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# CHARACTERIZING STREAM REACHES OCCUPIED BY EASTERN HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS ALLEGANIENSIS*): INSIGHTS FROM A PREVIOUSLY UNDOCUMENTED WESTERN NORTH CAROLINA STREAM POPULATION

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*Abstract:* The Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) is a large aquatic salamander found in cool, highly oxygenated rivers and streams of the eastern United States. Hellbender populations have been steeply declining over the past century, and they are a protected species in many states, including North Carolina where they are listed as a species of special concern. North Carolina contains over 2,000 waterways that could potentially support hellbender populations. It is vital to survey these waterways to better understand the distribution of the Eastern Hellbender and the environmental factors necessary to support populations. Although small streams could potentially act as refugia for both larval and adult hellbenders, most surveys have focused on large rivers, generally using substrate composition and cover rock presence as site selection determinants. In early summer of 2013, we surveyed Bent Creek, a small (25.3 $km^2$) catchment in the Pisgah National Forest beginning at the confluence with the French Broad River and concluding upstream to the Lake Powhatan dam. We documented four adult Eastern Hellbenders, including two captures and two tactile encounters/escapes. These represent the first records for *C. a. alleganiensis* in this catchment. In October 2013, we compared the number of cover rocks, water temperature, dissolved oxygen and substrate composition of three occupied and three unoccupied stream reaches. Although temperature and dissolved oxygen did not vary among reaches, occupied stream reaches had coarser substrates and a much higher occurrence of cover rocks than unoccupied reaches. Our findings suggest that commonly used surveying techniques relying on potential cover rocks and substrate composition are effective methods for selecting survey sites. Future research could examine whether the Eastern Hellbender population at Bent Creek is, or has the potential to become, a viable breeding population, as well as assessing population size upstream from Lake Powhatan. Suitability of stream conditions to consistent larval recruitment should also be evaluated. Our findings suggest that smaller streams such as Bent Creek can support viable populations of *C. a. alleganiensis*, and that restricting surveys to larger watersheds has the potential to miss important populations of this imperiled species.

*Key Words:* Bent Creek; habitat fragmentation; cover rock; substrate size; eDNA; French Broad River.

## INTRODUCTION

Hellbenders (*Cryptobranchus* spp.) are North America's largest salamanders, reaching up to 74 cm in total length (TL) (Petranka 1998). They are nocturnal, territorial generalist predators in cool, clear, highly oxygenated streams where they use flat-bottomed rocks for shelter, feeding, and reproduction (Williams et al. 1981; Humphries and Pauley 2005). Hellbenders use these rocks to ambush prey, hiding with only their head exposed until a crayfish (their primary food) or other small aquatic organism moves within striking range (Hillis and Bellis 1971). Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) in North Carolina's upper French Broad River drainage strongly prefer the largest available flat-bottomed rocks (Rossell et al. 2013). These large, flat rocks, generally ranging in size from 38–137 cm at their widest point (Hillis and Bellis 1971), are an important focus when surveying an area for hellbenders. Consequently, local geology can have a strong impact on determining hellbender presence or absence within a watershed (Jachowski et al. 2013; Quinn et al. 2013).

Distribution of hellbender populations is patchy, and overall abundance is declining across its range (Gates et al. 1985; Wheeler et al. 2003; Foster et al. 2009; Graham et al. 2011; Keitzer et al. 2013; Olson et al.

2013; Jachowski and Hopkins 2018). Currently listed as a species of special concern in North Carolina, the Eastern Hellbender was petitioned for listing under the Endangered Species Act (USFWS 2011), but the U.S. Fish and Wildlife Service declined to list this species in 2019 (USFWS 2019). At the watershed scale, variables associated with human activity such as catchment forest cover, stream canopy cover, and riparian buffer width often determine hellbender presence, driving smaller-scale differences in local habitat measures such as conductance, water quality, and substrate composition (Keitzer et al. 2013; Quinn et al. 2013; Pugh et al. 2016; Pitt et al. 2017; Jachowski and Hopkins 2018). Sedimentation, where fine substrate particles fill interstitial spaces between gravel and under large rocks, is considered a leading cause of habitat degradation for Eastern Hellbender populations (Nickerson et al. 2003; Quinn et al. 2013). Analyzing substrate quality and composition along with the presence of potential cover rocks could thus be instrumental in determining habitat suitability for this vulnerable salamander.

The continuing decline of the Eastern Hellbender makes population censuses and corresponding habitat analyses particularly vital today. Due to their cryptic nature, hellbenders can be difficult to document, and new populations are still being discovered (e.g., Albanese et al. 2011; Pugh et al. 2013). While most surveys are focused on large rivers, Bent Creek, a small stream in western North Carolina's Pisgah National Forest, offers an opportunity to survey a complete small catchment tributary (Fig. 1). There had been no documented record of this species in Bent Creek until the senior author (AL) and companion observed one diurnally active adult hellbender (>30 cm TL) in 2010. This observation was followed by a one-day survey in July 2012 where no hellbenders were captured, but surveyors made probable tactile contact (without visual confirmation). Then in 2013, environmental DNA (eDNA) testing of water samples from Bent Creek, below the Lake Powhatan dam, by the North Carolina Wildlife Resources Commission returned a positive result for the presence of Eastern Hellbender (Spear et al. 2015). The present study was undertaken in summer 2013 to survey a 5 km section of Bent Creek from its confluence with the French Broad River upstream to the Lake Powhatan dam. In-stream habitat was measured at six sites within this section in October 2013.

## METHODS

Bent Creek, located in the northeastern tip of the Pisgah National Forest in Buncombe County, North Carolina, runs through the oldest federal experimental forest east of the Mississippi River (Fig. 1). The Bent Creek catchment encompasses 25 $km^2$, consisting of 6% developed land, 1% agricultural land, and 93% least-developed land (Homer et al. 2015). Least-developed land is primarily deciduous hardwood forest, with some evergreen forest at higher elevations. With the exception of one downstream tributary, the catchment is protected from development within Pisgah National Forest. However, the catchment experiences heavy recreational use, with trails and gravel roads maintained for hiking, mountain biking, and horseback riding. Bent Creek is impounded to form Lake Powhatan, a small recreational reservoir. The dam at Lake Powhatan is 7 m in height, and likely serves as a barrier to movement of aquatic organisms, including the Eastern Hellbender. Because of its high percentage of forested cover and protection from development, we anticipated that Bent Creek was likely to contain a healthy Eastern Hellbender population.

Surveys for hellbender presence were completed between May and July 2013. Beginning at the confluence with the French Broad River, teams of four to seven people spread across the width of the creek and walked upstream. Most participants visually surveyed the creek from above the water's surface, though two used a mask and snorkel to survey underwater. While scanning the stream for any hellbenders out in the open, surveyors looked for rocks greater than 38 cm across at their widest point (Hillis and Bellis 1971). Rocks not completely embedded in substrate were slowly lifted with logging tools (e.g., a peavey or cant hook), while one to two people gently felt under the rock for the presence of hellbenders (Browne et al. 2011). Other team members positioned themselves with nets downstream to catch potential escapees. All rocks were then returned to the same position in which they were found.

Any hellbenders caught were placed in a dip net for processing. Snout-to-vent length (SVL) and total length (TL) were measured in a modified, ruled PVC pipe, then individuals were weighed using Pesola® spring scales and sexed. Tail width was also measured with dial calipers, and a 3 mm tail clipping was taken for future DNA analysis. Processed hellbenders were released at their capture sites and the site coordinates recorded using handheld GPS units. Locations of all non-capture tactile encounters were also noted.

For all rocks where hellbenders were captured, as well as for the suitable cover rock nearest to hellbenders encountered in the open, rock dimensions, shape, and percentage embedded in substrate were recorded. Rock length and width were obtained using measuring tape. Rock shape was visually described by dominant features as "thin and flat," "angular and blocky," or "smooth and round." Percent embeddedness was visually estimated by surveyors who snorkeled and inspected each cover rock prior to lifting; surveyors used cues of substrate deposits on rocks and qualitative assessment of the presence of an opening underneath to assign shelter rocks to embeddedness categories of <5%, 5–25%, 26–50%, or >50%. These visual estimates were based on methods by Platts et al. (1983).

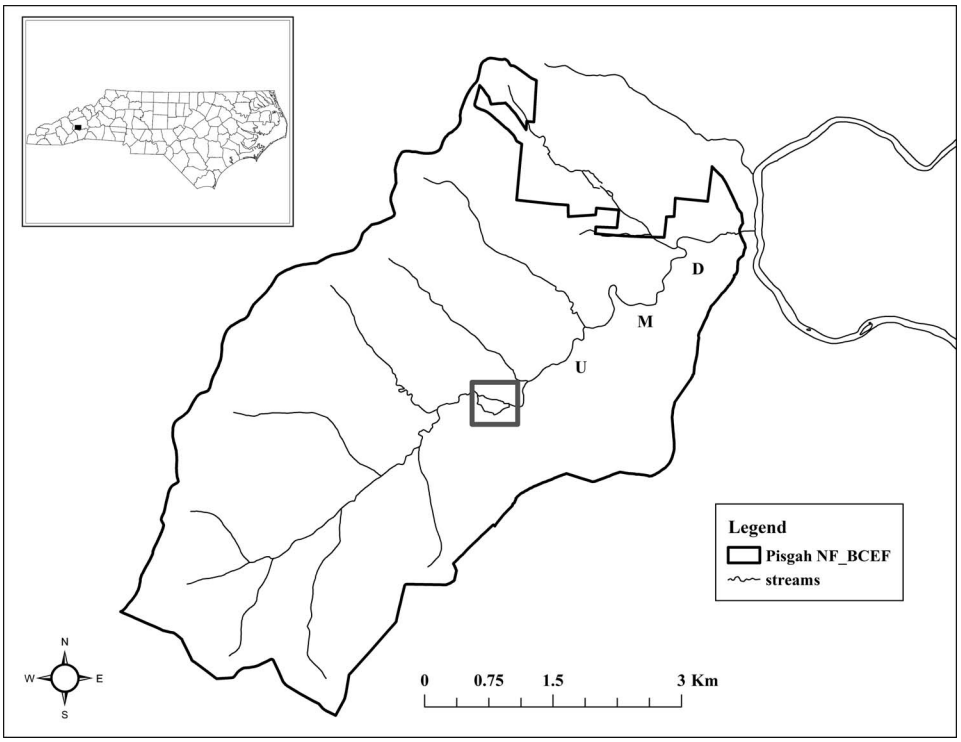


FIG. 1. Map of Bent Creek catchment, within Bent Creek Experimental Forest, Pisgah National Forest, NC; gray box indicates Lake Powhatan and dam, an upstream barrier for Eastern Hellbenders (*Cryptobranchus a. alleganiensis*). Reach locations: D = downstream, M = middle, U = upstream.

After field sampling was completed for the entire 5 km length of stream, we used ArcGIS (ESRI, Redlands, CA, version 10.5) software to create a map of Bent Creek marked with each hellbender capture and encounter location. The patchy distribution of sites occupied by hellbenders indicated three unoccupied reaches: one near the mouth of the creek near the French Broad River ("Downstream Unoccupied Reach"), one near the Lake Powhatan dam ("Upstream Unoccupied Reach"), and one between capture sites roughly in the middle of Bent Creek ("Middle Unoccupied Reach"). We used 100 m lengths to compare occupied and unoccupied stream reaches, as this number is well within the variation of home ranges sizes documented by Humphries and Pauley (2005). Occupied reaches were determined by measuring 50 m upstream and downstream from the point of encounter and marking both ends of the reach with flags.

One 100 m territory was randomly selected within each of the three aforementioned unoccupied reaches (Upstream, Downstream, and Middle). Each of these 100 m territories was then surveyed for the total number of suitable cover rocks. We defined suitable cover rocks as those with an opening large enough for an adult hellbender to enter, and width over 38 cm at their widest point (Hillis and Bellis 1971; Rossell et al. 2013). To compare substrate composition between occupied and unoccupied reaches, we completed a pebble count in each reach using the bank-to-bank zigzag method (Bevenger and King 1995), and classified substrate based on particle diameter using a modified Wentworth scale (Cummins 1962) as Clay/Silt (<0.0625 mm), Sand (0.0625–2 mm), Gravel (2–16 mm), Pebble (16–64 mm), Cobble (64–256 mm), Boulder (>256 mm) or Bedrock. We also measured water temperature and dissolved oxygen at the upstream end of each reach using a HACH® HQ40D meter.

Finally, we collected two water samples to test for the presence of hellbender eDNA upstream of Lake Powhatan. We used the same methods as previously used for downstream samples (Spear et al. 2015). One sample was collected at the mouth of the upstream portion of Bent Creek where it enters Lake Powhatan, and a second approximately 1.25 km upstream from Lake Powhatan. One liter of stream water was pumped through a 0.45 micron cellulose filter in a 100 mL disposable funnel attached to a vacuum flask. Using forceps, filter paper was transferred to a 1.5 mL vial containing 95% ethanol for transport to the lab at Tangled Bank Conservation, LLC, in Asheville, NC. Before processing each sample in the field, we donned clean rubber gloves, and equipment was treated with DNA Away® solution and dried. To test for cross-contamination, we processed 1 L of store-bought mineral water in the field, and brought the filter paper back to the lab. In the lab, DNA was amplified using qPCR, then screened for presence of Eastern Hellbender DNA using the same primers as Spear et al. (2015).

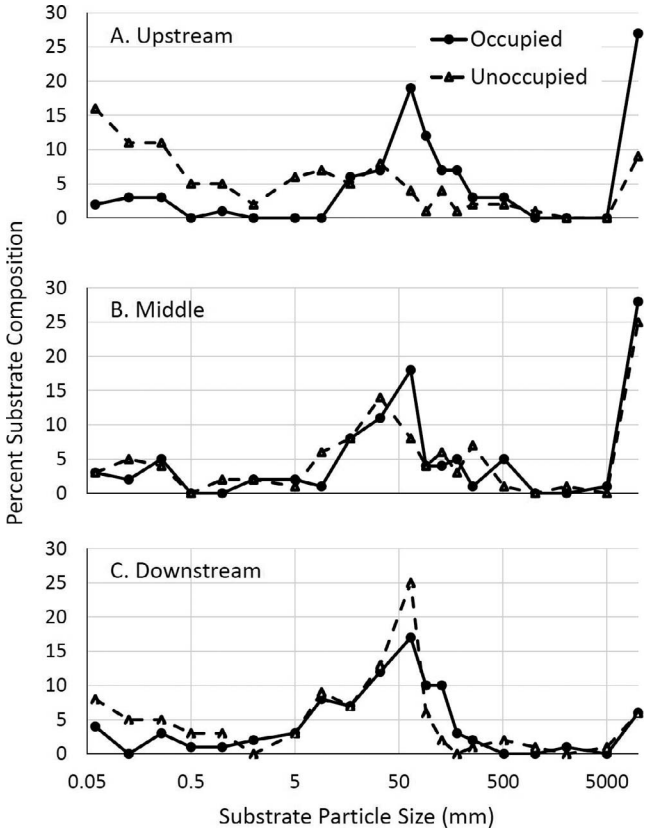


FIG. 2. Percent substrate particle size composition at occupied and unoccupied stream reaches in Bent Creek, Pisgah National Forest, NC. Largest substrate particle size indicates bedrock.

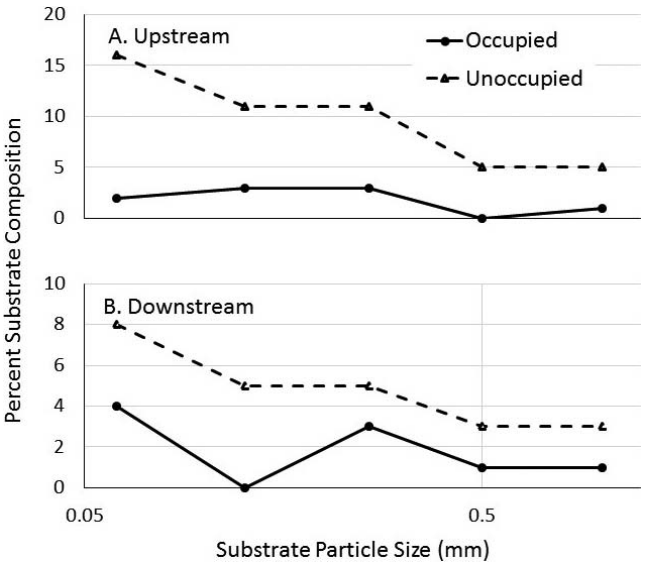


FIG. 3. Percent fine substrate (≤1 mm diameter) composition at occupied and unoccupied upstream and downstream study reaches in Bent Creek, Pisgah National Forest, NC. X-axis follows logarithmic scale; largest substrate particle size shown is 1 mm diameter.

## RESULTS

We captured two hellbenders and encountered two more that escaped. Given the small territory sizes of this species and the fact that escapees were between 50 and 100 m from captured individuals, we do not believe the escapees were re-captures; however, we cannot be completely certain. Captured individuals included one adult female (51 cm TL) and one adult male (37 cm TL). At 595 g and with a tail width of 30 mm, the female captured was thin, with no visible injuries or physical anomalies. The male weighed 330 g with a tail width of 35.7 mm and no injuries or anomalies. Both individuals appeared to be in good health, with body weights within the values typical of healthy populations of this species documented over the past decade (L. Williams and J. Groves, unpubl. data). Sample size was too small to perform statistical analysis on captures.

Occupied and unoccupied stream reaches differed in number of shelter rocks available and in dominant substrate category (Fig. 2 and Table 1). Mean number of cover rocks in occupied reaches was twice that of unoccupied reaches. Mean substrate size classes in the 25th, 50th (median) and 75th percentiles were two to five size categories larger at occupied sites than unoccupied ones (Table 1). Differences in particle size composition for occupied and unoccupied reaches varied by stream section. In the upstream and downstream sections, occupied reaches had lower percentage composition of fine substrata (≤1 mm diameter) than unoccupied reaches (Fig. 3). This difference was most pronounced in the upstream section, where overall fine substrate levels are high; all occupied reaches had less than 5% silt substrate composition. Substrate composition and abundance of suitable cover rocks was similar between occupied and unoccupied middle reaches, suggesting that this unoccupied reach may provide suitable hellbender habitat. Dissolved oxygen varied from 8.82 mg/L to 8.96 mg/L with a mean of 8.92 at unoccupied sites and 8.90 at occupied sites. Temperatures averaged 15.4°C at unoccupied sites and 15.5°C at occupied sites. All four cover rocks utilized at encounter sites were greater than 38 cm at their widest points (Table 2). Three of the four cover rocks were thin and flat. Embeddedness was less than 25% for all rocks and less than 5% for two of them. Water depth at encounter sites ranged from 5 cm to 31 cm and the distance from cover rocks ranged from 0 to 1.8 m.

The eDNA sample taken just above the start of cove waters of Lake Powhatan was negative for hellbender presence. However, the second sample farther upstream did return a weak signal positive for the presence of hellbender. This sample was re-run in lab analysis because it was potentially dehydrated, rendering results questionable. On a second attempt at analysis, the weak positive signal was replicated. This result can be interpreted as possibly a single individual hellbender existing high in the Bent Creek system or in a smaller tributary upstream of the water collection point. It is also likely that we were a considerable distance downstream from

Table 1. Habitat metrics for reaches of Bent Creek, Pisgah National Forest, North Carolina, unoccupied and occupied by Eastern Hellbenders (*Cryptobranchus a. alleganiensis*). Occupied reaches were those in which Eastern Hellbenders were encountered, and either escaped or were captured. Substrate particle size categories follow Cummins (1962); see text for size cutoffs for substrate categories.

| | | Substrate Category | | | | |
|---|---|---|---|---|---|---|
| Stream Reach | # Cover Rocks | 25th Percentile | 50th Percentile | 75th Percentile | Dissolved Oxygen (mg/L) | Temperature (°C) |
| Downstream Unoccupied | 11 | Gravel | Gravel | Gravel | 8.95 | 15.3 |
| Downstream Occupied | 47 | Gravel | Gravel | Cobble | 8.93 | 15.4 |
| Mid Unoccupied | 47 | Gravel | Gravel | Boulder | 8.96 | 15.2 |
| Mid Occupied | 45 | Gravel | Gravel | Bedrock | 8.94 | 15.3 |
| Upstream Unoccupied | 28 | Sand | Sand | Gravel | 8.86 | 15.7 |
| Upstream Occupied | 101 | Gravel | Cobble | Bedrock | 8.82 | 15.8 |
| Unoccupied Mean (±SE) | 29 (±10.4) | Sand | Gravel | Cobble | 8.92 (±0.03) | 15.4 (±0.15) |
| Occupied Mean (±SE) | 64 (±18.3) | Coarse Gravel | Very Coarse Gravel | Medium Boulder | 8.90 (±0.04) | 15.5 (±0.15) |

a source animal(s) (J. Apodaca, Tangled Bank Conservation, LLC, pers. comm.). No hellbender DNA was detected from field or PCR negative controls, and no cross-contamination was detected.

## DISCUSSION

Documenting the presence of four adult Eastern Hellbenders in Bent Creek confirms that this tributary stream system supports a small population of this imperiled salamander. However, the relatively undisturbed, forested catchment of Bent Creek does not appear to provide the high-quality refuge we had anticipated. While finding these individuals was encouraging, encountering only older adults may be reflective of a general trend toward reduced recruitment across the Eastern Hellbender's range (Wheeler et al. 2003). The presence of one adult female and at least one adult male in Bent Creek does indicate that this small population has the potential to reproduce. Since the completion of our study, one Eastern Hellbender larva was found in Bent Creek by North Carolina Arboretum staff (L. Williams, NCWRC, unpubl. data).

In order to encourage success of this species, efforts could be undertaken in the Bent Creek Experimental Forest to reduce sedimentation. Although this catchment is largely undeveloped, it is heavily used recreationally, including both horseback riding and mountain biking, and many bare soil trails cut through the riparian area to cross the stream. The impacts of numerous unbuffered footpaths, gravel roads, and bridges in close proximity to the creek likely include elevated rates of erosion and sediment deposition, particularly during periods of heavy rain (Witmer et al. 2009). Areas of extreme sedimentation just downstream from the Lake Powhatan dam might also provide a source for continued sediment deposition further downstream. The dam itself is also a barrier to hellbender movement (Philips and Humphries 2005), isolating individuals still persisting upstream of the lake. However, our values for cover rock embeddedness suggest that sediment input does not limit the number of suitable cover rocks. Sedimentation, however, likely decreases suitable interstitial habitat for prey species such as crayfish, other stream invertebrates, and smaller amphibians (Bilotta and Brazier 2008). Sedimentation could also negatively affect the ability of hellbenders to respire transcutaneously, and lead to suffocation of hellbender eggs and larvae.

The positive eDNA sample for hellbender above Lake Powhatan was surprising given the height of the Lake Powhatan dam that very likely serves as a movement barrier. However, this result suggests that efforts should be made to survey Bent Creek above the lake to assess habitat availability and estimate the number of hellbenders that may persist near the headwaters of this system.

Our results documenting larger substrates in occupied versus unoccupied stream reaches suggest that erosion remediation practices, such as installation of bridges to allow paths to cross the stream without increasing sediment flux, could improve overall habitat suitability of Bent Creek for Eastern Hellbenders. The patchiness of

Table 2. Eastern Hellbender (*Cryptobranchus a. alleganiensis*) encounter site habitat data, Bent Creek, Pisgah National Forest, North Carolina. (D = Downstream Section, M = Middle Section, U = Upstream Section).

| Hellbender Encounter (Section; Date) | Cover Rock Size (cm) | Cover Rock Shape | Cover Rock % Embeddedness | Water Depth (cm) | Distance from Cover Rock (m) |
|---|---|---|---|---|---|
| 1 (D; 21 May 2013) | 122 × 76 | Thin and Flat | 5–25 | 15 | 1.8 |
| 2 (D; 29 June 2013) | 56 × 25 | Angular/Blocky | <5 | 11 | 0 |
| 3 (M; 30 June 2013) | 100 × 61 | Thin and Flat | 5–25 | 5 | 0 |
| 4 (U; 21 July 2013) | 102 × 64 | Thin and Flat | <5 | 30 | 0.9 |

suitable shelter rocks also appears to drive hellbender distribution within the stream. Our results in this regard are similar to those from Pennsylvania, where intensive survey efforts yielded two animals found only in patch habitats where suitable, unembedded shelters existed (Quinn et al. 2013).

Our analysis indicated differences between unoccupied and occupied reaches in Bent Creek. While dissolved oxygen and temperature did not noticeably vary, the median substrate sizes in areas occupied by Eastern Hellbenders were larger than those of unoccupied sites, which is a finding similar to other studies quantifying substrate sizes in stream reaches with hellbenders (Burgmeier et al. 2011a; Quinn et al. 2013; Rossell et al. 2013.) Even more striking was the difference in cover rock abundance, with the mean number of cover rocks in occupied stream reaches more than doubling the number found in unoccupied reaches. Whereas the middle unoccupied reaches closely resembled two of the three occupied reaches in terms of cover rock abundance and substrate composition, the unoccupied reaches both upstream and downstream exhibited more fine substrates and far fewer cover rocks than the rest of the system. Studies in Indiana showed a similar pattern with larger, more abundant rock shelters available in hellbender occupied stream reaches (Burgmeier et al. 2011a,b). While Lake Powhatan dam prevents hellbenders moving further upstream, the lack of coarse substrate and suitable cover rocks downstream might also act as a deterrent for hellbenders entering or leaving Bent Creek, thus diminishing its value as a refugia tributary of the French Broad River.

Lying within heavily forested Pisgah National Forest, Bent Creek would seem to be a relatively protected area for the Eastern Hellbender. Indeed, protected areas are an important aspect of hellbender conservation (Freake and DePerno 2017). However, the small number of individuals found within this system, coupled with signs of anthropogenic stressors, indicate that the health of such seemingly sheltered streams should not be taken for granted. Other issues also need investigation, such as the determination of upstream limits to the hellbender population of Bent Creek, and the role of factors such as productivity in driving presence or absence of this top carnivore. As potential refugia and breeding grounds for hellbenders, North Carolina's smaller streams and headwater tributaries require increased survey efforts to help define how local hellbender populations utilize them and how individuals are distributed within them. In this regard, an important conservation implication of our study is the realization that these small streams do indeed hold the potential to harbor viable hellbender populations. Surveys for hellbenders often focus in large rivers, and, while such rivers may support larger populations, documenting this population in Bent Creek provides hope that some of the numerous smaller catchments in western North Carolina, particularly those draining forested landscapes, may harbor as yet undocumented populations of hellbenders. Our research also confirms that survey techniques which focus upon stream reaches with coarse substrates, limited siltation, and a high occurrence of larger shelter rocks are effective, particularly when covering large areas or systems with low hellbender population densities.

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