

**EFFECTS OF PHYSICOCHEMICAL PARAMETERS AND LAND-USE COMPOSITION ON THE  
ABUNDANCE AND OCCURRENCE OF EASTERN HELLBENDERS (*CRYPTOBRANCHUS  
ALLEGANIENSIS ALLEGANIENSIS*)**

A Thesis  
by  
MANLEY WORTH PUGH

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at Appalachian State University  
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Department of Biology

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## **Abstract**

### **Effects of physicochemical parameters and land-use composition on the abundance and occurrence of eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*)**

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Altered landscapes have negative effects on stream habitats through altering hydrologic, sediment, and nutrient cycling regimes. These changes often reduce or displace populations of sensitive biota. The hellbender (*Cryptobranchus alleganiensis*) is an imperiled salamander endemic to eastern North American streams. Although once widespread, hellbender distributions have contracted and populations have declined in the past several decades. Many consider hellbenders indicators of stream health; however, few studies have empirically linked hellbender presence to habitat or water quality. I examined the utility of riparian and catchment-scale land-use and local physicochemical habitat parameters to predict hellbender occurrence in an Appalachian river drainage. Models suggest that both local habitat attributes and catchment-scale land-use/land-cover significantly predict hellbender occurrence and abundance. Because broad-scale land-use changes likely affect hellbender distributions, management and conservation efforts should focus on protecting

stream catchments. Localized changes are also likely important but the high economic value of other cold-water resources in the area and existing streamside management guidelines may help buffer land-use impacts. Lastly, extinction debt associated with historical or recent land-use changes in parts of this quickly changing watershed possibly has yet to be realized.

## **Dedication**

This work is dedicated to my parents whose love and support have allowed me to pursue my dreams. Without their knowledge, time, and patience I would not have been able to achieve this. Further, this work is also dedicated to the rest of my family and friends who have always believed in me.

## **Acknowledgements**

Firstly, I extend my utmost appreciation to Drs. Lynn Siefferman and Michael Gangloff whose guidance, support, and encouragement have allowed me to achieve goals in my life that I previously thought I was not capable of. I thank my other committee member Dr. Wayne Van Devender who, during my undergraduate career, sparked a long dormant passion for herpetology. Since then his criticism and seemingly comprehensive knowledge of herpetofauna has been a vital component of my master's research. I thank Lori Williams and John Groves who first inspired my fascination with hellbenders and have been pivotal in the execution of this project. I also extend a special thanks to the Gangloff-Siefferman lab who I have had the distinguished privilege of working with over the past few years. Through their assistance on field work, academic advice, and friendship they have made my graduate experience enjoyable. I extend appreciation to all other faculty and colleagues at Appalachian State University who have helped me with teaching, academics, and who have always been good friends. I am also grateful for the financial support provided by the Graduate School's Zigli Research Award and the Department of Biology at Appalachian State University.

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## Foreword

The research detailed in this thesis will be submitted to the peer-reviewed journal *Freshwater Biology*. The thesis has been prepared according to the style guide for the journal.

## INTRODUCTION

### Land-Use Effects on Streams and Lotic Fauna

Both current and historical land-use may greatly affect stream ecosystem function and integrity (Huston, 2005; Moore & Palmer, 2005; Krause *et al.*, 2008; Maloney *et al.*, 2008). The frequency and intensity of disturbance resulting from land-use change can reduce stream water quality, invertebrate and fish diversity, and ultimately, ecosystem services (Snyder *et al.*, 2003; Allan, 2004; Pan *et al.*, 2004; King *et al.*, 2005; Ahearn *et al.*, 2005; Weijters *et al.*, 2009). Land-use change may also intensify the effects of hydrologic events reducing substrate heterogeneity as well as increased nutrient and sediment inputs (Naiman & Decamps, 1997; Harding *et al.*, 1999; Gulis & Suberkropp, 2003; Strayer *et al.*, 2003; Arthington *et al.*, 2009).

Altered physicochemical conditions may negatively impact populations of lotic taxa including benthic insects, mollusks, fishes, and amphibians. Generally, it is understood that local species richness in these taxa is positively related to the stability and integrity of nutrient cycling, flow regimes and substrate composition (Heino *et al.*, 2002; Heino, Muotka & Paavola, 2003; Snyder *et al.*, 2003; Willson & Dorcas, 2003; Price *et al.*, 2006; Maloney *et al.*, 2008; Barrett & Guyer, 2008; Weijters *et al.*, 2009). Increased sediment loads and decreased substrate heterogeneity may reduce shelter and survivorship of interstitial organisms and have dramatic effects on other important components in stream ecosystems.

Numerous studies have examined land-use effects on stream amphibian populations and community composition (reviewed in Collins & Storfer, 2003; Beebee & Griffiths, 2005). Studies of

smaller streams indicate that increased land-use disturbance intensity reduces abundance, species richness, and body condition of aquatic amphibians (Houlahan & Findlay, 2003; Willson & Dorcas, 2003; Gray & Smith, 2005; Price *et al.*, 2006, 2011; Price, Browne & Dorcas, 2012; Barrett & Guyer, 2008; Barrett *et al.*, 2010). Aquatic and semi-aquatic salamanders are considered indicator species in stream ecosystems because they are long-lived while concomitantly having physiologies that are largely open to the environment (Duellman & Trueb, 1985). Moreover, many Appalachian salamanders are regional endemics and are likely adapted to specific local environmental conditions (Petranka, 1998).

### **The Hellbender**

Hellbenders (*Cryptobranchus alleganiensis*) are large salamanders (>40 cm total length) that are fully aquatic and endemic to upland streams of the Appalachian and Ozark Mountains (Smith, 1907; Nickerson & Mays, 1973; Petranka, 1998). Currently, there are two sub-species recognized: the eastern hellbender (*C. a. alleganiensis*) and the Ozark hellbender (*C. a. bishopi*) (Nickerson & Mays, 1973); however, phylogenetic analyses suggest that these taxon designations are problematic and may be artificial (Routman, Wu & Templeton, 1994; Sabatino & Routman, 2009; Tonione, Johnson & Routman, 2011).

Hellbenders are long-lived which require specific habitat and prey items to maintain viable populations in streams (Smith, 1907; Nickerson & Mays, 1973). Hellbenders are often considered to be indicators of high water quality (Hillis & Bellis, 1971; Nickerson & Mays, 1973; Nickerson, Krysko & Owen, 2003); however, few studies have quantified or linked the effects of habitat and water quality on hellbenders empirically. Recent studies suggest that hellbender populations are declining in the majority of their range (Mayasich, Grandmaison

& Phillips, 2003; Wheeler *et al.*, 2003; Briggler *et al.*, 2007; Foster, McMillan & Roblee, 2009; Burgmeier *et al.*, 2011; Graham *et al.*, 2011). While there are many plausible causes of these declines, the most likely factor is habitat degradation of hellbender habitat through changes in local and regional land-use because of the negative effects land-use change has on stream habitats and water quality.

### **Goals of Thesis**

In this study I quantify hellbender abundance and both local (e.g. reach-scale) physicochemical and landscape (e.g. land-use/land-cover) parameters in a forested and sparsely populated river drainage in Western NC and Eastern TN. I examine the utility of habitat parameters to predict hellbender abundance and occurrence. I predicted that hellbender presence and abundance would share positive relationships with higher water quality, heterogeneous substrate composition, and more forested catchments.

## MATERIALS AND METHODS

### Hellbender Surveys

I sampled hellbenders from May-August in 2011 and 2012 at 20 sites in the Watauga River Drainage (Fig. 1). All sites consisted of a 150 m stream reach divided by cross-channel transects at 10 m intervals ( $n = 16$  per site). Sites were selected based on available access to tributaries, historical reports, and sites where previous researchers conducted studies on hellbenders. I scouted all sites prior to selection to insure that they contained suitable hellbender habitat (i.e., large to medium sized rocks, deep pools, fast-flowing riffles) (Hillis & Bellis, 1971; Nickerson & Mays, 1973). Field teams used timed visual-tactile surveys and snorkeled in an upstream direction systematically turning rocks by hand or using log peaveys to detect hellbenders (Nickerson & Krysko, 2003). I calculated catch per unit effort (CPUE) as the number of hellbenders captured per person hour (number of people searching x search time) at the site scale.

For all captured hellbenders, I determined sex (if possible) using the presence or absence of cloacal swelling (Petranka, 1998) and measured total length (TL), snout-to-vent length (SVL) (within 1 cm), and tail width (TW) (within 1 mm). Animals were classified as larvae if they had free gills, juveniles if they lacked free gills but with a TL < 22 cm, and adults if TL > 22 cm. Adult hellbenders were injected with Passive Integrative Transponder

(PIT) tags in subcutaneous tissue at the dorsum of the base of the tail. PIT numbers were stored in a PIT tag reader (BioMark Inc, Boise ID, USA). Individuals with TL < 22 cm were tagged with Visible Implant Elastomers ((VIE) Northwest Marine Technology Inc., Shaw Island WA, USA). All animals were returned to their point of capture after being processed.

### **Habitat Characterization**

I recorded the width of the stream channel at each transect and selected five 0.25 m<sup>2</sup> quadrats within each transect ( $n = 80$  per site). For each quadrat, I recorded distance to bank, water depth, mid-water column current velocity, and substrate composition. I used a modified Wolman pebble count to estimate median substrate size and composition. I measured all lithic particles with diameters > 2.0 mm and classified boulders as particles > 2 m diameter. I classified non-lithic particles as: bedrock, silt, sand, organic matter, and woody debris. I then used these data to calculate medians of particle size and means of stream width, current velocity, depth, and percent non-measurable substrate for each study site.

Water chemistry was assessed 3 times during 2011 and 2012 by measuring DO (% saturation and mg/L), pH, and conductivity at each site using a YSI Pro Series multi-meter (YSI Inc., Yellow Springs, OH, USA). I quantified stream nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) concentrations by analyzing three to five water samples at each site. Samples were frozen and analyzed within one week of sample collection. Concentrations of  $\text{NH}_3$  and  $\text{NH}_4^+$  were determined using an ammonium determination assay (Keeney & Nelson, 1982; Parsons,

Maita & Lalli, 1984; Mulvaney, 1996) and  $\text{NO}_x^-$  concentration was determined using manual vanadium (III) reduction (Miranda, Espey & Wink, 2001; Doane & Horwarth, 2003). Although manual vanadium (III) reduction tests for all variants of  $\text{NO}_x^-$ , the major contributor to this concentration is  $\text{NO}_3^-$  and will be referred to as such hereafter. Individual samples were run in triplicate and averaged for each sample and then for each site.

### **Land-Use/Land-Cover Assessment**

I quantified upstream land-use and land-cover (LULC) at both the riparian and catchment scales. To quantify LULC percentages at the catchment scale, I used a Digital Elevation Model (6.1 m resolution) downloaded from the North Carolina Department of Transportation and merged this raster with a National Elevation Dataset (resolution 3 m) downloaded from the U.S. Geological Survey Geospatial Data Gateway. I used ArcHydro© 10.0 and Spatial Analysis Hydrology toolbox in ArcGIS© 10.0 to delineate upstream watersheds for each site (ESRI, Redlands, California). I used a 2006 National Land Cover Dataset (resolution 30 m) downloaded from the USGS Geospatial Data Gateway and clipped this raster to delineate LULC for each watershed. I then calculated percentages of each LULC category for individual watersheds. I quantified Riparian LULC for each site using buffers (100 m) of all upstream tributaries draining into a specific site locality. I clipped the same 2006 National Land Cover Dataset using these buffers and quantified LULC percentages for each site locality. I combined all LULC categories into % forest, % urban, % agriculture, and % grass/shrub prior to statistical analyses.

## Statistical Analysis

Parameters were grouped into four classes: habitat (depth, stream velocity, stream width, and substrate composition), water quality (DO (mg/L), pH, conductivity,  $\text{NO}_3^-$ ), riparian LULC, and catchment LULC. I did not include  $\text{NH}_4^+$  concentration in analyses because  $\text{NH}_4^+$  was only found at a detectable level at two sites ( $< 0.1 \mu\text{g/mL}$ ). Principal components analysis (PCA) was performed separately on all four parameter classes. Using PCA, I reduced these variables into orthogonal variables (PCs). I selected this analysis because these types of datasets are often intercorrelated and this analysis allows for more standardized variables. I used linear regression to examine the degree to which LULC PCs predict physical and water chemical PCs. A backward step-wise multivariate regression model was used to determine which PCs predict hellbender CPUE and backwards step-wise logistic regression to determine which PCs best predict hellbender presence or absence. I conducted statistical analyses using SPSS 20.0 (SPSS Inc., Chicago, IL. USA).

## Results

### Hellbenders

Hellbenders were found in 8 of 20 sites (Fig. 1). I detected larvae at the two sites which yielded the largest abundances of hellbenders ( $> 12$  captures/150 m) (Table 1). I tagged 64 hellbenders over two field seasons with seven recaptures from two sites. Recaptured animals did not grow significantly from 2011 to 2012 ( $p > 0.05$ ). I found body-sizes ranging from 6-53 cm TL and TLs were skewed toward larger size classes. One present site generated no captures during my study. However, in addition to several anecdotal reports of hellbender sightings at this locality, a North Carolina Wildlife Resources Commission (NCWRC) search team captured a hellbender in the same site (L. A. Williams, NCWRC per. comm.; 15 July 2009); therefore, I classified it as a hellbender present site.

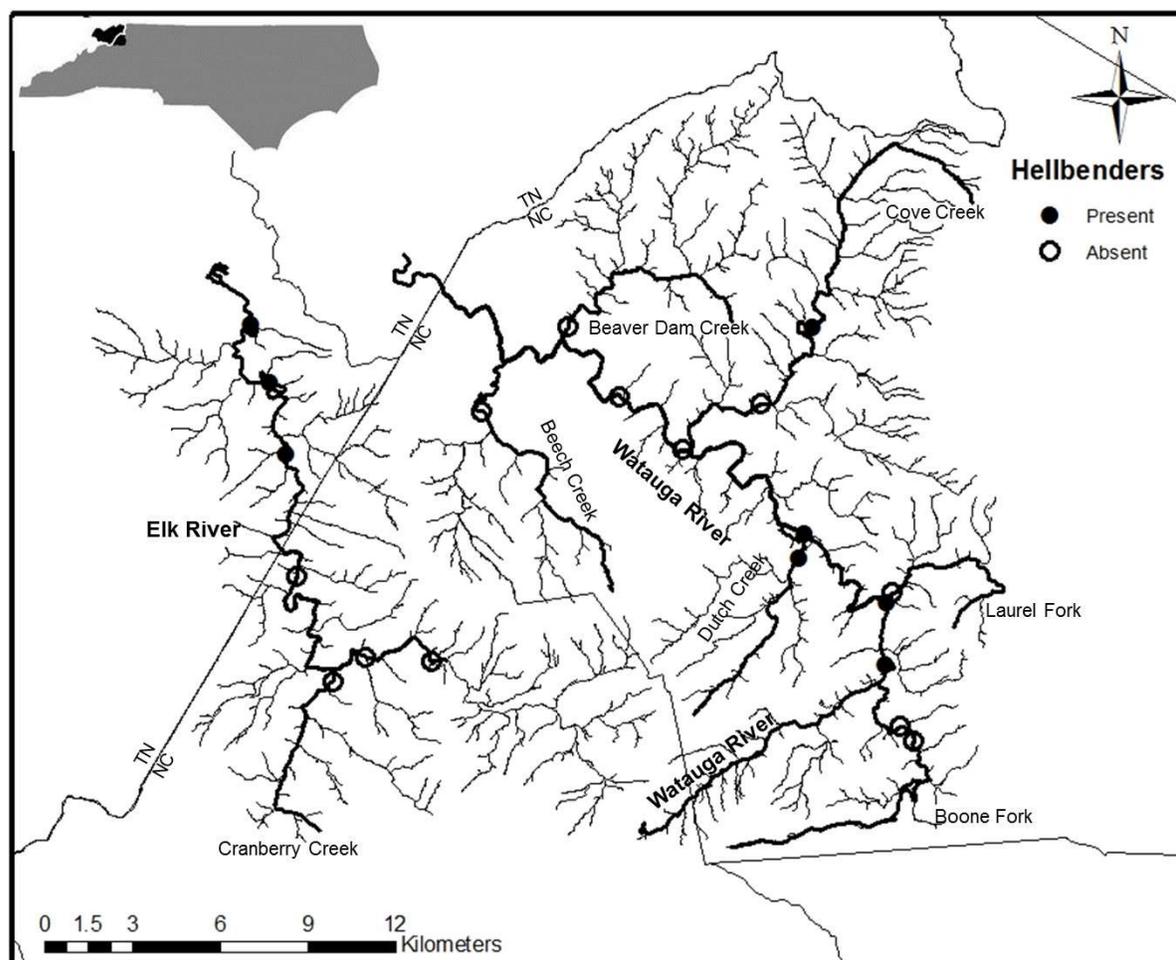


Figure 1: : Map of study streams and site localities in the Watauga River Drainage in northwestern North Carolina and northeastern Tennessee; sites where hellbenders were detected are represented by filled circles and sites that did not produce hellbender captures are represented by unfilled circles.

Table 1: Number, catch-per-unit-effort (CPUE), and mean (standard deviation) and range of hellbender total lengths (TL) collected from 7 sites in the Watauga River Drainage in 2011 and 2012.

Site Number	No. Hellbenders		Total Length (TL)		Recaptures	Larvae/ Juveniles	CPUE
	2011	2012	$\bar{x}$ (SD)	Range			
1	13	20	40.49 (9.82)	6-53	4	5	0.748
2	1	2	49 (-)	49	0	0	0.126
3	0	1	53	53	0	0	0.111
4	1	2	47 (2.83)	45-49	0	0	0.267
5	0	0	-	-	-	-	-
6	15	17	37.41 (7.93)	8-46	3	3	0.831
7	1	0	52 (-)	52	0	0	0.133
8	1	0	18 (-)	18	0	0	0.118
Total	32	42			7	8	

### Principal Components Analysis

All habitat and LULC data were normally distributed (Shapiro-Wilk  $p > 0.05$ ). Principal components analysis of habitat data produced four PCs that cumulatively explained 72.2% of the variation in the habitat data (Table 2). Habitat PC<sub>1</sub> explained 27.7% of the overall variation and % fine substrates loaded strongly negatively on PC<sub>1</sub> while stream width, depth, stream velocity, and median substrate size loaded strongly positively. Habitat PC<sub>2</sub> explained 17.2% of the overall variation in habitat. Percent organic and fine substrates loaded positively on habitat PC<sub>2</sub> while median substrate size loaded negatively. Habitat PC<sub>3</sub> explained 14.1% of the overall variation. Current velocity loaded positively on habitat PC<sub>3</sub> and % bedrock loaded negatively. Habitat PC<sub>4</sub> explained 13.2% of the overall habitat

variability. Stream velocity loaded positively on Habitat PC<sub>4</sub> and % boulder loaded negatively.

Principal components analysis of water quality parameters produced only one PC (water quality PC<sub>1</sub>) that explained 50% of the total variation (Table 2). Conductivity and NO<sub>3</sub><sup>-</sup> concentration loaded strongly positively and pH loaded negatively on water quality PC<sub>1</sub>. Principal components analysis of riparian LULC created two PCs that explained 83.16% of the variability of riparian LULC. Riparian PC<sub>1</sub> explained 57% of the variation and % forest cover loaded strongly negatively on PC<sub>1</sub>. Riparian PC<sub>2</sub> explained 26% of the variation in riparian LULC and % urban cover loaded negatively on PC<sub>2</sub>. PC analysis of catchment LULC revealed two PCs that explained 81% of the variability in catchment LULC. Catchment PC<sub>1</sub> explained 48% of the total variation in catchment LULC and % forest cover had a strong negative loading score for PC<sub>1</sub>. Catchment PC<sub>2</sub> explained 33% of the variability in catchment LULC. Percent agricultural cover loaded negatively while % urban cover loaded positively on PC<sub>2</sub>.

Table 2: Loading factors and percent variance explained for principal components analysis of all PC groups. Underlined values represent loading factors with absolute values > 0.5 and bolded percent variance explained values represent PCs included in multivariate and logistic regression models.

Physical Habitat	Variable	PC <sub>1</sub>	PC <sub>2</sub>	PC <sub>3</sub>	PC <sub>4</sub>
	Stream Width	<u>0.884</u>	0.276	-0.018	-0.200
	Depth	<u>0.585</u>	0.479	-0.350	0.309
	Stream Velocity	<u>0.618</u>	-0.163	<u>0.608</u>	0.115
	Median Substrate	<u>0.555</u>	<u>-0.571</u>	-0.227	-0.240
	%Wood	-0.412	-0.105	0.486	-0.088
	% Bedrock	0.095	-0.107	<u>-0.579</u>	-0.197
	% Organic	0.327	<u>0.710</u>	0.341	-0.303
	% Boulder	-0.028	0.146	-0.041	<u>0.882</u>
	% Fine Substrates	<u>-0.626</u>	<u>0.587</u>	-0.180	-0.264
	%Variation Explained	<b>27.68</b>	<b>17.23</b>	<b>14.07</b>	<b>13.24</b>
Water Chemistry					
	NO <sub>3</sub> <sup>-</sup>	<u>0.830</u>	-	-	-
	DO (% Saturation)	0.386	-	-	-
	Conductivity	<u>0.836</u>	-	-	-
	pH	<u>-0.558</u>	-	-	-
	% Variation Explained	<b>49.95</b>			
Riparian LULC					
	% Urban	<u>0.543</u>	<u>-0.792</u>	-	-
	% Forest	<u>-0.973</u>	0.158	-	-
	% Agriculture	<u>0.846</u>	0.333	-	-
	% Grass/Shrub	<u>0.570</u>	0.529	-	-
	% Variation Explained	<b>57.07</b>	26.09		
Catchment LULC					
	% Urban	<u>0.571</u>	<u>0.677</u>	-	-
	% Forest	<u>-0.979</u>	0.147	-	-
	% Agriculture	<u>0.583</u>	<u>-0.812</u>	-	-
	% Grass/Shrub	<u>0.542</u>	0.425	-	-
	% Variation Explained	<b>47.94</b>	33.03		

## Regression Analyses

Linear regression showed a significant positive relationship between Water Quality PC<sub>1</sub> and both Riparian PC<sub>1</sub> (riparian LULC) ( $R^2 = 0.38$ ;  $p = 0.004$ ) and Catchment PC<sub>1</sub> ( $R^2 = 0.44$ ;  $p = 0.001$ ) (Fig. 2). This suggests that reductions in forest cover at the riparian and catchment scales increase NO<sub>3</sub><sup>-</sup> concentration and conductivity in streams. The multivariate regression model showed a significant positive relationship between Habitat PC<sub>1</sub> and hellbender CPUE ( $R^2 = 0.20$ ;  $p = 0.04$ ) (Fig. 3). The logistic regression model found that catchment PC<sub>1</sub> and Habitat PC<sub>1</sub> and PC<sub>3</sub> form a significant predictive model of hellbender presence and absence ( $R^2 = 0.49$ ;  $\chi^2 = 8.93$ ;  $p = 0.03$ ). Overall model classification success was 90% and correctly predicted hellbender absence at 100% and presence at 75% of all sites respectively. The coefficient with the highest influence on the logistic model was catchment PC<sub>1</sub> ( $Wald \chi^2 = 2.93$ ;  $p = 0.09$ ) (Table 3) (Fig. 4).

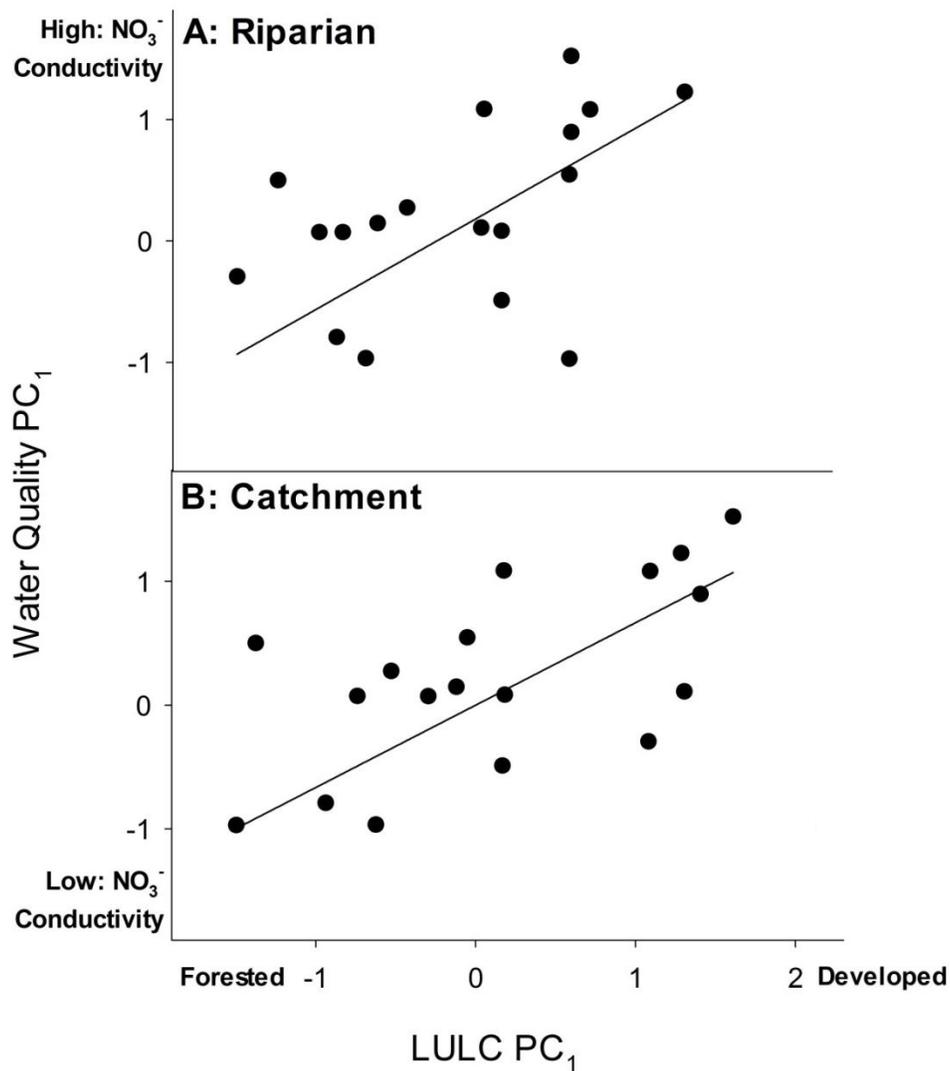


Figure 2: Relationships between water quality PC<sub>1</sub> and (a) riparian PC<sub>1</sub> ( $R^2 = 0.379$ ;  $p = 0.004$ ) (b) catchment PC<sub>1</sub> ( $R^2 = 0.443$ ;  $p = 0.001$ ). Percent upstream forest cover loads negatively on riparian PC<sub>1</sub> and catchment PC<sub>1</sub>. Conductivity and NO<sub>3</sub><sup>-</sup> concentration load positively on water quality PC<sub>1</sub>.

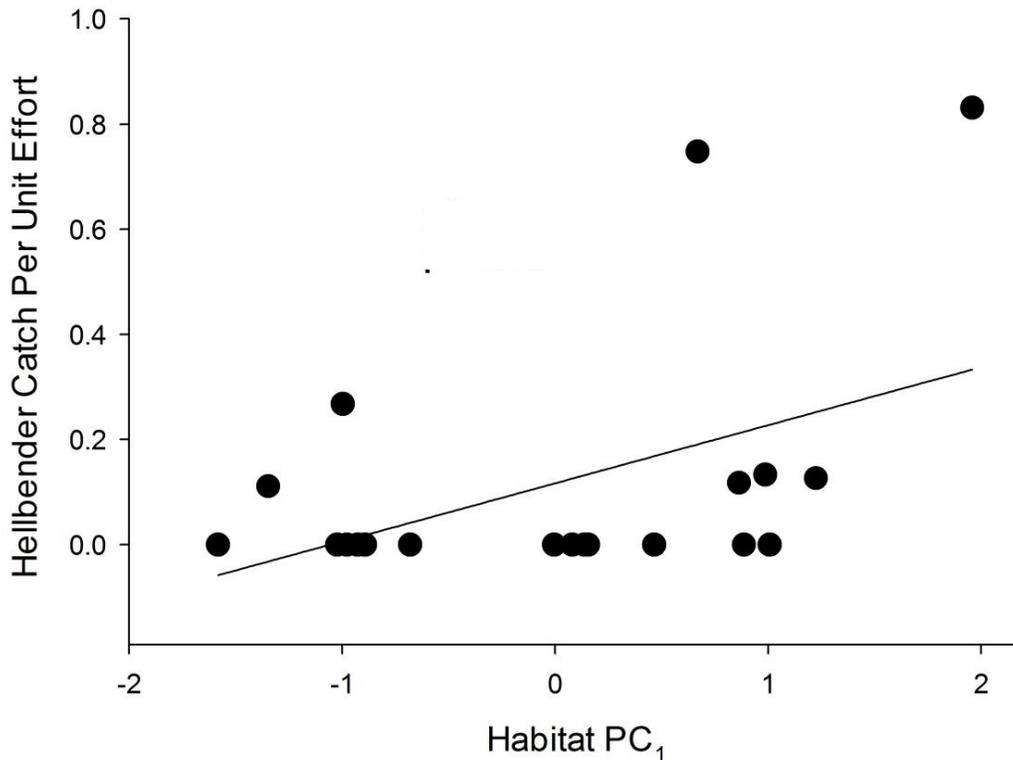


Figure 3: Scatter-plot displaying relationship between hellbender catch per unit effort and Habitat PC<sub>1</sub> ( $R^2 = 0.201$ ;  $p = 0.043$ ). CPUE was calculated as the number of animals captured divided by the number of person search hours. Stream size, flow, and median substrate size load positively while percent of fine substrates loads strongly negatively on habitat PC<sub>1</sub>.

Table 3: Estimated regression coefficients (B), Wald  $\chi^2$ , Explanatory power of (B), and p values from backward step-wise logistic regression model.

Coefficient	Hellbender Present/Absent Model			
	<i>B</i>	<i>Wald</i>	Exp( <i>B</i> ), 95% CI	<i>P</i>
Habitat PC <sub>1</sub>	1.003	2.368	2.728	0.124
Habitat PC <sub>3</sub>	-1.059	2.200	0.347	0.138
Catchment PC <sub>1</sub>	-1.249	2.926	0.287	0.087
Constant	-0.552	0.767	0.576	0.381

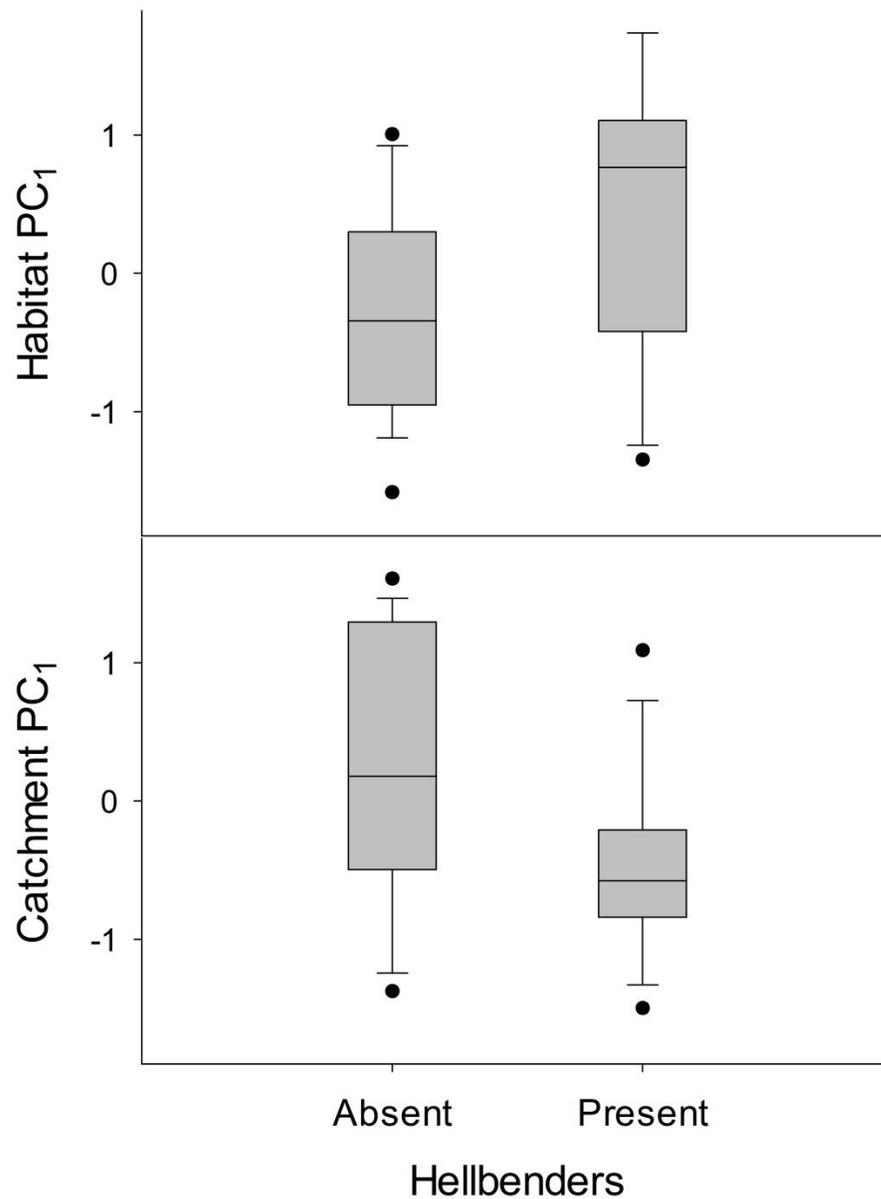


Figure 4: Box plots of habitat PC<sub>1</sub> and catchment PC<sub>1</sub> based on hellbender presence/absence. The line in the box represents the median, the boxes are the 25-75<sup>th</sup> percentiles while the whiskers are the 10<sup>th</sup> and 90<sup>th</sup> percentiles. Stream size, flow, and median substrate size load positively while percent of fine substrates loads negatively on Habitat PC<sub>1</sub>. Percent upstream forest cover loads negatively on catchment PC<sub>1</sub>.

## Discussion

Analysis of LULC data suggests that hellbenders are very sensitive to changes in forest cover. Forested catchments and riparian zones protect water quality by slowing nutrient export and spiraling rates and attenuating the power of hydrologic events (Peterjohn & Correll, 1984; Gregory *et al.*, 1991; Naiman, Decamps & Pollock, 1993; Naiman & Decamps, 1997; Nilsson & Berggren, 2000; Snyder *et al.*, 2003; Allan, 2004; Ahearn *et al.*, 2005; Krause *et al.*, 2008; Arthington *et al.*, 2009). Few hellbenders were found in catchments with < 80% forest cover. Although this level of sensitivity is alarming, it also suggests that buffer zones and selective re-forestation may help mediate the effects of recent ex-urban development and that re-forestation is one of the most promising strategies to restore degraded catchments and adjoining hellbender streams.

Linear regression models suggest that LULC and water quality are strongly linked in the Watauga Drainage. Both multivariate and logistic models support the hypothesis that physical habitat and LULC are strong predictors of hellbender abundance and occurrence. The strongest predictor of hellbender presence was catchment PC<sub>1</sub> (Table 3), which supports the speculations of Pugh *et al.*, (2013) that broad-scale LULC likely influences hellbender occurrence. Although numerous studies have shown the positive effects of riparian forests on stream habitats (Gregory *et al.*, 1991; Naiman *et al.*, 1993; Naiman & Decamps, 1997; Nilsson & Berggren, 2000; Snyder *et al.*, 2003), my models found little

evidence that riparian LULC affects hellbender distribution and abundance. This could represent a high sensitivity of hellbenders to changes in habitat and water quality or could be a result of the coarse resolution used to conduct the riparian analysis. Local physical habitat attributes, primarily habitat PC<sub>1</sub> (larger stream size and particles with reduced fine substrates), were also strong predictors of hellbender occurrence and influenced both multivariate and logistic models.

Hellbender occurrence is highly variable in the Watauga Drainage (Fig. 1). The two largest tributaries, the Watauga and Elk Rivers, had contrasting distribution patterns. In the Watauga River, the majority of hellbender captures occurred in headwater reaches whereas all hellbender captures in the Elk River occurred in downstream reaches closer to its confluence with the Watauga River. This pattern coincides with general land-use patterns in this region. The upper Watauga River drains protected lands on the Blue Ridge Parkway and Grandfather Mountain. Further, small-scale agriculture (primarily pasture) dominate land use along the lower Watauga River Valley. Forest clearing frequently extends into riparian zones for these operations. Although  $\text{NH}_4^+$  was too low to detect at most sites and  $\text{NO}_3^-$  concentrations in the Watauga Drainage are low ( $< 1.0 \mu\text{g/mL}$ ) compared to Piedmont streams, they have likely increased with expanding ex-urban and agricultural development in this drainage. The headwaters of the Elk River originate within the ski resort towns of Sugar Mountain and Banner Elk. Much of the Elk River's headwaters have been developed and this may impact water quality in the upper Elk River. Downstream from Banner Elk, the river flows through Pisgah and Cherokee National Forest lands. Riparian and catchment

forest protection on these federal lands may mediate upstream water quality impacts and help improve hellbender habitat quality in the Elk River.

The patchy distribution of hellbenders in the Watauga River Drainage may also indicate land-use mediated extinction debt within this drainage (Kuussaari *et al.*, 2009; Jackson & Sax, 2010). The majority of captures occurred at two sites (Table 1). Other sites produced only one to three captures of large, presumably older animals during repeated sampling events. Because hellbenders do not appear to be reproducing (i.e., lack of larvae and juveniles) in many reaches, these sub-populations are unlikely to persist without the addition of new individuals. In the multivariate regression model, habitat PC<sub>1</sub> was positively related to hellbender CPUE but the relationship though significant is not very strong. Two hellbender present sites received low habitat PC<sub>1</sub> scores which may suggest that these habitats are degrading but not past the threshold of sustaining hellbenders at this point in time. Perhaps the addition of sites with intermediate capture rates this relationship would become less obscure and provide evidence to test for extinction debt in this drainage. Further, the logistic regression model misclassified 25% of hellbender present sites as absent sites most likely because of because a few had low habitat PC<sub>1</sub> and high catchment PC<sub>1</sub> scores. The remaining hellbenders in these sites may represent a lag period between habitat degradation and hellbender extirpation from these reaches hindering the predictive power of the model. Regardless, further research will be required to confirm these speculations through creating more accurate habitat and land-use models.

My results demonstrate two distinct patterns in hellbender distribution and habitat parameters. First, stream physicochemical habitat parameters are significant predictors of

both hellbender presence and abundance. Second, LULC is a strong predictor of hellbender occurrence at the catchment scale and forest cover, at both riparian and catchment scales, influences local water quality in our study drainage. Sites in stream reaches with lower proportions of upstream forest cover tended to have higher nutrient and conductivity levels compared to more forested reaches. Together, my data suggest that relatively subtle changes to stream physicochemical habitat parameters are a likely source of hellbender declines throughout their range. Moreover, my data corroborate the work of others demonstrating that hellbenders are effective indicators of stream habitat and water quality (Smith, 1907; Hillis & Bellis, 1971; Nickerson & Mays, 1973; Nickerson *et al.*, 2003; Wheeler *et al.*, 2003; Briggler *et al.*, 2007; Hopkins & DuRant, 2011).

A preponderance of evidence suggests that hellbender declines are recent, dramatic and geographically widespread (Mayasich *et al.*, 2003; Wheeler *et al.*, 2003; Briggler *et al.*, 2007; Foster *et al.*, 2009; Burgmeier *et al.*, 2011; Graham *et al.*, 2011) leading to the classification of the Ozark hellbender (*C. a. bishopi*) as “endangered” under the U.S. Endangered Species Act (United States Fish Wildlife Service 2011). Hellbender decline has many plausible sources including prevalence of disease (i.e., *Ranavirus* and *Batrachochytrium dendrobatidis*) (Geng *et al.*, 2011; Bodinof *et al.*, 2011; Souza *et al.*, 2012) introduction of non-native predatory fishes (i.e., Brown and Rainbow Trout) (Gall & Mathis, 2010), and some notable instances of illegal collection (Nickerson & Briggler, 2007). My data provide the first quantitative link between landscape (LULC) parameters, local-scale habitat conditions, and hellbender abundance and occurrence. These relationships suggest that

recent land-use change in Central and Eastern U.S. has led to modification in stream physicochemical parameters and hellbender habitats.

Hellbender decline is alarming because hellbenders are important components of stream communities and likely impact the trophic stability of lotic systems (Smith, 1907; Hillis & Bellis, 1971; Nickerson & Mays, 1973; Humphries & Pauley, 2005). Additionally, declines suggest deteriorating water quality in many headwater systems of the Central and Eastern U.S.. Because headwaters significantly influence downstream water quality and quantity, degraded water quality may interfere with the quality of downstream ecosystem services available to human populations. (Ward, 1989; Pringle, 2001, 2003; Pan *et al.*, 2004; Freeman, Pringle & Jackson, 2007; Nadeau & Rains, 2007; Alexander *et al.*, 2007).

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### **Biographical Sketch**

Manley Worth Pugh was born in Raleigh NC but then moved to Asheboro NC. He attended grade schools in Asheboro and graduated from Asheboro High School in 2006. He graduated with a Bachelor of Science degree in Ecology, Evolution, and Environmental Biology at Appalachian State University in 2010. During his undergraduate studies, Pugh took a special interest in herpetology and traveled to Vietnam, Costa Rica, and Peru on trips sponsored by Appalachian State International Programs where he searched for these organisms along side international researchers from these countries. He started a master of science program in August of 2011 at Appalachian State and completed his master's in August of 2013.