rotunda*, *P. punctata* is reported only from San Marcos and Barton Springs. However, we found only a single shell of this species in a sample from Sessom Creek. A similar situation occurs for *P. imitata*, reported from the Verstraeten well (Bexar County). We encountered individuals that loosely resemble this species only in a Bell County spring. Resampling Verstraeten and nearby wells and hyporheic sites is needed to confirm whether this species still occurs at historic sites, as well as to undertake taxonomic work to determine if the Bell county population is *P. imitata*. Finally, with these samples, we report the rediscovery of *Stygopyrgus bartonensis*, previously known from only a few shells from one heavily human-impacted site. This species appears to persist in Austin-area springs and hyporheic zones, and additional hyporheic sampling in the region could greatly expand its known range.

Groundwater fauna typically have narrow ranges, often

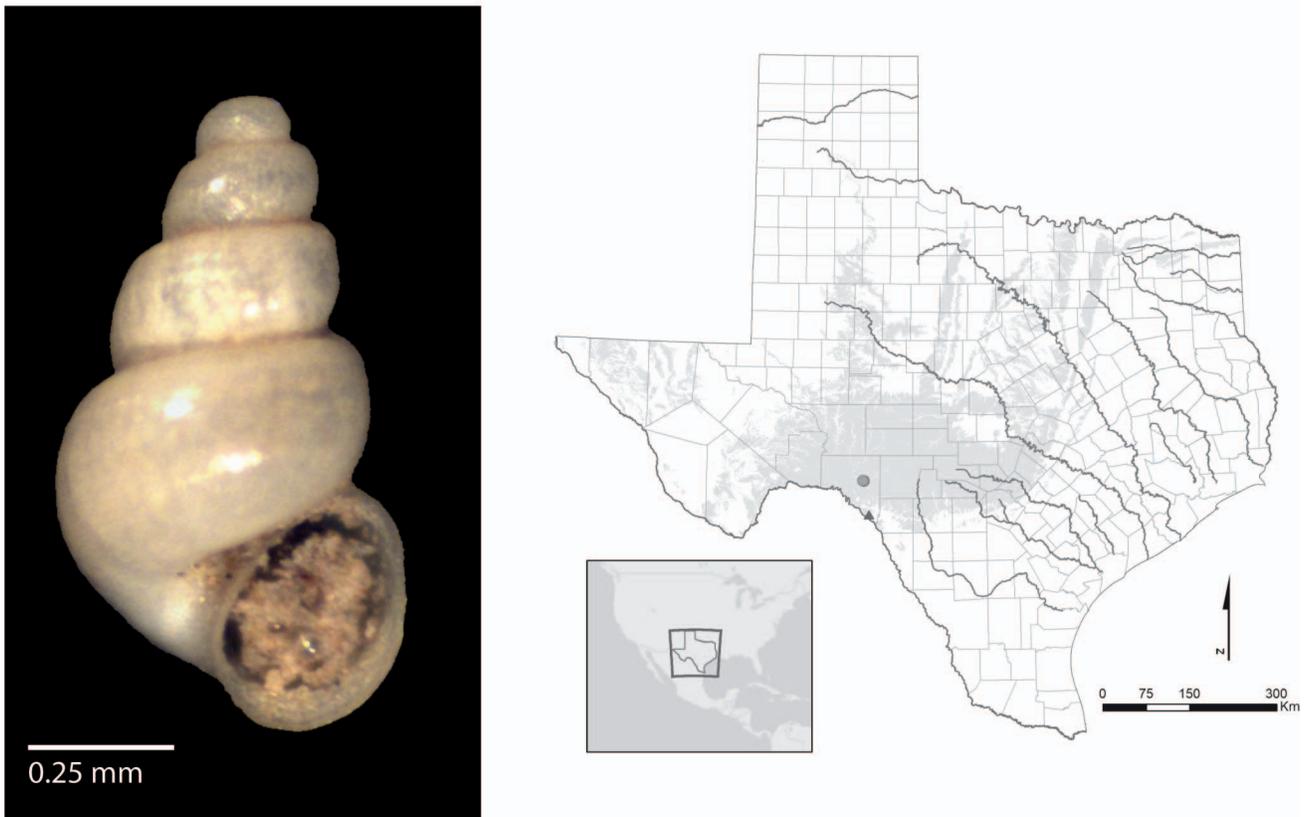
Tryonia diaboli Pilsbry and Ferriss 1906

Figure 16. *Tryonia diaboli*, apertural view (1.09 mm length; Dolan Springs, Val Verde County, Texas). Map of Texas with Edwards-Trinity Aquifer System highlighted in gray. Counties and major river drainages are also illustrated. Literature records are indicated with triangles and new records with circles.

known from one or a few sites (Falniowski et al. 2008), and it is expected that different groundwater units or catchments form hydrological barriers to gene flow (Barr and Holsinger 1985). A review of genetic connectivity among stygobitic animals found that 94% of the species examined had ranges <200 km in length (Trontel et al. 2009). Regional groundwater flow paths, the relative permeability and connectedness of karst systems, and whether there are stratigraphic and/or structural barriers separating groundwater units could all affect the frequency of gene flow (Barr and Holsinger 1985). Species that occupy both the hyporheic zone and connected aquifer habitats would be expected to have more opportunities for gene flow, thus resulting in potentially wider species ranges with sufficient mixing to preserve a more-or-less connected gene pool (Ward and Palmer 1994; Finston and Johnson 2004). In subterranean amphipods, for example, unique sets of species were found to occur in distinct catchment areas and show significant genetic differentiation over short geographical distances (Finston and Johnson 2004), and similar patterns have been observed in numerous groundwater-dependent gastropods (Perez et al. 2005; Trontel et al. 2009; but see Richling et al. 2017). In this study, assigning new collections of phreatic snails to species based on shell morphological characters, we find that some taxa (particularly *Phreatodrobia*

nugax, *P. micra*, and *Ph. taylori*) have surprisingly extensive ranges compared to our expectations for phreatic taxa. Based on findings in other groups (Stoch 1995; Trontel et al. 2009), and the observed morphological variability of these species, it is likely that these taxa include multiple cryptic lineages.

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REGULAR ARTICLE

MESOHABITAT ASSOCIATIONS OF THE DEVIL TRYONIA, *TRYONIA DIABOLI* (GASTROPODA: TRUNCATELLOIDEA: COCHLIOPIDAE)

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ABSTRACT

The Cochliopidae of Texas include both stygobitic species, those that occupy only underground habitats, and epigeal species, those living only in aboveground habitats. The devil tryonia, *Tryonia diaboli*, was described from the Devils River of Texas from river wrack, without additional habitat information. This species has been largely ignored since its description, so details of its habitat and ecology are obscure. In Dolan Springs and Finegan Springs, flowing into the Devils River, we sampled macroinvertebrates at five sites that form a gradient from the aquifer to the mainstem Devils River. We found the highest abundances of *T. diaboli* in aquifer samples, decreasing sharply downstream from the spring orifice. Our findings indicate that *T. diaboli* is stygophilic, occupying a transitional area including the aquifer as well as aboveground portions of springs.

KEY WORDS: stygophilic, spring snail, Devils River, aquifer, conservation

INTRODUCTION

Texas has a largely endemic groundwater- and aquifer-dependent snail fauna (Hershler and Thompson 1992; Hutchins 2018) that are of conservation concern (Johnson et al. 2013). Many species occupy different mesohabitats within the aquatic ecosystems and consequently have varied habitat requirements. They also may vary in their susceptibility to changes in spring flow, such as decreases or cessation of flow. Animals that occupy only groundwater, cave, and aquifer systems are referred to as stygobionts. Stygobitic gastropods typically have morphological adaptations to underground habitats, such as reduced size or pale-colored shells and depigmented bodies (Hershler and Liu 2017). In contrast, stygophiles such as *Fontigens nickliniana* (Hershler et al. 1990) and *Cochliopina riograndensis* may occupy both surface (epigeal) and underground habitats and may vary in these morphological features. A recent review on the origins of stygobitic gastropods (Osikowski et al. 2017) summarized many independent and ongoing invasions of subterranean

aquatic habitats; however, gastropods occupying both above- and belowground aquatic habitats are uncommon, although this may reflect sampling methods rather than the actual habitat occupancy of the organisms.

The stygobitic snail fauna of Texas includes ~12 extant species (Johnson et al. 2013; Hutchins 2018), all now considered members of the Cochliopidae (Clark 2019): *Balconorbis uvaldensis*, *Phreatoceras taylori*, eight species of *Phreatodrobia*, *Stygopyrgus bartonensis*, and *Texapyrgus longleyi* (Thompson and Hershler 1991). No members of *Tryonia* are reportedly stygobites. *Tryonia* includes 31 extant species that are broadly distributed across the southwestern United States and Mexico, one species in Florida, one species in Guatemala, and five species endemic to Texas springs. Most *Tryonia* are found in thermal, mineralized springs although a few are found in lakes and one in hypersaline, coastal waters (Hershler 2001). The *Tryonia* of Texas are found in mineralized, but not thermal or hot, springs (Hershler 2001). However, the generic placement of *Tryonia diaboli* has not been reevaluated since it was described in 1906 and former members of the genus *Tryonia* have been removed to

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Figure 1. Image of *Tryonia diaboli* from Finegan Spring, Devils River State Natural Area, Val Verde County, Texas, August 2009. Collected by Randy Gibson. Individual is 1.09 mm in length.

Ipnobius, *Pseudotryonia*, and *Juturnia* (Liu et al. 2001; Hershler et al. 2011b). *Tryonia diaboli* was not included or considered in the most recent systematic revision of the *Tryonia* (Hershler 2001) so its generic placement is uncertain.

The focus of this study is *T. diaboli*, a smooth-shelled species by original description (Pilsbry and Ferris 1906); however, there are several similar gastropods described from the region, differing in shell sculpture. *Texapyrgus longleyi* (Thompson and Hershler 1991) was described as a fully stygobitic species with the animal blind and unpigmented. This species was characterized by having strong spiral lines on the shell and was described from an unnamed spring on the Devils River, just downstream from Slaughter Bend, ~32 km N of Del Rio. Snails were collected using a net across the spring outflow and were not collected on the surface, although the surface rheocrene was sampled (Thompson and Hershler

1991). Another similar and nearby species with strong spiral shell lines is *Tryonia circumstriata* (Leonard and Ho 1960), described from late Pleistocene fossil deposits in the Pecos River, 0.25 mi N of Independence Creek and later discovered living in the lower Diamond Y spring and draw (Pecos River drainage) (Taylor 1987). *Tryonia circumstriata* is distinguished by distinctive shell sculpture of raised spiral threads; however, this trait is variable with some individuals having no spiral sculpture present (Taylor 1987; Hershler and Thompson 1992). This species was documented as epigeal, living on soft mud, in a spring run, and it was described as having a pigmented body and head. In this paper, we do not conduct a taxonomic study, but rather describe the habitat associations of individuals that conform to the original description of *T. diaboli*. Therefore, we included in this study only shells that are minute, smooth in sculpture (no spiral lines), with rounded, deeply incised whorls, and with a continuous lip that is barely adnate (touching) with the body whorl at the upper end (Fig. 1). All *T. diaboli* collections were made from the same watershed as the original description (Pilsbry and Ferris 1906). Sampling sites were about 26 km by air or 37 km by river upstream from the type locality of *Te. longleyi* and 220 km SW of the known localities for *T. circumstriata* in the Pecos River drainage.

Except for inclusion on taxon lists (e.g., Burch 1982; Johnson et al. 2013), *T. diaboli* has been ignored since its description, so the details of its habitat are obscure. It was described from “drift débris of the Devil’s River, about four miles from its mouth,” Val Verde County, Texas, providing no aquatic habitat information. A second locality “on the Rio San Felipe near Del Rio same county” also was mentioned (Pilsbry and Ferris 1906). The shells were described as dead and bleached, from river drift (meaning river wrack, not in drift nets), leaving the mesohabitat associations of the snail unknown.

Tryonia diaboli has a NatureServe conservation status rank of G1 indicating it is globally imperiled (Johnson et al. 2013), but it does not have U.S. federal protection and lacks all pertinent information to support consideration for listing. A recent review of the conservation status of groundwater-dependent Texas invertebrates concluded that spring sites along the Devils River did not have good viability (Hutchins 2018) primarily due to regional groundwater extraction for proposed municipal (Diaz et al. 2018) and industrial purposes (Industrial Economics Incorporated 2008), although elevated nutrient concentrations are also a concern (Moring 2012). In addition, declining flows in nearby springs have led to the extinction (Hershler et al. 2014) of one cochliopid snail, *Juturnia brunei* (Taylor 1987), and the recent federal listing of gastropods at those sites (U.S. Fish and Wildlife Service 2013). Another nearby species, *Tryonia oasiensis*, may be extinct (Hershler et al. 2011a). Spring sites along a 5-kilometer stretch of the lower Devils River and San Felipe Springs in Del Rio are the entirety of the known range of the devil tryonia. In this study, we present localized occurrence records and describe mesohabitat associations for *T. diaboli*.

METHODS

Sampling was conducted in the Devils River, Dolan Creek, and along Finegan and Dolan spring complexes (Fig. 2); the latter are permanent springs flowing into the Devils River in Val Verde County, Texas. Vouchers from Dolan aquifer samples are deposited in the Smithsonian Institution, USNM 1571310. To examine the habitat occupied by *T. diaboli* we sampled across five hydrologically connected ground and surface water zones: aquifer (water flow emerging from spring orifice), spring orifice substrate (substrate up to 1 m directly below the spring orifice), transition zone (the part of the spring run below the spring orifice and traveling toward a river or creek), Dolan Creek, and Devils River. Each one of these zones was considered a mesohabitat, or biologically and physicochemically distinct habitat (Pardo and Armitage 1997), within the overall ecosystem.

Each mesohabitat was sampled using the most appropriate method to accurately quantify its invertebrate community. Compared to epigeal habitats, an aquifer provides restricted access to the available habitats within the system. Therefore, sampling for aquifer species requires passive collection of animals over a time interval. The animals captured using this method are living at some unknown depth of the aquifer and have been dislodged or otherwise caught in the water flow exiting the aquifer. Epigeal substrates are more easily accessible and require “snapshot” (not time-interval) methods using a frame and net (Surber and Hess samplers [Bioquip Products, Inc., Rancho Dominguez, CA, USA]) to capture benthic aquatic invertebrates living in and on the substrate.

We sampled the aquifer community (stygo-bites) using 250- μm mesh drift nets installed over the water flow of an undisturbed spring orifice for about 3 d in August, October, and November 2016. Locations for drift netting were selected randomly. All spring sites were mapped using a Trimble Nomad and a Pro XT receiver (Trimble Navigation Limited, Corvallis, OR, USA). Benthic aquatic invertebrate samples were taken using Surber or Hess samplers, as appropriate depending on water depth. Spring orifice substrate and transition zone samples were taken using a Surber sampler with 500- μm mesh and 0.092- m^2 area in February, April, May, October, and November 2016. Spring orifice substrate and transition zone samples were collected at nearby spring openings that were different than the aquifer samples and were distributed along the length of each spring complex. These springs were nearby but not identical due to disturbance from other collection activity conducted at the same time as this sampling. Spring orifice substrate samples were collected directly below the orifice and up to 1 m downstream. For sampling, large cobbles were cleaned inside the sampler and removed from inside the sampled area. Then, for 45 s, the substrate was disturbed to dislodge invertebrates into the net. Finally, due to their increased depth, creek and river samples (Dolan Creek, Upper and Lower Devils River) were taken repeatedly from within the same riffle using Hess samplers with 500- μm mesh in February, April, July, and November 2016. Creek samples were collected starting in the down-

stream section of a riffle or shallow run and were taken from cobble and gravel substrates.

For each creek sample, basic water chemistry was collected using a Hydrotech compact DS5 (Hydrotech ZS Consulting, Round Rock, TX, USA). Flow (FH950; Hach, Loveland, CO, USA) and depth were also recorded at each creek sample. All samples were collected and placed into 95% isopropyl alcohol and sorted under microscopes. We counted or measured only shells with tissue. To compare shell sizes, sets of *T. diaboli* from aquifer ($n = 34$) and creek ($n = 30$) samples were photographed and measured. The shell measurements taken were maximum length along the columellar axis and maximum width at right angles to the columellar axis. The shell measurements were compared using a *t*-test in JMP Pro 13.0.0 (SAS Institute Inc., Cary, NC, USA).

To determine mesohabitat associations of *T. diaboli* and other common community taxa, the mesohabitats were coded 1–5 (1 = aquifer sample, 2 = spring orifice substrate sample, and continuing downstream) and an indicator analysis (Dufrene and Legendre 1997) was conducted in R with the package “labdsv” (Roberts 2013).

RESULTS

Over 72,000 invertebrates, including 640 *T. diaboli*, were collected from 132 samples from all mesohabitats (Table 1). The contribution of aquatic invertebrates from each mesohabitat is as follows: aquifer = 13,175; spring orifice substrate = 6,734; transition zone = 11,678; Dolan Creek = 23,237; and Devils River = 16,787. *Tryonia diaboli* were most abundant in aquifer samples, with an average of 9.35 individuals per sample, comprising 2.62% of macroinvertebrates collected (Table 1). Spring orifice substrate and transition samples had lower abundances, averaging 4.22 and 4.26 individuals per sample, respectively. The lowest abundances, at 3.75 individuals per sample or 0.14 per site, were found in the Devils River sample. Creek samples from Finegan Springs resulted only in three individuals and were not analyzed or further presented here. Indicator analysis found *T. diaboli* to be significantly ($P < 0.05$) associated with the aquifer samples (Table 2). The aquifer and creek sites where *T. diaboli* were collected had similar water chemistry values (Table 3).

Aquifer community sampling occurred in late summer through fall (August, October, and November) of 2016 and creek samples were taken in late winter through early summer (February, April, and July) of that year. The average count of *T. diaboli* from both creek and aquifer for Dolan Springs across all sampling periods is shown in Table 4. *Tryonia diaboli* shells from Dolan Springs creek samples taken in winter to early summer were significantly smaller in both length ($P = 0.01$) and width ($P = 0.0034$) than those from the aquifer taken in late summer to fall. In addition, the aquifer (late summer to fall) shells included a larger range of sizes, up to 2.04 mm total length, compared to the largest creek (winter to early summer) snail, measuring 1.34 mm. The average length of individuals sampled from the aquifer (late summer to

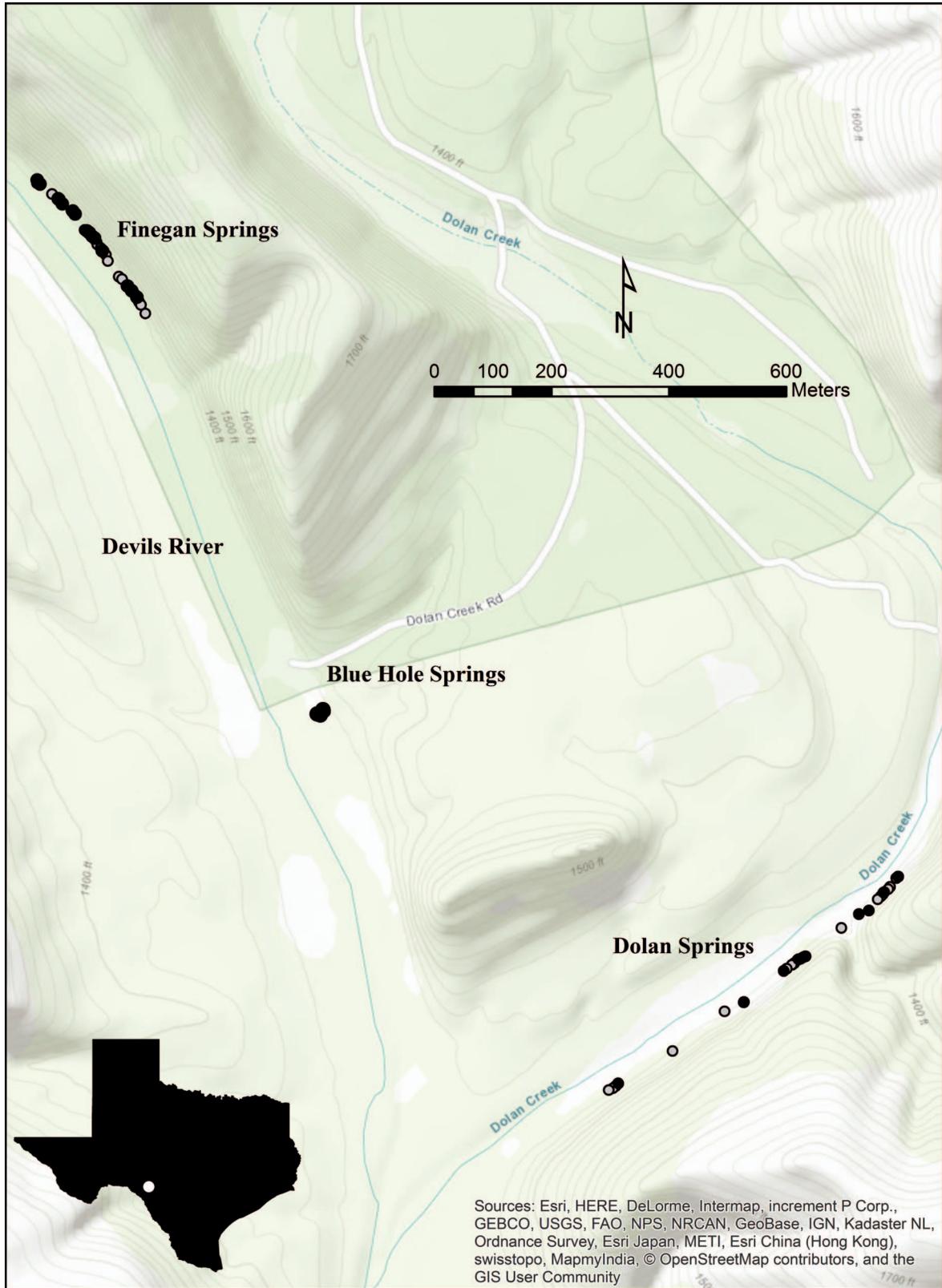


Figure 2. Map of sites. Sites sampled are in black and all other spring outlets are presented in gray.

Table 1. Total number of *Tryonia diabolii* from 132 benthic aquatic invertebrate samples collected in 2016 from the Devils River and Dolan Creek along with their associated spring complexes. Each column represents a specific ecosystem component sampled to examine mesohabitat associations.

	Spring Orifice				
	Aquifer	Substrate	Transitional	Dolan Creek	Devils River
No. of individuals	346	135	81	75	3
% of individuals collected	54.06	21.09	12.66	11.72	0.47
% of invertebrate samples	2.62	2.04	0.69	0.32	0.03
Sample size	37	32	19	20	21

fall) was 1.25 mm (SEM = 0.06 mm) and the length of individuals from creek samples (winter through fall) was 1.09 mm (SEM = 0.02 mm). Average widths from creek and aquifer samples were 0.675 (SEM = 0.026 mm), and 0.588 mm (SEM = 0.0085 mm), respectively. There were no observed differences in animal or shell pigmentation among the samples collected.

DISCUSSION

In this study, macroinvertebrate samples were taken from five mesohabitats on a gradient from aquifer to creek, with the unexpected result that *T. diabolii* was most common (54% of

Table 2. Mesohabitat associations of aquatic invertebrates from indicator species analysis of different ecosystem components. Taxa are ordered by each ecosystem component, from aquifer samples toward downstream, then alphabetically within ecosystem component category.

Taxa	Zone	Indicator	P value
<i>Lirceolus</i> sp.	Aquifer	0.921	0.001
<i>Psephenus</i> sp.	Aquifer	0.259	0.067
<i>Tryonia diabolii</i>	Aquifer	0.363	0.005
<i>Heterelmis</i> cf. <i>glabra</i>	Spring orifice substrate	0.357	0.005
<i>Heliocopsyche</i> sp.	Transition	0.710	0.001
<i>Hyaella</i> sp.	Transition	0.609	0.001
<i>Microcyloepus pusillus</i>	Transition	0.573	0.002
<i>Phanocerus clavicornis</i>	Transition	0.345	0.034
<i>Allenhyphes vesus</i>	Dolan	0.657	0.001
Chironomini	Dolan	0.650	0.001
<i>Cochliopina riograndensis</i>	Dolan	0.471	0.001
<i>Elimia</i> sp.	Dolan	0.496	0.001
<i>Melanoides tuberculata</i>	Dolan	0.343	0.004
<i>Metrichia</i> sp.	Dolan	0.644	0.001
Orthoclaudiinae	Dolan	0.534	0.002
Simulium	Dolan	0.547	0.001
Tanytarsini	Dolan	0.491	0.001
Turbellaria	Dolan	0.383	0.004
<i>Chimarra</i> sp.	Devils	0.494	0.001
<i>Corbicula</i> sp.	Devils	0.734	0.001
<i>Fallceon quilleri</i>	Devils	0.487	0.001
<i>Hexacyloepus ferrugineus</i>	Devils	0.800	0.001
<i>Macrelmis</i> sp.	Devils	0.555	0.001
<i>Thraulodes gonzalezi</i>	Devils	0.635	0.001

individuals) in samples taken using drift nets to collect invertebrates exiting the aquifer. Mesohabitats farther from the aquifer and spring source had declining abundances of *T. diabolii*. This finding is supported by both the count and indicator analyses. Indicator analyses determined that the typical stygobitic species *Lirceolus* sp. (water slater) and the spring species *Heterelmis* cf. *glabra* (riffle beetle) also were significantly associated with the aquifer or spring orifice substrate samples. This is a surprising finding as no other *Tryonia* species has been documented occupying stygobitic habitats.

In general, other *Tryonia* species are most abundant at spring heads (Brown et al. 2008), with some species found in the spring run or creek up to about 1 km downstream. In this study, we found *T. diabolii* acting as a stygophile, occupying both stygobitic (aquifer) and near-spring sites, declining in abundance in the creeks, and at very low densities in the main channel. This could be due to a physiological requirement for water characteristics associated with proximity to the aquifer, such as thermal stability or oxygen concentration, or to biotic characteristics such as a preferred food source. It is possible that the decline in abundance that we observe downstream is related to our sampling method, with the cumulative collection of animals over 3 d in aquifer samples being compared to “snapshot” sampling methods in the downstream samples. However, we think the general trend of decreasing abundance downstream is broadly accurate for two reasons. First, the abundance of individuals sampled from the aquifer is two times higher per sample, representing 54% of the total individuals collected across all five mesohabitats. This finding seems unlikely to be entirely due to sampling method. Second, the trend of reduced abundance downstream is also seen across the “snapshot” sampling methods, with reduced abundance in creek and river samples compared to spring orifice substrate and transition zones.

Tryonia typically live in highly mineralized, sometimes thermal, (Hershler and Sada 1987; Hershler 1999; Liu et al. 2001) and even hypersaline environments (Kitting 2015), usually on mud or gravel substrate as well as on vegetation and detritus (Hershler et al. 2011a). If water flow is retained, some species (e.g., *T. chuviscarae*) are able to persist in highly human-impacted springs and streams (Hershler and Sada 1987; Hershler et al. 2011a; Kitting 2015), although at lower abundances (Hershler et al. 2011a). In the epigeal sites sampled in this study, we found *T. diabolii* occupying sites that

Table 3. Average and standard deviation (in parentheses) of water physicochemical values for Dolan Springs ($n = 7$ sites, 3 sampling periods) and Finegan Springs ($n = 7$, 3 sampling periods) aquifer creek sites ($n = 6$, 4 sampling periods) where *Tryonia diabolii* was collected.

		Temp. (°C)	Conductivity (mS/cm)	DO (mg/L)	pH	TDS (g/L)	Flow (m/s)
Dolan Springs	Aquifer	22.619 (0.10)	458.460 (3.27)	7.528 (0.43)	7.347 (0.12)	0.291 (0.00)	0.82 (0.29)
	Creek	23.200 (2.97)	444.500 (18.53)	8.120 (1.16)	7.880 (0.07)	0.281 (0.01)	0.55 (0.28)
Finegan Springs	Aquifer	22.472 (0.07)	475.207 (3.93)	7.604 (0.11)	7.307 (0.08)	0.304 (0.00)	1.00 (0.34)

DO = dissolved oxygen; TDS = total dissolved solids.

were characterized as having the following substrates: cobble, bedrock, gravel, rubble, and organic debris. The spring sites occupied by *T. diabolii* were more moderate in temperature, ~22–23°C. Altogether, *T. diabolii* is unusual among the *Tryonia* as it occupies habitats that are nonthermal, not heavily mineralized, and both stygobitic and epigeal.

Snail population abundance could vary seasonally for a variety of reasons, for instance, due to seasonal patterns of reproduction or to water level fluctuations. While there is not comparative data for other *Tryonia* species, hydrobioids are generally annual with seasonal recruitment in cold springs (Brown et al. 2008). In *Juturnia kosteri*, there is a three- to four-fold increase in snail density in summer, potentially due to recruitment, decline in water level, or both (Johnson et al. 2019). Aquifer sampling in this study was conducted in summer and fall, with the highest average abundance across sites of 11.9 in August and a drop to 2.2 in November. Creek samples farther downstream and earlier in the year (January through July) found lower abundances, with the highest abundance of 5.5 in April. This finding suggests an increase in abundance in spring and summer and a decline in winter, which aligns with the typical expectation for hydrobioid gastropods. These data on *T. diabolii* are not conclusive, in part because our sampling is not complete across seasons. In addition, while the methods used are the best available to accurately sample these mesohabitats, comparisons between findings using a “snapshot” sampling method and those using a time-interval sampling method must be made with caution.

In the absence of a modern taxonomic reappraisal of *T. diabolii*, *Te. longleyi*, and *T. circumstriata*, we focused this description of mesohabitat associations on individuals conforming to the original description of *T. diabolii*. It is possible that *T. diabolii* and *Te. longleyi* and/or *T. circumstriata* are valid species which are similar in size and shell morphology and which occur in the same watershed or region. Indeed, spring snails tend to be extreme narrow-range endemics. On the other hand, if any of these species were synonymized with

T. diabolii (the name with taxonomic priority), the mesohabitat associations described would still apply and the main finding of this study would still be novel, as neither *Te. longleyi* nor *T. circumstriata* is reportedly stygophilic.

Tryonia diabolii was ranked as imperiled by the most recent evaluation of conservation status of North American freshwater snails (Johnson et al. 2013), based on the limited population extent, as the species was documented only from a small stretch of the Devils River and nearby San Felipe Springs (Pilsbry and Ferris 1906). Except for the current study, there has been a complete lack of research on this organism. The presence of *T. diabolii* in both the aquifer and spring run indicates it could occupy a tremendously greater habitat area than previously understood. Over half the individuals encountered during this sampling came from the aquifer (drift net) samples, eluding to higher numbers underground than at the surface. However, the full extent of the habitat of this species is unclear as we have not determined if this population occupies only the shallower hyporheic zone rather than deeper in the phreatic zone of the aquifer. Future work would need to determine if the species is present in well samples in addition to those underground habitats closely associated with springs.

The question of the potential habitat this species can occupy leads to an important corollary for species conservation; if this species occupies significant belowground habitat, it is possible that it could survive deeper in the aquifer during cessation of flows. Two other closely related, fully epigeal species, *Juturnia brunei* and reportedly *T. oasiensis* (Hershler et al. 2011a), have recently gone extinct due to cessation of spring flow (Hershler et al. 2014). *Juturnia brunei* had persisted in the Phantom Lake spring system through extensive human modification of the spring and creek channels for irrigation; however, it appears to have gone extinct when local groundwater levels dropped sufficiently such that the spring where it resided went completely dry (Hershler et al. 2014). This study is a small first step in understanding the

Table 4. Average and standard deviation (in parentheses) of *Tryonia diabolii* individuals collected across sampling period (2016) in Dolan (DS) and Finegan Springs (FS) in aquifer and/or creek samples. Values for n are as follows for aquifer samples (not individuals): August: DS and FS, $n = 7$; October: DS, $n = 7$, FS, $n = 6$; November: DS, $n = 3$, FS, $n = 2$. Values for n are as follows for creek samples (not individuals) at DS: February, $n = 6$; April, $n = 6$; July, $n = 6$.

		February	April	July	August	October	November
Dolan Springs	Aquifer	—	—	—	10.43 (12.53)	8.00 (13.22)	0.67 (0.58)
	Creek	4.17 (6.21)	5.50 (8.48)	2.33 (5.72)	—	—	0.50 (0.55)
Finegan Springs	Aquifer	—	—	—	13.29 (18.74)	5.17 (4.26)	4.5 (2.12)

ecology of *T. diaboli*, a task with some urgency due to local extinctions of related species in similar habitat.

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REGULAR ARTICLE

PROVENANCE AND DISPOSITION OF SPECIMENS APPEARING IN COLOR PLATES OF *THE FRESHWATER MUSSELS OF TENNESSEE*

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ABSTRACT

A total of 260 specimens of native freshwater mussels and numerous specimens of three introduced bivalves were photographed and published in *The Freshwater Mussels of Tennessee*, but the provenance and disposition for these specimens were not included. All but nine of the 260 bookplate freshwater mussel specimens and all of the introduced bivalve specimens used in the figures were recently found. Information on length, catalogue number, collection locality, date of collection, and collector(s) is provided herein.

KEY WORDS: freshwater mussels, Tennessee, provenance, disposition

INTRODUCTION

In 1998, Paul W. Parmalee and Arthur E. Bogan published *The Freshwater Mussels of Tennessee*, which features individual species accounts for 129 native species, state and USA distribution maps, and color photographs of individual specimens featuring the interior and exterior of the shell. It also presents individual chapters on various topics, including structure, development and growth, taxonomy, classification, ecology, and aboriginal exploitation. For each native species treated in *The Freshwater Mussels of Tennessee*, two representative specimens are depicted to show subtle variations in shell characteristics and sexual dimorphism (where it exists). Three nonnative species, *Corbicula fluminea* (Müller, 1774), *Dreissena polymorpha* (Pallas, 1771), and *Mytilopsis leucophaeata* (Conrad, 1831), also are depicted; these show two to nearly one dozen specimens. Unfortunately, provenance (location where the specimen originated) and disposition (museum and catalogue number) of figured specimens are not provided. Subsequent to 1998, several books and reports added significantly to the body of knowledge on freshwater mussels in the USA. These include the mussel faunas of Alabama (Williams et al. 2008), Ohio (Watters et al. 2009),

and Florida (Williams et al. 2014). Each monograph presents two or more color photographs for all species and provides provenance and disposition for the figured specimens.

During a recent reorganization of the mollusk collection at the University of Tennessee McClung Museum of Natural History and Culture (UTMM), most of the book plate specimens used in *The Freshwater Mussels of Tennessee* were found. Fortunately, nearly all of the figured specimens bear a small inscription on one valve indicating the book plate in which it appeared; otherwise it would have been difficult to discern which individuals were used as figured specimens, at least for some of the more common species from which there are several thousand available specimens. Although the UTMM collection contains all species treated in *The Freshwater Mussels of Tennessee*, specimens were borrowed from other institutions for photographic purposes or to gain a clearer understanding of various shell characteristics presented in the text. Institutions from which shell material was borrowed in preparation for *The Freshwater Mussels of Tennessee* include the Illinois Natural History Survey (INHS), Ohio State University Museum of Biological Diversity (OSUM), University of Michigan Museum of Zoology (UMMZ), Carnegie Museum of Natural History (CM), and the Academy of Natural Sciences of Drexel University

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(ANSP). Several of these borrowed specimens appear as book plate specimens in *The Freshwater Mussels of Tennessee*.

Properly referenced voucher material and voucher collections are valuable to researchers and natural resource managers. Although reference material can be used in the field to compare with live specimens and assist with identification, it often is not possible or convenient to have reference shells on hand during fieldwork. In these circumstances, a book with high-quality color plates can be particularly useful. West Virginia and Ohio now require each applicant for a state collecting permit to pass an identification test (Clayton et al. 2018; Ohio Department of Natural Resources and U.S. Fish and Wildlife Service 2018), and several other states are considering a similar requirement. In Ohio, the test is administered “open book” and the applicant is allowed to bring any outside source to use while taking the test, including reference books (J. Navarro, Ohio Department of Natural Resources, personal communication). Identifying live mussels can be challenging and shell characteristics for many of our native species vary considerably across their ranges or even within the same drainage (i.e., headwaters to big river), a concept described by Ortmann (1920). Many of the native species of the USA are protected by the U.S. Endangered Species Act and dead shells of federally protected species cannot be retained legally unless the collector has been issued a federal salvage permit. Sometimes, a dead shell is the only proof a species existed or continues to exist in a particular stream or river. In Tennessee, 51 freshwater mussel species are protected by federal law, and the Tennessee Wildlife Resources Agency does not allow live native mussels to be taken without prior permission, even species that are not under federal protection. For these reasons, it is useful (for identification purposes) and historically important to understand the origin, history, and size of book plate specimens appearing in *The Freshwater Mussels of Tennessee*.

METHODS

Of the 260 specimens of native species depicted in the Tennessee book, 232 were taken from the UTMM collection; the remainder were borrowed from the INHS (10 specimens) and the UMMZ (nine specimens). Each figured specimen in the UTMM collection has now been segregated from the general collection and placed in an archival, acid-free foam package. A notation has been made in the electronic database accompanying each figured specimen. Book plate specimens were measured to the nearest millimeter (greatest length) using Vernier calipers; their collection locality was verified using U.S. Geological Survey quadrangle maps, although a few specimens lacked a specific provenance. Species accounts presented herein are as presented in *The Freshwater Mussels of Tennessee* (plate number, scientific name, authority, and common name). Catalogue number follows the abbreviation for the collection from which the specimen originated. The use of river mile (RM), creek mile (CM), and air mile in the collection locality was retained because this is the increment

used in quadrangle maps. State route and county road are abbreviated as SR and CR, respectively. When the collector(s), collection locality, or date of collection was unknown or could not be inferred from other specimens, it was left blank. Museum acronyms follow Sabaj (2016).

RESULTS

Book plate specimens used in *The Freshwater Mussels of Tennessee* were found in the mollusk collections at UTMM, INHS, and UMMZ. Despite a careful search of each mollusk collection where figured specimens were known to have been borrowed, nine could not be located: *Epioblasma biemarginata* (plate 21, lower), *Epioblasma flexuosa* (plate 24, upper and lower), *Epioblasma lenior* (plate 28, upper), *Epioblasma lewisii* (plate 29, upper and lower), *Pleurobema rubellum* (plate 90, upper and lower), and *Quadrula fragosa* (plate 103, upper).

Since 1998, there have been numerous revisions to the taxonomy of freshwater mussels in the USA. Of the 129 species treated in *The Freshwater Mussels of Tennessee*, 38 have been revised (Williams et al. 2017). These revisions range from relatively minor emendations such as correcting the species name to conform to the gender of the genus, to significant changes on the basis of phylogenetic analysis. A summary of these revisions is provided in Table 1 and is based on the list of species in Williams et al. (2017) unless otherwise noted. Taxonomy used in the species accounts is as it appears in *The Freshwater Mussels of Tennessee*.

Provenance and Disposition of Book Plate Specimens Appearing in *The Freshwater Mussels of Tennessee*

Plate 1. *Cumberlandia monodonta* (Say, 1829), *Spectacle-case*.—Upper specimen: 125 mm (right valve), 129 mm (left valve). UTMM 2229. Clinch River, U.S. Highway 25E, RM 151.9, Claiborne Co., TN. March 1, 1975 (the two valves in the upper figure of plate 1 are from different individuals).

Lower specimen: 113 mm. UTMM 2227. Clinch River, Kyles Ford, RM 190, Hancock Co., TN. October 21, 1973.

Plate 2. *Actinonaias ligamentina* (Lamarck, 1819), *Mucket*.—Upper specimen: 83 mm. UTMM 1823. Clinch River, Kyles Ford, RM 190, Hancock Co., TN. September 15, 1973.

Lower specimen: 105 mm. UTMM 1803. Cumberland River, Rome Landing, Smith Co., TN. September 10, 1977.

Plate 3. *Actinonaias pectorosa* (Conrad, 1834), *Pheasant-shell*.—Upper specimen: 96 mm. UTMM 1789. Red River, Community of Dot where SR 96 runs adjacent to river, Logan Co., KY. August 27, 1977.

Lower specimen: 108 mm. UTMM 1835. Powell River, Buchanan Ford, RM 99.2, Claiborne Co., TN. July 17, 1983.

Plate 4. *Alasmidonta atropurpurea* (Rafinesque, 1831), *Cumberland Elktoe*.—Upper specimen: 77 mm. UTMM 4197.

Lower specimen: 64 mm. Same as preceding.

Plate 5. *Alasmidonta marginata* Say, 1818. *Elktoe*.—Upper

Table 1. Taxonomic status of species presented in Parmalee and Bogan (1998). Only species that have changed since 1998 are presented herein.

Name in Parmalee and Bogan (1998)	Changes Since 1998	Current Name(s) if Different from Parmalee and Bogan (1998)
<i>Anodonta suborbiculata</i> Say, 1831 Flat Floater	Reassigned to <i>Utterbackiana</i>	<i>Utterbackiana suborbiculata</i> (Say, 1831) Flat Floater
<i>Anodontoides ferussacianus</i> (Lea, 1834) Cylindrical Papershell	Populations above Cumberland Falls elevated from synonymy	<i>Anodontoides ferussacianus</i> (Lea, 1834), <i>Anodontoides denigrata</i> (Lea, 1852), Cumberland Papershell
<i>Elliptio dilatata</i> (Rafinesque, 1820) Spike	Reassigned to <i>Eurynia</i>	<i>Eurynia dilatata</i> Rafinesque 1820, Spike
<i>Epioblasma capsaeformis</i> (Lea, 1834) Oyster Mussel	New species described from lower TN drainage	<i>Epioblasma capsaeformis</i> (Lea, 1834) Oyster Mussel <i>Epioblasma ahlstedti</i> Jones and Neves, 2010, Duck River Dartersnapper
<i>Epioblasma florentina florentina</i> (Lea, 1857) Yellow Blossom	Nominotypical subspecies not required	<i>Epioblasma florentina</i> (Lea, 1857), Yellow Blossom
<i>Epioblasma florentina walkeri</i> (Wilson and Clark, 1914) Tan Riffleshell	Elevated to species; new subspecies described from upper TN drainage and elevated to species	<i>Epioblasma walkeri</i> (Wilson and Clark, 1914), Tan Riffleshell <i>Epioblasma aureola</i> Jones and Neves, 2010, Golden Riffleshell
<i>Epioblasma torulosa torulosa</i> (Rafinesque, 1820) Tubercled Blossom	Nominotypical subspecies not required	<i>Epioblasma torulosa</i> (Rafinesque 1820), Tubercled Blossom
<i>Epioblasma torulosa gubernaculum</i> (Reeve, 1865) Green Blossom	Elevated to species	<i>Epioblasma gubernaculum</i> (Reeve, 1865), Green Blossom
<i>Fusconaia barnesiana</i> (Lea, 1838) Tennessee Pigtoe	Reassigned to <i>Pleuronaia</i>	<i>Pleuronaia barnesiana</i> (Lea, 1838), Tennessee Pigtoe
<i>Fusconaia ebena</i> (Lea, 1831) Ebonyshell	Reassigned to <i>Reginaia</i> ; species name corrected	<i>Reginaia ebenus</i> (Lea, 1831), Ebonyshell
<i>Lampsilis altilis</i> (Conrad, 1834) Finelined Pocketbook	Reassigned to <i>Hamiota</i>	<i>Hamiota altilis</i> (Conrad, 1834), Finelined Pocketbook
<i>Lampsilis straminea claibornensis</i> (Lea, 1838) Southern Fatmucket	Subspecies no longer recognized	<i>Lampsilis straminea</i> (Conrad, 1834), Southern Fatmucket
<i>Lampsilis teres</i> (Rafinesque, 1820) Yellow Sandshell	Two geographically overlapping phenotypes elevated to species ¹	<i>Lampsilis teres</i> (Rafinesque, 1820) Yellow Sandshell <i>Lampsilis sietmani</i> Keogh and Simons 2019, Canary Kingshell
<i>Lasmigona holstonia</i> (Lea, 1838) Tennessee Heelsplitter	Mobile Basin populations elevated to species	<i>Lasmigona holstonia</i> (Lea, 1838), Tennessee Heelsplitter <i>Lasmigona etowaensis</i> (Conrad, 1849), Etowah Heelsplitter
<i>Lexingtonia dolabelloides</i> (Lea, 1840) Slabside Pearlymussel	Reassigned to <i>Pleuronaia</i>	<i>Pleuronaia dolabelloides</i> (Lea, 1840), Slabside Pearlymussel
<i>Obovaria jacksoniana</i> (Frierson, 1912) Southern Hickorynut	Synonymized with <i>Obovaria arkansasensis</i>	<i>Obovaria arkansasensis</i> (Lea, 1862), Southern Hickorynut
<i>Pleurobema chattanoogaense</i> (Lea, 1858) Painted Clubshell	Synonymized with <i>Pleurobema decisum</i>	<i>Pleurobema decisum</i> (Lea, 1831), Southern Clubshell
<i>Pleurobema gibberum</i> (Lea, 1838) Cumberland Pigtoe	Reassigned to <i>Pleuronaia</i> ; species name corrected	<i>Pleuronaia gibber</i> (Lea, 1838), Cumberland Pigtoe
<i>Pleurobema johannis</i> (Lea, 1859) Alabama Pigtoe	Synonymized with <i>Pleurobema perovatium</i> ; not part of TN fauna	<i>Pleurobema perovatium</i> (Conrad, 1834), Ovate Clubshell

Table 1, continued.

Name in Parmalee and Bogan (1998)	Changes Since 1998	Current Name(s) if Different from Parmalee and Bogan (1998)
<i>Pleurobema perovatum</i> (Conrad, 1834) Ovate Clubshell	Range in Alabama River drainage redefined; not part of TN fauna	
<i>Pleurobema rubellum</i> (Conrad, 1834) Warrior Pigtoe	Range in Alabama River drainage redefined; not part of TN fauna	
<i>Pleurobema troschelium</i> (Lea, 1852) Alabama Clubshell	Synonymized with <i>Pleurobema georgianum</i>	<i>Pleurobema georgianum</i> (Lea, 1841), Southern Pigtoe
<i>Ptychobranthus greeni</i> (Conrad, 1834) Triangular Kidneyshell	Subspecies elevated from synonymy; range of <i>Ptychobranthus greenii</i> redefined (not part of TN fauna)	<i>Ptychobranthus foremanianus</i> (Lea, 1842), Rayed Kidneyshell
<i>Ptychobranthus subtentum</i> (Say, 1825) Fluted Kidneyshell	Species name corrected	<i>Ptychobranthus subtentus</i> (Say, 1825), Fluted Kidneyshell
<i>Quadrula cylindrica</i> (Say, 1817) Rabbitsfoot	Reassigned to <i>Theliderma</i>	<i>Theliderma cylindrica</i> (Say, 1817), Rabbitsfoot
<i>Quadrula intermedia</i> (Conrad, 1836) Cumberland Monkeyface	Reassigned to <i>Theliderma</i>	<i>Theliderma intermedia</i> (Conrad, 1836), Cumberland Monkeyface
<i>Quadrula metanevra</i> (Rafinesque, 1820) Monkeyface	Reassigned to <i>Theliderma</i>	<i>Theliderma metanevra</i> (Rafinesque, 1820), Monkeyface
<i>Quadrula nodulata</i> (Rafinesque, 1820) Wartyback	Reassigned to <i>Cyclonaias</i>	<i>Cyclonaias nodulata</i> (Rafinesque, 1820), Wartyback
<i>Quadrula pustulosa</i> (Lea, 1831) Pimpleback	Reassigned to <i>Cyclonaias</i>	<i>Cyclonaias pustulosa</i> (Lea, 1831), Pimpleback
<i>Quadrula sparsa</i> (Lea, 1841) Appalachian Monkeyface	Reassigned to <i>Theliderma</i>	<i>Theliderma sparsa</i> (Lea, 1841), Appalachian Monkeyface
<i>Strophitus connasaugaensis</i> (Lea, 1858) Alabama Creekshell	Reassigned to <i>Pseudodontoideus</i> ²	<i>Pseudodontoideus connasaugaensis</i> (Lea, 1858) Alabama Creekshell
<i>Toxolasma lividus</i> Rafinesque, 1831 Purple Lilliput	Species name corrected	<i>Toxolasma lividum</i> Rafinesque, 1831, Purple Lilliput
<i>Toxolasma parvum</i> (Barnes, 1823) Lilliput	Species name corrected	<i>Toxolasma parvum</i> (Barnes, 1823), Lilliput
<i>Toxolasma texasensis</i> (Lea, 1857) Texas Lilliput	Species name corrected	<i>Toxolasma texasiense</i> (Lea, 1857), Texas Lilliput
<i>Villosa iris</i> (Lea, 1829) Rainbow	Caney Fork population elevated from synonymy	<i>Villosa iris</i> (Lea, 1829) Rainbow <i>Villosa sima</i> (Lea, 1838) Caney Fork Rainbow
<i>Villosa perpurpurea</i> (Lea, 1861) Purple Bean	Synonymized with <i>Venustaconcha trabalis</i>	<i>Venustaconcha trabalis</i> (Conrad, 1834), Tennessee Bean
<i>Villosa trabalis</i> (Conrad, 1834) Cumberland Bean	Reassigned to <i>Venustaconcha</i> ; split into two species	<i>Venustaconcha trabalis</i> (Conrad, 1834), Tennessee Bean <i>Venustaconcha troostensis</i> (Lea, 1834), Cumberland Bean
<i>Villosa vanuxemensis</i> (Lea, 1838) Mountain Creekshell	Subspecies elevated to species	<i>Villosa vanuxemensis</i> (Lea, 1838), Mountain Creekshell <i>Villosa umbrans</i> (Lea, 1857), Coosa Creekshell

¹Keogh, S. M. and A. M. Simons (2019).²Smith et al. (2018).

specimen: 79 mm. UTMM 1905. Clinch River, Kyles Ford, RM 190, Hancock County, TN. October 21, 1973. Paul Parmalee.

Lower specimen: 75 mm. Same as preceding (length of specimen in bottom figure estimated because tip of posterior margin is missing).

Plate 6. *Alasmidonta raveneliana* (Lea, 1834), *Appalachian Elktoe*.—Upper specimen: 70 mm. UTMM 1848. Little Tennessee River, CR 1456, Macon Co., NC. September 13, 1989. Richard Biggins.

Lower specimen: 81 mm. Same as preceding.

Plate 7. *Alasmidonta viridis* (Rafinesque, 1820), *Slipper-shell*.—Upper specimen: 39 mm. UTMM 1851. Floyds Fork of Salt River, 3.4 miles NW of Ft. Washington, Bullitt Co., KY. June 26, 1983. Glen Fallo.

Lower specimen: 49 mm. Same as preceding.

Plate 8. *Amblema plicata* (Say, 1817), *Threeridge*.—Upper specimen: 86 mm. UTMM 1932. Mississippi River, lock and dam no. 18, Henderson Co., IL. November 14, 1988.

Lower specimen: 114 mm. UTMM 1984. Harpeth River, 1 mile E of Pegram, Davidson Co., TN. December 12, 1973.

Plate 9. *Anodonta suborbiculata* Say, 1831. *Flat Floater*.—Upper specimen: 117 mm. UTMM 2137. Reelfoot Lake, Buzzard Slough, Obion Co., TN. August 1985. Paul Parmalee.

Lower specimen: 127 mm. UTMM 2141. Marais de St. Friol Slough of Mississippi River, RM 635.2, Crawford Co., WI.

Plate 10. *Anodontoides ferussacianus* (Lea, 1834), *Cylindrical Papershell*.—Upper specimen: 65 mm. UTMM 3356. Horicon Marsh, Dodge Co., WI. July 26, 1972. Harold Mathiak.

Lower specimen: 65 mm. UTMM 3355. Rock River, Allentown, Washington Co., WI. August 1971. Harold Mathiak.

Plate 11. *Arcidens confragosus* (Say, 1829), *Rock Pocketbook*.—Upper specimen: 103 mm. UTMM 3327. Mississippi River, 1 mile south of lock and dam 22, Ralls Co., MO. July 12, 1977.

Lower specimen: 118 mm. UTMM 2214. Lake Springfield, Sangamon Co., IL. March 1977.

Plate 12. *Cyclonaias tuberculata* (Rafinesque, 1820), *Purple Wartyback*.—Upper specimen: 71 mm. UTMM 2264. Tennessee River, approximately 5 miles downstream of Pickwick Dam, Hardin Co., TN. December 21, 1985. Paul Parmalee.

Lower specimen: 93 mm. UTMM 2252. Duck River, Lillard Mill, Marshall Co., TN. April 12, 1985. Paul Parmalee.

Plate 13. *Cyprogenia stegaria* (Rafinesque, 1820), *Fan-shell*.—Upper specimen: 57 mm. UTMM 2268. Cumberland River, Rome, between RM 293 and 296, Smith Co., TN. June 8, 1980.

Lower specimen: 54 mm. UTMM 2271. Licking River, Butler, Pendleton Co., KY. August 23, 1971.

Plate 14. *Dromus dromas* (Lea, 1834), *Dromedary Pearlymussel*.—Upper specimen: 66 mm. UTMM 2311. Cumberland River, Celina, Clay Co., TN. November 14, 1930.

Lower specimen: 68 mm. UTMM 2310. Clinch River, Frost Ford, Hancock Co., TN.

Plate 15. *Ellipsaria lineolata* (Rafinesque, 1820), *Butterfly*.—Upper specimen: 62 mm. UTMM 1281. Green River, lock and dam no. 5, Butler/Warren cos., KY. August 9, 1983. Glen Fallo.

Lower specimen: 91 mm. UTMM 1283. Mississippi River, 1 mile south of lock and dam no. 22, Ralls Co., MO. July 12, 1977.

Plate 16. *Elliptio arca* (Conrad, 1834), *Alabama Spike*.—Upper specimen: 49 mm. UTMM 8969. Conasauga River, upstream of SR 74, Polk Co., TN. July 11, 1977.

Lower specimen: 61 mm. UTMM 10876. Conasauga River, downstream of SR 74, Bradley Co., TN. October 11, 1988.

Plate 17. *Elliptio arctata* (Conrad, 1834), *Delicate Spike*.—Upper specimen: 77 mm. UTMM 3359. Kinchafoonee Creek, Webster Co., GA. April 27, 1995. Gerald Dinkins, Paul Parmalee, and Mark Hughes.

Lower specimen: 76 mm. UTMM 3360. Tombigbee River, Vienna Landing, Pickens Co., AL. July 25, 1977. Paul Parmalee.

Plate 18. *Elliptio crassidens* (Lamarck, 1819), *Elephant-tear*.—Upper specimen: 94 mm. UTMM 2623. Tennessee River, 1 mile E of Faxon, Hardin Co., TN. August 24, 1984.

Lower specimen: 113 mm. Same as preceding.

Plate 19. *Elliptio dilatata* (Rafinesque, 1820), *Spike*.—Upper specimen: 109 mm. UTMM 8205. Cumberland River, Rome, Smith Co., TN. July 12, 1978.

Lower specimen: 98 mm. UTMM 10879. Cumberland River, Rome, Smith Co., TN. September 10, 1977.

Plate 20. *Epioblasma arcaeformis* (Lea, 1831), *Sugar-spoon*.—Upper specimen: Female, 43 mm. UTMM 3880. Cumberland River, TN (specimen has barely legible writing that appears to indicate its origin was the Cumberland River, TN).

Lower specimen: Male, 47 mm. UTMM 3894. Holston River, Austin Mills, Hawkins Co., TN. September 9, 1927.

Plate 21. *Epioblasma biemarginata* (Lea, 1857), *Angled Riffleshell*.—Upper specimen: Female, 36 mm. INHS 20271.4. Tennessee River, Florence, Lauderdale Co., AL.

Lower specimen: Male. Provenance and disposition unknown.

Plate 22. *Epioblasma brevidens* (Lea, 1831), *Cumberlandian Combshell*.—Upper specimen: Female, 54 mm. UTMM 4111. Powell River, 1 mile upstream of Riverside, CR 2548, Claiborne Co., TN. March 1, 1975.

Lower specimen: Male, 53 mm. UTMM 4110. Powell River, McDowell Ford, RM 107, Hancock Co., TN. June 24, 1980.

Plate 23. *Epioblasma capsaeformis* (Lea, 1834), *Oyster Mussel*.—Upper specimen: Female, 50 mm. UTMM 9370. West Prong Little Pigeon River, Sevierville, Sevier Co., TN. December 17, 1985.

Lower specimen: Male, 54 mm. UTMM 4118. West Prong

Little Pigeon River, Sevierville, Sevier Co., TN. September 4, 1985.

Plate 24. Epioblasma flexuosa (Rafinesque, 1820), Leafshell.—Upper specimen: Provenance and disposition unknown.

Lower specimen: Provenance and disposition unknown.

Plate 25. Epioblasma florentina florentina (Lea, 1857), Yellow Blossom.—Upper specimen: Female, 51 mm. UMMZ 58249. Stones River at Walter, Rutherford Co., TN. 1933. William Clench and Henry van der Schalie.

Lower specimen: Male, 60 mm. Same as preceding.

Plate 26. Epioblasma florentina walkeri (Wilson and Clark, 1914), Tan Riffleshell.—Upper specimen: Female, 45 mm. UTMM 3874. Hiwassee River, RM 54, Polk Co., TN. August 5, 1992. Paul Parmalee and Mark Hughes.

Lower specimen: Male, 47 mm. UTMM 9593. Hiwassee River, RM 54, Polk Co., TN. August 15, 1992. Paul Parmalee and Mark Hughes.

Plate 27. Epioblasma haysiana (Lea, 1834), Acornshell.—Upper specimen: Female, 29 mm. UTMM 3887. Holston River, Austin Mills, Hawkins Co., TN.

Lower specimen: Male, 37 mm. UTMM 3884. Elk River, Franklin Co., TN. October 11, 1963.

Plate 28. Epioblasma lenior (Lea, 1842), Narrow Catspaw.—Upper specimen: Provenance and disposition unknown.

Lower specimen: Male, 41 mm. INHS 20298. North Fork Clinch River, TN.

Plate 29. Epioblasma lewisii (Walker, 1910), Forkshell.—Upper specimen: Provenance and disposition unknown.

Lower specimen: Provenance and disposition unknown.

Plate 30. Epioblasma metastrata (Conrad, 1838), Upland Combshell.—Upper specimen: Female, 32 mm. INHS 20296.4. Black Warrior River, AL.

Lower specimen: Male, 30 mm. INHS 20296.3. Black Warrior River, AL.

Plate 31. Epioblasma obliquata (Rafinesque, 1820), Catspaw.—Upper specimen: Female, 51 mm. UTMM 10863. Cumberland River, Rome, RM 296.8, Smith Co., TN. June 10, 1980. Steven Ahlstedt.

Lower specimen: Male, 65 mm. Same as preceding.

Plate 32. Epioblasma othcaloogensis (Lea, 1857), Southern Acornshell.—Upper specimen: Male, 30 mm. UMMZ 91452. Chattooga River, GA.

Lower specimen: Male, 32 mm. Same as preceding.

Plate 33. Epioblasma personata (Say, 1829), Round Combshell.—Upper specimen: Female, 45 mm. UTMM 3881. Alabama.

Lower specimen: Male, 44 mm. Same as preceding.

Plate 34. Epioblasma propinqua (Lea, 1857), Tennessee Riffleshell.—Upper specimen: Female, 52 mm. INHS 20306.12. Tennessee River, Florence, Lauderdale Co., AL.

Lower specimen: Male, 56 mm. INHS 20306.13. Same as preceding.

Plate 35. Epioblasma stewardsonii (Lea, 1852), Cumber-

land Leafshell.—Upper specimen: Female, 40 mm. UTMM 10761. Clinch River, Edywood, TN.

Lower specimen: Male, 42 mm. UTMM 3875. Holston River, Austin Mills, Hawkins Co., TN. September 9, 1927.

Plate 36. Epioblasma torulosa torulosa (Rafinesque, 1820), Tubercled Blossom.—Upper specimen: Female, 57 mm. UTMM 4191. Provenance unknown.

Lower specimen: Male, 61 mm. Same as preceding.

Plate 37. Epioblasma triquetra (Rafinesque, 1820), Snuffbox.—Upper specimen: Female, 41 mm. UTMM 10842. Clinch River, Kyles Ford, Hancock Co., TN. September 15, 1973.

Lower specimen: Male, 54 mm. UTMM 9737. Clinch River, Kyles Ford, Hancock Co., TN. October 21, 1973.

Plate 38. Epioblasma turgidula (Lea, 1858), Turgid Blossom.—Upper specimen: 46 mm. INHS 20295.10. Duck River, Columbia, Maury Co., TN.

Lower specimen: 38 mm. INHS 20295.9. Duck River, Columbia, Maury Co., TN.

Plate 39. Fusconaia barnesiana (Lea, 1838), Tennessee Pigtoe.—Upper specimen: 57 mm. UTMM 10796. West Prong Little Pigeon River, Sevierville, Sevier Co., TN. March 14, 1987. Paul Parmalee.

Lower specimen: 69 mm. UTMM 10953. West Prong Little Pigeon River, Sevierville, Sevier Co., TN. August 14, 1985. Paul Parmalee.

Plate 40. Fusconaia cor (Conrad, 1834), Shiny Pigtoe.—Upper specimen: 34 mm. UTMM 1404. Paint Rock River, 2 miles E of Trenton, Jackson Co., AL. August 18, 1976. Paul Parmalee.

Lower specimen: 69 mm. Same as preceding.

Plate 41. Fusconaia cuneolus (Lea, 1840), Finerayed Pigtoe.—Upper specimen: 59 mm. UTMM 12125. Clinch River, Frost Ford, Hancock Co., TN. September 29, 1974. Paul Parmalee.

Lower specimen: 63 mm. UTMM 12126. Clinch River, Kyles Ford, Hancock Co., TN. September 15, 1973. Paul Parmalee.

Plate 42. Fusconaia ebena (Lea, 1831), Ebonyshell.—Upper specimen: 73 mm. UTMM 1298. Cumberland River, near Hartsville, Trousdale Co., TN. November 19, 1982. Paul Parmalee.

Lower specimen: 75 mm. UTMM 1291. Tennessee River, Crump, Hardin Co., TN (commercial sheller cull pile). August 26, 1977.

Plate 43. Fusconaia flava (Rafinesque, 1820), Wabash Pigtoe.—Upper specimen: 71 mm. UTMM 1346. Cumberland River, Rome, Smith Co., TN. September 10, 1977.

Lower specimen: 65 mm. UTMM 3354. Harpeth River, near substation off I-40, Davidson Co., TN. May 18, 1974. E. Breitburg.

Plate 44. Fusconaia subrotunda (Lea, 1831), Longsolid.—Upper specimen: 62 mm. UTMM 1455. Cumberland River at Rome, Smith Co., TN. June 18, 1978.

Lower specimen: 80 mm. UTMM 1304. Powell River, McDowell Ford, RM 107, Hancock Co., TN. August 4, 1979.

Plate 45. *Hemistena lata* (Rafinesque, 1820), *Cracking Pearlymussel*.—Upper specimen: 78 mm. UTMM 12127. Clinch River, RM 179.8, Hancock Co., TN. November 17, 1978.

Lower specimen: 71 mm. UTMM 12128. Clinch River, Frost Ford, Hancock Co., TN. September 29, 1974.

Plate 46. *Lampsilis abrupta* (Say, 1831), *Pink Mucket*.—Upper specimen: Female, 94 mm. UTMM 3352. Tennessee River, Kentucky Lake, KY. August 19, 1982. Clyde Brown.

Lower specimen: Male, 93 mm. UTMM 0323. Cumberland River, Hartsville, Trousdale Co., TN. September 11, 1977.

Plate 47. *Lampsilis atilis* (Conrad, 1834), *Finelined pocketbook*.—Upper specimen: Female, 73 mm. UTMM 1132. Conasauga River, SR 74, Bradley Co., TN. October 8, 1978.

Lower specimen: Male, 76 mm. UTMM 12129. Conasauga River, SR 74, Bradley Co., TN. May 4, 1975.

Plate 48. *Lampsilis cardium* Rafinesque, 1820, *Plain Pocketbook*.—Upper specimen: Female, 102 mm. UTMM 3347. Mississippi River, RM 633.2, Crawford Co., WI. September 9, 1986. James Theler.

Lower specimen: Male, 126 mm. UTMM 3346. Muskingum River, Lowell, Washington Co., OH. September 9, 1973. Bob Winters.

Plate 49. *Lampsilis fasciola* Rafinesque, 1820, *Wavyrayed Lampmussel*.—Upper specimen: Female, 67 mm. UTMM 12130. West Prong Little Pigeon River, Sevierville, Sevier Co., TN. June 22, 1985. Paul Parmalee.

Lower specimen: Male, 75 mm. UTMM 12131. West Prong Little Pigeon River, Sevierville, Sevier Co., TN. December 17, 1985. Paul Parmalee.

Plate 50. *Lampsilis ornata* (Conrad, 1835), *Southern Pocketbook*.—Upper specimen: Male, 82 mm. UTMM 12132. Conasauga River, SR 2, Murray Co., GA. October 4, 1978. Paul Parmalee.

Lower specimen: Male, 93 mm. UTMM 12133. Conasauga River, SR 286, Murray Co., GA. October 8, 1978. Paul Parmalee.

Plate 51. *Lampsilis ovata* (Say, 1817), *Pocketbook*.—Upper specimen: Female, 93 mm. UTMM 0305. Little River, U.S. Highway 411, Blount Co., TN. July 30, 1976.

Lower specimen: Male, 122 mm. UTMM 0303. Tennessee River, Hardin Co., TN. August 1984. Ed Hunt.

Plate 52. *Lampsilis siliquoidea* (Barnes, 1823), *Fatmucket*.—Upper specimen: Female, 98 mm. UTMM 0948. Wolf River, E of Moscow, Fayette Co., TN. October 31, 1994. Don Manning.

Lower specimen: Male, 100 mm. Same as preceding.

Plate 53. *Lampsilis straminea claibornensis* (Lea, 1838), *Southern Fatmucket*.—Upper specimen: Female, 82 mm. UTMM 1136. Tombigbee River, Vienna Landing, Pickens Co., AL. July 25, 1977.

Lower specimen: Male, 95 mm. Same as preceding.

Plate 54. *Lampsilis teres* (Rafinesque, 1820), *Yellow Sandshell*.—Upper specimen: Female, 105 mm. UTMM

0156. Tennessee River, below Pickwick Dam, Hardin Co., TN. August 24, 1984. Paul Parmalee and Ed Hunt.

Lower specimen: Male, 101 mm. Same as preceding.

Plate 55. *Lampsilis virescens* (Lea, 1858), *Alabama Lampmussel*.—Upper specimen: Female, 52 mm. UTMM 4076. Hurricane Creek, CM 2.9, Jackson Co., AL. May 29, 1980.

Lower specimen: Male, 61 mm. UTMM 4075. Paint Rock River, Jackson Co., AL.

Plate 56. *Lasmigona complanata* (Barnes, 1823), *White Heelsplitter*.—Upper specimen: 117 mm. UTMM 3350. Cumberland River, Trousdale Co., TN (commercial sheller cull pile). November 19, 1982. Paul Parmalee.

Lower specimen: 135 mm. UTMM 3351. Little Tennessee River (Tellico Reservoir), silo along northern end of Tellico Parkway, Loudon Co., TN. February 25, 1990. Paul Parmalee and Mark Hughes.

Plate 57. *Lasmigona costata* (Rafinesque, 1820), *Fluted-shell*.—Upper specimen: 85 mm. UTMM 3358. Little South Fork Cumberland River, Freedom Church Ford, Wayne Co., KY. October 30, 1977. Arthur Bogan.

Lower specimen: 118 mm. UTMM 0446. Clinch River, SR 23, 3 miles SW of Clinchport, Scott Co., VA. September 2, 1977.

Plate 58. *Lasmigona holstonia* (Lea, 1838), *Tennessee Heelsplitter*.—Upper specimen: 66 mm. UTMM 0474. Pinhook Creek, SR 225, Murray Co., GA. March 22, 1988. Paul Parmalee.

Lower specimen: 72 mm. Same as preceding.

Plate 59. *Lasmigona subviridis* (Conrad, 1835), *Green Floater*.—Upper specimen: 42 mm. UMMZ 104159. Reed Creek, Wythe Co., VA. September 16, 1912. Arnold Ortmann.

Lower specimen: 49 mm. Same as preceding.

Plate 60. *Lemiox rimosus* (Rafinesque, 1831), *Birdwing Pearlymussel*.—Upper specimen: Female, 33 mm. UTMM 0497. Duck River, Lillard Mill, RM 177.2, Marshall Co., TN. August 6, 1979. Steven Ahlstedt.

Lower specimen: Male, 44 mm. Same as preceding.

Plate 61. *Leptodea fragilis* (Rafinesque, 1820), *Fragile Papershell*.—Upper specimen: 124 mm. UTMM 0520. Harpeth River, 1 mile S-SE of Pegram, Cheatham Co., TN. August 13, 1988. Paul Parmalee.

Lower specimen: 119 mm. UTMM 0535. Muskingum River, Lowell, Washington Co., OH. June 28, 1975. Robert Winter.

Plate 62. *Leptodea leptodon* (Rafinesque, 1820), *Scale-shell*.—Upper specimen: 78 mm. UTMM 1236. Meramec River, Missouri. Ronald Oesch.

Lower specimen: 67 mm. UTMM 1237. Meramec River, above Times Beach, Franklin Co., Missouri. June 24, 1972. Ronald Oesch.

Plate 63. *Lexingtonia dolabelloides* (Lea, 1840), *Slabside Pearlymussel*.—Upper specimen: 65 mm. UTMM 0554. Duck River, RM 174, Marshall Co., TN. July 6, 1979.

Lower specimen: 51 mm. UTMM 5538. Provenance

unknown (specimen donated to UTMM from William Strode Collection).

Plate 64. Ligumia recta (Lamarck, 1819), Black Sandshell.—Upper specimen: Female, 115 mm. UTMM 0573. Tennessee River, Hardin Co., TN. August 24, 1984. Paul Parmalee.

Lower specimen: Male, 119 mm. UTMM 0591. Mississippi River, 1 mile S of lock and dam number 22, Ralls Co., MO. July 12, 1977.

Plate 65. Ligumia subrostrata (Say, 1831), Pondmussel.—Upper specimen: Female, 93 mm. UTMM 3353. Hatchie River drainage, TN. Don Manning.

Lower specimen: Male, 113 mm. Same as preceding.

Plate 66. Medionidus acutissimus (Lea, 1831), Alabama Moccasinshell.—Upper specimen: 47 mm. UTMM 0971. Conasauga River, 1.5 miles W of Tenna, Polk Co., TN. October 8, 1978. John Hurd.

Lower specimen: 43 mm. UTMM 0970. Conasauga River, SR 74, Bradley Co., TN. October 8, 1978. John Hurd.

Plate 67. Medionidus conradicus (Lea, 1834), Cumberland Moccasinshell.—Upper specimen: 53 mm. UTMM 0664. Clinch River, Kyles Ford, Hancock Co., TN. September 15, 1973. Paul Parmalee.

Lower specimen: 60 mm. UTMM 0666. West Prong Little Pigeon River, Sevierville, Sevier Co., TN. December 6, 1986.

Plate 68. Medionidus parvulus (Lea, 1860), Coosa Moccasinshell.—Upper specimen: 48 mm. UTMM 0645. Conasauga River, SR 74, Bradley Co., TN. October 8, 1978. John Hurd.

Lower specimen: 51 mm. Same as preceding.

Plate 69. Megaloniais nervosa (Rafinesque, 1820), Washboard.—Upper specimen: 103 mm. UTMM 12134. Tennessee River, approximately 0.5 miles downstream of Pickwick Dam, RM 206.3, Hardin Co., TN. December 21, 1985.

Lower specimen: 112 mm. UTMM 12135. Tennessee River, RM 198.5, Hardin Co., TN. May 1, 1987. Paul Parmalee.

Plate 70. Obliquaria reflexa Rafinesque, 1820, Threehorn Wartyback.—Upper specimen: 52 mm. UTMM 0740. Little Tennessee River (Tellico Reservoir), approximately 1.6 miles S of Little Tennessee River bridge, Loudon Co., TN. February 25, 1990. Paul Parmalee and Mark Hughes.

Lower specimen: 53 mm. Same as preceding.

Plate 71. Obovaria jacksoniana (Frierson, 1912), Southern Hickorynut.—Upper specimen: Female, 33 mm. UTMM 3335. Provenance unknown.

Lower specimen: Male, 40 mm. UTMM 3335. Provenance unknown.

Plate 72. Obovaria olivaria (Rafinesque, 1820), Hickorynut.—Upper specimen: Female, 63 mm. UTMM 0212. Mississippi River, 1 mile S of lock and dam 22, Ralls Co., MO. July 12, 1977. Paul Parmalee.

Lower specimen: Male, 75 mm. UTMM 0220. Cumberland River, Rome, RM 296, Smith Co., TN. July 12, 1978.

Plate 73. Obovaria retusa (Lamarck, 1819), Ringpink.—

Upper specimen: Female, 50 mm. UTMM 3332. Ohio River, Falls of the Ohio, Louisville, KY. E.W. Payne.

Lower specimen: Female, 49 mm. UTMM 3333. Alabama.

Plate 74. Obovaria subrotunda (Rafinesque, 1820), Round Hickorynut.—Upper specimen: 44 mm. UTMM 0240. Duck River, RM 174, Marshall Co., TN. July 6, 1979.

Lower specimen: 58 mm. UTMM 3334. Embarrass River, 3 miles SE of Sainte Marie, Jasper Co., IL. August 21, 1955.

Plate 75. Pegias fabula (Lea, 1838), Littlewing Pearlymussel.—Upper specimen: Female, 28 mm. UTMM 12136. Little South Fork Cumberland River, Freedom Church Road, Wayne Co., KY. October 30, 1977.

Lower specimen: Male, 28 mm. UTMM 12137. Little South Fork Cumberland River, Freedom Church Road, Wayne Co., KY. September 7, 1979.

Plate 76. Plectomerus dombeyanus (Valenciennes, 1827), Bankclimber.—Upper specimen: 102 mm. UTMM 0249. Hatchie River, E-SE of SR 51, Lauderdale Co., TN. August 25, 1984. Paul Parmalee and Don Manning.

Lower specimen: 109 mm. Same as preceding.

Plate 77. Plethobasus cicatricosus (Say, 1829), White Wartyback.—Upper specimen: 88 mm. UMMZ 81933. Cumberland River, Nashville, TN. Albert G. Wetherby.

Lower specimen: 79 mm. Same as preceding.

Plate 78. Plethobasus cooperianus (Lea, 1834), Orangefoot Pimpleback.—Upper specimen: 75 mm. UTMM 0288. Tennessee River, RM 198.5, Hardin Co., TN (commercial sheller cull pile). Spring 1987. Paul Parmalee.

Lower specimen: 83 mm. UTMM 12138. Tennessee River, RM 198.5, Hardin Co., TN (commercial sheller cull pile). December 26, 1992. Paul Parmalee.

Plate 79. Plethobasus cyphus (Rafinesque, 1820), Sheepnose.—Upper specimen: 77 mm. UTMM 0270. Clinch River, Kyles Ford, Hancock Co., TN. July 29, 1974.

Lower specimen: 89 mm. UTMM 0264. Cumberland River, Rome, Smith Co., TN. July 12, 1978.

Plate 80. Pleurobema chattanoogaense (Lea, 1858), Painted Clubshell.—Upper specimen: 62 mm. UTMM 9515. Conasauga River, Murray Co., GA. October 4, 1978.

Lower specimen: 62 mm. Same as preceding.

Plate 81. Pleurobema clava (Lamarck, 1819), Clubshell.—Upper specimen: 57 mm. UTMM 12139. Allegheny River, West Hickory, Forest Co., PA. July 6, 1973.

Lower specimen: 52 mm. UTMM 12140. Allegheny River, West Hickory, Forest Co., PA. August 14, 1974.

Plate 82. Pleurobema cordatum (Rafinesque, 1820), Ohio Pigtoe.—Upper specimen: 86 mm. UTMM 12141. Tennessee River, Crump, Hardin Co., TN (commercial sheller cull pile). August 26, 1977.

Lower specimen: 85 mm. UTMM 12142. Tennessee River, Crump, Hardin Co., TN (commercial sheller cull pile). August 24, 1984.

Plate 83. Pleurobema georgianum (Lea, 1841), Southern Pigtoe.—Upper specimen: 55 mm. UTMM 10963. Conasauga River, Mitchell Bridge, Whitfield/Murray cos., GA. November 12, 1978. John Hurd.

Lower specimen: 45 mm. UTMM 0960. Conasauga River, SR 74, Bradley Co., TN. October 8, 1978. John Hurd.

Plate 84. Pleurobema gibberum (Lea, 1838), Cumberland Pigtoe.—Upper specimen: 46 mm. UTMM 0957. Collins River, SR 56, 1 mile E of Mt. Olive, Grundy Co., TN. September 3, 1978. Arthur Bogan.

Lower specimen: 52 mm. Same as preceding.

Plate 85. Pleurobema hanleyianum (Lea, 1852), Georgia Pigtoe.—Upper specimen: 55 mm. UTMM 7420. Conasauga River, SR 74, Bradley Co., TN. October 8, 1978. John Hurd.

Lower specimen: 49 mm. Same as preceding.

Plate 86. Pleurobema johannis (Lea, 1859), Alabama Pigtoe.—Upper specimen: 27 mm. INHS 24411.2. Cahaba River, AL.

Lower specimen: 24 mm. INHS 24411.1. Cahaba River, AL.

Plate 87. Pleurobema oviforme (Conrad, 1834), Tennessee Clubshell.—Upper specimen: 66 mm. UTMM 0993. Tellico River, RM 25.4, Monroe Co., TN. November 26, 1983.

Lower specimen: 64 mm. UTMM 0977. West Prong Little Pigeon River, Sevierville, Sevier Co., TN. March 31, 1986.

Plate 88. Pleurobema perovatum (Conrad, 1834), Ovate Clubshell.—Upper specimen: 49 mm. UTMM 3336. Chewacla Creek, 4 miles S-SW of Tuskegee, Macon Co., AL. June 18, 1995. Doug Shelton.

Lower specimen: 55 mm. Same as preceding.

Plate 89. Pleurobema plenum (Lea, 1840), Rough Pigtoe.—Upper specimen: 62 mm. UTMM 1041. Cumberland River, Rome, Smith Co., TN. June 5, 1979.

Lower specimen: 55 mm. UTMM 1043. Green River, Honakers Ferry, Butler Co., KY. August 20, 1971.

Plate 90. Pleurobema rubellum (Conrad, 1834), Warrior Pigtoe.—Upper specimen: Provenance and disposition unknown.

Lower specimen: Provenance and disposition unknown.

Plate 91. Pleurobema rubrum (Rafinesque, 1820) Pyramid Pigtoe.—Upper specimen: 66 mm. UTMM 4443. Clinch River, Frost Ford, Hancock Co., TN. November 20, 1992. Mark Hughes.

Lower specimen: 64 mm. UTMM 3337. Cumberland River, Rome, Smith Co., TN. September 10, 1977.

Plate 92. Pleurobema sintoxia (Rafinesque, 1820), Round Pigtoe.—Upper specimen: 92 mm. UTMM 1039. Cumberland River, Trousdale Co., TN. November 19, 1982.

Lower specimen: 102 mm. UTMM 1065. Big South Fork, RM 62, Scott Co., TN. September 1982. R. Hoffman.

Plate 93. Pleurobema troschelianum (Lea, 1852), Alabama Clubshell.—Upper specimen: 41 mm. UTMM 0998. Conasauga River, SR 74, Bradley Co., TN. October 1, 1978.

Lower specimen: 46 mm. UTMM 0961. Conasauga River, approximately 1.5 miles downriver of U.S. Highway 411, Polk Co., TN. October 8, 1983. Paul Parmalee.

Plate 94. Potamilus alatus (Say, 1817), Pink Heelsplitter.—Upper specimen: 165 mm. UTMM 3330. Cumberland River, RM 141-142, Montgomery Co., TN. July 8, 1983.

Lower specimen: 140 mm. UTMM 0765. Clinch River, Kyles Ford, Hancock Co., TN. July 29, 1974.

Plate 95. Potamilus ohiensis (Rafinesque, 1820), Pink Papershell.—Upper specimen: 134 mm. UTMM 0764. Iowa River, near Hills, Johnson Co., IA. August 1983.

Lower specimen: 157 mm. Same as preceding.

Plate 96. Potamilus purpuratus (Lamarck, 1819), Bleu-fer.—Upper specimen: Female, 94 mm. UTMM 0835. Hatchie River, E-SE of SR 51, Lauderdale Co., TN. August 25, 1984.

Lower specimen: Male, 132 mm. UTMM 0836. Old St. Francis River, above mouth of Tyronza River, RM 81.5, Cross Co., AR. September 30, 1986. Steven Ahlstedt.

Plate 97. Ptychobranthus fasciolaris (Rafinesque, 1820), Kidneyshell.—Upper specimen: 120 mm. UTMM 0890. Powell River, U.S. Highway 25E, Claiborne Co., TN. October 21, 1973.

Lower specimen: 102 mm. UTMM 0933. Cumberland River, Trousdale Co., TN. November 19, 1982. Jake Petty.

Plate 98. Ptychobranthus greeni (Conrad, 1834), Triangular Kidneyshell.—Upper specimen: 66 mm. UTMM 12143. Conasauga River, SR 2, Murray Co., GA. October 4, 1978. Paul Parmalee.

Lower specimen: 56 mm. UTMM 12144. Conasauga River, SR 2, Murray Co., GA. October 8, 1978. David Stansbery.

Plate 99. Ptychobranthus subtentum (Say, 1825), Fluted Kidneyshell.—Upper specimen: 77 mm. UTMM 0869. Clinch River, Frost Ford, Hancock Co., TN. October 1, 1992.

Lower specimen: 87 mm. UTMM 3331. Middle Fork Holston River, 0.5 miles E of Seven Mile Ford, Smyth Co., VA. August 4, 1972.

Plate 100. Pyganodon grandis (Say, 1829), Giant Floater.—Upper specimen: 167 mm. UTMM 3348. Watauga Reservoir, near Wagnor Island, Johnson Co., TN. February 4, 1984. Paul Parmalee.

Lower specimen: 129 mm. UTMM 3349. Rhea Springs Hatchery, NE Knoxville, Knox Co., TN. January 16, 1974.

Plate 101. Quadrula apiculata (Say, 1829), Southern Mapleleaf.—Upper specimen: 87 mm. UTMM 1543. Coosa River (Lay Reservoir), Shelby Co., AL. June 15, 1993. U.S. Shell Company, Hollywood, AL.

Lower specimen: 93 mm. UTMM 3329. Tennessee River (Kentucky Reservoir), TN. 1991.

Plate 102. Quadrula cylindrica (Say, 1817), Rabbitsfoot.—Upper specimen: 95 mm. UTMM 1644. Powell River, Buchanan Ford, RM 99, Claiborne Co., TN. April 30, 1976.

Lower specimen: 101 mm. UTMM 1648. Tennessee River, RM 198.5, Hardin Co., TN. Spring 1987.

Plate 103. Quadrula fragosa (Conrad, 1835), Winged Mapleleaf.—Upper specimen: Provenance and disposition unknown.

Lower specimen: 87 mm. UMMZ 75809. Duck River, Columbia, Maury Co., TN. Albert G. Wetherby.

Plate 104. Quadrula intermedia (Conrad, 1836), Cumberland Monkeyface.—Upper specimen: Female, 72 mm. UTMM

1640. Powell River, McDowell Ford, RM 107, Hancock Co., TN. June 24, 1980.

Lower specimen: Male, 60 mm. Same as preceding.

Plate 105. *Quadrula metanevra* (*Rafinesque, 1820*), *Monkeyface*.—Upper specimen: 72 mm. UTMM 3328. Tennessee River, Reynoldsburg Island, 6 miles S of New Johnsonville, Humphreys Co., TN. June 1983.

Lower specimen: 67 mm. UTMM 1538. Tennessee River, RM 191, Hardin Co., TN. November 8, 1980.

Plate 106. *Quadrula nodulata* (*Rafinesque, 1820*), *Wartyback*.—Upper specimen: 59 mm. UTMM 1518. Sangamon River, Sangamon Co., IL. November 29, 1975.

Lower specimen: 49 mm. UTMM 1499. Licking River, Butler, Pendleton Co., KY. August 23, 1971.

Plate 107. *Quadrula pustulosa* (*Lea, 1831*), *Pimpleback*.—Upper specimen: 68 mm. UTMM 1526. Cumberland River, Rome, Smith Co., TN. September 7, 1978.

Lower specimen: 57 mm. UTMM 1632. Tennessee River (Watts Bar Reservoir), Roane Co., TN. March 14, 1992. Paul Parmalee and Mark Hughes.

Plate 108. *Quadrula quadrula* (*Rafinesque, 1820*), *Mapleleaf*.—Upper specimen: 70 mm. UTMM 4509. Provenance unknown.

Lower specimen: 72 mm. UTMM 1531. Cumberland River, RM 141-142, Montgomery Co., TN. July 8, 1983.

Plate 109. *Quadrula sparsa* (*Lea, 1841*), *Appalachian Monkeyface*.—Upper specimen: 55 mm. UTMM 12145. Powell River, Fugate Ford, RM 100, Claiborne Co., TN. August 29, 1975.

Lower specimen: 64 mm. UTMM 1639. Powell River, Buchanan Ford, RM 99, Claiborne Co., TN. April 30, 1976.

Plate 110. *Simpsonaias ambigua* (*Say, 1825*), *Salamander Mussel*.—Upper specimen: 46 mm. UTMM 3325. Provenance unknown.

Lower specimen: 46 mm. UTMM 3325. Provenance unknown.

Plate 111. *Strophitus connasaugaensis* (*Lea, 1858*), *Alabama Creekmussel*.—Upper specimen: 70 mm. UTMM 2729. Conasauga River, U.S. 411, Polk Co., TN.

Lower specimen: 85 mm. UTMM 2728. Conasauga River, SR 74, Bradley Co., TN. January 26, 1989. Paul Parmalee.

Plate 112. *Strophitus undulatus* (*Say, 1817*), *Creeper*.—Upper specimen: 79 mm. UTMM 2716. Licking River, Butler, Pendleton Co., KY. August 23, 1971.

Lower specimen: 81 mm (right valve), 79 mm (left valve). UTMM 2709. Rock River, Allenton, Washington Co., WI. August 9, 1971 (the two valves are from different individuals).

Plate 113. *Toxolasma cylindrellus* (*Lea, 1868*), *Pale Lilliput*.—Upper specimen: Male, 29 mm. UTMM 3386. Estill Fork Paint Rock River, Estill Fork, 18.1 miles NW of Scottsboro, Jackson Co., AL. September 28, 1976. David Stansbery and Kathy Borrer.

Lower specimen: Male, 30 mm. UTMM 4215. Paint Rock River, AL.

Plate 114. *Toxolasma lividus* *Rafinesque, 1831*, *Purple Lilliput*.—Upper specimen: Female, 36 mm. UTMM 12146.

West Prong Little Pigeon River, Sevierville, Sevier Co., TN. March 31, 1986.

Lower specimen: Male, 43 mm. UTMM 12147. West Prong Little Pigeon River, Sevierville, Sevier Co., TN. February 1, 1986.

Plate 115. *Toxolasma parvus* (*Barnes, 1823*), *Lilliput*.—Upper specimen: Female, 34 mm. UTMM 2756. Lake Springfield, Sangamon Co., IL. March 1977. Walter Klippel.

Lower specimen: Male, 37 mm. Same as preceding.

Plate 116. *Toxolasma texasensis* (*Lea, 1857*), *Texas Lilliput*.—Upper specimen: Female, 52 mm. UTMM 3324. Tulot Seep Ditch No. 123, above bridge S of Anderson Tully (Stewart), Poinsett Co., AR. August 8, 1987. Steven Ahlstedt.

Lower specimen: Male, 48 mm. UTMM 2772. South Fork Ouachita River, SR 379, Montgomery Co., AR. June 6, 1980. John Harris.

Plate 117. *Tritogonia verrucosa* (*Rafinesque, 1820*), *Pistolgrip*.—Upper specimen: Male, 111 mm. UTMM 2826. Paint Rock River, 1.2 miles N of Princeton, Jackson Co., AL. August 18, 1976.

Lower specimen: Female, 127 mm. UTMM 3326. Little South Fork Cumberland River, Freedom Ford, Wayne Co., KY. October 30, 1977.

Plate 118. *Truncilla donaciformis* (*Lea, 1828*), *Fawnsfoot*.—Upper specimen: 37 mm. UTMM 2885. Licking River, Butler, Pendleton Co., KY. August 23, 1971.

Lower specimen: 43 mm. UTMM 2888. Iowa River, Hills, Johnson Co., IA. August 1983.

Plate 119. *Truncilla truncata* (*Rafinesque, 1820*), *Deer-toe*.—Upper specimen: 54 mm. UTMM 2891. Sangamon River, approximately 2 miles E of Salisbury, Sangamon Co., IL. November 29, 1975.

Lower specimen: 52 mm. UTMM 2884. Licking River, 328 feet upstream of mouth of Steer Creek, Pendleton Co., KY. July 21, 1984. Glen Fallo.

Plate 120. *Uniomerus declivis* (*Say, 1831*), *Tapered Pondhorn*.—Upper specimen: 70 mm. UTMM 2954. Bear Creek, Hatchie National Wildlife Refuge, S of Brownsville, Haywood Co., TN. August 27, 1983. Don Manning.

Lower specimen: 86 mm. Same as preceding.

Plate 121. *Uniomerus tetralasmus* (*Say, 1831*), *Pondhorn*.—Upper specimen: 81 mm. UTMM 2956. South Fork Forked Deer River, S of Bells, Haywood Co., TN. November 9, 1982. Don Manning.

Lower specimen: 80 mm. UTMM 2952. Creek on Crestview Golf Course, Wichita, Sedgwick Co., KS. July 28, 1983. Kent Vickery.

Plate 122. *Utterbackia imbecillis* (*Say, 1829*), *Paper Pondshell*.—Upper specimen: 78 mm. UTMM 4187. Provenance unknown.

Lower specimen: 71 mm. UTMM 3357. Buffalo River, 0.25 miles E of Beardstown, Perry Co., TN. December 12, 1973. Paul Parmalee.

Plate 123. *Villosa fabalis* (*Lea, 1831*), *Rayed Bean*.—Upper specimen: Female, 30 mm. UTMM 3015. French Creek, Utica, Venango Co., PA. June 1, 1986.

Lower specimen: Male, 38 mm. UTMM 3125. French Creek, Saegertown, Crawford Co., PA. August 22, 1970. Walter Klippel.

Plate 124. *Villosa iris* (Lea, 1829), *Rainbow*.—Upper specimen: Female, 63 mm. UTMM 3027. East Fork Stones River, 0.5 miles N of Sharpville, Rutherford Co., TN. September 7, 1978. Arthur Bogan and Paul Parmalee.

Lower specimen: Male, 76 mm. UTMM 3086. North Fork Holston River, 1 mile upstream of SR 23, Scott Co., VA. August 25, 1977.

Plate 125. *Villosa lienosa* (Conrad, 1834), *Little Spectacle-case*.—Upper specimen: Female, 60 mm. UTMM 12148. Ouachita River, McGuire Access, SR 88, Polk Co., AR. September 12, 1978. Wayne Starnes.

Lower specimen: Male, 70 mm. UTMM 12149. Ouachita River, McGuire Access, SR 88, Polk Co., AR. September 11, 1978. Wayne Starnes.

Plate 126. *Villosa perpurpurea* (Lea, 1861), *Purple Bean*.—Upper specimen: Female, 37 mm. UTMM 3041. Copper Creek, CM 4.0, Scott Co., VA. October 19, 1979.

Lower specimen: Male, 42 mm. Same as preceding.

Plate 127. *Villosa taeniata* (Conrad, 1834), *Painted Creekshell*.—Upper specimen: Female, 66 mm. UTMM 3064. Little Jack Creek, 4 miles NE of Fairview, Fentress Co., TN. May 7, 1980. David Etnier and Bruce Bauer.

Lower specimen: Male, 72 mm. UTMM 3042. Duck River, 2 miles E of Fairview, Coffee Co., TN. October 24, 1973. Paul Parmalee.

Plate 128. *Villosa trabalis* (Conrad, 1834), *Cumberland Bean*.—Upper specimen: Male, 50 mm. UTMM 12150. Rockcastle River, Rockcastle Co., KY. June 1975.

Lower specimen: Female, 49 mm. UTMM 3018. Rockcastle River, SR 80, Rockcastle Co., KY. August 4, 1970.

Plate 129. *Villosa vanuxemensis* (Lea, 1838), *Mountain Creekshell*.—Upper specimen: Female, 64 mm. UTMM 3246. West Prong Little Pigeon River, Sevierville, Sevier Co., TN. January 1, 1986.

Lower specimen: Male, 69 mm. UTMM 3244. Little Pigeon River, SR 66, Sevierville, Sevier Co., TN. March 14, 1987. Paul Parmalee.

Plate 130. *Villosa vibex* (Conrad, 1834), *Southern Rainbow*.—Upper specimen: Female, 70 mm. UTMM 3323. Provenance unknown.

Lower specimen: Male, 66 mm. Same as preceding.

Plate 131. *Corbicula fluminea* (Muller, 1774), *Asian Clam*.—Upper specimen: 50 mm. UTMM 5566. Tennessee River (Fort Loudoun Reservoir), TN.

Middle right specimen: 35 mm. Same as preceding.

Middle left specimen: 34 mm. Same as preceding.

Lower specimen: 43 mm. Same as preceding.

Plate 132A. *Dreissena polymorpha* (Pallas, 1771), *Zebra Mussel*.—(clockwise from top, in mm) 18, 15, 15, 16, 17, 17, 15, 15, 16, 17, 16. UTMM 5567. Provenance unknown.

Plate 132B. *Dreissena polymorpha* (Pallas, 1771), *Zebra Mussel*.—UTMM 5568. Provenance unknown. Attached to *Leptodea fragilis* specimen approximately 87 mm in length.

Plate 133. *Mytilopsis leucophaeata* (Conrad, 1831), *Dark Falsemussel*.—(mm) 23, 22, and 19. UTMM 5569. Provenance unknown (only four of the 12 figured specimens could be located in the UTMM collection).

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NOTE

FRESHWATER MUSSEL ASSEMBLAGE STRUCTURE IN A SMALL EDWARDS PLATEAU IMPOUNDMENT WITH COMMENTS ON CONSERVATION IMPLICATIONS FOR TEXAS FATMUCKET, *LAMPSILIS BRACTEATA* (GOULD 1855)

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ABSTRACT

While freshwater mussels are often negatively impacted by large reservoirs, the influence of smaller low-head dams on resident mussel fauna is variable. A 2017 planned dewatering of Robinson Lake, a small water-supply reservoir located in the Llano River, Texas, presented an opportunity to quantify the native unionid community. We also compared unionid communities between Robinson Lake and a riverine portion of the mainstem Llano River to assess how impoundments may influence assemblage structure, and we evaluated the conservation implications for two Endangered Species Act (ESA) candidate species. In total, we salvaged and relocated 1,012 live unionids representing five species from Robinson Lake, including ESA-candidate species *Lampsilis bracteata* and *Cyclonaias petrina*. Lentic specialists were observed exclusively in Robinson Lake, while lotic specialists and habitat generalists occurred in the Llano River. Though community composition differed, we did observe overlap among sites, suggesting that Robinson Lake contains a subset of the unionid community within nearby riverine reaches, and it supports more lentic-adapted species. Contrary to previous habitat assessments, observations of *L. bracteata* reproduction in Robinson Lake suggests that this species is able to adapt to lacustrine environments and establish populations within small impoundments, though catch rates suggest higher densities in lotic habitats. As increased utilization of water resources and changing climatic patterns continue to impact spring-fed river systems of the Edwards Plateau region, such impoundments may become important conservation units for *L. bracteata* during major drought conditions.

KEY WORDS: community structure, freshwater mussels, Llano River, Texas fatmucket, reservoir, Unionidae

INTRODUCTION

Since the early 20th century, the decline of freshwater mussels has been largely ascribed to the modification and impoundment of rivers and streams (Haag and Williams 2014). Large reservoirs have drastically altered the hydrology and morphology of North American rivers, inundating and fragmenting habitat for many unionids and their host fishes (Bogan 1993; Neves et al. 1997; Watters 2000). Along with the inundation of upstream lotic habitats, large reservoirs may alter the natural hydrology, temperature, and sediment dynamics downstream, which can be harmful to mussels at multiple ontogenetic stages (Layzer and Madison 1995; Haag 2012). Effects of large reservoirs have resulted in the localized extirpation or extinction of multiple freshwater mussel species across the USA (Vaughn and Taylor 1999; Garner and McGregor 2001; Parmalee and Polhemus, 2004; Haag 2009).

While large impoundments pose a major threat to many native unionids through alteration of natural hydrology, temperature, and sediment transport regimes, the influence of smaller low-head dams and associated reservoirs on freshwater mussel populations are more variable (Hart et al. 2002). Low-head dams in several North American Midwest rivers have acted as barriers to freshwater mussels and host fish species, decreasing freshwater mussel occurrence in upstream areas (Watters 1996). Similarly, low-head dams on the Neosho River, Kansas, are thought to influence mussel assemblages within inundated areas, decreasing species richness (Dean et al. 2002). In contrast, several small dams in Alabama have been shown to improve downstream mussel habitat (Gangloff et al. 2011) and actually enhance downstream mussel growth (Singer and Gangloff 2011). Similarly, in Mississippi, diverse and healthy mussel assemblages have been documented downstream of a low-head dam, due to the presence of stable lotic habitats (Haag and Warren 2007). These studies suggest that the influence of small dams on freshwater mussel

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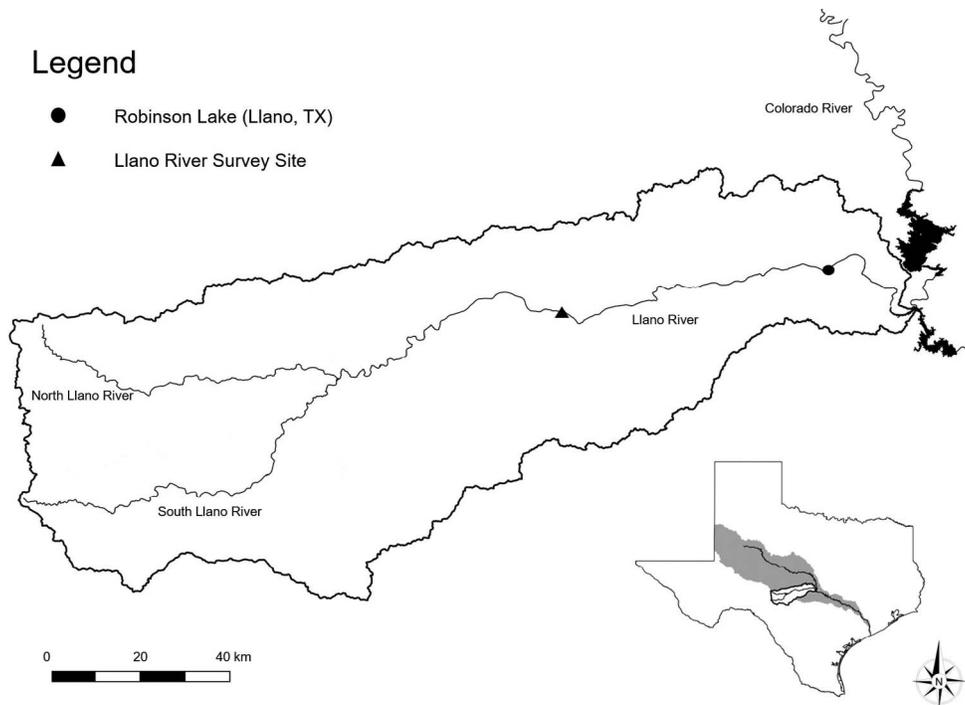


Figure 1. Map of the Llano River drainage area showing both the Robinson Lake salvage site and Llano River qualitative survey site.

populations may differ on a regional, site-specific, or reach scale.

Texas has more dams than any other U.S. state (Shuman 1995), with flood control and water storage impoundments of various sizes located in every major river basin (Zhang and Wurbs 2018). As in other areas, large reservoirs have been shown to negatively impact downstream unionid assemblages (Randklev et al. 2013, 2016; Tsakiris and Randklev 2016), but little information is available on the effects of small low-head dams and reservoirs, including the effects on the mussel communities that inhabit them. Given the number of such dams in the state, this insufficiency represents a critical data gap in the current understanding of Texas freshwater mussel populations.

For this study, a planned dewatering event at a small water supply reservoir in Llano, Texas, presented an opportunity to quantify the existing mussel community. Robinson Lake, also known as Llano Park Lake, is an impoundment created by a low-head dam on the Llano River (COLT 2018). Robinson Lake, along with another similarly sized reservoir downstream, is used by the City of Llano for municipal water supply, serving over 3,000 people (COLT 2018). During recent droughts, the Llano River at Llano stopped flowing, leaving the city's water supply dependent upon storage in the reservoirs until flows returned. As a result, Robinson Lake was purposefully drained in the fall of 2017 to dredge sand that had accumulated over the years, reducing storage capacity. A preliminary survey documented Texas fatmucket *Lampsilis bracteata* (Gould 1855) in Robinson Lake. Therefore, an aquatic resource relocation was conducted to minimize

impacts to unionids during the dewatering process. Data collected during the drawdown summarize the unionid community present in this small reservoir, and supplement the available information on habitat utilization of *L. bracteata*, an Edwards Plateau endemic and Endangered Species Act (ESA) candidate species (USFWS 2011).

METHODS

The Llano River is a major tributary of the Colorado River in the Edwards Plateau region of central Texas (Heltmuller and Hudson 2009; Perkin et al. 2010). It begins near Junction, Texas (Kimble County), at the confluence of the North Llano River and South Llano River, flowing approximately 161 km east until draining into Lake Lyndon B. Johnson, a mainstem impoundment of the Colorado River (Perkin et al. 2010). Robinson Lake is an approximately 0.40-km² reservoir located in the lower portion of the basin (Fig. 1). The climate in the watershed from west to east exhibits a semiarid to subhumid gradient (Heltmuller and Hudson 2009). Karstic spring discharges in the North Llano and South Llano rivers provide perennial flows to the mainstem Llano River (Heltmuller and Hudson 2009; Perkin et al. 2010). Despite the perennial spring sources, the Llano River at Llano, Texas, which is distant from spring sources, has experienced zero-flow conditions during recent droughts (BBEST 2011).

From November 29 through December 1, 2017, as water levels in Robinson Lake were drawn down, a four- to eight-person crew systematically surveyed for emerged unionids for 95 person-h along the water's edge in recently desiccated

portions of the reservoir, totaling 0.35 km² of search area. Salvaged unionids were placed into transport containers with water that was changed periodically. All unionids were compiled intermittently, identified to species, enumerated, and measured to the nearest millimeter. Following mussel collections, the majority of the unionids were translocated to the previously identified relocation site downstream. The site was located approximately 2.5 km downstream of Robinson Lake, after the Llano River transitions back to riverine conditions. This reach of the Llano River is expected to exhibit more perennial flows than areas immediately upstream of the reservoir and contains habitat that was consistent with previously documented occupied reaches (Randklev et al. 2017). A small portion of *L. bracteata* collected were shipped alive to Auburn University and U.S. Fish and Wildlife hatcheries as part of an ongoing research project (Bonner et al. 2018).

Additionally, we compared the Robinson Lake unionid community with a nearby riverine mussel community to explore differences in assemblages among lacustrine and riverine habitats. These data were based on a March 2017 qualitative survey we conducted in the mainstem Llano River, upstream of Robinson Lake (Fig. 1). This site was located in a perennial reach sustained by spring discharges (Randklev et al. 2017), with shallow run and pool sequences dominated by limestone bedrock. The main goal of this survey was to identify potential broodstock populations for the propagation of ESA candidate species Texas pimpleback *Cyclonaias petrina* (Gould 1855) and *L. bracteata*, and secondarily to identify what other taxa might be present. We used visual and tactile search methods, focusing our efforts in high-quality unionid habitat within all available mesohabitat types. Unionid sampling was completed based on when surveyors were confident that all mesohabitats were thoroughly searched, resulting in a 3-person-h search effort within an approximately 1,500-m² area. To summarize the Robinson Lake and Llano River mussel assemblages, we present raw abundance, relative abundance ([species total/total catch] • 100), catch per unit effort (mussels collected/person-h), and length frequency distributions.

RESULTS

We collected and salvaged a total of 1,012 live unionids representing five species from Robinson Lake. *Lampsilis bracteata* and paper pondshell *Utterbackia imbecillis* (Say 1829) were the most abundant species observed, accounting for 62.7% ($n = 635$) and 36.2% ($n = 366$) of the community, respectively. We also observed *C. petrina*, pimpleback *Cyclonaias pustulosa* (Conrad 1835), and lilliput *Toxolasma parvum* (Barnes 1823), which in aggregate accounted for 1.1% ($n = 11$) of the Robinson Lake community (Table 1). Moreover, we observed 15 gravid female *L. bracteata*, which were translocated to U.S. Fish and Wildlife hatcheries for propagation efforts. The overall size distribution of *L. bracteata* exhibited a left-skewed distribution, with about

Table 1. Relative abundance (raw abundance in parentheses) and catch per unit effort (CPUE; mussels/person-h) of live unionids collected during a 2017 salvage effort conducted in Robinson Lake.

Scientific Name	Common Name	Relative Abundance	CPUE
<i>Cyclonaias petrina</i>	Texas pimpleback	0.1 (1)	0.01
<i>Cyclonaias pustulosa</i>	Pimpleback	0.9 (9)	0.10
<i>Lampsilis bracteata</i>	Texas fatmucket	62.7 (635)	6.68
<i>Toxolasma parvum</i>	Lilliput	0.1 (1)	0.01
<i>Utterbackia imbecillis</i>	Paper pondshell	36.2 (366)	3.85
	Totals	1,012	10.65

50% ($n = 310$) ranging from 55 mm to 60 mm. We observed approximately 6% ($n = 35$) of *L. bracteata* collected at lengths 30 mm or less, including several individuals ranging from 12 mm to 15 mm. *Utterbackia imbecillis* exhibited a right-skewed distribution, with about 50% ($n = 59$) ranging from 45 mm to 55 mm. We also frequently observed individuals ranging from 60 mm to 90 mm, but we did not observe many individuals 30 mm or less. *Cyclonaias pustulosa* ranged from 36 mm to 57 mm, while the single *C. petrina* and *T. parvum* lengths were 51 mm and 21 mm, respectively (Fig. 2).

We found a total of 102 mussels representing four species at the Llano River site. *Cyclonaias petrina* and *L. bracteata* were the dominant species, comprising 61.8% ($n = 63$) and 21.6% ($n = 22$) of the mussels observed, respectively. Creeper *Strophitus undulatus* (Rafineque 1820) represented 15.7% ($n = 16$) of unionids collected, while pistolgrip *Tritogonia verrucosa* (Rafineque 1820) was the least abundant at 1.0% ($n = 1$) (Table 2). In general, the size distribution of the species observed displayed a left-skewed distribution. Approximately 75% of *C. petrina* ($n = 46$) and *L. bracteata* ($n = 16$) ranged from 40 mm to 50 mm; similarly, 75% of *S. undulatus* had shell lengths between 50 mm and 60 mm (Fig. 3). Lastly, the shell length of the single *T. verrucosa* was 52 mm.

We observed greater overall catch rates at the Llano River (33.99 mussels/person-h) than at Robinson Lake (10.65 mussels/person-h). Only *C. petrina* and *L. bracteata* were observed at both sites. Catch rates of *C. petrina* were much higher at the Llano River compared to Robinson Lake, totaling 21.00 mussels/person-h and 0.01 mussels/person-h, respectively. In contrast, *Lampsilis bracteata* catch rates were

Table 2. Relative abundance (raw abundance in parentheses) and catch per unit effort (CPUE; mussels/person-h) of live unionids collected during a 2017 freshwater mussel survey in the Llano River.

Scientific Name	Common Name	Relative Abundance	CPUE
<i>Cyclonaias petrina</i>	Texas pimpleback	61.8 (63)	21.00
<i>Lampsilis bracteata</i>	Texas fatmucket	21.6 (22)	7.33
<i>Strophitus undulatus</i>	Creeper	15.7 (16)	5.33
<i>Tritogonia verrucosa</i>	Pistolgrip	1.0 (1)	0.33
	Totals	102	33.99

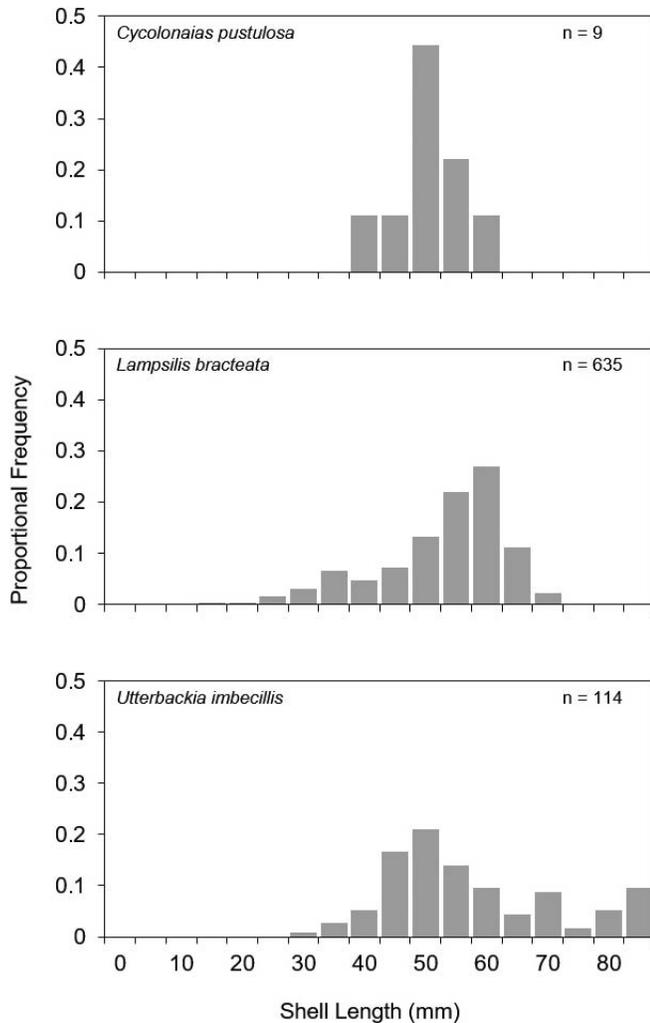


Figure 2. Histograms comparing proportional frequency of shell length for *Cyclonaias pustulosa*, *Lampsilis bracteata*, and *Utterbackia imbecillis* in Robinson Lake. Shell lengths are grouped into 5-mm bins.

generally similar among sites, with 6.68 mussels/person-h at Robinson Lake and 7.33 mussels/person-h at the Llano River (Tables 1 and 2).

DISCUSSION

We observed five species of unionids and multiple size classes in Robinson Lake. Species richness was similar to Llano River observations, though community composition differed. *Cyclonaias petrina* and *L. bracteata* were the only species that occurred at both study sites. *Cyclonaias petrina* was the dominant species at the Llano River but was represented by only a single individual at Robinson Lake. In contrast, *L. bracteata* represented a larger percentage of the Robinson Lake community. The core difference in community composition was the variation in occurrence among the nonoverlapping species. Lentic specialist *U. imbecillis* was observed exclusively in Robinson Lake and characterized over a third of the community. We also observed the lentic-adapted

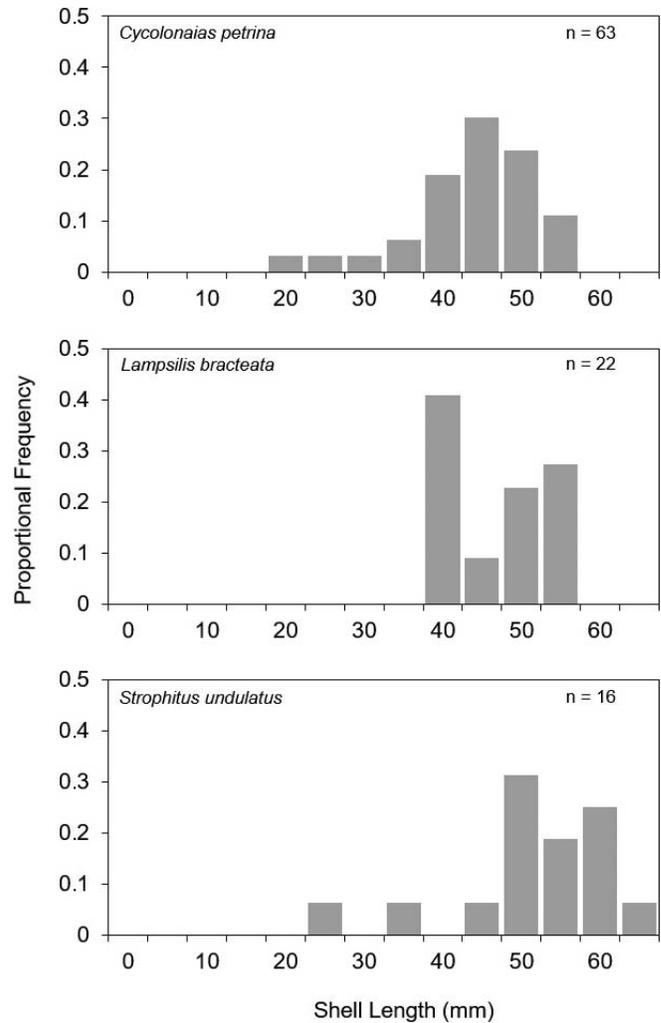


Figure 3. Histograms comparing proportional frequency of shell length for *Cyclonaias petrina*, *Lampsilis bracteata*, and *Strophitus undulatus* in the Llano River. Shell lengths are grouped into 5-mm bins.

T. parvum in Robinson Lake, though we found only a single individual. *Strophitus undulatus* and *T. verrucosa* were observed only in the Llano River. *Strophitus undulatus* is adapted to both lentic and lotic conditions (Howells 2014), and only a single *T. verrucosa* was observed. These differences support previous studies showing that small riverine impoundments contain a subset of the unionid community within surrounding riverine reaches, with the addition of more lentic-adapted species (Ahlstedt and McDonough 1993; Haag 2012; Pilger and Gido 2012). At Wheeler Reservoir, Tennessee, Ahlstedt and McDonough (1993) observed an incursion of lentic-adapted species, congruent with our observations in Robinson Lake.

Although *L. bracteata* exhibited a higher raw abundance and relative abundance at Robinson Lake, catch rates of the species were proportionate between Robinson Lake and the Llano River. However, the area surveyed was substantially greater in Robinson Lake compared to the Llano River. Unionid surveys in the Pedernales, Llano, and San Saba rivers

exhibited *L. bracteata* catch rates similar to those observed here, but in an even smaller search area of 150 m² (Randklev et al. 2017), which supports the finding that *L. bracteata* occur in higher densities in lotic systems. Despite this, the presence of gravid females and juveniles 30 mm or less suggests a reproducing population of *L. bracteata* in Robinson Lake. A previous study on population demographics of the wavyrayed lampmussel *Lampsilis fasciola* (Rafinesque 1820), a congener of *L. bracteata* (Inoue et al. 2019), aged individuals with shell lengths 20 mm to 30 mm as year-1 and year-2 juveniles, and individuals 20 mm or less as year-0 juveniles (Jones and Neves 2011). This supports recent recruitment of *L. bracteata* in Robinson Lake, though additional research on shell length–age relationships specific to *L. bracteata* are warranted.

Observations of successful spawning and recruitment suggests that *L. bracteata* can persist in lacustrine environments and establish populations within small impoundments. Similar observations of *Lampsilis* species have been observed within Midwest and Southeast U.S. drainages (Coker et al. 1921; Bogan 2002). Based on our results, impoundments may serve as potentially important conservation units for this species, and may be especially important given recent droughts in the Llano River basin.

Recent research of the effects of desiccation on *L. bracteata* documented that once completely emerged, 50% of *L. bracteata* died within approximately 2 d at an exposure temperature of 25°C, confirming that desiccation during extended periods of low flow may be detrimental to this species (Bonner et al. 2018). Increased utilization of water resources may exacerbate future drought conditions in the Llano River basin, increasing the intensity and duration of extreme low-flow events and potentially increasing the number of *L. bracteata* impacted by desiccation. Drought contingency plans typically outline hierarchical steps to decide if intervention and relocation of unionids is warranted for populations at risk of extirpation. Unionid relocation in situ is preferable, with ex situ (e.g., hatchery) relocation suggested as a last resort due to observed declines in unionid body condition and survival in hatchery environments (Newton et al. 2001). Robinson Lake could serve as a replacement for ex situ translocation for *L. bracteata* in the event that the main channel of the river is mostly desiccated and the reservoir maintains water. It should be emphasized that Robinson Lake would not be appropriate refugia for other rare species. For example, we observed only a single *C. petrina* at Robinson Lake, strongly supporting the idea that impoundments are detrimental to this species. Further investigation on why some species exhibit habitat pliability, while others do not, is warranted, and may be important for future habitat conservation of rare species.

In conclusion, results of this salvage effort offer valuable insight into the unionid assemblage within a small Edwards Plateau impoundment and how species composition compares to nearby riverine assemblages. These data refine the current knowledge on habitat utilization of two ESA candidates, *C. petrina* and *L. bracteata*. *Cyclonaias petrina* was far more

common in riverine collections, while *L. bracteata* was well represented in both Robinson Lake and the Llano River. Contrary to previous habitat assessments (Howells et al. 1996; Howells 2014), this finding suggests that small reservoirs could serve as habitat for *L. bracteata*. As climate change and increased groundwater and surface water withdrawal (Bowles and Arsuffi 1993) continue to affect spring-fed river systems of the Edwards Plateau region, such small reservoirs could become crucial conservation units in the effort to preserve rare and endemic species.

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REGULAR ARTICLE

TRANSGENERATIONAL EFFECTS OF COPPER ON A FRESHWATER GASTROPOD, *PLANORBELLA PILSBRYI*

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ABSTRACT

Although much less common in ecotoxicology than traditional single-generation studies, multigenerational studies may offer a deeper understanding of the chronic and population-level effects caused by contaminants. To evaluate the potential utility of multigenerational contaminant studies and develop a feasible generational test design, we conducted a two-generational toxicity test using the freshwater File Ramshorn snail (*Planorbella pilsbryi*). Adults were exposed to five sublethal concentrations of copper, which resulted in significant delays in reproduction with increasing copper exposure and complete reproductive inhibition at the highest concentration (75.0 µg/L). Mortality and inhibition of reproduction were not observed in the control and three lowest concentrations (4.7, 9.4, and 18.8 µg/L Cu) over the course of the exposure and during recovery in clean water, indicating no lasting adverse effects. However, subsequent exposure of the unexposed juveniles that were produced during the recovery period (i.e. those not directly exposed to copper) showed that juveniles born to copper-exposed parents (LC50: 11.57 µg/L Cu; 95% CI: 3.71–19.43 µg/L Cu) were significantly less tolerant to copper exposure than juveniles born to unexposed parents (LC50: 29.25 µg/L Cu; 95% CI: 22.17–36.32 µg/L Cu). Despite no obvious changes in parental reproductive success, the fitness of unexposed juveniles was compromised due to parental exposure.

KEY WORDS: molluscs, multigenerational, ecotoxicology, copper, snails

INTRODUCTION

In the environment, an organism may be exposed to numerous contaminants over its lifetime. Due to the size and longevity of some organisms, as well as constraints on time and space in laboratory studies, single-generation/single-lifestage toxicity tests are far more common for ecotoxicological risk assessment (Kimberly and Salice 2015). However, it is now understood that exposure to certain stressors can have impacts extending far beyond the generation of exposed individuals. Single-generation/single-lifestage tests may not reveal chronic effects that may occur in the environment. Environmental stressors, both natural and anthropogenic, can be strong drivers for change within a population, and over time, they may trigger acclimation, cause genetic adaptation, or weaken the population to the point of extinction (Lagisz and

Laskowski 2008). Exposure to contaminants or stressors may lead to changes in an organism's behavior, physiology, or diet or in its interactions with the environment, which may directly alter the organism's fitness and lead to subsequent changes in the fitness of its unexposed offspring (Plautz and Salice 2013). As a result, exposure history may influence the risks posed by a contaminant to the current population. The alteration of risk due to past exposure events may intensify with increasing frequency or duration of exposure, thus limiting the predictive power of a single-generation risk assessment (Kimberly and Salice 2014).

These changes, termed multigenerational effects, are nongenotypic alterations in life history traits that may be passed on as a result of one or more of the following factors: changes in parental diet or environment; transfer of immune system factors, hormones, or organelles; phenotypic plasticity; and epigenetic effects, which are defined as heritable changes in gene function or expression in the absence of changes to the

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Table 1. Summary of a range of observed changes in offspring fitness due to parental exposure as reported in various multigenerational studies.

Species	Parental Stressor	Multigenerational Endpoint	Effect	Source
Increased offspring fitness due to parental exposure to stressor				
<i>Biomphalaria glabrata</i> (freshwater pulmonate snail)	Predation	Cd tolerance	Decreased	Plautz and Salice 2013
<i>Culicoides furens</i> (biting midge)	Metal exposure Zn, Cr, Ag, Ni, Hg, Pb, Cu, Mn, Cd	Metal tolerance	Decreased	Vedamanikam and Shazilli 2008
<i>Lymnaea stagnalis</i> (freshwater pulmonate snail)	Cd exposure	Cd tolerance	Increased	Reategui-Zirena et al. 2017
<i>Protophormia terraenovae</i> (blow fly)	Cu exposure	Innate immunity	Increased	Pölkki et al. 2012
Decreased offspring fitness due to parental exposure to stressor				
<i>Daphnia magna</i> (water flea)	High temperatures	Oxidative stress	Increased	Bae et al. 2016
<i>Daphnia magna</i> (water flea)	Cd/ high temperature	Cd/ high temperature tolerance	Decreased	Kimberly and Salice 2015
<i>Chironomus tepperi</i> (nonbiting midge)	Poor diet	Zn tolerance	Decreased	Colombo et al. 2014
<i>Orizyas latipes</i> (medaka fish)	Bisphenol-A	Reproduction/embryo mortality	Decreased/ Increased	Bhandari et al. 2015
<i>Physa pomilia</i> (freshwater pulmonate snail)	Cd exposure	Reproduction	Decreased	Kimberly and Salice 2014
No change in offspring fitness due to parental exposure to stressor				
<i>Enchytraeus albidus</i> (potworm)	Metal (Zn, Cd, Cu, Pb)	Metal tolerance (Zn, Cd, Cu, Pb)	No change	Lock and Janssen 2002

DNA sequence (Morgan et al. 2007; Plautz and Salice 2013). These changes in life history may be induced by environmental or anthropogenic stressors and can cause secondary phenotypic changes in organisms not directly exposed to the stressor (Anway et al. 2005; Skinner 2014). The resulting transgenerational impacts are variable and may have positive or negative effects on the fitness of subsequent generations depending on the parental stressor and the multigenerational endpoint used (Table 1). Continued investigation into multigeneration toxicity for a variety of chemicals, stressors, species, and endpoints will be critical in ensuring accurate risk assessment of environmental pollutants.

While research regarding the transgenerational impacts of contaminants is limited, there are even fewer transgenerational studies involving molluscs, especially gastropods (Oehlmann 2007). After arthropods, the Molluscan phylum is the second most species-rich taxonomic group, and the most species-rich Molluscan class is the gastropods (Strong 2008). Additionally, freshwater gastropods make up 80% of freshwater molluscs by species (Brown 2001; Strong 2008). Molluscs are often at higher risk of exposure to elevated concentrations of contaminants because of their sedentary lifestyle, resulting in prolonged exposure compared to more motile organisms (Gao et al. 2017). Additionally, pulmonate gastropods are known to be especially susceptible to accumulating metals from the environment relative to other aquatic organisms (Pyatt et al. 2002). Benthic invertebrates, such as gastropods, are abundant

in freshwater systems and play an important role in nutrient cycling and microbial activity through the breakdown of organic matter and the mixing of surface sediments (Covich et al. 1999). Gastropods and their eggs also represent an important food source for other aquatic organisms and waterfowl, representing up to 60% of the benthic invertebrate biomass and abundance in some freshwater ecosystems (Habdija et al. 1995). Despite their considerable diversity and importance, gastropods are still underrepresented in ecotoxicological studies, and little is known about the multigenerational impacts of contaminants on gastropod populations or the possible cascading impact that transgenerational effects may have on the greater freshwater ecosystem (Lysne et al. 2008; Tallarico 2016).

The objective of this study was to evaluate possible multigenerational effects of contaminants on freshwater gastropods. We used a two-generation study design to follow the influence of a sublethal copper (Cu) pulse exposure on adult freshwater snails and the subsequent generation of unexposed offspring. Copper is a widespread waterborne contaminant that may enter aquatic systems from a range of anthropogenic sources, including mining, municipal and industrial wastewater effluents, and road runoff (CCME 1999). Copper is also a common ingredient in agricultural fertilizers and pesticides, and it can enter the water column via overland runoff or when applied directly to water bodies as an algaecide or molluscicide. In small concentrations, Cu is

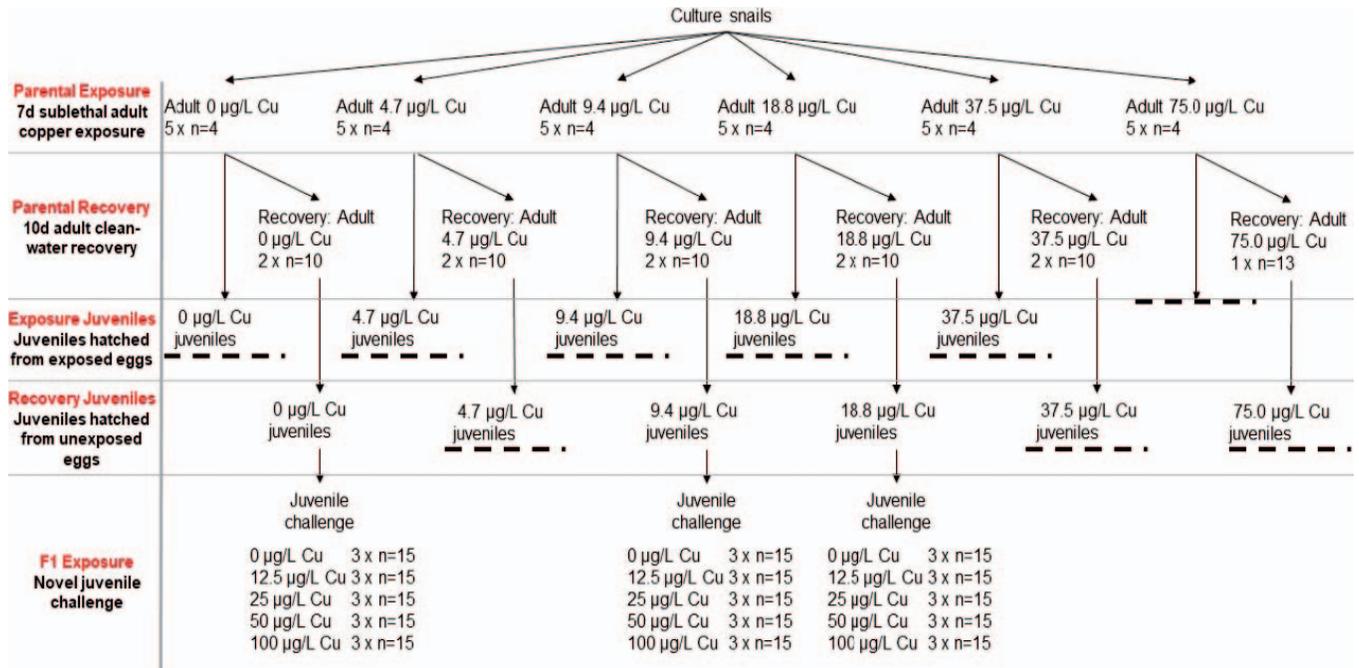


Figure 1. The multigenerational test design used in the present study involved three stages: the Parental Exposure, the Parental Recovery, and the F1 Exposure. The Parental Exposure involved an acute adult copper exposure and resulted in eggs laid during exposure. Following the exposure, adults were moved to clean water for the Parental Recovery phase, which resulted in unexposed offspring born to exposed parents. Finally, these unexposed offspring were subject to a novel juvenile challenge in the F1 Exposure to assess the sensitivity of unexposed juveniles according to their Parental Exposure levels. The dashed lines indicate the termination of a trial. Reproduction was inhibited completely at the highest concentration during the Parental Exposure resulting in no Exposure juveniles. Only juveniles produced during the Recovery stage from the control, 9.4, and 18.8 µg/L Cu treatments were used in the F1 Exposure. The other Recovery juveniles as well as all the Exposure juveniles were omitted due to lack of survivorship and logistical constraints.

essential to metabolic function including mitochondrial activity in most organisms (Gao et al. 2017), and therefore, it must be obtained from the environment. However, above normal background concentrations, copper can cause significant damage to metabolic pathways (Ng et al. 2011). Using Cu as a reference toxicant, this study evaluates whether multigenerational effects can be induced by contaminant exposure and whether fitness and contaminant sensitivity in unexposed offspring are affected by parental exposure.

METHODS

Test Organism

The freshwater gastropod used in this study, *Planorbella pilsbryi*, or File Ramshorn, inhabits lakes, ponds, and slow-moving streams throughout south-central Canada and the northern United States (Clarke 1981; Burch 1982; Johnson et al. 2013). *Planorbella pilsbryi* is pulmonate and hermaphroditic, meaning it has a modified mantle and can breathe air directly and it possesses both male and female genitalia (Clarke 1981). The generation time of *P. pilsbryi* is relatively short (typically 56–63 d), and, under ideal conditions, adults are prolific, making them an excellent model organism for multigenerational studies.

Planorbella pilsbryi were obtained from a culture held at

the Canadian Centre for Inland Waters in Burlington, Ontario, Canada, for several years. The culture was maintained at room temperature in a 16:8 light-dark cycle in several large aquaria fitted with circulatory pumps and aeration. Aquaria water was changed weekly, and snails were fed continuously a diet of organic spinach in excess supplemented with dried shrimp pellets (Shrimp Pellets, Cobalt Aquatics, Rock Hill, SC, USA), algae pellets (Algae Grazers, Cobalt Aquatics, Rock Hill, SC, USA), and calcium carbonate (> 99% purity, Fisher Scientific, Ottawa, ON, Canada) for shell health. Similar-sized adults were randomly selected from culture. The shell length of subsampled snails was measured to characterize the adult snail population used in the test (mean shell length of 11.96 ± 1.04 mm [$n = 20$]). Aquaria, or culturing, water was dechlorinated Lake Ontario water and had a hardness of 126 mg CaCO₃, dissolved organic carbon (DOC) of 0.9 mg/L, and a pH of 8.13. Full characterization of the physicochemical properties of culture and copper exposure water is provided in the Supplemental Information (Tables S1–S4).

Test Design

A multigenerational test, summarized in Figure 1, comprises three stages: a 7-d adult sublethal Cu exposure (Parental Exposure), a 10-d recovery and egg-laying period in clean water (Parental Recovery), and a 72-h juvenile Cu

exposure for the F1 offspring (F1 Exposure). At each stage, several endpoints were observed including: qualitative behavior and feeding; reproductive timing including oviposition periodicity and rate over time; reproductive outputs including number of egg masses and egg counts; developmental markers including egg viability and hatch time; and finally, juvenile mortality as a measure of partial-exposure influence.

Incubators were maintained at 21°C and subject to a 16:8 light-dark cycle. Test solutions were made using a concentrated stock solution of copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Fisher Scientific) diluted with dechlorinated culture water to nominal Cu concentrations of 0, 4.7, 9.4, 18.8, 37.5, and 75.0 $\mu\text{g/L}$ for the 7-d adult exposure and 0, 12.5, 25, 50, and 100 $\mu\text{g/L}$ for the 72-h juvenile Cu challenge. Nominal concentrations were within $\pm 10.8\%$ of measured concentrations, and therefore all concentrations reported in this study are nominal (Table S4 and S5). The Parental Exposure included five replicates of each concentration and four snails per replicate. For the Parental Recovery period, the snails from each parental treatment were randomly reassigned into two replicate test vessels per exposure with 10 snails per replicate for all treatments except the highest (75.0 $\mu\text{g/L}$). In the 75.0 $\mu\text{g/L}$ treatment, seven snails died during exposure, so the 13 remaining were placed in a single test vessel for the Parental Recovery. Finally, the F1 Exposure was a juvenile Cu challenge that involved three replicates per concentration with five juvenile snails per replicate. This format for the F1 Exposure was repeated for the juveniles born during the Parental Recovery phase under pristine conditions to the control, 9.4, and 18.8 $\mu\text{g/L}$ Cu parental exposure groups. The 37.5 and 75.0 $\mu\text{g/L}$ Cu parental exposure groups did not contain enough juveniles to include them in the F1 Exposure because all concentration exposures were simultaneous. The 4.7 $\mu\text{g/L}$ Cu parental exposure group was omitted as well.

Following the initial 7-d Parental Exposure, the adult snails were removed, rinsed, and placed in new 1-L beakers with clean water according to their original exposure level. Adults were kept in the new beakers for the Parental Recovery, a 10-d recovery period during which they laid eggs under pristine conditions. Adults were then removed, and the eggs were left to develop for 3.5 wk. Both the eggs laid during the Parental Exposure in copper-contaminated water, and those laid during the Parental Recovery were rinsed after the removal of the adults and subsequently maintained in dechlorinated culture water that was changed weekly until they hatched. Resulting juveniles born from adults in the Parental Exposure and Parental Recovery were maintained in the same containers under similar conditions and fed spinach for 4 wk.

The final stage involved a juvenile challenge in which 1-mo-old F1 offspring were exposed to Cu for the first time. The F1 Exposure was limited to juveniles that were laid by adults in clean water during the Parental Recovery phase. The juvenile challenge was designed to assess whether parental Cu exposure influenced offspring sensitivity to Cu. This experiment involved a 72-h acute Cu exposure in 250-mL jars under similar conditions as the Parental Exposure. In contrast to the

adult exposure, juveniles in the F1 Exposure were unfed during the exposure as 250-mL beakers were not aerated due to the short duration and lower oxygen demand of the juveniles. This exposure was designed as a factorial mortality test (Fig. 1) in which juveniles born to exposed parents in the Parental Recovery phase were subject to three identical copper toxicity tests in concentrations ranging from 0 to 100 $\mu\text{g/L}$. For example, juveniles born to parents in the Parental Exposure control treatment were randomly assigned to one of the F1 Exposure treatment levels (0–100 $\mu\text{g/L}$) and exposed for 72 h. This was done for juveniles born to parents from the control, 9.4 $\mu\text{g/L}$, and 18.8 $\mu\text{g/L}$ Cu treatments. Juveniles born to the 4.7 $\mu\text{g/L}$, 37.5 $\mu\text{g/L}$, and 75.0 $\mu\text{g/L}$ parental treatment groups were omitted due to logistical constraints and few surviving juveniles in the two highest Parental Exposure groups. Mortality data collected from the juvenile challenge was used to calculate an LC50 value for the juveniles born to each of the Parental Exposure groups tested.

Macrophotography Analysis

To assess the impact of copper on reproduction, several endpoints were measured using macrophotography and digital image processing. The number of egg masses, number of eggs, number of eggs per mass, viability, time to oviposition, and time to hatch were quantified by taking photographs of masses at regular intervals throughout development using an Apple iPhone® 6S (Apple Inc., Cupertino, CA, USA) equipped with an Ollo® Clip macro lens (olloclip; Foothill Ranch, CA, USA). Images were analyzed using ImageJ to count and track output and development (Schneider et al. 2012). Photographs and subsequent measurements were recorded for all eggs produced during both Parental Exposure and Recovery trials across all Cu treatments.

Copper Analysis

Exposure solution samples were taken at the start of the Parental Exposure and pooled across replicates and concentration. A subset of samples was taken at the end of the Parental Exposure to characterize any change in Cu concentration (Table S4). Samples were acidified with HNO_3 (Reagent Grade, Fischer Scientific) to approximately a pH of 1, filtered to 0.45 μm using a syringe-tip filter, and stored at 4°C until analyzed. Concentrations of Cu in water samples were measured using inductively coupled plasma mass spectrometry at Environment and Climate Change Canada's National Laboratory for Environmental Testing (ECCC NLET) using methods developed by the ECCC NLET (Environment Canada 2014). The limit of detection for Cu was 0.02 $\mu\text{g/L}$.

Statistical Analysis

SigmaPlot v14.0 (Systat Software, San Jose, CA, USA) was used to analyze data for distribution, outliers, and

significant differences between treatments and to generate figures.

Adult survival and qualitative behavior.—Adult survival over time was recorded as well as observations on behavior and feeding during the Parental Exposure and Parental Recovery.

Reproductive timing, output, and quality.—Reproductive endpoints (time to oviposition, oviposition rate over time, number of egg masses, number of eggs, number of eggs/mass, egg viability, and time to hatch) were measured for eggs laid during the Parental Exposure and Recovery. Due to the random reassignment of adults into fewer replicate test vessels for the Recovery, the endpoints for both the Parental Exposure and Recovery trials cannot be compared statistically. For the Parental Exposure endpoints, a one-way ANOVA ($\alpha = 0.05$, d.f. = 5) was conducted to compare endpoints among Cu treatments. When the assumptions for a one-way ANOVA could not be met (normality and homoscedasticity), a one-way ANOVA on ranks (Kruskal-Wallis) was conducted instead ($\alpha = 0.05$, d.f. = 5). Where significant differences between treatments were found, a Dunnett's post-hoc test was used to compare treatments to the control treatment ($\alpha = 0.05$). For the Parental Recovery, there were insufficient replicates to perform statistical analyses (0–37.5 $\mu\text{g/L}$ d.f. = 2; 75.0 $\mu\text{g/L}$ d.f. = 1).

The time to oviposition was measured by recording date of oviposition for each egg mass across all replicates and treatments. To incorporate the replicates in which no egg masses were laid throughout the test, time to oviposition was measured as the delay in oviposition by percent of the duration of the test. For example, replicates in which no egg masses were laid received a value of 100% for time to oviposition as no egg masses were laid for the entire duration of the test. Mean time to oviposition as percent test duration was calculated for all egg masses and replicates.

For the purpose of this test, nonviable eggs were defined as those that showed no sign of development after 10 d. In viable eggs, early signs of development were observed between 3 and 5 d. Typical hatching time for eggs laid by unstressed adults at room temperature ($\sim 22^\circ\text{C}$) is approximately 12 d, and no development after 10 d suggests that hatching success is unlikely. Figure S1 illustrates the development of a viable and a nonviable egg.

Juvenile survival and copper sensitivity.—Juvenile sensitivity was assessed in RStudio v.1.1.456 (Ritz et al. 2015; RStudio Team 2016) using the drc library to generate dose–response curves and to calculate the LC50s and 95% confidence intervals for juveniles born to each Parental Exposure grouping (0, 9.4, and 18.8 $\mu\text{g/L}$ Cu). Sample code used in RStudio is provided in the Supplemental Information (Table S6).

RESULTS

Adult Survival and Qualitative Behavior

Parental exposure survival and behavior.—Adult survival was 100% in each of the 0, 4.7, 9.4, 18.8, and 37.5 $\mu\text{g/L}$

treatments and 65% in the 75.0 $\mu\text{g/L}$ treatment (13/20 individuals survived).

Within minutes of the initiation of the sublethal adult exposure, all individuals within the 75.0 $\mu\text{g/L}$ Cu treatment were rendered immobile as well as most individuals in the 37.5 $\mu\text{g/L}$ exposure. These findings are in contrast with those seen in the individuals exposed to lower concentrations, which were active and began eating immediately. Immobility was observed only in the three highest treatment groups and in the first 24 h was 40%, 65%, and 75% for the 18.8, 37.5, and 75.0 $\mu\text{g/L}$ Cu treatments, respectively. Upon completion of the 7-d exposure, all individuals in the 18.8 $\mu\text{g/L}$ Cu treatment, and 95% of those in the 37.5 $\mu\text{g/L}$ Cu treatment, had recovered and had begun eating and laying eggs. None of the individuals in the highest treatment showed any sign of recovery throughout the 7-d exposure. To determine survival in the highest treatment after the 7-d exposure, the retracted foot of each snail was touched with a probe to gauge response. Of the 20 individuals in the 75.0 $\mu\text{g/L}$ Cu treatment, seven individuals were unresponsive and were removed from the study.

Parental recovery survival and behavior.—All individuals that survived the 7-d exposure also survived the 10-d recovery period. In the first 24 h of the recovery period, all snails had resumed eating and oviposition.

F1 exposure survival.—Survivorship between eggs laid during the Parental Recovery and successfully hatched juveniles was not significantly different between exposures, resulting in a mean hatching success rate of 90.6% after 3 wk across all exposures. The highest hatching success occurred in the 75.0 $\mu\text{g/L}$ Cu parental treatment group with 93.6% success, and the lowest hatching success was 88.1% in the 9.4 $\mu\text{g/L}$ Cu parental treatment level. As juveniles laid during the Parental Exposure were not used in the F1 Exposure, the hatchability and survivorship for that cohort was not assessed.

Reproductive Timing

Parental exposure time to oviposition.—An increasing delay in oviposition was observed with increasing copper concentration, likely a consequence of immobility. Delay in oviposition was measured as the number of days to the first oviposition from the beginning of the exposure. No delay in oviposition in the control treatment was observed, but a 2-d, 4-d, and 7-d delay in oviposition was observed in the 4.7–18.8, 37.5, and 75.0 $\mu\text{g/L}$ Cu treatments, respectively (Fig. 2). The 7-d delay observed in the 75.0 $\mu\text{g/L}$ Cu treatment resulted in complete inhibition of oviposition throughout the 7-d exposure (Fig. 2).

Time to oviposition was measured as the percent delay of the total 7-d adult Cu exposure time to account for replicates with no egg masses. In general, mean time to oviposition increased slightly with increasing Cu exposure concentration for eggs laid during the parental exposure (Fig. 2). No egg masses were laid in any of the replicates of the highest

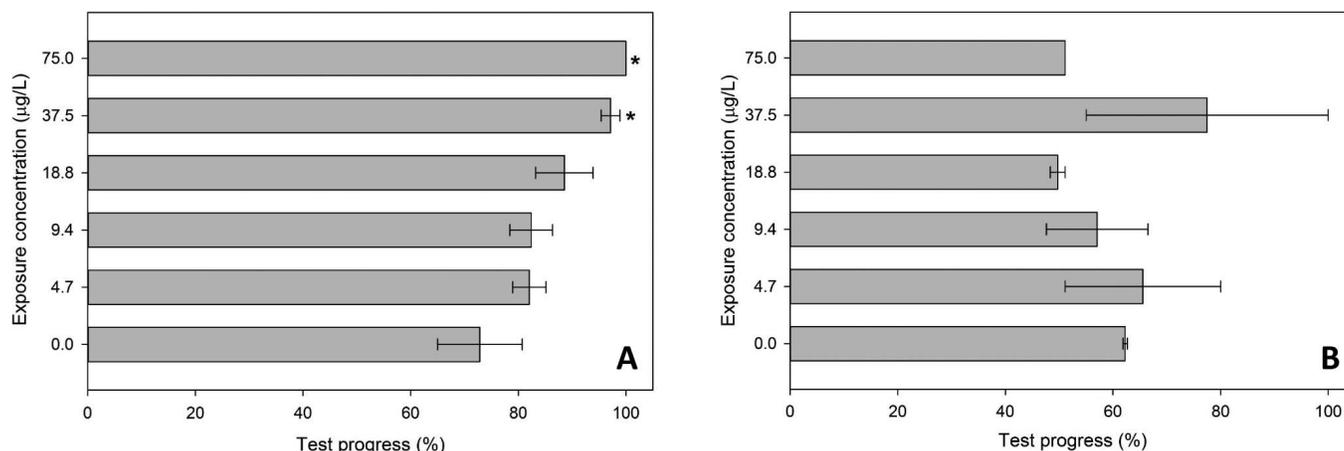


Figure 2. Mean (S1: $n = 4$, S2: $n = 2$) delay in oviposition during the sublethal Parental Exposure (A) and Parental Recovery (B) phases measured as the delay in the percent of the test's progress. Bars represent the mean time along the test progress at which oviposition occurred. Due to complete inhibition of oviposition at the highest treatment level, delay in test progress was used to be able to include these data in our analysis. Since no egg masses were laid in the Parental Exposure 75.0 $\mu\text{g/L}$ (A) treatment, oviposition was delayed by 100% of the test duration. During the Parental Exposure (A), delays in oviposition increase with increasing exposure and statistically significant delays in oviposition were observed at 37.5 $\mu\text{g/L}$ ($P = 0.037$) and 75.0 $\mu\text{g/L}$ ($P = 0.008$). Asterisks indicate statistically significant differences from the control treatment group (Kruskal-Wallis one-way ANOVA on ranks and Dunnnett's post-hoc: $\alpha = 0.05$, $H = 17.83$, d.f. = 5).

treatment (75.0 $\mu\text{g/L}$ Cu), and two replicates in the 18.8 and 37.5 $\mu\text{g/L}$ Cu treatments had no egg masses. Time to oviposition was significantly delayed in the 37.5 and 75.0 $\mu\text{g/L}$ Cu treatments relative to the control ($H = 17.83$; $P = 0.037$ and $P = 0.008$, respectively).

Parental recovery time to oviposition.—Within the first 24 h of the recovery phase, snails in all treatments were laying eggs. In contrast to time to oviposition during the Parental Exposure, there was a slight negative correlation between exposure concentration and mean time to oviposition in clean water during the Parental Recovery (Fig. 2). No obvious delays in oviposition occurred, although a slight delay was noted in the 37.5 $\mu\text{g/L}$ Cu treatment, likely due to the influence of a single replicate with no egg masses. Interestingly, after a complete inhibition of egg laying during the Parental Exposure, adults from the 75.0 $\mu\text{g/L}$ Cu treatment had a slightly lower delay in oviposition than the control treatment during the 10-d recovery period (Fig. 2), which could suggest that snails in the highest treatment accelerated output in comparison to the controls once removed from the Cu exposure.

Reproductive Output

Parental exposure reproductive output.—There were no significant differences in the number of egg masses laid between treatments during Cu exposure (Fig. 3). A slight increase in oviposition in the 4.7 $\mu\text{g/L}$ Cu treatment was observed and mean oviposition tended to decrease with increasing Cu concentration (Fig. 3). Complete inhibition of oviposition was observed in the 75.0 $\mu\text{g/L}$ Cu treatment and, although not significant, was very close to significance ($H = 14.033$; $P = 0.055$) (Fig. 3).

The number of eggs laid per snail was considerably more variable than egg masses per snail, and poor correlation was observed with eggs/snail and Cu exposure concentration (Fig. 3). However, a noticeable decrease in egg production per snail was observed in the 37.5 and 75.0 $\mu\text{g/L}$ treatments, with a 63% and 100% reduction in egg production, respectively (Fig. 3).

Within each egg mass, individual eggs were counted and measured (Fig. 3). As no eggs were produced in the 75.0 $\mu\text{g/L}$ treatment, it was omitted from this analysis. Although no statistically significant differences were observed, there was a trend of increasing eggs per egg mass with increasing copper concentration up to the 18.8 $\mu\text{g/L}$ Cu treatment (Fig. 3). Interestingly, despite a reduction in mean egg masses per snail, the 18.8 $\mu\text{g/L}$ Cu had the highest number of eggs/egg mass or the largest egg masses.

Parental recovery reproductive output.—There was no apparent trend in the number of egg masses laid per snail during the recovery, although treatments with higher outputs tended to coincide with faster mean oviposition (Fig. 3). The two treatments with the slowest mean oviposition were 4.7 and 37.5 $\mu\text{g/L}$ Cu, and they also had the fewest mean egg masses/snail (Fig. 3). Similarly, the 9.4 and 18.8 $\mu\text{g/L}$ Cu treatments had the fastest mean time to oviposition and ultimately resulted in the highest mean number of egg masses produced per snail (Figs. 2, 3). However, despite having faster time to oviposition than the control, the 75.0 $\mu\text{g/L}$ Cu treatment resulted in 57% fewer egg masses produced per snail, indicating that gastropods did not compensate for previous reproductive inhibition during exposure by increasing oviposition during the recovery phase (Figs. 2, 3). The same trends observed for egg masses per snail also applied for the number of eggs laid per snail (Fig. 3).

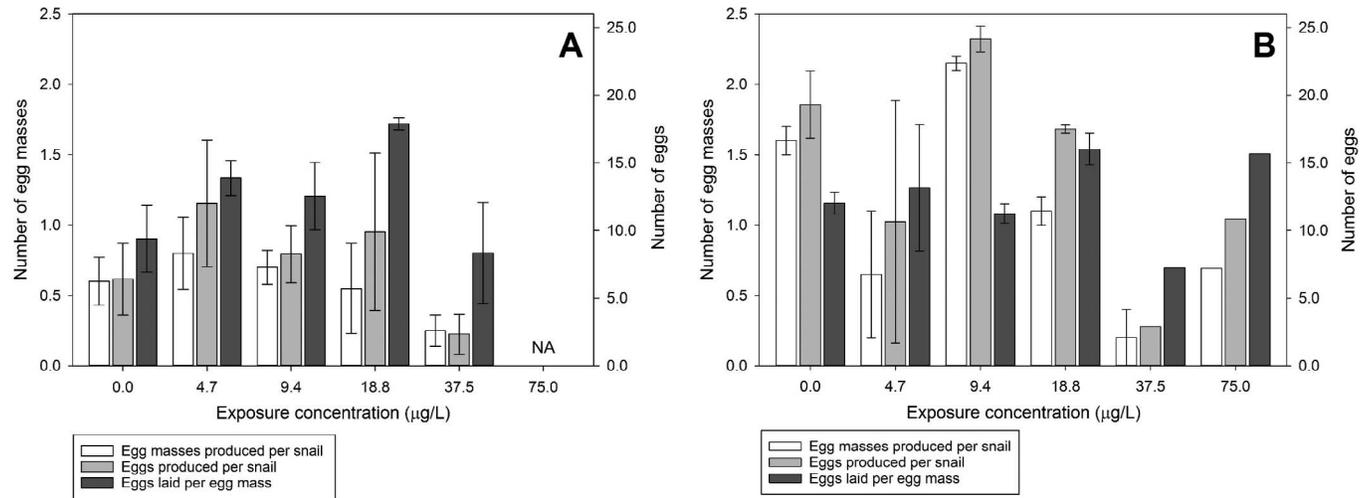


Figure 3. Three measurements of reproductive output for adults during the Parental Exposure (A) and Parental Recovery (B) phases. Mean (Exposure: $n = 4$, Recovery: $n = 2$) number of egg masses laid per snail (white bars) and mean number of eggs laid per snail (gray bars) decreased with increasing Cu exposure in the Parental Exposure with no egg masses or eggs laid at 75.0 µg/L (A) but was not strongly affected once adults recovered (B). The mean (Exposure: $n = 4$, Recovery: $n = 2$) number of eggs laid per egg mass (black bars) was not calculated for the Exposure 75.0 µg/L treatment (A) as no egg masses were laid. In both the Exposure and Recovery reproduction tests, number of eggs per egg mass was not strongly affected by copper exposure. Error bars indicate the standard error of the mean and could not be calculated for the Recovery 37.5 and 75.0 µg/L treatments (B) due to no egg production in some replicates.

The mean number of eggs per mass did not correlate with time to oviposition, unlike the number of egg masses and eggs per snail (Fig. 3). Although a trend was not evident, we observed that the highest treatment (75.0 µg/L Cu) had the highest egg-per-egg-mass ratio (largest egg masses) despite decreased egg production relative to the control and lower treatment groups.

Reproductive Output Quality

Parental exposure viability and time to hatch.—For egg masses laid during the Parental Exposure period, parental exposure, gamete exposure within the parents, and exposure of the egg masses themselves (in ovo exposure) are factors in the health of the F1 juveniles. As the eggs were laid throughout the parental exposure, duration of in ovo Cu exposure depended on time of oviposition and was typically longer at lower treatments due to inhibition of reproduction and delay in oviposition caused at the higher treatments. The maximum in ovo exposure duration for each treatment (Fig. S2) was 4, 5, 4, 1 and 0 d, respectively (Table S5). Mean exposure duration of egg masses was 2 d for the three lower treatments, 1 d for the 37.5 µg/L Cu treatment, and 0 d for the 75.0 µg/L Cu treatment (as no egg masses were laid).

Percent viability remained relatively consistent across all treatments, with greatest variability observed in the control treatment ($71.2\% \pm 18.1$) (Fig. 4). The lower mean and greater variability in egg viability in the control treatment are due to there being one replicate with only one egg mass that contained one nonviable egg, resulting in a value of 0% viability for that entire replicate. This anomalous replicate is

considered a significant outlier ($>3 \cdot \text{IQR}$), and when omitted, the mean percent viability of the control treatment is $89.0\% \pm 4.1$, consistent with the other treatment groups. Time to hatch was calculated for each egg mass as the difference in days between oviposition date and hatch date. No significant differences were observed in time to hatch among the treatments (Fig. 4).

Parental recovery viability and time to hatch.—As the egg masses were laid in clean water during the Parental Recovery phase, parental exposure was the only factor as in ovo exposure did not occur. Viability remained consistent, regardless of parental exposure level for eggs laid during the adult recovery period (Fig. 4).

Time to hatch was relatively consistent and demonstrated no obvious influence of parental Cu exposure level on time to hatch (Fig. S4). However, a slight decrease in time to hatch was observed in the highest concentration (75.0 µg/L Cu) (Fig. 4).

F1 exposure juvenile Cu sensitivity.—Juveniles born to the parental control group, the parental 9.4 µg/L Cu treatment, and the parental 18.8 µg/L Cu treatment were randomly selected and subjected to the same range of Cu concentrations for the F1 Exposure. From these tests, a dose–response curve was created, and LC50s were calculated for juvenile snails based on their parental exposure level. Juvenile Cu sensitivity increased with greater parental exposure. The juvenile LC50 of the highest-tested parental exposure group (18.8 µg/L Cu) was significantly lower ($11.57 \mu\text{g/L Cu}$; 95% CI: 3.71–19.43 µg/L Cu) than that of the parental control group ($29.25 \mu\text{g/L Cu}$; 95% CI: 22.17–36.32 µg/L Cu), demonstrating a significant increase in juvenile sensitivity caused by increased parental exposure to Cu (Table 2).

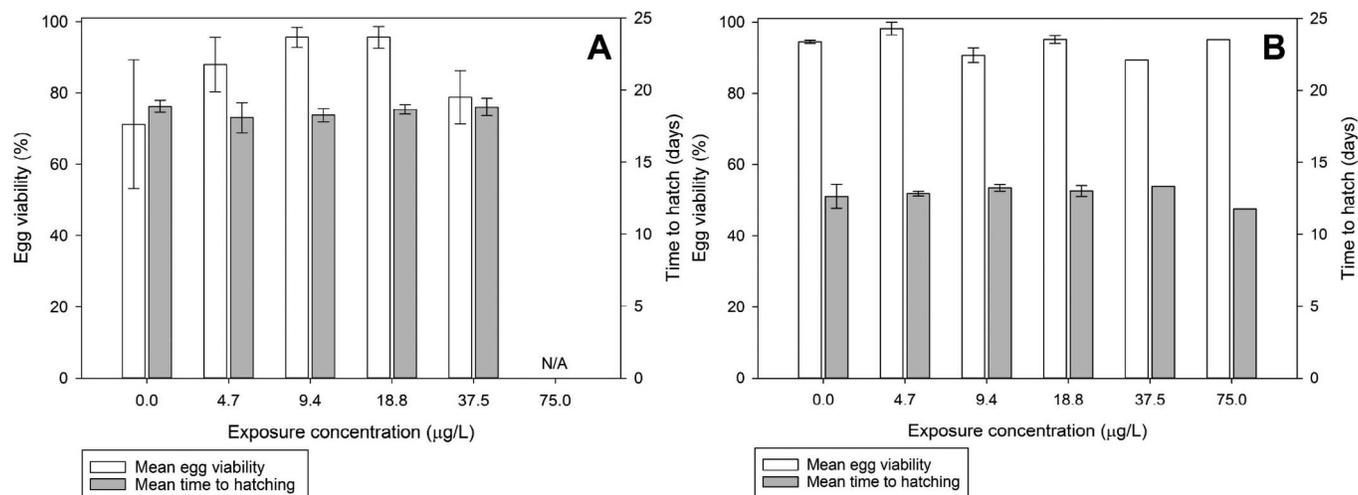


Figure 4. Two measures of reproductive success for juveniles born during the Parental Exposure (A) and Parental Recovery (B) phases. Mean (Exposure: $n = 4$, Recovery: $n = 2$) percent viability of eggs (white bars) was close to 90% across all treatments and was unaffected by the combination of parental exposure and in ovo exposure (A) as well as parental exposure alone (B). Egg viability for this study is defined in Figure S1. Egg viability could not be calculated for the Exposure 75.0 $\mu\text{g/L}$ treatment as no egg masses or eggs were laid. Mean (Exposure: $n = 4$, Recovery: $n = 2$) time to hatch (gray bars) also was unaffected by in ovo and parental Cu exposure for eggs laid during both the Parental Exposure (A) and Parental Recovery (B) phases. Error bars indicate standard error of the mean and could not be calculated for the Recovery 37.5 and 75.0 $\mu\text{g/L}$ treatments (B) due to no egg production in some replicates.

DISCUSSION

Adult Survival and Qualitative Behavior

Despite the inhibition of mobility, feeding, and reproduction in the adults exposed to copper concentrations over 9.4 $\mu\text{g/L}$, all exposed individuals quickly recovered once removed from Cu exposure, and the initial exposure concentration had no influence on recovery time. While Cu is an essential element at very low concentrations, even slight elevations above background Cu levels can have significant adverse effects on freshwater gastropods (Gao et al. 2017). For example, in *Lymnaea stagnalis* juveniles, exposure to increasing concentrations of Cu for 96 h was proportional to decreases in Na and Ca concentrations in the soft tissues, which was hypothesized to cause adverse effects on the nervous and muscular systems (Ng et al. 2011). This mechanism is consistent with the observed inhibition of

Table 2. Estimated LC50 values, standard error, and 95% confidence intervals for the F1 Exposure. Juveniles born during the Parental Recovery to Cu-exposed parents had increased sensitivity to Cu with increasing parental exposure. Parental treatment level is indicated in the first column. As the confidence intervals of the LC50 for juveniles born to the 18.8 $\mu\text{g/L}$ treatment and the control treatment do not overlap, the 18.8 $\mu\text{g/L}$ juveniles are considered to be significantly more sensitive to Cu.

Parental Exposure Level ($\mu\text{g/L}$ Cu)	LC50 ($\mu\text{g/L}$ Cu)	Standard Error	95% CI Lower Boundary	95% CI Upper Boundary
0.0	29.25	3.609	22.17	36.32
9.4	26.65	2.494	21.77	31.55
18.8	11.57	4.011	3.710	19.43

mobility, feeding, reproduction, and eventually death seen in the Parental Exposure phase of our study.

Dissolved organic carbon (DOC) and other sources of organic material can bind to aqueous Cu, reduce its bioavailability, and ameliorate toxicity (Allen et al. 1980; Erickson et al. 1996; Schwartz et al. 2004). Increasing levels of DOC in laboratory exposures have been shown to reduce Cu toxicity to molluscs (Gillis et al. 2008, 2010; Wang et al. 2009). In the Parental Exposure, all replicates were fed identical quantities of spinach and contained the same number of snails, in an effort to limit variation in organic matter and thus variation in bioavailable Cu. The possible sources of organic material included the test organisms, spinach feed, egg masses laid during the test, and feces. However, due to the differing responses to Cu toxicity (inhibition of reproduction and feeding), the organic matter produced and present in each vessel also changed throughout the test. For example, in lower treatments, reproduction was not inhibited, and a number of egg masses were produced which could act as a possible Cu sink. In contrast, reproduction was inhibited in higher concentrations, meaning that there were no egg masses to act as a potential Cu-binding sink. Due to feeding inhibition in higher Cu treatments, uneaten spinach would potentially bind Cu, in contrast to the lower treatments in which snails consumed most of the spinach. Despite possible variations in organic matter present in each vessel and over time, a clear concentration-response relationship was observed for the inhibition of reproduction and feeding. Increasing Cu was bioavailable in proportion to the exposure concentrations and not likely ameliorated in any one treatment due to increased feeding or reproduction.

Reproductive Timing

Exposure to sublethal concentrations of Cu for 7 d caused significant delays in oviposition and inhibition of reproduction with increasing concentration (Figs. 2, 3). However, once Cu exposure was terminated, these effects dissipated, and oviposition returned to levels within the control range. Similar behavioral responses were seen by Gao et al. (2017) with exposure of the freshwater pulmonate snail *Physella acuta* to Cu. Exposure to 40 µg Cu/L resulted in inhibition of movement and reproduction in the first 24 h of exposure, and complete inhibition was observed at 80 µg Cu/L in water with a hardness of 84.8 mg/L as CaCO₃ (Gao et al. 2017).

In the Parental Recovery, snails in the highest treatment (75.0 µg/L Cu) were the first to begin laying egg masses, possibly indicating an overcompensation mechanism after the week-long inhibition of mobility and reproduction. The same species (*P. pilsbryi*) demonstrated recovery with overcompensation in reproduction when exposed to sublethal concentrations of the surfactant MON 0818, resulting in significantly higher egg production in treatment groups exposed to the highest concentrations compared with those that were not exposed (Prosser et al. 2017). This response is similar to what was observed in the current study with exposure to sublethal concentrations of Cu resulting in faster oviposition (Fig. 2; 11% faster in 75.0 µg/L treatment compared with control treatment during the recovery phase) and more eggs produced per mass (Fig. 3; 30% more eggs/mass than control). However, we observed that sublethal Cu exposure (>18.8 µg/L Cu) caused reduced egg production compared with the control even after recovery (Figs. 3, 4; 57% fewer egg masses/snail and 44% fewer eggs/snail).

Additionally, there is possible evidence for an energetic trade-off between number of egg masses laid and the number of eggs per mass. During the Parental Exposure, the minimum egg mass size (measured as number of eggs/mass) increased with increasing Cu concentration until the highest two treatments, in which a significant decrease in egg mass production was observed. For Parental Recovery egg masses, this trend was less pronounced, but elevated egg-to-mass ratios were observed in the highest treatment, despite relatively lower reproductive output compared with control and lower exposures. It is possible that, rather than laying more egg masses (which would require more laying events), individuals instead invested energy toward increasing egg numbers laid during each event to maximize offspring survival under Cu-contaminated conditions. It is widely accepted that body size is the primary driver of reproductive output, with larger individuals tending to produce more eggs (DeWitt 1954; Norton and Bronson 2006). In the closely related freshwater pulmonate snail *Helisoma trivolis*, Norton and Bronson (2006) observed that while there was a significant positive relationship between body size and egg production, body size accounted for only 24% of the variability in eggs laid per mass. The clear growth-reproduction trade-off described by Norton and Bronson (2006) is further supported by Koene and Maat's (2004) calculation of energy use in growth and

reproduction in *Lymnaea stagnalis*. Snails reared in isolation had a mean dry weight 11.3% higher than those raised in groups that were actively reproducing, and 9.5% of total energy intake by group-reared animals was allotted to egg production, accounting for observed differences in final average dry weight between the two test groups (Koene and Maat 2004). Additionally, Ng et al. (2011) suggested that the lack of growth in *L. stagnalis* exposed to sublethal concentrations of Cu was due to energy use for detoxification rather than growth. These studies demonstrate that snails in stressful conditions employ energetic trade-offs relating to reproduction and toxicity. It is possible that the variability in egg mass production observed in the current study is controlled by factors related to resource availability rather than body size alone, and that under stressed conditions, resources may be allocated to survival and detoxification rather than reproduction or growth.

Reproductive Output and Quality

Although reproductive output quantity was affected by exposure to Cu both during exposure and in recovery, the viability of eggs laid during both the Parental Exposure and Parental Recovery phases was unaffected by copper concentration (Fig. 4). During the Parental Exposure stage, some eggs also experienced in ovo exposure for a maximum of 5 d, depending on date of oviposition. For example, eggs laid toward the beginning of the Parental Exposure remained in the exposure solutions with the parents for the remainder of the 7-d test. However, viability remained consistently close to 90% for all treatments, with the exception of one anomalous egg mass in the control treatment of the Parental Exposure (Fig. 4). All eggs laid during Parental Recovery were unexposed and influenced only by the treatment of the Parental Exposure. Viability of eggs was constant among all treatment groups whether the eggs were laid during Cu exposure or recovery.

As a measure of developmental delays, time to hatch also appeared to be unaffected by both parental exposure and in ovo exposure, remaining constant across all treatment for eggs laid during both the Parental Exposure and Recovery (Fig. 4). This suggests that for sublethal Cu exposure, if egg masses are successfully laid, there are minimal effects on the hatching success of the embryo due to parental exposure and in ovo exposure. In contrast, Cd exposure in another pulmonate, *L. stagnalis*, resulted in decreased viability and increased time to hatch under exposure and recovery conditions (Reategui-Zirena et al. 2017). Similarly, both parental exposure and in ovo exposure to environmentally relevant concentrations of Cd in *Physa pomilia* influenced offspring tolerance to Cd with in ovo exposure being a stronger driver of change in offspring tolerance (Plautz and Salice 2013). This difference in response to Cd could be influenced by a number of factors, including variability in the sensitivity of the two species or variability in the protection offered by the gelatinous matrix of the egg mass between species and metals (Pechenik 1979; Przeslawski 2004).

Although time to hatch was relatively consistent within each test stage, hatch time varied slightly for eggs laid during the Parental Exposure and those laid during Parental Recovery (Fig. 4). Mean hatch time across all treatments for the Parental Exposure was 18.3 ± 1.4 d, compared with 12.9 ± 0.6 d for Parental Recovery. While temperature and light regime were the same for both Parental Exposure and Parental Recovery, the two experiments were conducted in different incubators. However, no direct comparisons between hatch time or other endpoints were made between eggs laid in the Parental Exposure and those laid during the Parental Recovery.

Juvenile Survival and Copper Sensitivity

Results from the parental exposure to Cu and recovery alone suggest that waterborne Cu exposure in lab-exposed pulmonate snails does not induce lasting effects on assessed endpoints, if a population survives initial exposure. Survival, feeding, behavioral, and reproductive endpoints measured during the Parental Exposure clearly demonstrate a dose-response relationship with increasing Cu exposure causing increasing adverse effects. These endpoints returned to control levels during the Parental Recovery, and the quality of reproductive output (viability and time to hatch) was unaffected by both parental exposure and in ovo exposure.

Additionally, parental treatment level appears to have minimal impact on hatching success and early juvenile survivorship in the generation laid during the Parental Recovery period. However, the subsequent juvenile Cu challenge clearly demonstrates a reduction in Cu tolerance due to parental Cu exposure (Fig. S3). Copper sensitivity of juveniles raised under pristine conditions increased with increasing parental exposure and was significantly higher in juveniles born to the highest-tested parental treatment group compared with juveniles born to unexposed control parents (Table 2).

While in ovo exposure of the egg masses laid during the Parental Recovery did not occur, it is possible that the gametes experienced copper exposure during the sublethal Parental Exposure before being laid in clean water during the Recovery. Pulmonate snails, including our test species, receive sperm and may store it for long periods of time, with the potential for both eggs and sperm to be exposed to a contaminant within the parent (Norton and Bronson 2006). While fertilization is internal, to the best of our knowledge, egg masses are deposited fairly quickly after fertilization, given that in several developmental studies involving pulmonates, the earliest stages of development can be seen occurring outside of the parent (Brown 2001; Khangarot and Das 2010; Bandow and Weltje 2012). As such, we conclude that negligible in ovo exposure occurs in the developing embryo except when the embryo itself is exposed to a contaminant.

Although the juveniles used in the F1 Exposure likely experienced negligible in ovo exposure before being laid during the Parental Recovery, their gametes were potentially

exposed to Cu within the parents during the sublethal exposure. However, since egg viability and time to hatch were unaffected by the Parental Exposure concentration, we can conclude that any internal copper exposure did not prevent the gametes from successfully fertilizing and developing into a viable offspring. This suggests that the significant difference in juvenile Cu sensitivity may be attributed to either latent influence of gamete exposure, indirect effects caused by parental exposure, or a combination of both. Additional studies are needed to discern the possible influence of each of these exposure routes on juvenile fitness and sensitivity, including the use of a depuration period between exposure and recovery to minimize the influence of gamete exposure as well as further toxicity testing using embryos to allow us to better account for effects due to in ovo exposure.

Stream water quality monitoring data collected by the Ontario Provincial Water Quality Monitoring Network (PWQMN) throughout 2016 demonstrate that Cu is ubiquitous in the environment and that concentrations can reach or exceed the reproductive and multigenerational effect concentrations produced in the present study (Ontario Ministry of the Environment 2016). As this is not an exhaustive evaluation of Cu in the environment, it is important to note other modifying factors that can affect bioavailability and thus the toxicity of Cu in natural aquatic systems. For example, Gillis et al. (2010) reported that Cu EC50s were up to three-fold higher when freshwater mussel glochidia were exposed in Cu-augmented natural water compared to Cu-augmented reconstituted laboratory water. While we cannot comment on how frequently Cu concentrations in the environment may exceed the multigenerational effect concentrations reported here without accounting for possible modifying factors, our study demonstrates that even one pulse event at potentially environmentally relevant concentrations can cause significant detriment to the next generation.

Multigenerational Ecotoxicological Studies

Both historical exposure as well as developmental exposure to environmental stressors can cause latent or transgenerational effects later in life or even in future generations (Salice et al. 2010; Kimberly and Salice 2014). Multigenerational studies of these effects are much less common in ecotoxicology than the standard single-generation and single-lifestage tests. One major reason for the lack of multigenerational research is that studies spanning multiple generations can involve substantial logistical challenges especially for larger or long-lived organisms. However, our study, among other recent investigations, demonstrates that transgenerational effects caused by previous contaminant exposure may pose additional risks to future generations of offspring, a reality that adds complexity to environmental risk assessment. There is evidence that gastropods are prone to experiencing transgenerational effects as seen in Cd exposure of *Physella pomilia* and *Biomphalaria glabrata*, as well as increased multigenerational Cu burden in *Pomacea paludosa*

(Rogevich et al. 2009; Salice 2010; Kimberly and Salice 2014).

Although a number of mechanisms are commonly associated with inducing transgenerational effects, DNA methylation is the most well-studied mechanism and considered to be the most important (Bombail et al. 2004; Vandegehuchte and Janssen 2011). Exposure of adult *P. pomilia* to sublethal concentrations of the pharmaceutical prednisolone resulted in reduced fecundity and increased juvenile developmental abnormalities and mortality of the F1 with increasing exposure concentration and duration (Bal et al. 2017). With subsequent prednisolone exposure in the F2 generation, developmental abnormalities occurred at lower concentrations than in the F1 (Bal et al. 2017). Additionally, this study demonstrated the presence of a relationship between DNA methylation and multigenerational effects, with DNA methylation decreasing with increasing prednisolone concentration in the F1 (Bal et al. 2017). Furthermore, metals such as cadmium have been shown to induce DNA methylation in terrestrial snails, although minimal literature exists linking DNA methylation caused by metal exposure with multigenerational effects (Nica et al. 2017).

While it is possible that the multigenerational effects we observed could be evidence of epigenetics, it is important to note that some authors suggest continuation of induced changes must be observed in the F3 to confirm the role of DNA methylation or epigenetics (Youngson and Whitelaw 2008). We believe that it is more likely that the primary driver of the observed multigenerational effect in our study is related to energetic trade-offs between survival and reproduction under stressed conditions in the parental generation. Gastropods have detoxification, resistance, and repair mechanisms to improve chances of survival under contaminant stress, including the use of metallothioneins (Dallinger and Berger 1993). There is some evidence to suggest that these mechanisms in invertebrates have an associated energetic cost that may impede growth and reproduction to prioritize survival (Walker et al. 2012). Production of gametes, especially eggs, is also an energetically expensive process, and the encapsulation of eggs in a gelatinous matrix has been shown to have considerable energetic costs in gastropods (Stickle 1973). It is possible that under extreme contaminant stress, less energy is allocated to reproduction to improve the individual's chance of survival. Additionally, the cost of producing the gelatinous egg mass may contribute to the trend of increasing number of eggs per egg mass seen during the Parental Exposure at the three lower treatments, which were not experiencing significant reductions in total oviposition due to Cu exposure.

Additionally, it is important to note that there is a potential influence of latent effects due to gamete exposure within the exposed parents in multigenerational studies like this one. However, the potential routes of exposure, both direct and indirect, that were modeled in this study reflect the potential multigenerational hazard posed by certain contaminants, such as copper, in the environment. In a real pulse scenario, Cu

concentrations easily may reach levels capable of inducing severe parental effects but not mortality, as seen in our study. While we are not able to distinguish between the direct effect of gamete exposure in the parents and the indirect effects due to parental exposure, both would be present in an actual environmental exposure and, in light of our results, could still potentially cause serious latent changes to the sensitivity of the F1 generation, which ultimately could affect the survival of the entire population in the event of a subsequent exposure.

In conclusion, sublethal Cu exposure to adult *P. pilsbryi* caused significant reduction in reproductive output but did not affect the hatching success of the egg masses that were laid. However, a subsequent novel exposure of juveniles reared under pristine conditions but born to Cu-exposed parents demonstrated that parental Cu exposure decreased juvenile tolerance. A clear dose–response relationship was observed with increasing parental exposure causing significant decreases in juvenile Cu tolerance later in life. Contaminants, in this case Cu, can induce latent effects in freshwater gastropods that manifest a generation after exposure has ended. Multigenerational studies such as this one, reveal the added complexity of the transgenerational risks of contaminants in a population that traditional single-lifestage toxicity tests do not capture. As transgenerational effects may significantly alter the tolerance of subsequent generations to future stressors, multigenerational studies have important implications for accurate and protective risk assessment. Given the abundance and importance of gastropods in freshwater ecosystems and food webs, chronic population-level transgenerational effects in gastropods may have larger impacts on the overall health of the ecosystems they inhabit (Bal et al. 2017). This study demonstrates that sublethal pulse exposures to contaminants such as Cu can induce effects that are not evident in the exposed generation, once recovered, but that continue to negatively impact the health and success of a subsequent unexposed generation and should therefore be considered for their potential to cause additional population-level risk.

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