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Determining the Presence of the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis)* and Differentiators of Occupied vs. Unoccupied Habitats in Bent Creek, Buncombe County, North Carolina

Alexander Levine
Department of Environmental Studies
The University of North Carolina at Asheville
One University Heights
Asheville, North Carolina 28804 USA

Faculty Advisor: Dr. David Gillette

Abstract

The Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis)* is a large aquatic salamander found in cool, clean, highly oxygenated rivers and streams within the eastern United States. Hellbender populations have been steeply declining over the past century and are a protected species in most states where they are found, including North Carolina where they are listed as a species of special concern. North Carolina contains approximately 3000 waterways that could potentially support hellbender populations. It is vital to survey these waterways to better understand the distribution of the Eastern Hellbender and what environmental factors enable these systems to support threatened hellbender populations. Although smaller tributaries could potentially act as important refugia for both larval and adult hellbenders[1], most surveys had thus far been focused upon scattered sections of larger waterways, generally using substrate quality and the presence of large cover rocks as primary determinants for site selection. Until this project was completed, no survey in western North Carolina had ever covered an entire stream system. During May, June and July of 2013, the entirety of Bent Creek was surveyed, beginning at the mouth (the French Broad River) and concluding at the Lake Powhatan dam. Four adult Eastern Hellbenders were found, with two captures and two tactile encounters/escapes. In October 2013, three occupied sites and three unoccupied sites were examined, determining the number of cover rocks, the temperature, dissolved oxygen and dominant substrates at each site. While temperature and dissolved oxygen did not vary significantly, occupied stream sections had significantly coarser substrates and a much higher occurrence of cover rocks than unoccupied sections. This data indicated that commonly used surveying techniques relying on cover rocks and substrate composition are likely the most effective means of selecting survey sites in large aquatic systems. Future research might examine whether the Eastern Hellbender population at Bent Creek is or has the potential to become a viable breeding population, if the stream conditions are amenable to larval recruitment, and whether breeding could be encouraged with the use of habitat improvement and artificial nesting rocks.

1. Introduction

Hellbenders are one of North America's largest salamanders, reaching up to 74 cm in length[2]. They are nocturnal and territorial, requiring large, flat-bottomed rocks for shelter and reproductive purposes. Hellbenders also use these rocks to ambush their prey, hiding with only their head exposed until a crayfish (their primary food) or some other small aquatic organism moves within striking range[3]. These large flat rocks, generally ranging in size from 38 - 137 cm at their widest point are thus an important focal point when surveying an area for hellbenders[3]. Eastern Hellbenders in the upper French Broad River drainage were recently shown to have a strong preference for the largest available flat-bottomed rocks[4]. Currently listed as a species of special concern in North Carolina, the Eastern

Hellbender is not a federally protected species and thus currently relies upon state legislation for protection against being illegally collected, killed, harmed or otherwise harassed[5].

Distribution of hellbender populations across its range is patchy and on the decline[6]. Due to this amphibian's sensitivity to changes in aquatic systems, habitat degradation is thought to be the most serious current threat to Eastern Hellbender populations. Sedimentation, where soft fine particles such as silt fill the spaces between gravel and under large rocks, is widely considered the leading type of stream degradation related to diminishing hellbender populations[7]. With sedimentation in many stream systems accelerating due to anthropogenic land use and alterations, analyzing substrate quality and composition along with the presence of potential cover rocks could be instrumental in determining habitat suitability for this vulnerable salamander.

The continuing decline of the Eastern Hellbender makes censuses and corresponding habitat analyses particularly vital today. Currently listed as Endangered in Maryland, Ohio, Illinois and Indiana, the Eastern Hellbender's species of special concern status in North Carolina is currently under review[8]. While most surveys focus on segments of larger stream systems, Bent Creek offered a rare opportunity to survey a complete small tributary where a hellbender had been recently sighted, but never caught. After the initial sighting in 2010 by the author and companion, a one-day survey of Bent Creek took place in July, 2012 where no hellbenders were captured, but probable tactile contact (without visual confirmation) was made. eDNA testing in Bent Creek was conducted by the North Carolina Wildlife Resources Commission in 2013. Water samples were taken from the system and sent to a laboratory where DNA was filtered, extracted and tested for genetic markers unique to Eastern Hellbenders. This test returned a positive result for the presence of hellbenders. In this study, the entirety of Bent Creek - from its mouth at the French Broad River to its headwaters at the Lake Powhatan dam - was surveyed for Eastern Hellbenders in the summer of 2013. Select in-stream metrics were subsequently taken at six sites in October, 2013.

2. Methods

This survey was completed at Bent Creek, in Buncombe County North Carolina, between May and July, 2013. Bent Creek, located in the northern tip of the Pisgah National Forest River District, runs through the oldest federal experimental forest east of the Mississippi River. Beginning at 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 any rocks roughly greater than 38 cm. across at their widest point[3]. These rocks were then either manually or visually checked to see if they were completely embedded in the substrate. If the rock had enough purchase to be flipped, it was slowly lifted by hand or peavey while one to two people gently felt under the rock for the presence of hellbenders. The others 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. This procedure involved measuring snout to vent length and total length in a modified, ruled PVC pipe as well as sexing individuals, measuring maximum tail width, and taking a small tail clipping for later DNA analysis. Each capture was also weighed on-site while inside the pre-weighed mesh bag. Processed hellbenders were released at their capture sites and the site locations were recorded using a portable GPS. All non-capture tactile encounters were recorded as well.

Once Lake Powhatan dam was reached upstream, GIS software was employed to create a map of Bent Creek marked with each hellbender capture and encounter site. The patchy distribution of sites occupied by hellbenders created three unoccupied zones - one near the mouth of the creek (downstream), one near Lake Powhatan dam (upstream), and one sandwiched between capture sites roughly in the middle of Bent Creek (middle). There has been a wide range of estimates regarding the size of hellbender territories. In deciding upon what length of stream section to consider a "territory," two factors were taken into account. While related research has produced varying results, an oft-cited 2005 study found an average home range of hellbenders in a West Virginia stream to be 198 m^2 [9]. However, hellbender territories seem to be at least partially density-dependent, with smaller populations sometimes utilizing larger territories[10]. Therefore, for this study, 100-meter lengths of stream section were used to compare occupied and unoccupied territories. Two of the occupied territories were determined by measuring 50 meters in either direction of the point of encounter and marking both ends of the zone with flags. The third occupied territory encompassed two tactile encounters (individuals grasped and viewed but not captured) which were less than 20 meters apart. As it was impossible to determine whether these tightly-spaced encounters represented a territory boundary or overlap, this section of Bent Creek was treated as a single territory and measured from the midpoint of the two encounters. One unoccupied territory was then randomly selected from each of the three aforementioned

unoccupied zones and flagged at both ends of a measured 100-meter section. Each territory was then surveyed for total number of unembedded cover rocks over 38 cm at their widest point, using a meter stick for confirmation. A pebble count was then completed in each section[11]. Substrates were defined based on the size of particles collected, with 20 size classes ranging from silt/clay to bedrock. Temperature and dissolved oxygen content were measured once at each territory's upstream terminus in mid-afternoon within a span of 30 minutes. Finally, T-tests with two degrees of freedom were applied to cover rock sizes, dissolved oxygen contents, and temperatures to determine statistical significance between occupied and unoccupied stream sections.

3. Results

Two hellbenders were caught. One was an adult female weighing 595 g, 51 cm in total length, with a maximum tail width of 30 cm. The other was an adult male weighing 330 g, 37 cm in total length, with a maximum tail width of 35.7 cm. Two other hellbenders were tactile encounters only. All four cover rocks utilized at encounter sites were greater than 38 cm. at their widest points. Three of the four cover rocks were thin and flat, while embeddedness was less than 25% at two sites and less than 5% at the remaining sites. Water depth at encounter sites ranged from 5 to 30.5 cm and the distance from cover rocks ranged from zero to 183 cm (Table 1).

Table 1. Eastern Hellbender (*Cryptobranchus a. alleganiensis)* encounter site habitat data from Bent Creek survey, summer 2013

Hellbender Encounter	**Cover Rock Size (cm)**	**Cover Rock Shape**	**Cover Rock % Embeddedness**	**Water Depth at Encounter Site (cm)**	**Distance from Cover Rock (cm)**
1 05-21-2013	122 x 76	Thin and Flat	5-25	15	183
2 06-29-2013	56 x 25	Angular / Blocky	< 5	11.5	0
3 06-30-2013	99 x 61	Thin and Flat	5-25	5	0
4 07-21-2013	102 x 64	Thin and Flat	< 5	30.5	91

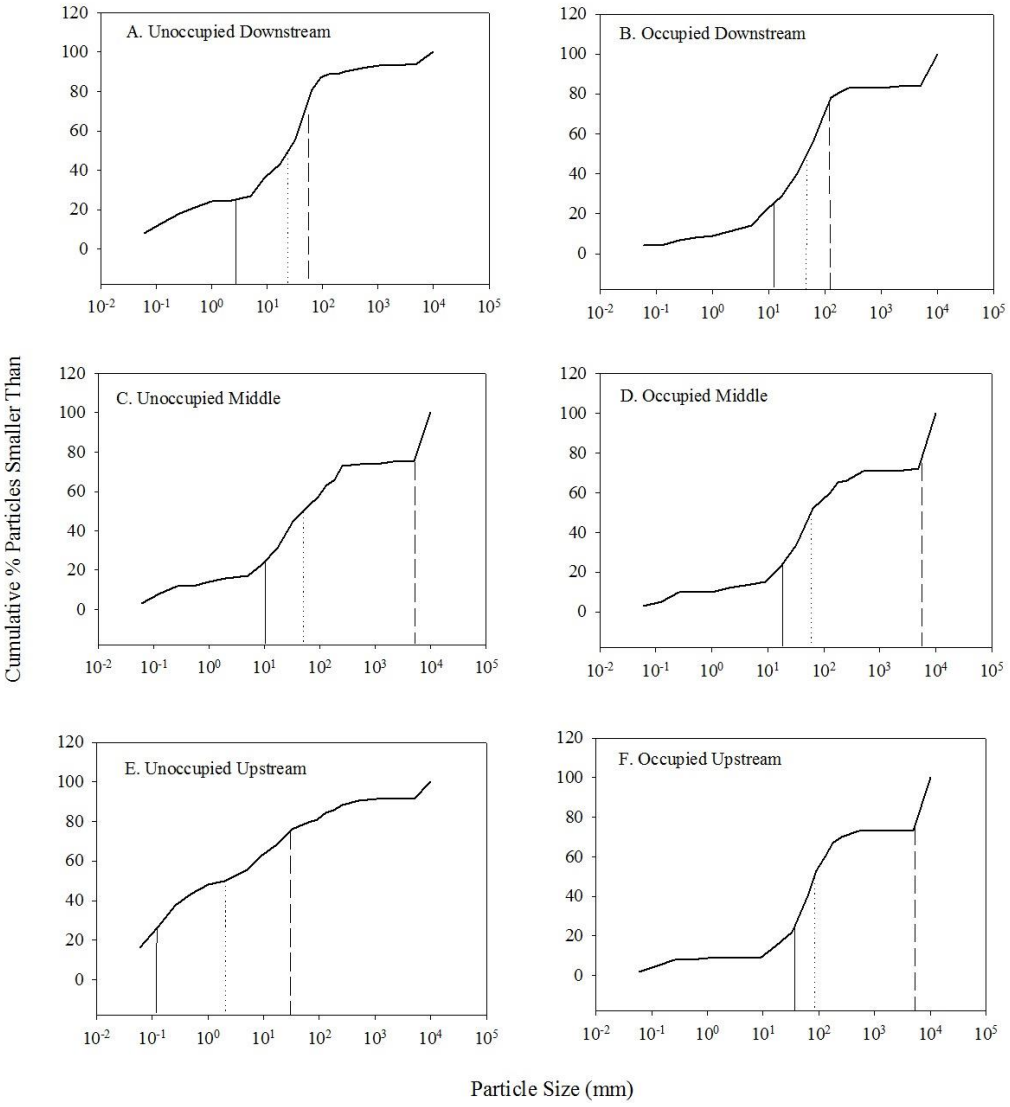
Dissolved oxygen varied from 8.82 mg/L to 8.96 mg/L with a mean of 8.92 at unoccupied sites and 8.90 at occupied sites. Temperatures averaged 15.4°C at unoccupied sites and 15.5°C at occupied sites. Mean substrate size classes in the 25th, 50th (median) and 75th percentiles were two to five categories larger at occupied sites than unoccupied ones. There were, on average, over twice as many cover rocks in occupied stream sections than there were in unoccupied territories. T-tests yielded p-values of 0.17 for cover rock abundance, 0.62 for dissolved oxygen, and 0.67 for temperature (Table 2).

Table 2. Bent Creek stream metrics for unoccupied and occupied Eastern Hellbender (*Cryptobranchus a. alleganiensis)* sites, summer 2013

Stream Site	**Number of Cover Rocks**	**Substrate Size *25th Percentile***	**Substrate Size *Median***	**Substrate Size *75th Percentile***	**Dissolved Oxygen mg/L**	**Temperature °C**
Unoccupied Mean (± Standard Error)	29 (± 10.4)	Very Coarse Sand (1-2 mm)	Medium Gravel (9-16 mm)	Medium Cobble (91-128 mm)	8.92 (± 0.0318)	15.4 (± 0.153)
Occupied Mean (± Standard Error)	64 (± 18.3)	Coarse Gravel (17-32 mm)	Very Coarse Gravel (33-64 mm)	Medium Boulder (513-1024 mm)	8.90 (± 0.0384)	15.5 (± 0.153)
T-test p-value	0.17	NA	NA	NA	0.62	0.67

The unoccupied downstream (A) and unoccupied upstream (C) sites contained, on average, a higher percentage of fine substrate particles between 0.10 and 1.0 mm when compared to the three occupied stream sections. The unoccupied middle site (B) closely resembled the substrate profiles of the three occupied sites (Chart 1).

Chart 1. Distribution of particles according to size in occupied and unoccupied sections of Bent Creek, summer 2013.(Each vertical line in the graphs below represents a substrate size percentile - solid lines are 25th percentile, dotted lines are 50th percentile (median) and dashed lines are 75th percentile.)



4. Discussion

Documenting the presence of four adult Eastern Hellbenders in Bent Creek confirms that this stream system supports a small population of the threatened salamander. While finding these individuals was encouraging, encountering only adult hellbenders seemed to reflect a general trend toward reduced recruitment across the Eastern Hellbender's range[12]. However, the presence of one adult female and at least one adult male in Bent Creek indicates that this population, although small, has the potential to reproduce. In order to encourage breeding success, certain efforts could be undertaken in the Bent Creek Experimental Forest to help reduce sedimentation. The impacts of having numerous unbuffered footpaths, roads and bridges in such close proximity to the creek might include elevated rates of erosion and sediment deposition, particularly during periods of heavy rain[13]. Areas of extreme sedimentation near Lake Powhatan dam might also provide a source for continued sediment deposition further downstream. Silt removal in these stream sections could help remediate sedimentation in areas otherwise suitable for Eastern Hellbender larvae. In conjunction with habitat improvements, Bent Creek could also be utilized as a site for artificial nest rocks. Generally constructed with a combination of chicken-wire and concrete, these manmade dens have already been successfully used in captive breeding efforts with the closely-related Ozark Hellbender (*Cryptobranchus alleganiensis bishopi*)[14]. These structures have just begun to be employed in Eastern Hellbender habitats to encourage nesting and to aid in the collection of hellbender eggs for captive rearing programs[1].

Statistical comparisons of unoccupied and occupied sites in Bent Creek indicated that there was no significant difference between measured habitat differentiators. While low statistical power due to this small system's limited number of sample sites restricted the ability to accurately detect statistical significance, comparisons of means and p-values did indicate some notable differences between habitats. Although dissolved oxygen and temperature did not noticeably vary between sites, the 25th, median, and 75th percentile substrates in areas occupied by Eastern Hellbenders were all several classes larger than those within unoccupied sites. Even more striking was the difference in cover rock abundance when comparing unoccupied and occupied areas, with the mean number of cover rocks in occupied stream sections more than doubling the number found in unoccupied sections. The p-value of 0.17 for cover rock abundance indicated that this difference can be considered a nonsignificant trend. Furthermore, while the middle unoccupied site closely resembled two of the three occupied sites in terms of cover rock abundance and substrate composition, the unoccupied sites bookending Bent Creek exhibited much finer substrates and far fewer cover rocks than the rest of the system. While Lake Powhatan dam would necessarily prevent hellbenders moving further upstream, the lack of coarse substrate and suitable cover rocks downstream might act as a deterrent for hellbenders entering or leaving Bent Creek, particularly larvae and smaller juveniles.

The Eastern Hellbender is often associated with forested streams, where vegetative shade aids in maintaining the lower water temperatures upon which these salamanders rely[15]. Lying within a national forest and largely surrounded by the North Carolina Arboretum, Bent Creek would appear to be a relatively protected area for hellbenders. However, the small number of individuals found within this system, coupled with obvious signs of anthropogenic stressors, indicate that the health of such seemingly sheltered streams ought not be taken for granted. As potential refugia and breeding grounds for hellbenders, North Carolina's small tributaries require increased survey efforts to help define how local hellbender populations utilize them and how heavily populated these systems are. This research supported the notion that survey techniques which focus upon stream sections with coarse substrates, limited siltation and a high occurrence of cover rocks are most effective, particularly when covering large areas or systems with low hellbender population densities. Comparing habitat differentiators in a larger system could provide vital supporting data with greater statistical power.

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