

TROPHIC ECOLOGY OF AN IMPERILED GIANT SALAMANDER
(*CRYPTOBRANCHUS A. ALLEGANIENSIS*) IN SOUTHERN APPALACHIAN STREAMS

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by
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Abstract

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Eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) are large, aquatic amphibians that represent a significant portion of vertebrate predator biomass in many systems, yet their trophic ecology and role in aquatic foodwebs has remained largely speculative. Although past gut content and observational studies of hellbenders suggest that they predominantly eat crayfish, such methodological approaches may be biased toward prey items that remain in the stomachs of consumers longer (e.g., crayfish chelae) compared to other food items (e.g., fish tissues). Additionally, no hellbender dietary studies have been conducted in streams where crayfish abundances are very low. I determined variation in the trophic position of eastern hellbenders in three streams in western North Carolina along a gradient of land-use from a pristine to a highly impacted site, using stable isotope analysis of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. I found that although eastern hellbender trophic position did not vary among sites eastern hellbenders in the South Toe River (a site characterized by extremely low crayfish biomass) appear to have a diet comprised of both crayfish and benthic fish species.

Additionally, eastern hellbenders in the South Toe occupy a significantly higher trophic position than a top fish predator, Rock bass (*Ambloplites rupestris*). These results suggest that, in some streams, eastern hellbenders may act as apex predators and could potentially exhibit top-down control of aquatic community structure. The differences in diet across these three river systems suggest that these ancient amphibians are do not feed exclusively on crayfishes and that they appear capable of altering their dietary strategies to meet energy demands when resource availability varies.

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and may you serve and protect the incredible biodiversity of our streams and skies. I thank Dr. Ed Burrell, who always found time to answer all of my silly questions and helped me understand the ins and outs of stable isotope analysis, and Dr. Madritch, who gave me access to the equipment and materials that I needed to complete my project. To my dog, Sky, who joined my small animal family the day before I started grad school and guarded both myself and my sanity throughout the entire process. Finally, I would like to thank Appalachian State University for funding my project and making this research possible.

Dedication

This thesis is dedicated to Jimmy “Papa” Yaun (1941-2016) and Richard “Sam” Jakiel (1941-2017). One taught me to love nature, the other to love science. Both taught me to never give up.

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Introduction

Foodwebs are deeply intertwined with patterns of biodiversity and ecosystem function (Thompson et al., 2012) and have been used to describe ecosystem characteristics such as community structure (Cohen & Newman, 1985), competition (Paine, 1966), and consumer effects on ecosystem functions (Carpenter, Kitchell, & Hodgson, 1985; Power, 1990). An important aspect of foodwebs is trophic position, which describes a consumer's average feeding level relative to primary producers and can be used to classify species into functional groups (Layman, Winemiller, Arrington, & Jepsen, 2005). Alterations to the distribution of organisms within functional groups may lead to changes in ecosystem function and have cascading effects on other trophic levels (Rall et al., 2012).

Lotic systems are strongly influenced by surrounding landscapes (Allan, 2004; Vannote, Minshall, Cummins, Sedell, & Cushing, 1980). Alterations to these landscapes can have dramatic impacts on community structure and ecosystem function (Allan, 2004). Streams in agricultural areas tend to be characterized by higher water temperatures and nutrient concentrations (Karr & Schlosser, 1978; Allan, 2004). Nutrient loading can result in eutrophication, altering the composition of basal resource assemblages and the replacement of native, sensitive species with non-native, intolerant species (Lenat & Crawford, 1994). Urbanization (i.e., increases in impervious surfaces) typically results in increased runoff, leading to increases in nutrients and pollutants, and ultimately can alter the function and structure of aquatic communities through changes in the abundance and diversity of basal sources and fish and invertebrate diversity (Paul & Meyer, 2001). The impacts of

urbanization can cause drastic changes to stream communities even when urban land-use occurs at relatively low levels (<15%).

Changes in land-use such as agriculture and urbanization can affect the structure and functioning of aquatic foodwebs, and can ultimately alter the abundance and distribution of prey sources available to consumers (Allan, 2004). Characterizing the variation of a species' diet is a key component to understanding the consequential implications of ecological and evolutionary processes (Zerba & Collins, 1992). If diet varies substantially enough between populations of a given species, they may occupy different trophic positions within their respective foodwebs (Trippel & Beamish, 1993; Vander Zanden & Rasmussen, 1996; Vander Zanden, Shuter, Lester, & Rasmussen, 2000). Historically, a species' trophic position was defined using behavioral observations and examination of stomach contents, two labor-intensive methods that are often limited by small sample sizes and that may be unsuitable for working with sensitive taxa or endangered species (Bryan & Larkin, 1972; Zerba & Collins, 1992). Recently, however, ecologists have used isotope ratios to quantitatively define trophic position on a continuous, rather than discrete, scale and trace the flow of energy and matter through communities (Vander Zanden & Rasmussen, 1996). Variation in consumer diets has also been linked to variation in prey abundances (e.g., Allan, 1981; Greenstreet, McMillan, & Armstrong, 1998). Variation in trophic position among populations can be inferred using stable isotopes because this method provides a time-integrated measure of trophic position for individuals (Vander Zanden & Rasmussen, 1999).

Eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) are large (up to 2.2 kg), aquatic and increasingly rare amphibians that represent a significant portion of vertebrate predator biomass in many systems, yet their trophic ecology and role in aquatic foodwebs is poorly understood. This is likely due to the secretive behavior of hellbenders which makes dietary studies of these amphibians difficult. Hellbenders are thought to be nocturnal and use refugia such as large, flat boulders and submerged logs during the day (Noeske & Nickerson, 1979), making it difficult to observe foraging behavior. Humphries and Pauley (2005) have speculated that hellbenders occupy high trophic positions, although this postulation has not yet been empirically studied.

Past observational and gut content studies of hellbenders have found that they feed primarily on crayfishes (Smith, 1907; Netting, 1929; Green, 1935; Bishop, 1941; Swanson, 1948; Nickerson & Mays, 1973; Peterson, Reed, & Wilkinson, 1989), which can comprise as much as 100% of their diet (Smith, 1907; Green, 1935; Peterson et al., 1989). The geographic range of hellbenders is congruent with the highest biodiversity of freshwater crayfish in the world (Taylor et al., 1996). When found in high abundances, hellbenders may influence populations of ecologically important primary and secondary consumers such as crayfish. Because crayfish are keystone species and ecosystem engineers in some systems (Creed, 1994; Creed & Reed, 2004; Usio & Townsend, 2004), hellbenders may indirectly affect stream trophic pathways and ecosystem functions through their influence on invertebrate populations (Peterson et al., 1989; Humphries & Pauley, 2005). The importance of crayfish in hellbender diets is further supported by qualitative data that suggests crayfish availability

may limit the abundance and fitness of hellbenders (Nickerson, Krysko, & Owen, 2003; Hecht-Kardasz, Nickerson, Freake, & Coclough, 2012). Fish are the second most-cited food source in hellbender diet studies. Peterson et al. (1989) found that fish were present in the stomachs of 20% of the 108 hellbenders sampled. Fishes in hellbender stomachs included *Cottus* spp., *Oncorhynchus mykiss*, *Campostoma anomalum*, and lampreys (family Petromyzontidae; Nickerson, Ashton, & Braswell, 1983; Peterson et al., 1989). Other food items include snails and aquatic insect larvae (Nickerson et al., 1983; Peterson et al., 1989) and non-food items such as fishing line, hooks, plastic bait, and rocks (Peterson et al., 1989) have also been recovered from hellbender stomachs. Finally, several studies show that hellbenders are cannibalistic (Smith, 1907; Bishop, 1941; Nickerson & Mays, 1973; Peterson, Metter, Miller, Wilkinson, & Topping, 1988) starting as larvae (Smith, 1907); adults will cannibalize oocytes, eggs, and juveniles (Nickerson & Mays, 1973; Humphries, Solis, Cardwell, & Salveter, 2005; Miller & Miller, 2005).

Although researchers have used stomach content studies to examine hellbender diet, stomach content analysis has several major limitations. First, different prey items have varying digestion rates in salamanders (Jaeger, 1990) and fish (Moyle & Cech, 2000). For example, Wiggs (1976) fed captive hellbenders a variety of food items and found that crayfish exoskeletal elements, such as chelae, remain in the digestive tract longer than do the tissues of fish. Second, analyses of stomach contents also only show what an animal has ingested, which is not always necessarily representative of assimilation or contribution to the consumer's nutrition profile (Bearhop et al., 1999; Votier, Bearhop, MacCormick, Ratcliffe,

& Furness, 2003). For instance, hellbenders are ambush predators that feed using buccal suction (Cundall, Lorenz-Elwood, & Groves, 1987) and thus may ingest non-target materials.

Another method used to examine the diet of consumers is body condition, or the deviance of expected mass from a given body length. Body condition correlates with fat storage (Pope & Matthews, 2002), which can influence health, fecundity (Girish & Saidapur, 2000), growth, and mortality (Anderson, 1996). Factors that can influence body condition include land-use (Karraker & Welsh, 2006), stress, disease (Bodinof et al., 2012), food availability (Pope & Matthews, 2002), temperature, and population density (Reading & Clarke, 1995). Hecht, Nickerson, Freake, Colclough, and Stofer (2019) found that hellbender body condition is positively correlated with relative frequencies of crayfish. Therefore, in systems that support high crayfish abundances, hellbender body condition can be expected to be higher than in streams with low abundances of crayfish.

In this study, I compared eastern hellbender trophic position among three populations in the New and Tennessee drainages and then examined the influence of biological and abiotic factors on the trophic position of eastern hellbenders in each stream. I hypothesized that the trophic position of eastern hellbenders would vary along a land-use gradient ranging from sites categorized as pristine (South Toe River) to intermediate- (Watauga River) and high-impacted streams (South Fork New River). I predicted that eastern hellbenders in the South Toe River will have significantly higher trophic positions than eastern hellbenders in the South Fork New and the Watauga River will be somewhere in between. Additional variation in trophic position may be explained by differences in size/age class, where larger

adult eastern hellbenders will hold higher trophic positions than smaller juvenile eastern hellbenders.

I hypothesized that diet will also vary with prey availability among sites and predicted that eastern hellbenders in the South Fork New and Watauga rivers will have a diet consisting almost entirely of crayfish, whereas the population in the South Toe River may eat some crayfish, but crayfish are uncommon in this stream and so a substantial portion of eastern hellbender diets in this stream may be comprised of fish. Because crayfish productivity in the South Fork New and Watauga rivers is high, I predict that these populations will have higher body conditions compared to the South Toe River population.

Methods

Study Sites

I collected foodweb data from three sites (i.e., eastern hellbender populations) from June to October 2017. I sampled eastern hellbender tissue and collected specimens for trophic analyses from one site in the New River Drainage (South Fork New River, Watauga County, North Carolina) and two sites in the Tennessee River Drainage (Watauga River, Watauga County, North Carolina, and South Toe River, Yancey County, North Carolina). Sites were selected using historical eastern hellbender records acquired through the work of former graduate students at Appalachian State University (Pugh, Groves, Williams, & Gangloff, 2013; Pugh, Hutchins, Madritch, Siefferman, & Gangloff, 2016; Franklin, 2016). I

targeted sites where at least four eastern hellbenders were detected during recent surveys to obtain tissue samples from 5-6 individuals per site.

The New River flows for 515 km north-northeast from its headwaters in the mountains of Watauga County, North Carolina into Virginia and West Virginia, where its confluence with the Gauley River forms the Kanawha River, a tributary of the Ohio River (VADEQ, 2015). In North Carolina, the New River Basin drains 1950 km² across the Blue Ridge physiographic province of the southern Appalachian Mountains in Watauga, Ashe, and Alleghany counties (NCDWQ, 2011). Developed land is comprised of impervious cover along major transportation routes and within six municipalities in the three counties:

1) Watauga County: the towns of Boone and Blowing Rock, 2) Ashe County: Jefferson and West Jefferson, and 3) Alleghany County: Sparta. The South Fork New River watershed comprises an area of 909 km² and contains seven of the eight impaired stream segments within the New River Basin (NCDWQ, 2011). Land cover within this watershed is mostly forested and has the highest amount of disturbed/urban and agricultural land-cover, as well as the largest population in the New River Basin.

The Watauga River Basin is the second smallest basin in North Carolina and contains ~435 km of the Watauga and Elk Rivers with their tributaries, and spans an area of 531 km² (NCDENR, 2005). Located in the Blue Ridge physiographic province, the Watauga River Basin is characterized by forested slopes across a rugged mountainous terrain, with a geology consisting mostly of metamorphic rocks with some igneous and sedimentary areas (Griffith et al., 2002). The Watauga and Elk Rivers are impounded to form Watauga Reservoir, and

downstream the lower Watauga joins the Holston and Tennessee rivers (NCWRC, 2015). The basin's boundaries encompass six municipalities in Watauga and Avery Counties, North Carolina: Banner Elk, Beech Mountain, Seven Devils, Sugar Mountain, Valley Crucis, and the western portion of Boone.

Lastly, the South Toe River is a pristine, high-elevation stream that flows through Mitchell, Yancey, and McDowell Counties, North Carolina in the Nolichucky River Basin. From its headwaters on the eastern slope of Mount Mitchell at an elevation of 2,037 m, the South Toe River flows north to Celo, NC to its confluence with North Toe River in Kona, NC. The upper South Toe River is considered an Outstanding Resource Waters by North Carolina and maintains an "Excellent" rating by the Volunteer Water Information Network (EQI, 2013). The South Toe is regarded as a stronghold for eastern hellbender populations, with many anecdotal sightings reported from its headwaters near Celo, NC downstream to its confluence with the Toe River in Yancey County (M. Gangloff pers. obsv.).

Land Use and Land Cover

Land-use and land-cover (LULC) for the South Fork New River, Watauga, and Nolichucky River drainages were analyzed using ArcGIS 10.3 and the ArcHydro Toolset (ESRI, Redlands, CA). I delineated drainages following a modified version of the Merwade (2012) protocol using Digital Elevation Models (DEM) from the United States Geological Survey (USGS) National Elevation Dataset with 2013-2018 National Land Cover Dataset (30-m resolution). I then clipped the raster to the three watersheds encompassing the three

sites. I simplified USGS LULC categories by combining nine of the land-use types into three categories: forest cover (deciduous, evergreen, and mixed forest), disturbed (developed open space, and low, medium, and high intensity developed cover), and agriculture (hay/pasture, cultivated crops). The remaining five categories were labeled as miscellaneous. The percent cover of each of the three main categories was calculated at the sub-catchment scale (i.e., the entire watershed upstream from a site).

Data Collection

At each site, I conducted eastern hellbender surveys and measured instream habitat parameters within a 150-m reach. I subdivided reaches by transects at 10-m intervals (n = 16 transects per reach) and constrained searches to individual transects (Pugh, Franklin, Siefferman, & Gangloff, 2018). While moving in an upstream direction, I lifted cobble and smaller rocks by hand and used log peaveys to lift boulders and expose potential eastern hellbenders. I captured eastern hellbenders by hand and transferred to a dip net or mesh bag submerged in the stream, or placed in a water-filled bucket. At sites that had < 3 eastern hellbenders within the 150-m reach, I moved upstream until at least five animals were captured. I measured total length and mass, estimated sex and age class (when possible), and noted any abnormalities (i.e., missing digits, scars) of each animal. I then took a small tail clip (~ 5 mg) from the top portion of the end of the tail for stable isotope analyses before returning each animal to its capture location.

I collected fish and crayfishes using a backpack electro-fisher and seines. I identified fishes and measured total length, standard length, and mass (Table 1). I euthanized two to five individuals of different species previously reported in the stomachs of eastern hellbenders, as well as top predators (e.g., Rock bass, *Ambloplites rupestris*) in a bucket containing a solution of 300-500 mg/L of tricaine methanesulfate (MS-222) until opercular movement ceased (~ 10 minutes). Following euthanization, I stored fishes temporarily on ice in the field, and later transferred them to a -20°C freezer until processing.

Stable Isotope Sample Preparation

I prepared specimens for isotopic analysis using protocols developed by the Center for Applied Isotope Studies (CAIS) Stable Isotope Ecology Lab (http://cais.uga.edu/analysis_stable_iso.html). I dissected portions of the caudal muscle from fishes and abdomen muscles of crayfish to avoid lipid bias (Burress, Holcomb, & Armbruster, 2016). I only used snail soft tissues for analysis. Aquatic insects were identified to family and lyophilized whole. Following lyophilization, I used a pestle and mortar to grind the dried tissue samples into a homogeneous powder, weighed to three decimal places (mg) to a minimum of approximately 1.000 mg dry weight, and placed them into separate tin capsules. I did not grind the eastern hellbender tail clips for concern that I would lose too much material in the grinding process. Therefore, eastern hellbender tail clips consisted of both muscle tissue and a thin layer of skin tissue (Milanovich & Maerz, 2012). I weighed each individual into two separate tins for replication, except for eastern hellbender tail tissue

as the tail clips only met the minimum required weights. Samples were analyzed for natural abundance ^{15}N and ^{13}C by an isotope ratio mass spectrometer coupled with an elemental analyzer at the CAIS Stable Isotope Ecology Laboratory (University of Georgia, Athens, GA, USA).

Trophic Position

Trophic position (TP) was calculated for each animal using the formula

$$\text{TP} = ((\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}})/3.4) + 2$$

where 3.4 is the assumed trophic enrichment factor, and +2 is used to account for the baseline of a primary consumer rather than a primary producer (Vander Zanden & Rasmussen, 1999).

I analyzed eastern hellbender TP using SPSS v. 24 (IBM Corp, 2016). All tests were two-tailed and set to a significance level of $p \leq 0.05$. I used a Shapiro-Wilk test of normality and found that TP was normally distributed ($p > 0.05$) and had no extreme outliers. I then used a one-way ANOVA with Tukey post hoc tests to determine if TP was different among sites. To examine whether TP could be explained by age class (i.e., total length) I visualized my data with correlation scatter plots for each of the South Fork New, Watauga, and South Toe River sites (Figure 1). Finally, I compared eastern hellbender and Rock bass TP from South Fork New and South Toe river sites using a T-test. Watauga River *A. rupestris* samples were excluded from analyses due to insufficient sample sizes ($n = 2$).

Stable Isotope Mixing Models

Isotopes are elements that vary in the number of neutrons in their nuclei and, consequently, have different atomic masses (Fry, 2006). This difference in mass results in differing physiological reaction rates, a phenomenon known as isotope fractionation. Nitrogen exhibits a stepwise enrichment from one trophic level to the next due to the discrimination of the heavier isotope in chemical processes. This discrimination results in consumers having a higher ^{15}N value relative to their diet and makes nitrogen enrichment ratios a reliable inference of trophic position. Stable isotope ratios allow for better interpretation of dietary relationships than traditional gut content analyses because nitrogen is assimilated into tissues (Michener, 1994). Carbon isotopes, on the other hand, are conserved with trophic transfers from prey to consumers, and are used to trace energetic pathways from one trophic level to the next, and to pinpoint primary production sources (e.g., C3 or C4 photosynthetic pathways). The ratios of the heavier to lighter isotopes of carbon and nitrogen are denoted as $\delta^{13}\text{C}$ ($^{13}\text{C}/^{12}\text{C}$) and $\delta^{15}\text{N}$ ($^{15}\text{N}/^{14}\text{N}$), respectively. Past studies have shown that $\Delta\delta^{13}\text{C}$ (Δ denotes the change in isotope ratios between diet and consumer) is approximately +1‰, and $\Delta\delta^{15}\text{N}$ is around +3-4‰ (mean = 3.4‰, where ‰ indicates parts per thousand; DeNiro & Epstein, 1978, 1981).

Although earlier studies used stable isotopes of carbon and nitrogen to evaluate energy flow and foodweb structure in single systems (e.g., Peterson, Howarth, & Garritt, 1985; Keough, Sierszen, & Hagley, 1996; Hansson et al., 1997), more recent studies have found that comparisons across multiple systems are necessary to address many ecologically

important questions, such as the response of different populations of a species across gradients of anthropogenic impacts (e.g., Layman, Quattrochi, Peyer, & Allgeier, 2007) and variation in trophic position resulting from differences in habitat and resource availability (e.g., Vander Zanden et al., 2000). In freshwater environments, substantial temporal and spatial variation in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of basal resources where carbon renders an organism or species' absolute trophic position meaningless when comparing across multiple systems (Post, 2002). To resolve this issue, Vander Zanden and Rasmussen (1999) proposed using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of long-lived primary consumers as $\delta^{13}\text{C}_{\text{base}}$ and $\delta^{15}\text{N}_{\text{base}}$. Primary consumers, such as snails and mussels in aquatic systems, exhibit a lower temporal variance of their isotope signatures (Cabana & Rasmussen, 1996). Long-lived primary consumers should also reflect the spatial variance within and among systems, and are preferred indicators of basal resource inputs over primary producers (Cabana & Rasmussen, 1996; Vander Zanden & Rasmussen, 1999).

Haines (1976) first applied the use of stable isotopic composition of consumers to infer the assimilated diet of animals. The use of mixing models in trophic studies has become an essential item in the toolbox of many ecologists. Early stable isotope mixing models (SIMMs) produced point estimates for the dietary contributions of food sources to consumers, but sources of variation and uncertainty in isotopic values were not addressed until the introduction of the IsoError mixing model 25 years after Haines' classic study (Phillips & Gregg, 2001). While SIMMs is a useful tool in examining the diet of consumers, they are not a "magic bullet," and the assumptions and limitations of these models must be

accounted for. Stable isotopes vary across both temporal and spatial scales and the type of tissue collected can vary substantially in its representation of a given time period (Phillips et al., 2014). For example, bone collagen isotope ratios reflect dietary trends over an animal's lifetime (Radtke, Lenz, Showers, & Moksness, 1996; Dalerum & Angerbjorn, 2005), muscle and liver tissue represent a few months, and blood represents dietary items consumed during the last few days (Tieszen, Boutton, Tesdahl, & Slade, 1983).

A major assumption of SIMMs is acquisition of prior information on the diet of the species of interest, which is usually acquired through gut content analysis of each individual in the study (Phillips et al., 2014). However, gut content analysis was not a viable option for this study because eastern hellbenders are listed for protection by North Carolina, and gastric lavage is an invasive technique that has never been performed on a wild eastern hellbender in North Carolina. I instead referred to the results of other studies found in the literature, which is an acceptable option when stomach content data are unavailable. A second major assumption of SIMMs is trophic enrichment, or discrimination, factors (DF), which are measureable units of energy transfer between trophic levels (Post, 2002). Isotopic fractionation from source to mixture (i.e., $\Delta\delta^{15}\text{N}$) is an important assumption in mixing models and slight alterations to these fractionation values can substantially alter the posterior distribution results. A widely accepted DF across many taxa in different environments (marine, freshwater, terrestrial) is 3.4‰ for $\delta^{15}\text{N}$ and 0-1‰ for $\delta^{13}\text{C}$ (DeNiro & Epstein, 1978; Post, 2002) which are the base assumptions for most modeling software. However, this fractionation value may vary depending on the species of interest (Post, 2002). To determine

this DF, researchers sometimes feed captive animals a controlled diet, where the stable isotope signatures of the food item are known and can then use tissue from the captive consumer to determine the proportion of prey item isotopes assimilated into their own tissues (e.g., Burress et al., 2016; Gillespie, 2013). Because I was not able to experimentally determine a species-specific DF for eastern hellbenders, I ran my models with a DF of 0.5‰ for $\delta^{13}\text{C}$ and 3.4‰ for $\delta^{15}\text{N}$.

To examine food source contribution (%) to the consumer, I ran a three-source dual-isotope mixing model using the Bayesian Mixing Models package in R (MixSIAR GUI, version 3.1, Stock & Semmens, 2016). This package is a framework that uses multiple models to allow researchers to create mixing models of the source contribution to a mixture (i.e., consumer) based on data structure and research questions. MixSIAR is a popular tool for generating mixing models in ecological studies because it incorporates uncertainty in the discrimination factors (DF), as well as explicitly dealing with variability among consumer and prey isotopic values (Stock & Semmens, 2016). I used the graphical user interface (GUI) version of this package. The results of stable isotope mixing models are often reported as source proportion distributions with statistical bounds such as 95% credible intervals (Bayesian confidence intervals) rather than just summary values such as means or medians (Phillips et al., 2014). By reporting distributions, researchers can avoid misrepresenting the uniqueness of the mixing model results that single summary values can introduce (Phillips & Gregg, 2001).

Foodweb models included three prey items that have been historically reported in the stomachs of eastern hellbenders: benthic fishes (e.g., *Campostoma anomalum*, *Nocomis micropogon*, and *Etheostoma* spp.), crayfish (*Cambarus* and *Orconectes* spp.) and snails (*Leptoxis dilatata* and *Elimia proxima*). Benthic fishes were pooled together into a single category because they did not have statistically different $\delta^{15}\text{N}$ values from each other (Phillips et al., 2014). The three potential food sources were found to be isotopically distinct after pooling fish values and this allowed the mixing models to discriminate among dietary items.

Due to lack of stomach content (i.e., prior) data in this study, I used four different prior distributions. The first three are informative priors, $\alpha = c(1, 1, x)$, where $x = 0.25$ in Model 1, 0.5 in Model 2, and 0.75 in Model 3, all of which constrain the prior belief that snails may not contribute a significant portion to eastern hellbender diet. However, fish and crayfish still have an equal chance of being consumed. The fourth model used a generalist/uninformative prior, $\alpha = c(1, 1, 1)$, where each of my three food sources was assumed to have an equal chance of being eaten. I specified Markov Chain Monte Carlo (MCMC) parameters as a length of “very long” (chain length = 1,000,000; burn-in = 500,000; # chains = 3; Stock & Semmens, 2016). To determine if my three chains had converged on the posterior distributions, I used two default diagnostic tests: Gelman-Rubin and Geweke. MCMC also generates a deviance information criterion (DIC) to measure model complexity and fit, which is a common information criterion in Bayesian statistical analyses (Spiegelhalter, Best, Carlin, & van der Linde, 2003).

Eastern Hellbender Body Condition

I quantified body condition using standardized residuals of a linear regression with total length (cm) as the independent variable and mass (g) as the dependent variable (Jakob, Marshall, & Uetz, 1996; Figure 2). To determine whether body condition was different between streams, I used a one-way ANOVA with body condition as my dependent variable and drainage as the factor. Additionally, I used Tukey's honestly significance difference (HSD) post hoc tests to further examine where the differences occurred between my groups.

Results

Land Use and Land Cover

Of the three sites examined, the South Fork New River site's watershed was the most impacted by development and had the highest levels of agricultural (9.3%) and urban land-use (Table 2). The South Toe and the Watauga river watersheds are both >80% forested, although the Watauga site's watershed had a slightly higher percent (4.6%) of disturbed land compared to the South Toe River watershed. The South Toe Watershed had the highest percent forest (85.8%) and lowest levels of disturbed (7.2%) and agricultural (5.4%) land-use (Table 2).

Trophic Position

I analyzed 18 eastern hellbender tail clips from three sites, one in each of the South Fork New, Watauga, and South Toe river drainages. I found that isotope ratios in one of the

three juveniles (total length < 30 cm) sampled was a significant statistical outlier and drove a trend in the relationship between total length and trophic position in the South Toe River. I therefore removed this outlier from all other statistical analyses. The average TP of eastern hellbenders across the three sites included in the analyses was 2.9, and was not statistically different among sites ($F_{2,15} = 1.392$, $p = 0.279$), although eastern hellbender TP appeared to be slightly higher in the South Toe compared to the South Fork New and Watauga river sites (Figure 3).

Eastern hellbenders had similar TPs to Rock bass (*A. rupestris*) in the South Fork New River site ($t(8) = -1.729$, $p = 0.122$; Table 3, Figure 4), although Rock bass seemed to occupy higher TPs in this stream. In the South Toe River, eastern hellbenders occupied a significantly higher TP than Rock bass ($t(8) = 2.946$, $p = 0.019$; Figure 5).

Stable Isotope Mixing Models

In the most parsimonious mixing model basal consumer (i.e., snail) contributions were constrained in the prior distributions from the first model (Table 4). Although snail contributions to eastern hellbender diet were altered across the four mixing models, proportional contributions of both benthic fish and crayfish prey sources remained relatively stable across all models (Table 4). For example, fish contributed a mean of 47% to the diet of eastern hellbenders in the South Toe for every model, with very minimal changes in the 95% credible intervals. In contrast, the mean proportion of crayfish in the Watauga River site varied from 66% in Model 4 to 79% in Model 1, and from 68% in Model 4 to 80% in Model

1 for the South Fork New River site. Across all models, fish contributed the least amount to the diet of eastern hellbenders in the Watauga River (highest mean proportion = 12% in Model 1), and crayfish contributed relatively the same proportion to eastern hellbender diets at the South Fork New and Watauga river sites. In the South Toe, crayfish and fish each constituted nearly half of the diet of this population of eastern hellbenders and this was consistent across all models. I reported the visual component of the posterior distributions and 95% CI for the most parsimonious model (Model 1) for the South Fork New (Figure 6), Watauga (Figure 7), and South Toe (Figure 8) River sites.

Eastern hellbender Body Condition

Eastern hellbender body condition was statistically different among my three sites ($F_{2,15} = 3.624$, $p = 0.05$; Figure 9). A Tukey post hoc test revealed that eastern hellbender body condition in the South Fork New River was significantly greater than eastern hellbender body condition in both the Watauga ($p = 0.036$) and South Toe rivers ($p = 0.034$).

Discussion

I quantified trophic position and examined the proportional contribution of crayfish and benthic fishes to the diet of *Cryptobranchus alleganiensis alleganiensis* in three western North Carolina streams using the first stable isotope analysis of this uncommon and imperiled salamander. Although trophic position was not significantly different among sites, eastern hellbenders in the South Toe River (pristine) appear to occupy slightly higher trophic

positions than populations in both the South Fork New (highly-impacted) and Watauga (moderately-impacted) river sites. Further analysis of this unexpected result revealed that eastern hellbenders in the South Toe River occupied significantly higher trophic positions than Rock bass (*A. rupestris*), whereas the opposite trend was found in the South Fork New and Watauga rivers. As predicted, the results of the mixing models suggest that eastern hellbenders in the South Fork New and Watauga rivers primarily consume crayfish, whereas the South Toe population's diet appears to be comprised of nearly half crayfish and half benthic fishes. Although the diets of eastern hellbenders in both the South Fork New and Watauga River sites had similar proportions of crayfish, eastern hellbender body condition was similar in the Watauga and South Toe Rivers and both were significantly lower than the body conditions observed in the South Fork New River population.

Contrary to my hypothesis, I found that eastern hellbender trophic position did not vary significantly across the three hellbender populations. However, eastern hellbenders in the South Toe appear to occupy slightly higher trophic positions and are closer to being tertiary predators (i.e., occupy a distinctly higher trophic level) than populations in the South Fork New and Watauga rivers. To examine this unexpected result further, I compared the trophic position of eastern hellbenders to Rock bass, which are generally considered to occupy high trophic positions in foodwebs (Pound, Nowlin, Huffman, & Bonner, 2011). I found that eastern hellbenders in the South Toe River occupied significantly higher trophic positions than Rock bass. Rock bass trophic positions in both the South Fork New and Watauga rivers were not significantly different from eastern hellbender trophic positions at

these sites but are closer to tertiary predation levels than the eastern hellbender populations and thus appear to have at least slightly higher positions within the foodwebs.

Because eastern hellbenders in the South Toe River site occupy significantly higher positions than a predatory fish, they may act as apex predators in this system. However, this is highly speculative and future studies should include other predatory fishes including salmonids and Smallmouth bass. The South Toe River is considered a pristine headwater stream and land-use in its watershed is >85% forested with very low levels of disturbed and agricultural land cover (Table 2). Despite the scarcity of crayfish in this stream, eastern hellbender populations are widespread throughout much of the South Toe River watershed (M. Gangloff pers. obsv.). Studies examining the effects of land-use on fish assemblages have found that species richness and diversity correlate positively with highly forested land-use (Meador, Coles, & Zappia, 2005), whereas increases in urbanization (i.e., impervious surface cover) may lead to reduced overall species and endemic species diversity (Meador et al., 2005), as well as increased abundance of disturbance-tolerant species (Onorato, Angus, & Marion, 2000; Wang, Lyons, Kanehi, Bannerman, & Emmons, 2000; Scott & Helfman, 2001). It is possible that because the South Toe is relatively pristine, it may exhibit a foodweb structure that is similar to historical conditions with eastern hellbenders occupying high trophic levels relative to other stream predators. In contrast, in streams with foodwebs that are impacted by increased nutrient concentrations and invasive fishes, eastern hellbenders may occupy much lower trophic positions. For example, the New River Drainage has the highest proportion of introduced fishes among eastern United States river drainages

including 56 species that are considered introduced and established compared with 45 native species (Jenkins & Burkhead, 1994). Future studies should examine variability of isotopic composition and diet of predatory fishes among these streams, as well as incorporate methods to more clearly examine differences in foodweb structure across these sensitive montane ecosystems. Additionally, if eastern hellbenders occupy higher trophic positions relative to other top fish predators within the South Toe River, it is possible that they may potentially exhibit top-down control of other organisms and ecological processes (e.g., detritivory) in the South Toe River and other Appalachian headwater ecosystems where they remain abundant.

The results of the mixing models suggest that crayfish are the dominant food source of eastern hellbenders in the South Fork New and Watauga rivers, with proportional values as high as 94% in the South Fork New River and 98% in the Watauga River. These results are not surprising given the large numbers of crayfish encountered during eastern hellbender surveys in these rivers (A. Yaun pers. obs.). In some systems, crayfishes have been found to comprise almost half of the total invertebrate production (Momot, 1995). The South Fork New River has six species of freshwater crayfish and five species occur in the Watauga (NatureServe Explorer, 2019). These results are also congruent with past gut content analyses suggesting that *Cambarus* and *Orconectes* comprise between 70 and 100% of eastern hellbender diet by weight (Smith, 1907; Green, 1935; Peterson et al., 1989).

As predicted, crayfish were not the dominant prey of eastern hellbenders in the South Toe River. Although the 95% credible intervals indicate they can comprise as high as 65% of

eastern hellbender diet, eastern hellbenders in this river appear to consume almost equal proportions of crayfish and benthic fish. In contrastingly to the South Fork New and Watauga rivers, only two crayfish taxa have been reported from this high-gradient stream, both of which are considered “rare” according to NCPAWS reports, although no quantitative study on crayfish has occurred in this particular stream. Past gut content studies of eastern hellbenders collectively suggest that crayfish are the dominant food source, while other prey (i.e., fish) are far less important (Smith, 1907; Green, 1935; Peterson et al., 1989). However my analyses of South Toe River isotope data contrast these findings and is the first reported case of a population of eastern hellbenders whose diet is not dominated by crayfish. These results suggest that eastern hellbenders may therefore exhibit previously unreported foraging behaviors in systems where crayfish are not a readily available food source.

The trophic position of eastern hellbenders in the South Fork New River site declined significantly with increasing lengths and this trend contrasts strongly with the South Toe River site, where the difference between the smallest individual (30 cm total length) and largest individual (52 cm total length) spanned nearly an entire trophic position. Because trophic position is somewhat related to diet (animals feeding on prey higher up in the foodweb have higher trophic positions sensu DeNiro & Epstein, 1978, 1981), these trends may be a result of the high plasticity of eastern hellbender diets among study locations. Fishes are abundant but are also likely more difficult to capture than crayfish. Dunn (2016) found that eastern hellbenders exploiting chub-nest associate schooling cyprinids were not very successful at capturing free-swimming fishes. However eastern hellbender capture

efficiencies for more benthic-associated fishes are likely to be substantially higher. It also seems likely that younger/smaller eastern hellbenders are more likely feed more on small crayfish and predatory insect larvae (e.g., Megaloptera) in the South Fork New River and shift to adult crayfish late in life. Juvenile eastern hellbenders in the South Toe River are likely consuming mostly aquatic insect larvae and as the animals grow and become more effective predators, they may switch their diet to larger fish.

Eastern hellbenders in the South Fork New River were in significantly better body condition than animals in either of the Watauga and South Toe river sites. Due to the low sample sizes of eastern hellbenders in this study, I confirmed these findings with a larger dataset containing eastern hellbender morphology measurements at additional sites in the South Fork New, Watauga, and South Toe rivers collected by previous graduate students at Appalachian State (Figure S1). The high body condition of eastern hellbenders in the South Fork New River site may be a result of low eastern hellbender abundance combined with high crayfish production in this system. The amount of stream that I needed to search to obtain five eastern hellbenders was highly variable (400 m in the South Fork New versus 150 m in the Watauga and South Toe) and suggests that eastern hellbender abundance was lowest at the South Fork New River site. Thus, eastern hellbenders in the South Fork New River may be less abundant relative to that of their prey. Crayfish are considered to be a low-quality food resource relative to other benthic invertebrates and fish due to their low caloric content per gram (Rabeni, 1992). However, crayfish, like hellbenders, are primarily nocturnal

and are likely to become more vulnerable to predation at night when they are actively foraging on the streambed.

Eastern hellbenders in the Watauga River site had similar body condition compared to individuals in the South Toe, and populations in both rivers had significantly lower body condition compared to eastern hellbenders in the South Fork New River. The Watauga River supported the highest eastern hellbender abundance of this study, with 12 animals found within a 150-m reach. Intraspecific competition characteristic of populations of a species found in high densities can lead to lower body conditions (e.g., Reading & Clarke, 1995), which may explain the low body conditions found in the high-abundance eastern hellbender population at the Watauga River site.

The low body conditions observed in South Toe River eastern hellbenders may be a result of altered foraging behavior within this population. The South Toe has numerous reports of anecdotal sightings of eastern hellbenders roaming the streambed in daylight hours (M. Gangloff pers. obsv.). These sightings, combined with the results of surveys reporting low crayfish diversity and isotopic model data suggesting that benthic fishes comprise a high proportion of the diet of South Toe River eastern hellbenders suggest that in this system, eastern hellbenders are more likely to forage for fish during the day when fish are more active. When foraging during the day, capturing fish may require more effort than capturing crayfish, which could explain why eastern hellbender body condition is lower in the South Toe than in the South Fork New River site.

The comparison of data across the three populations in this study suggest that eastern hellbenders employ plastic dietary preferences and foraging behaviors in different populations. Future studies should investigate how eastern hellbender foraging behavior changes among systems with differing prey bases, habitat quality, and land-use practices. The foraging strategies of animals across spatial and temporal scales can have important ecological and evolutionary consequences, including selection against specialists when resource availability changes in response to environmental fluctuations or increased competition (Gillepsie, 2013). Examining how variability in habitat and land-use affects stream communities and predator foraging strategy and prey availability may help resource managers better understand spatial variability of eastern hellbender populations and reveal patterns contributing to their extirpation from nearly 50% of their historical range (Wheeler et al., 2003). Incorporating a trophic perspective into existing eastern hellbender management plans may identify populations that are resource-limited and lead to strategies such as improving basal resource availability or managing for invasive species that could be used to bolster eastern hellbender body condition which could, in turn, lead to increased egg production, larval production, and ultimately recruitment in resource-limited populations. However, given that resource availability is closely linked to both land-use-driven changes in habitat conditions as well as the introduction of exotic fishes it is critical to interpret trophic data within a holistic, watershed-focused framework in order to understand how local and regional processes influence the role of eastern hellbenders in stream ecosystems.

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Tables and Figures

Table 1. Fish species from the South Fork New, Watauga, and South Toe River sites with total length (cm), standard length (cm), and mass (g).

Site	Species	Total Length	Standard Length	Mass
South Fork New	<i>Etheostoma flabellare</i>	6.0	5.2	1.8
South Fork New	<i>Etheostoma flabellare</i>	5.6	4.0	1.0
South Fork New	<i>Etheostoma flabellare</i>	5.3	4.5	1.3
South Fork New	<i>Campostoma anomalum</i>	10.5	9.0	13.7
South Fork New	<i>Campostoma anomalum</i>	9.2	8.0	8.4
South Fork New	<i>Campostoma anomalum</i>	8.5	7.0	5.8
South Fork New	<i>Ambloplites rupestris</i>	12.0	9.0	31.2
South Fork New	<i>Ambloplites rupestris</i>	15.0	12.0	67.3
South Fork New	<i>Ambloplites rupestris</i>	11.0	9.0	25.2
South Fork New	<i>Ambloplites rupestris</i>	10.0	8.0	23.4
South Fork New	<i>Ambloplites rupestris</i>	9.0	7.0	17.9
Watauga	<i>Etheostoma chlorobranchium</i>	6.5	5.5	2.9
Watauga	<i>Etheostoma chlorobranchium</i>	6.0	5.0	2.5
Watauga	<i>Etheostoma chlorobranchium</i>	7.0	6.0	3.5
Watauga	<i>Nocomis micropogon</i>	8.0	7.0	4.4
Watauga	<i>Campostoma anomalum</i>	10.0	8.0	10.7
Watauga	<i>Campostoma anomalum</i>	10.5	9.0	9.2
South Toe	<i>Etheostoma blennioides</i>	10.0	9.0	9.6
South Toe	<i>Etheostoma blennioides</i>	9.0	8.0	7.6
South Toe	<i>Etheostoma blennioides</i>	10.0	8.5	8.7
South Toe	<i>Nocomis micropogon</i>	18.5	16.0	75.1
South Toe	<i>Campostoma anomalum</i>	17.0	15.0	54.9
South Toe	<i>Ambloplites rupestris</i>	16.0	12.0	87.9
South Toe	<i>Ambloplites rupestris</i>	15.0	11.0	50.8
South Toe	<i>Ambloplites rupestris</i>	12.5	10.0	39.6
South Toe	<i>Ambloplites rupestris</i>	11.0	9.0	25.0
South Toe	<i>Ambloplites rupestris</i>	11.0	9.0	23.5

Table 2. Percent land-use, total drainage area (km²) leading to each site, and site elevation (m) for the South Fork New, Watauga, and South Toe River sites. Percent land-use was determined for each site by using the ArcHydro Toolset in ArcGIS. Percent total forest, disturbed, and agricultural were generated by combining similar LULC classes.

USGS LULC Classes	LU Code	Site Names		
		South Fork New	Watauga	South Toe
Open Water	11	0.23	0.42	0.01
Developed, Open Space	21	17.59	9.42	6.19
Developed, Low Intensity	22	3.58	1.05	0.66
Developed, Medium Intensity	23	3.39	0.57	0.25
Developed, High Intensity	24	1.51	0.06	0.05
Barren Land	31	0.15	0.06	0.03
Deciduous Forest	41	57.50	76.50	77.06
Evergreen Forest	42	3.53	2.40	5.97
Mixed Forest	43	1.82	3.65	2.8
Shrub/Scrub	52	0.91	0.78	0.81
Herbaceous	71	0.45	0.35	0.67
Hay/Pasture	81	9.30	4.62	5.42
Woody Wetlands	90	0.05	0.13	0.08
Total Forest Cover	41-43	62.85	82.54	85.83
Total Disturbed Cover	21-24	26.07	11.09	7.16
Total Agricultural Cover	81	9.30	4.62	5.42
Drainage Area (km²)		80.3	57.8	216.0
Site Elevation (m)		1,528	1,476	1,330

Table 3. Isotopic ratios of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ and trophic position (mean \pm 1SD) for eastern hellbenders, fish, and crayfish in the South Fork New, Watauga, and South Toe River sites.

Site	Species	n	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	Trophic Position
South Fork New	<i>Cryptobranchus alleganiensis</i>	5	11.7+0.5	19.4+0.5	2.9+0.2
South Fork New	<i>Etheostoma flabellare</i>	3	12.5+0.8	21.6+0.7	3.0+0.2
South Fork New	<i>Campostoma anomalum</i>	3	11.8+0.1	20.0+1.2	2.9+0
South Fork New	<i>Ambloplites rupestris</i>	5	12.4+0.7	21.7+1.4	3.0+0.2
South Fork New	<i>Orconectes cristavarius</i>	5	8.1+0.2	21.7+0.2	1.8+0.1
Watauga	<i>Cryptobranchus alleganiensis</i>	7	12.0+1.0	20.0+1.4	2.8+0.2
Watauga	<i>Etheostoma chlorbranchium</i>	2	12.5+0.5	18.1+1.3	2.9+0.1
Watauga	<i>Nocomis micropogon</i>	2	12.4+0.6	18.1+1.9	3.0+0.1
Watauga	<i>Cambarus bartonii</i>	2	8.5+0.3	21.9+0.6	1.8+0.1
South Toe	<i>Cryptobranchus alleganiensis</i>	5	11.9+0.7	19.1+0.8	3.0+0.2
South Toe	<i>Etheostoma blennioides</i>	3	11.6+0.2	17.3+0.4	2.9+0.1
South Toe	<i>Nocomis micropogon</i>	2	9.9+0.5	19.4+2.6	2.4+0.2
South Toe	<i>Ambloplites rupestris</i>	5	10.9+0.2	19.4+1.4	2.7+0.1
South Toe	<i>Cambarus robustus</i>	2	6.5+0.3	23.2+0.3	1.4+0.1

Table 4. Results from the four MixSIAR mixing models. Values represent mean % contributions of dietary sources to different eastern hellbender populations. Values in parentheses are distributions of the 95% credible intervals. Deviance information criterion (DIC) allows for model comparisons.

Study Site	Model	DIC	Crayfish	Benthic Fish	Aquatic Snails
South Fork New	1	56.3	80 (50-94)	14 (4-29)	6 (0-36)
Watauga			79 (41-98)	12 (1-27)	10 (0-53.2)
South Toe			49 (26-65)	47 (32-64)	4 (0-23)
South Fork New	2	57.5	76 (41-93)	14 (4-28)	11 (0-47)
Watauga			74 (33-97)	11 (1-25)	16 (0-60)
South Toe			47 (31-64)	47 (31-64)	7 (0-26)
South Fork New	3	59.3	71 (34-91)	13 (4-29)	16 (0-55)
Watauga			69 (31-96)	10 (1-25)	21 (0-65)
South Toe			44 (19-64)	47 (30-66)	9 (0-31)
South Fork New	4	59.3	68 (30-90)	13 (4-27)	19 (1-58)
Watauga			66 (26-94)	9 (1-24)	25 (0-69)
South Toe			44 (19-62)	47 (30-63)	10 (0-31)

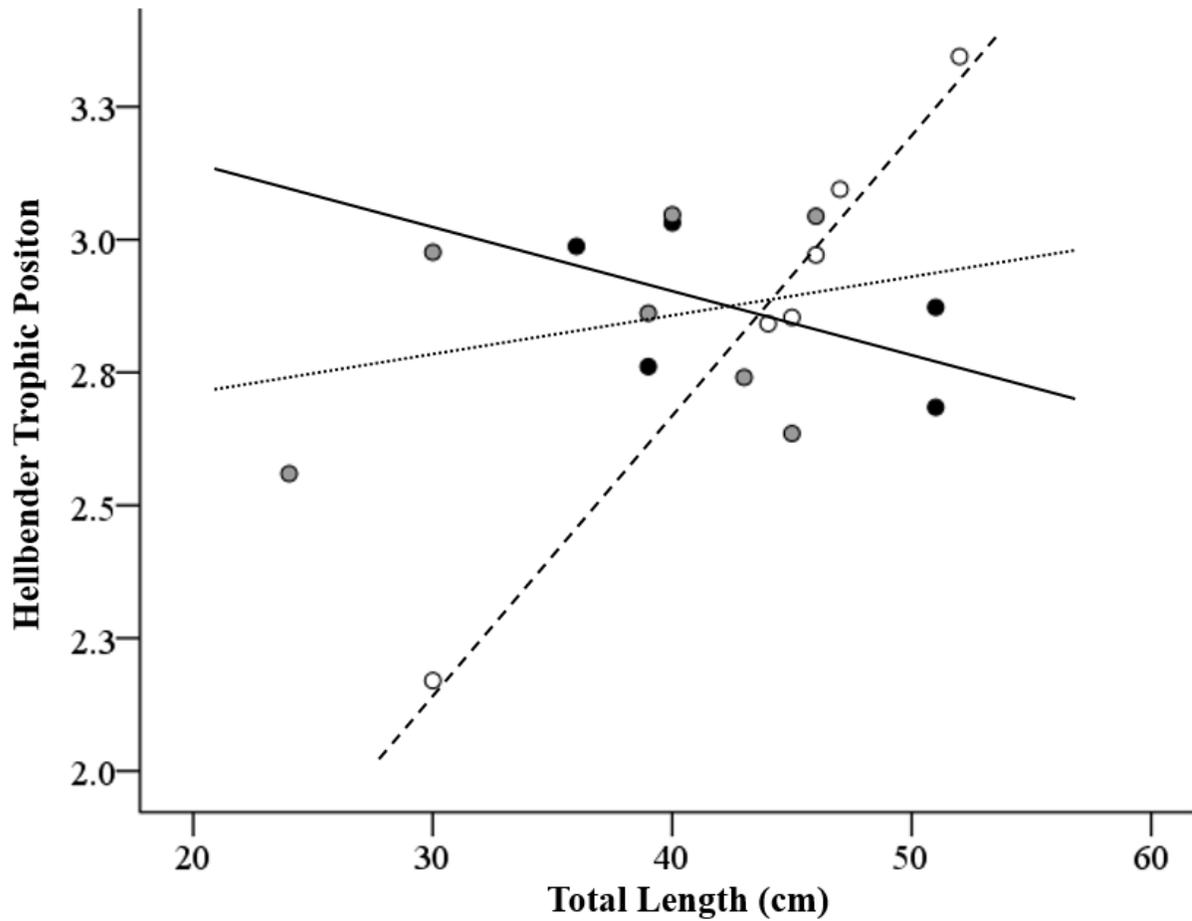


Figure 1. Regression of total length and trophic position of eastern hellbenders in the South Fork New River (black circles, solid line; $R^2 = 0.313$, $F_{1,3} = 28.796$, $p = 0.013$), Watauga River (grey circles, dotted line; $R^2 = 0.094$, $F_{1,5} = 25.156$, $p = 0.004$), and South Toe River (white circles, dashed line; $R^2 = 0.852$, $F_{1,4} = 65.769$, $p < 0.001$) sites.

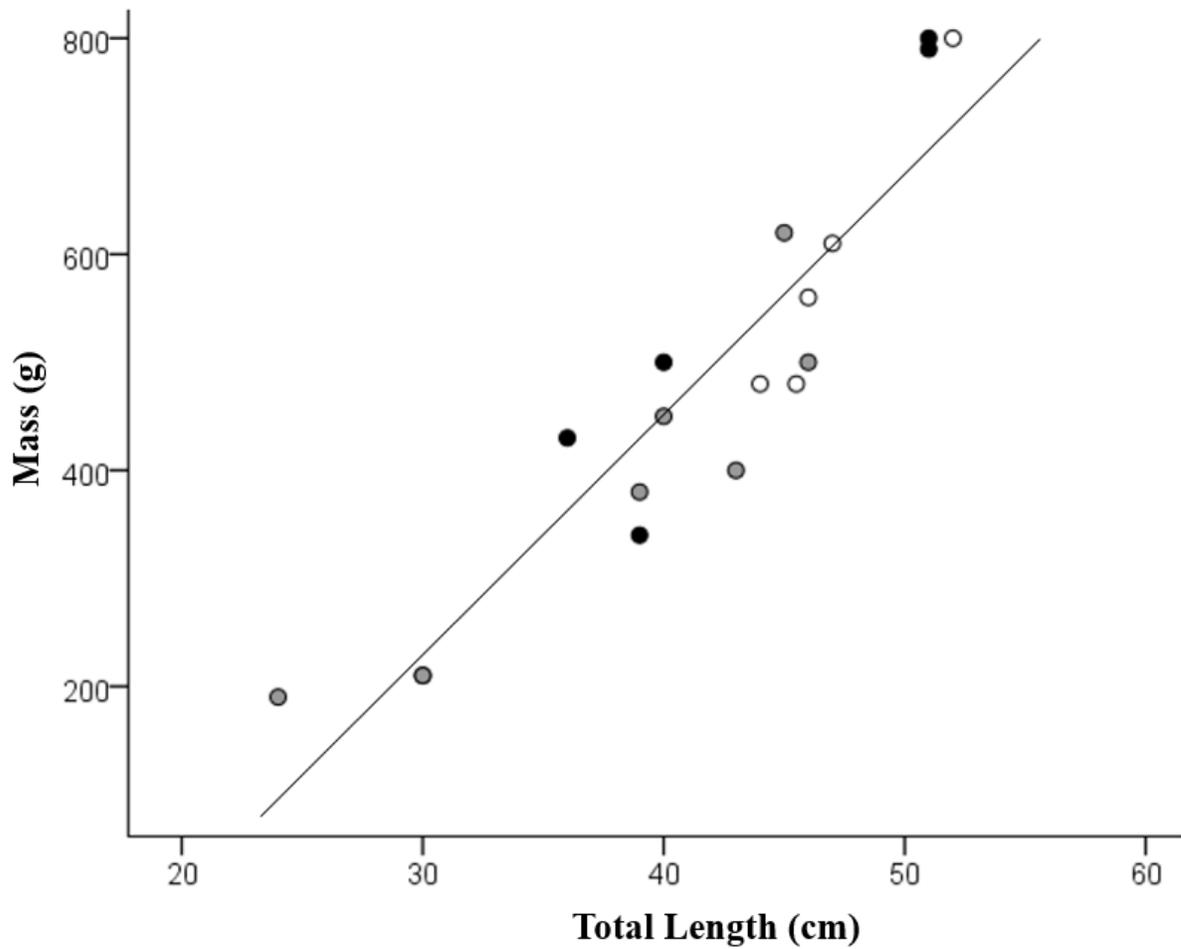


Figure 2. Regression of total length (cm) and mass (g) of eastern hellbenders in the South Fork New (black circles), Watauga (grey circles), and South Toe (white circles) River sites ($R^2 = 0.852$, $F_{1,16} = 92.361$, $p < 0.001$).

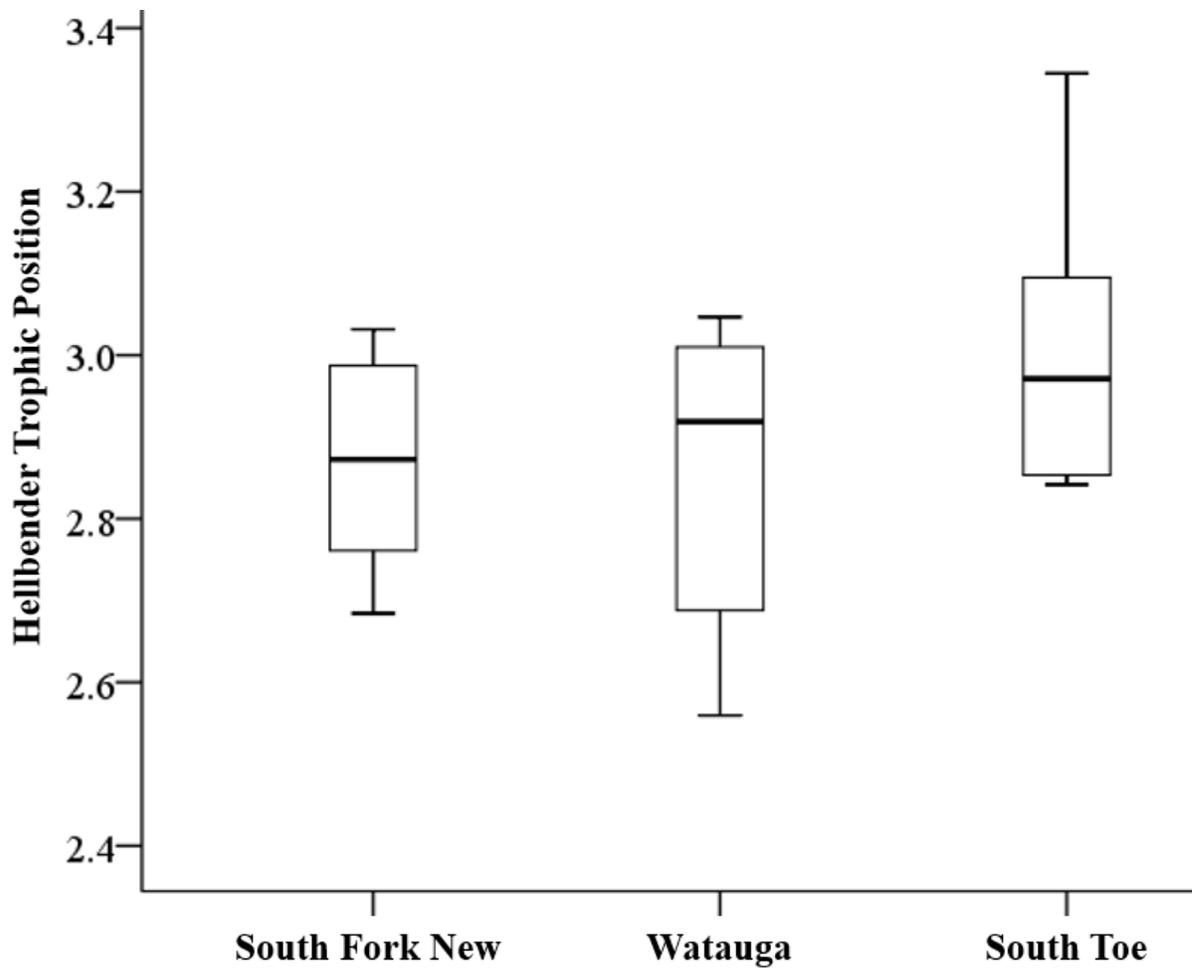


Figure 3. Trophic positions of eastern hellbenders in the South Fork New, Watauga, and South Toe River sites ($F_{2,15} = 1.392$, $p = 0.279$). The middle line represents the median with the first and third quartiles above and below, and the minimum and maximum values on the whiskers.

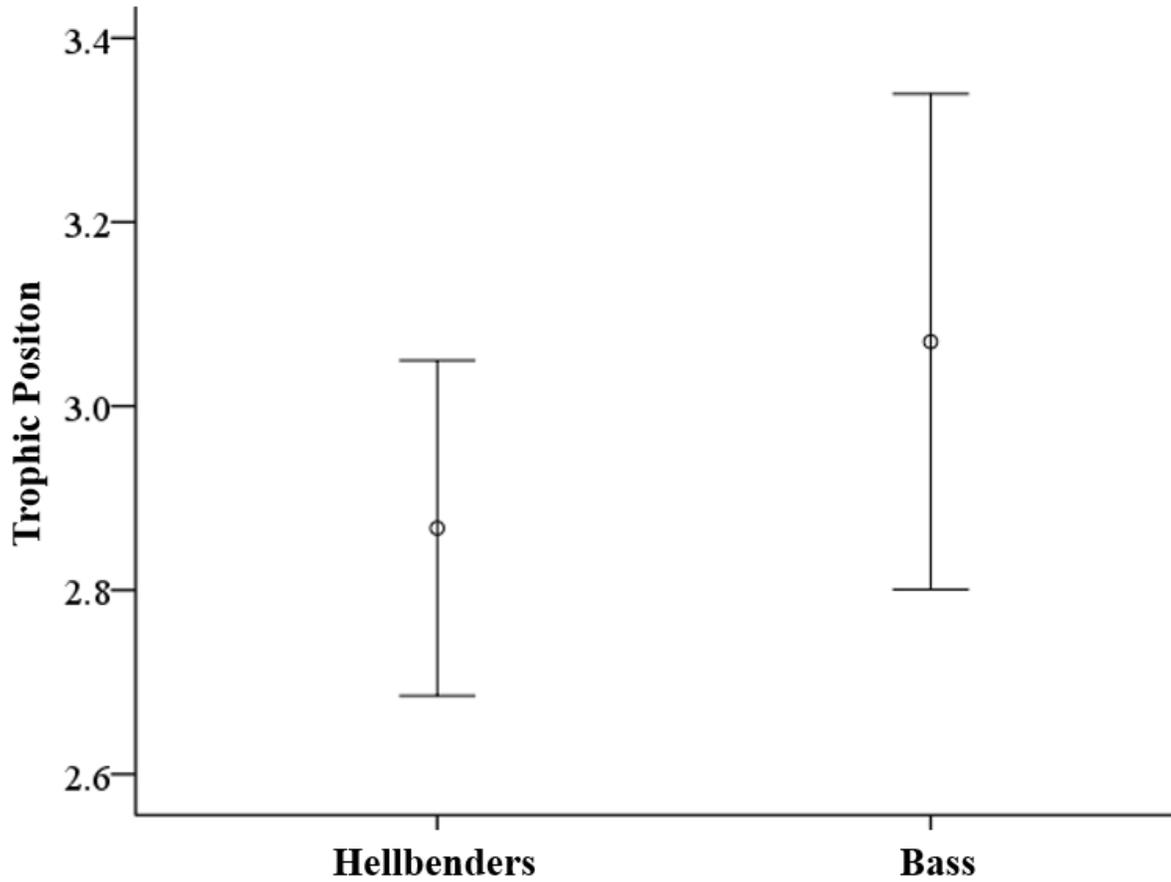


Figure 4. Trophic positions of eastern hellbenders and Rock bass (*Ambloplites rupestris*) in the South Fork New River site ($t(8) = -1.729$, $p = 0.122$). Error bars represent 95% CI.



Figure 5. Trophic positions of eastern hellbenders and Rock bass (*Ambloplites rupestris*) in the South Toe River site ($t(8) = 2.946$, $p = 0.019$). Error bars represent 95% CI.

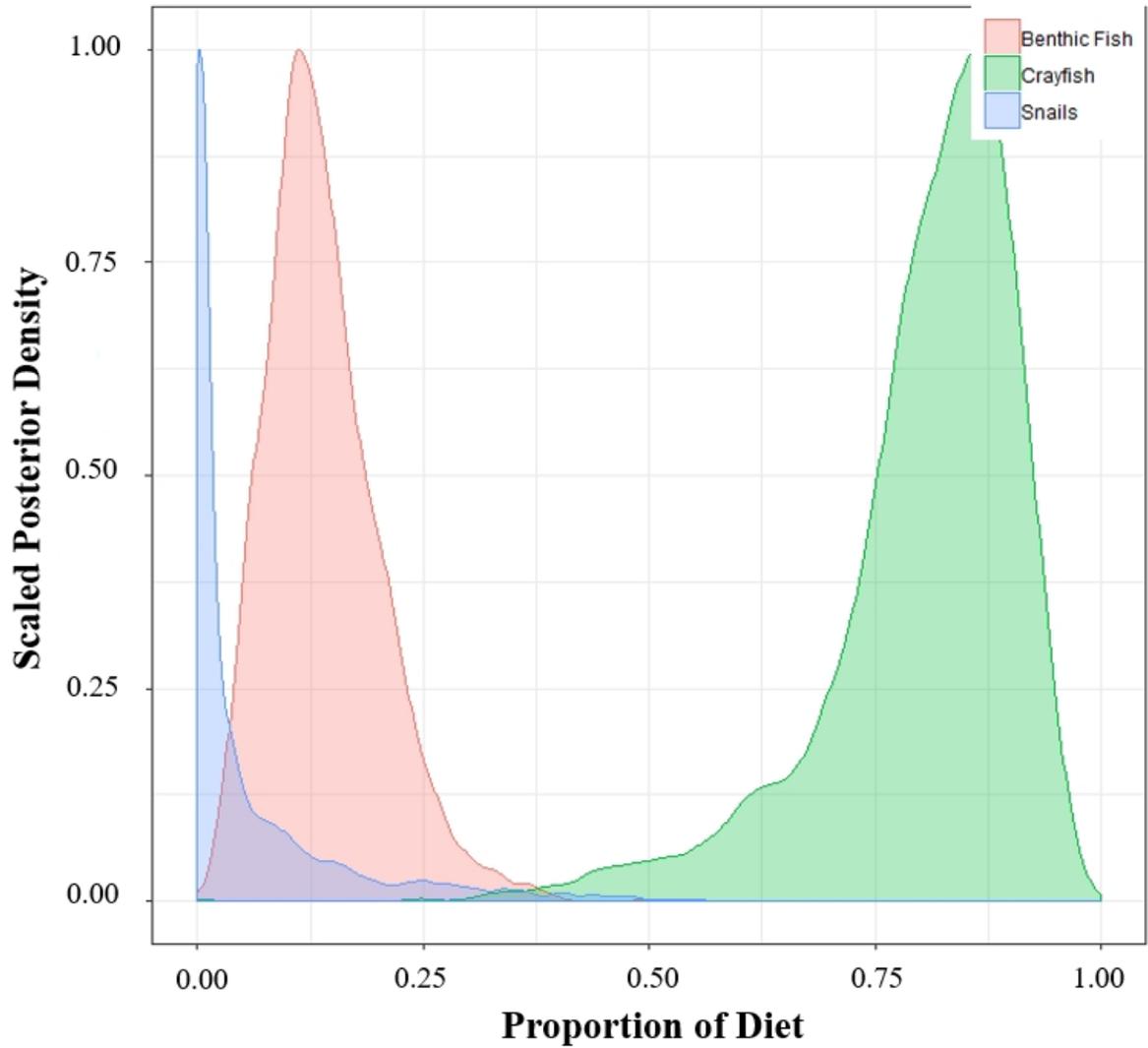


Figure 6. Posterior distribution plot of the proportion (%) of benthic fish (red; mean = 14%), crayfish (green; mean = 80%), and aquatic snails (blue; mean = 6%) produced by Model 1 for the South Fork New River site.

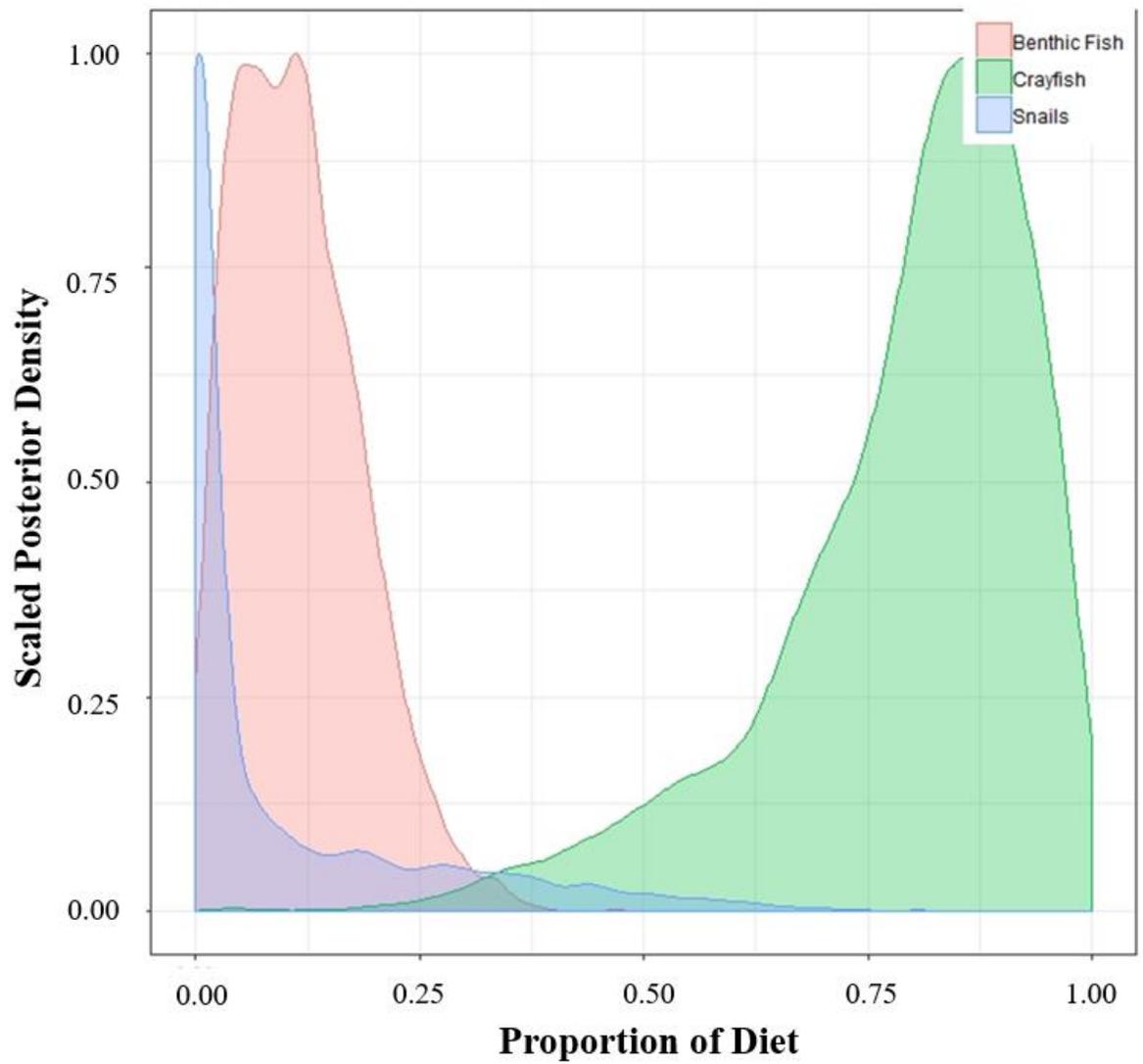


Figure 7. Posterior distribution plot of the proportion (%) of benthic fish (red; mean = 12%), crayfish (green; mean = 79%), and aquatic snails (blue; mean = 10%) produced by Model 1 for the Watauga River site.

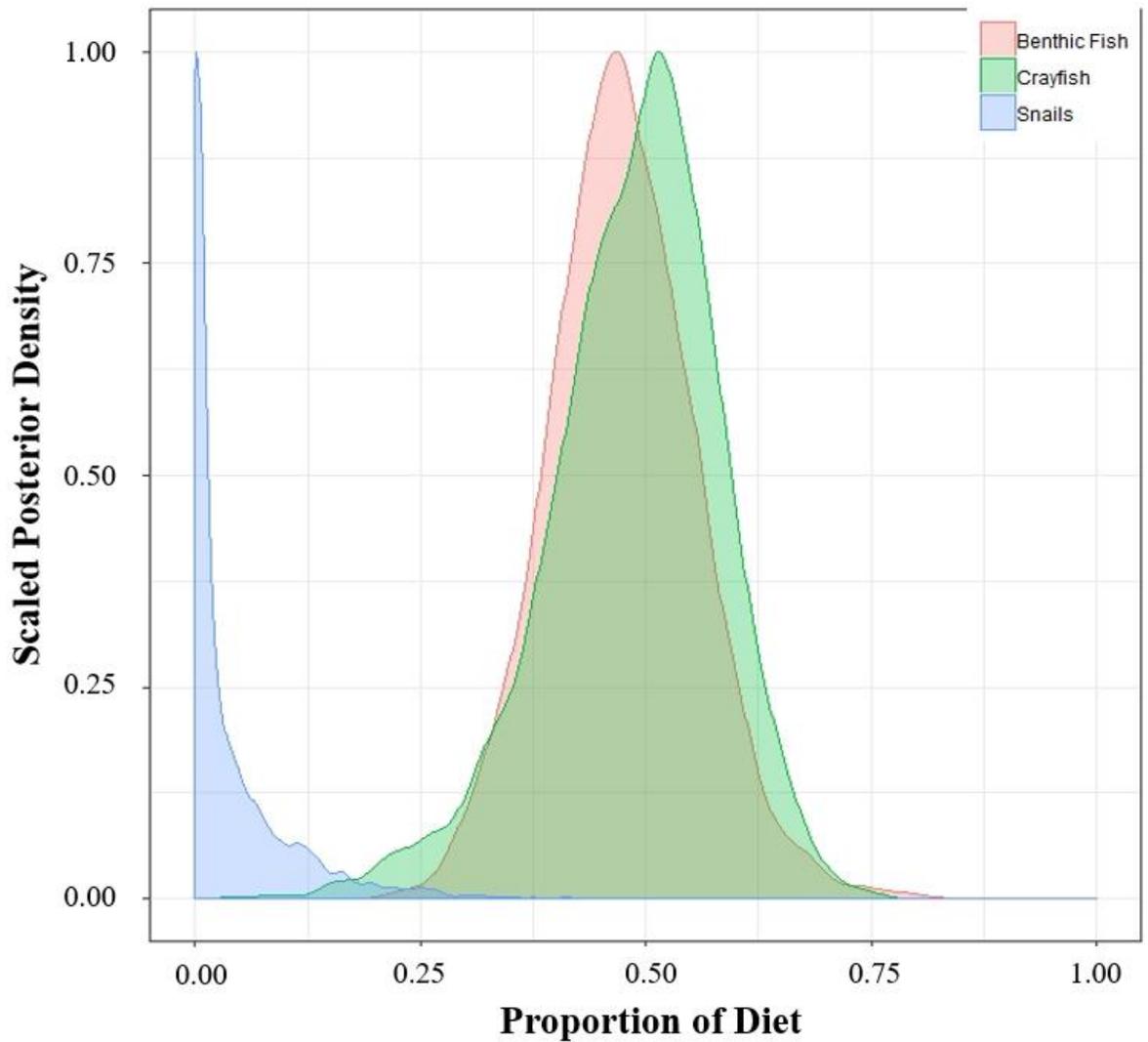


Figure 8. Posterior distribution plot of the proportion (%) of benthic fish (red; mean = 47%), crayfish (green; mean = 49%), and aquatic snails (blue; mean = 4%) produced by Model 1 for the South Toe River site.

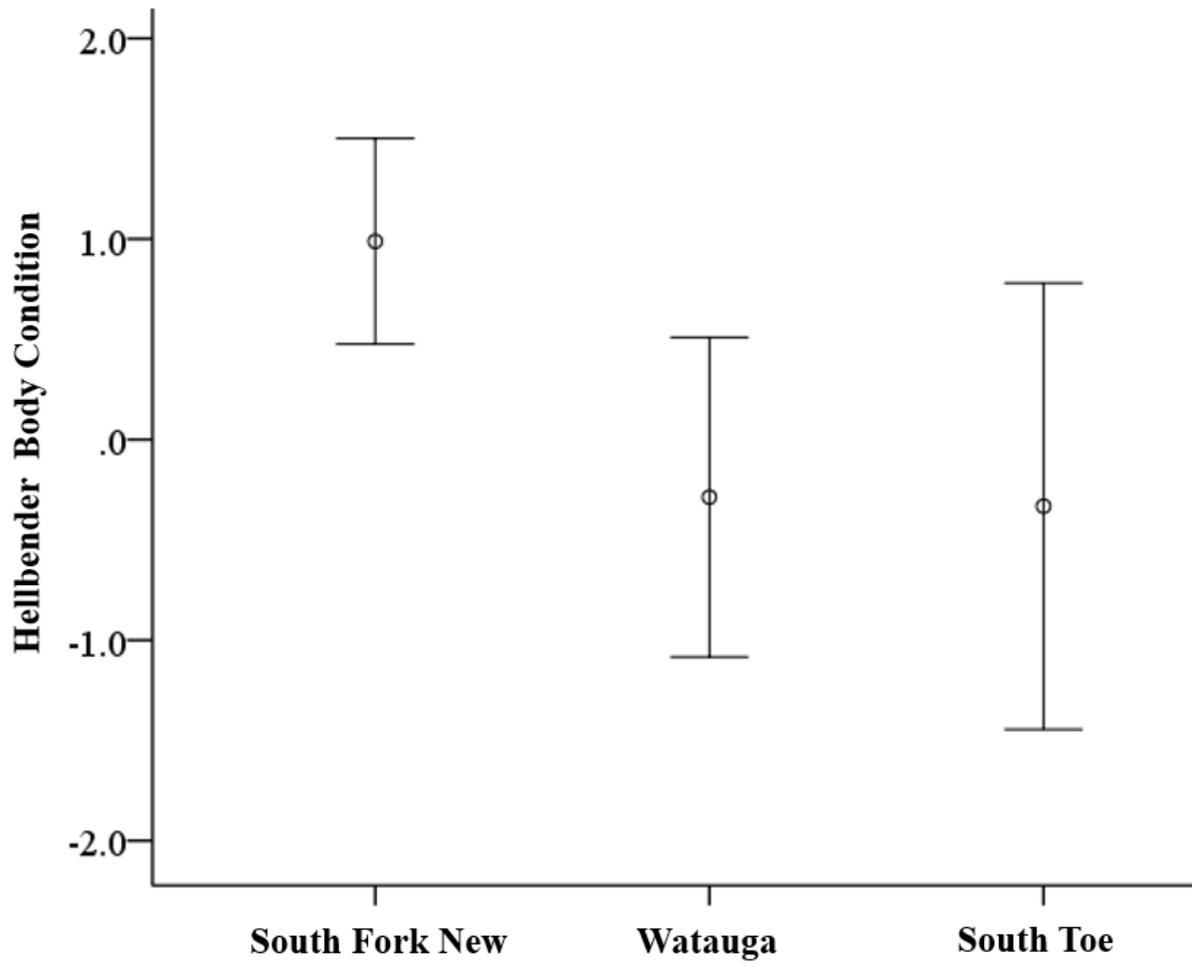


Figure 9. Body condition of eastern hellbenders in the South Fork New, Watauga, and South Toe River sites ($F_{2,15} = 3.624$, $p = 0.05$).

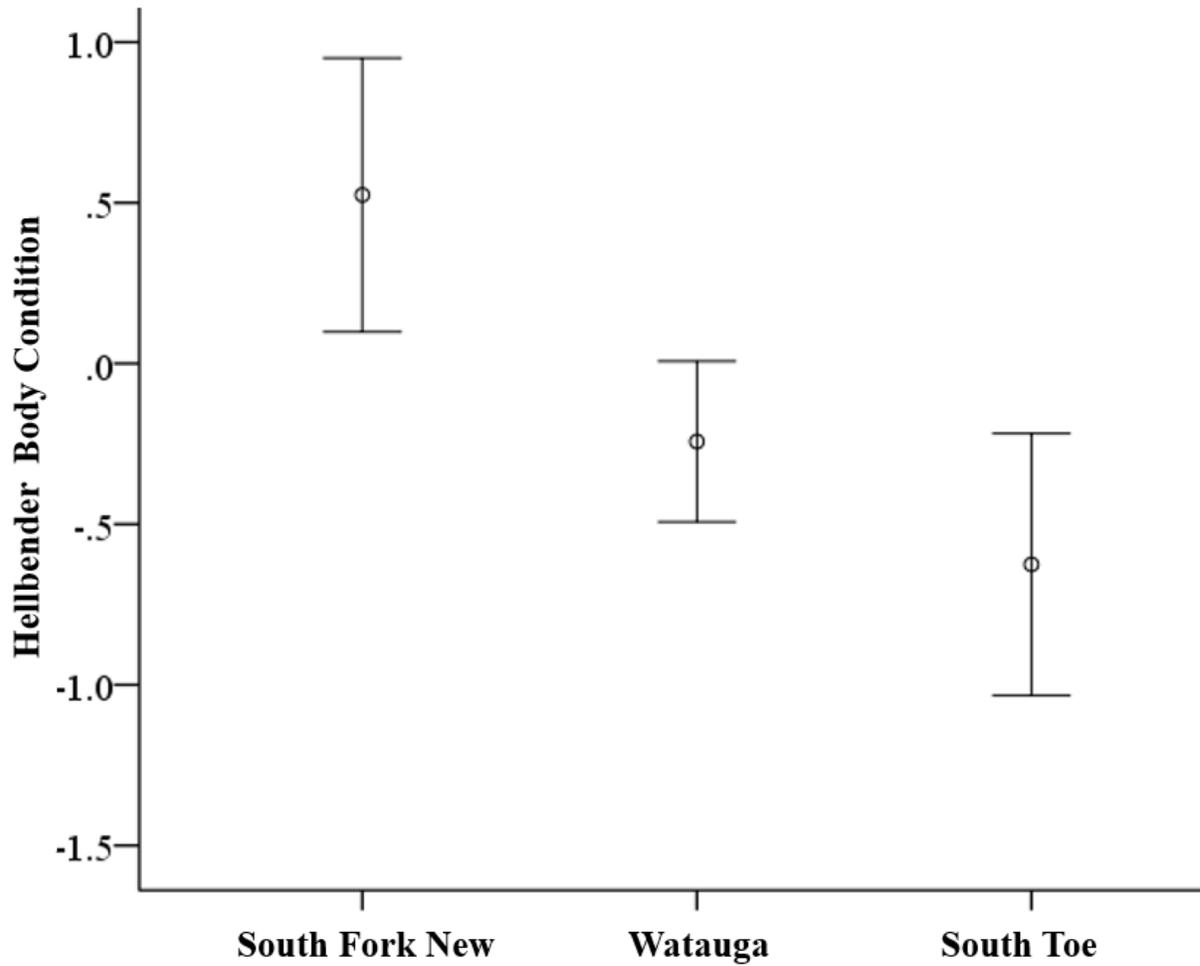


Figure S1. Body condition of eastern hellbenders at 10 sites in the South Fork New (n=29), 1 site in the Watauga (n=24), and 3 sites in the South Toe (n=18) Rivers using data from this study combined with data from two previous graduate students at Appalachian State ($F_{2,65}=9.773$, $p<0.001$).

Vita

Ashley Elizabeth Yaun was born in Atlanta, Georgia, on May 1, 1991, to Anne and Frank Yaun. Ashley grew up in the panhandle of Florida, where she developed a love for the outdoors at a young age and often brought home an assortment of lizards and other Florida critters, to her mother's dismay. She graduated Cum Laude from Brookwood School in 2010 and entered Caldwell Community College and Technical Institute in 2011 where she was awarded an Associate of Arts in May 2013. That autumn, she entered Appalachian State University to study Biology; in December 2015, she was awarded the Bachelor of Science degree. In the fall of 2016, she accepted a research assistantship in Biology at Appalachian State University and began study toward a Master of Science degree, which she received in August 2019.