Observations of the first stygobiont snail (Hydrobiidae, *Fontigens* sp.) in Tennessee

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Abstract

The Appalachian Valley and Ridge (AVR) region of East Tennessee (USA) is one of the most cave-rich areas of the state, with approximately 1,470 caves in a 6,379 km^2 area. Despite decades of exploration, only 5.1% of caves in the area were biologically inventoried prior to 2013 and basic data on species distributions were lacking. This sampling gap prompted the start of ongoing biological surveys in the AVR. One of the first AVR bioinventories took place in 2013 in a small cave in Knox County (TKN24). A surface stream enters the cave from the northeast and flows down-dip through the lower wet passage. The cave receives a high anthropogenic load in the form of surface runoff, physical debris (i.e., trash), and raw sewage. In 2015, a large sewage release flushed through the cave, but prior estimates of total aerobic bacterial loads in 2014 indicated an unhealthy waterway.

Despite the obvious human impact, during the bioinventory we identified 14 invertebrate and five vertebrate taxa. Of particular interest were the small (1–3 mm), white to translucent, aquatic hydrobiid snails attached to the sides and bottom of rocks in the stream, which represent the first stygobiotic snail discovered in Tennessee. Detailed monthly surveys were conducted from March to August 2014 to gain insight into the snail's life history, abundance, physical distribution within the cave stream, and habitat preferences. Snail abundance and density were estimated from visual-encounter surveys in a single area of the stream passage. Surfaces of ten rocks were examined during each survey. Most (65 to 100%) of the snails were observed on the bottom of rocks completely submerged in the stream water. Snail density negatively correlated with increasing temperature (R^2 = 0.61) and was greatest in March (1.11 snails per m^2) in 9 ˚C water and lowest in August (0.39 snails per m^2) in 15 °C water.

This new Tennessee hydrobiid has been identified as a member of the genus *Fontigens* based on morphology. Previously described stygobiotic hydrobiid snails from karst regions in the eastern United States include *Antrorbis breweri* from northeastern Alabama, *Fontigens bottimeri, F. morrisoni, F. tartarea, F. turritella*, and *Holsingeria unthanksensis* from the Virginias, as well as *F. cryptica* from Indiana, *F. antroectes* from Illinois, and *Antroselates spiralis* from Indiana and Kentucky. This new population extends the distribution of the genus into Tennessee.

Keywords: Hydrobiid, cavesnail, Appalachian, aquatic, invertebrate, mollusc

1. Introduction

Biological surveys of subsurface invertebrate diversity continue to provide a wealth of new information about cave fauna, including the discovery of previously undescribed species. The Appalachian Valley and Ridge (AVR) region of East Tennessee is particularly promising for the discovery of new invertebrate taxa, and for extending ranges of known invertebrate and vertebrate taxa. This region has been undersampled compared to neighboring karst areas like the Interior Low Plateau (Niemiller and Zigler 2013; Niemiller *et al.* 2016). Recent efforts to fill this sampling gap highlight the need for continued research in and exploration of this region (e.g., Keenan *et al.* 2014; Niemiller *et al.* 2016). One of the reasons for a sampling gap may be because biodiversity in AVR caves has been assumed to be lower than in other karst regions, due in part to the caves being smaller in length and size, and because smaller caves could have limited habitat availability (e.g., terrestrial and/or aquatic habitats) to support a more specious fauna. Another reason why AVR caves may have been ignored in the past for biodiversity studies is because many of the known cave systems have the potential to be impacted by human activities, such as contamination from road waste, agricultural waste, or urbanization, as well as from visitation by people and enhanced foot-traffic due to their proximity to urban centers.

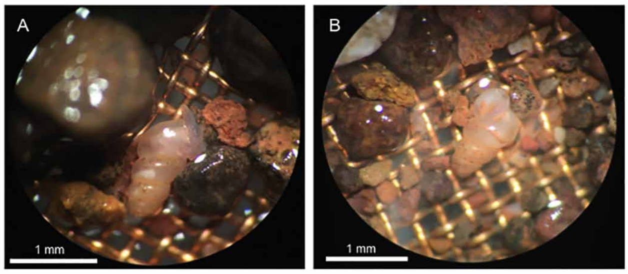

Figure 1. Photographs of snails recovered from Cruze Cave. (A) Lateral and (B) ventral views of two snails recovered from sieved cave sediment. The sieve mesh is 30 µm wide.

During a biosurvey of Cruze Cave, Knox County, Tennessee (TN24) in May 2013, tiny, white, aquatic snails were observed on several rocks within the cave stream, approximately 75

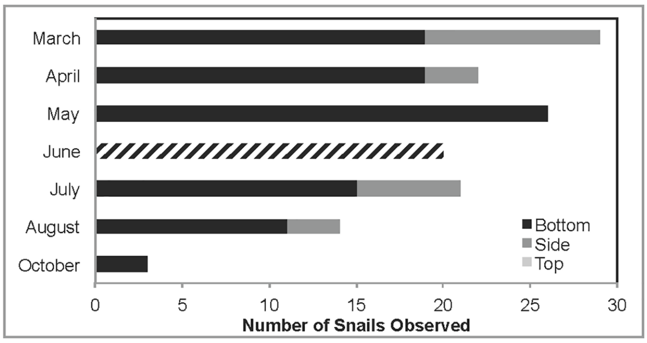

Figure 2. Physical distribution of snails on rocks during each monthly observation period. During the June observations, physical distribution data were not collected, but approximately 75% of snails were observed on the bottom of rocks and the remaining 25% were observed on sides of rocks. No snails were observed on the tops of rocks.

m from the cave entrance. There had been several previous accounts of terrestrial snails in cave systems in Tennessee, as well as aquatic snails likely transported by fluvial systems during high flow events (Hubricht 1940, 1964). However, to our knowledge, the presence of stygobiont snails in the state had not yet been reported. This absence in the literature has been surprising, especially considering the diversity of stygobiont snails known from adjacent states in the Appalachian region, including Virginia, Alabama, and Kentucky (Hershler 1989; Hershler and Thompson 1990; Hershler et al. 1990; Lewis 1994), as well as other bordering states, including Missouri (Peck 1998; Lewis et al. 1999) and Arkansas (Graening 2003).

Cruze Cave is developed along southeast-dipping bedding and fracture planes within the Holston Formation (Knox Group), which is comprised of Ordovician-aged carbonates and interbedded shales and sandstones. Cruze Cave is located within the city limits of Knoxville, and a dozen homes are within 500 m of the cave entrance. The cave is approximately 19 m in depth (total vertical extent), and the lower, main passage extends for a surveyed length of about 300 m. A surface stream enters the cave and flows through the length of navigable passage before ending in a narrow sump. Because of the direct connection to the surface, stream flow is subject to variable discharge during intense rainfall events. Anthropogenic debris (i.e., lawn chairs, bottles, trash, road waste) is wedged within passage fractures as high as 4 m above the base-flow streambed. During base-flow conditions, the stream is 12 cm deep or less. Sampling was only done during base-flow conditions. In addition to visible trash, the presence of pervasive brown- to- orange biofilms covering all underwater surfaces of the cave indicated a high organic load to the cave, such as from sewage. Such biofilms would provide a nutrient source for the snails and other macrofauna, even if the microbes were linked to sewage contamination. The aims of this study were to evaluate snail population densities over time, and to evaluate stream water quality, including confirming if there was continued sewage contamination into the cave.

2. Methods

Detailed monthly surveys occurred from March 2014 until August 2014. The purpose of the surveys was to gain insight into the life history, abundance, physical distribution, and habitat preference of snails within the cave stream. Snail abundance and densities were estimated from visual-encounter surveys in a single area of the stream passage, located approximately 150 m from the entrance. The surfaces of ten fully submerged rocks were examined during each survey for the presence of snails. Rock length, width, and height were measured to quantify surface area. When encountered, snail position on the rock was recorded as top, bottom, or side.

Stream physiochemistry, including water pH, conductivity, and temperature, was measured using standard field instruments. During one sampling event in October, total aerobic bacteria and total yeast and mold colony forming units (CFU) counts per mL were conducted using RIDA® COUNTs culture plates (R-Biopharm AG), according to manufacturer instructions.

3. Results

All of the observed snails were unpigmented and ranged in size from sub-mm to 3 mm in length (Figure 1). Their shells were turreted with 4-5 rounded whorls, and ranged in color from completely clear to orange/brown, and sometimes appeared spotted, which was likely due to variable colonization of microbial biofilms or food within the snail gut. The snails were identified as belonging to the genus Fontigens (Robert Hershler, personal communication), although the species is new and currently undescribed.

During the observation period, snails were restricted to the bottom (84%) and sides (16%) of rocks (Figure 2). Total snail density, presented as a function of rock surface area, ranged from 1.11 snails/m^2 in March to 0.39 snails/m^2 in August, the highest and lowest density time points, respectively (Table 1).

Snail density negatively correlated with increasing temperature (R^2 = 0.573), with the greatest number and highest density of snails observed in March (29 snails, 1.11 snails/m2) in 9 °C water, and the lowest in October (3 snails, 0.3 snails per rock) in almost 15 °C water (Figure 3). In addition, the snail density increased as the stream chemistry shifted from pH ~7.0 up to pH 7.7 (R^2 = 0.509). Snail density was not correlated with conductivity (Figure 3; R^2 = 0.001).

Total aerobic bacteria and total yeast and molds were quantified from water at three locations along the cave stream: upstream of the snails (approximately 50 m from the

Table 1. Number of observed snails, snail density, and stream physiochemistry at each observation point (2013).

	#of Snails	# of Rocks	Snail Density/ Surface Area (m2)	pH	Temp. (°C)	Conductivity (µS/cm)
March	29	6	1.11	7.72	9.7	426
April	22	10	0.73	7.66	10.9	437
May	26	12	0.48	7.51	11.1	401
June	20	13	0.62	7.3	11.7	142.8
July	21	10	0.72	7.25	12.6	362
August	14	10	0.39	6.98	13.7	504
October	3	10		7.04	14.7	297.4

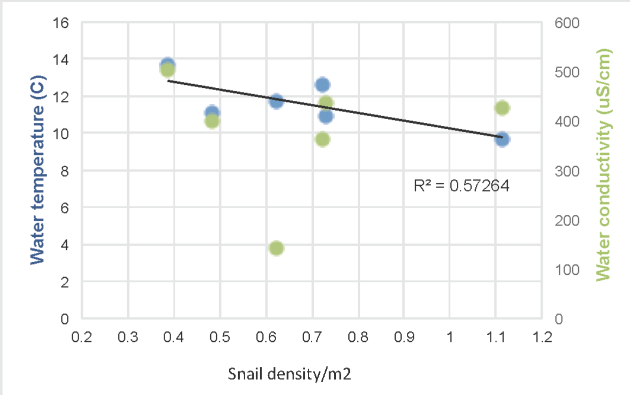

Figure 3. Snail density as a function of water conductivity (µS/cm) and water temperature (°C). The number of observed snails negatively correlated with temperature.

entrance), next to the snails, and downstream (approximately 250 m from the entrance). Aerobic bacterial CFU decreased along the length of the passage, as did the CFU per ml for yeast and molds (Table 2).

4. Discussion

The distribution, ecology, and systematics of hydrobiid snails within the genus *Fontigens*, particularly subterranean species, are largely understudied. In the years since the Cruze Cave discovery, several other individuals likely to be of the same species were discovered from two caves in nearby Roane and Sevier counties in Tennessee. As the first reported stygobiont snail from Tennessee, the Cruze Cave snails will help to fill a large gap in our understanding of hydrobiid distribution in the AVR because the current range of stygobiont hydrobiids includes the states that neighbor Tennessee: Virginia and Kentucky to the north and Alabama to the south (Hershler *et al.* 1990; Lewis 1994), as well as Missouri (Peck 1998; Lewis *et al.* 1999) and Arkansas (Graening 2003) to the west.

The Cruze Cave *Fontigens* sp. snails preferred to colonize the bottom and sides of rocks within the streambed rather than the top surfaces that could be exposed to flowing water and predators. The Enigmatic Cave snail (*Fontigens antroecetes*) from Illinois and Missouri has a similar niche preference for the bottom (~80%) and sides (~15%) of rocks in its cave stream (Taylor *et al.* 2013). It is likely that the snails graze the microbial biofilms on the rocks, and that the high load of microbial cells in the cave stream serve as a constant supply of microbes for biofilm growth. Additional research needs to be done to determine if the microbial load changes seasonally, which may affect the snail population densities. It is clear that seasonal changes in population density do correlate to temperature and/or pH changes in the stream water. The current dataset did not indicate a relationship with conductivity.

Table 2. Total aerobic bacteria and total yeast and mold counts from three locations along the Cruze Cave stream.

	Total aerobic bacteria (CFU per mL)	**Total yeast and mold (CFU per mL)**
Upstream	2600	181
Midstream (near snails)	1352.5	48
Downstream	1200	25.5

5. Conclusions

The discovery of stygobiontic hydrobiids in three caves (at present) in the AVR region of East Tennessee supports recent suggestions that there is high potential to discover new species in the region, as well as high potential for species range extensions (Niemiller and Zigler 2013). As Cruze Cave demonstrates, even caves that are heavily impacted by human activity have the potential to host diverse invertebrate (and vertebrate) communities, and more caves within urban watersheds should not be dismissed for future biosurveys. Assumptions that cave faunal diversity may be lower because a cave is small or because of anthropogenic impacts should not be used as criteria to guide biosurveys in the AVR region.

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