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Hellbenders (*Cryptobranchus alleganiensis*) May Exhibit an Ontogenetic Dietary Shift

Kirsten A. Hecht^{1,2,*}, Max A. Nickerson^{1,2}, and Phillip B. Colclough³

Abstract - Organisms in lotic habitats often experience dietary shifts over their lifetime. The diet of adult *Cryptobranchus alleganiensis* (Hellbender) is well studied throughout the species' range, but knowledge regarding the natural history of larval Hellbenders, including dietary information, remains scarce. We obtained non-lethal diet samples from 23 larval Hellbenders. Larval Hellbenders consumed primarily invertebrate prey including mayfly (Ephemeroptera) and caddisfly (Trichoptera) nymphs. Since these items do not comprise a large proportion of the adult diet, Hellbenders may undergo an ontogenetic dietary shift. Therefore, future management and conservation decisions regarding the Hellbender should consider the abundance and density of aquatic insect populations.

Introduction

Stream species that experience substantial changes in body size or type during their life cycle commonly experience ontogenetic shifts in diet (Giller and Malmqvist 1998, Petranka 1984). *Cryptobranchus alleganiensis* (Daudin) (Hellbender), an imperiled lotic amphibian, grows rapidly in the first few years of life (Smith 1907, Unger and Mathis 2013). Hatchlings emerge from eggs at approximately 20–30 mm total length (TL) with external gills and undeveloped limbs (Bishop 1941, Smith 1907). Hellbenders develop fully formed limbs when 2 months old and reabsorb their external gills within 2 years of hatching (Bishop 1941). After 5–7 years, Hellbenders become sexually mature, and adults range from 300 to 740 mm TL (Nickerson and Mays 1973).

Over 15 papers examining the Hellbender diet found that crayfish and fish are the main diet items of Hellbenders (e.g., Green 1933, 1935; Netting 1929; Nickerson and Mays 1973; Peterson et al. 1989). Adult Hellbenders were the focus of these previous studies, however, and the diet of wild Hellbender larvae remains largely unknown with the exception of 2 anecdotal reports (Pitt and Nickerson 2006, Smith 1907). A sample from a single Hellbender larvae collected by Pitt and Nickerson (2006) contained a mixture of aquatic insect remains including Megaloptera, Ephemeroptera, and Diptera. Smith (1907) collected a second-year larvae that regurgitated a smaller conspecific. In captivity, however, Hellbender larvae have been reared successfully on *Lumbriculus variegatus* (Müller) (Blackworm), mayfly nymphs (*Stenonema* spp.), cladocerans (*Ceriodaphnia* spp, *Simocephalus* spp.),

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and chopped *Lumbricus terrestris* L. (Nightcrawler), with crayfish and small fish added to their diet as they attain larger sizes (Unger and Mathis 2013). To increase knowledge of wild larval Hellbender feeding habits, we investigated the diet of larval Hellbenders in Little River, TN.

Methods

Due to the high number of larvae found during previous studies (Nickerson et al. 2003), we chose Little River as the study location (See Hecht-Kardasz et al. 2012 for additional site information). We conducted diurnal skin-diving surveys in July and August 2008, July and August 2009, and July, August, and September 2010 when river conditions were suitable (390 total survey hours). During 21 survey events, we located larval Hellbenders and secured them in moistened ziplock bags to measure TL and snout–vent length (SVL). Individuals under 125 mm TL, both gilled and non-gilled, were classified as larvae (Bishop 1941, Nickerson and Mays 1973). Using an Ohaus® CS2000 compact digital scale (accuracy ± 1.0 g; Ohaus Corporation, Parsippany, NJ), we measured mass in grams. We collected non-lethal diet samples from larval *C. alleganiensis* using a modified stomach flushing technique (Meehan and Miller 1978) by inserting a 14.2-g (0.5-oz) capacity Easy Feeder Nipple Tip Syringe (Four Paws Products, Ltd., Hauppauge, NY) filled with river water into the mouth and depressing the syringe to flush out stomach contents. A plastic container was placed under individuals to collect regurgitated stomach contents, which were then preserved in either 70% ethanol or buffered 10% dilution of concentrated formalin. We returned all Hellbenders to their capture site following sample collection. With the aid of a 0.75–3.0x binocular microscope (Bausch and Lomb, Bridgewater, NJ), we used a taxonomic key (Merritt and Cummins 1996) to identify diet items to the lowest possible taxonomic level, usually order, depending on the condition of item. We calculated numerical abundance and relative frequency of diet items in relation to both total sampled diet items and sampled individuals.

Results

We collected a total of 33 Hellbender larvae, but failed to acquire stomach contents from 30.3% of individuals that were therefore assumed to have empty stomachs. Successfully sampled larvae ($n = 23$) ranged from 40–118 mm TL (mean = $69.4 \text{ mm} \pm 13.95$) and weighed 2–9 g (mean = $2.8 \text{ g} \pm 1.5$). All but one Hellbender appeared to be first-year larvae. The mean number of prey items per individual was 1.96 ± 1.15 , and ranged from 1 to 6. Aquatic insects and crayfish comprised the majority of the 45 identifiable sampled diet items (Fig. 1). Ephemeroptera and Trichoptera were the most commonly identified insect orders consumed by Hellbender larvae (Table 1), with 43.5% and 26.1% of the sampled individuals in Little River having consumed these prey types respectively. We found a single vertebrate diet item, a *Eurycea* salamander, which is discussed further in Hecht-Kardasz and Nickerson (2013). 27% of sampled individuals had incidentally ingested items in their stomach, including plant matter and gravel. We were not able to identify

17.8% of diet items, primarily aquatic insects, to order due to the digested state of the specimens. We collected one additional sample, an Ephemeroptera nymph (Heptageniidae), from a sub-adult (204 mm TL).

Discussion

Based on our results, an ontogenetic shift in the Hellbender diet appears likely, although quickly digestible soft-bodied prey, such as fish, could have been missed during the study. Consistent with the findings of Pitt and Nickerson (2006), aquatic insects comprised 83.8% of identified dietary items collected from Little River larvae. While previous studies found that adult Hellbenders occasionally consume aquatic insects (Green 1935, Peterson et al. 1989), there is no evidence that insects make up a significant proportion of the adult diet. Decapods, which generally

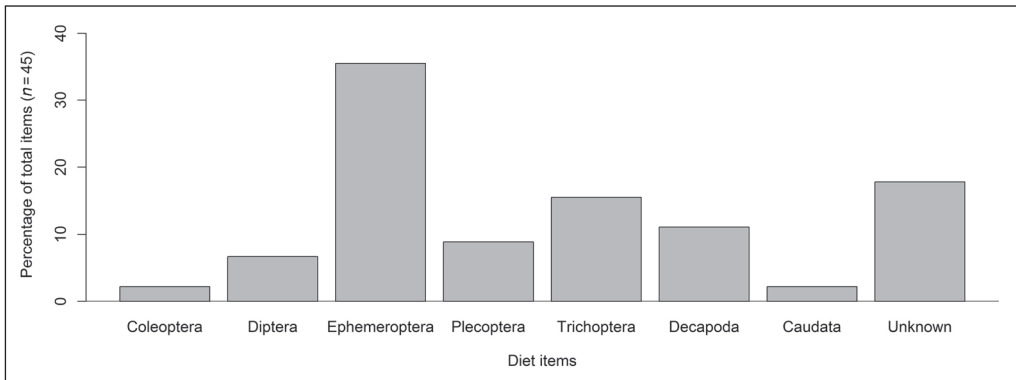


Figure 1. Composition of larval *Cryptobranchus alleganiensis* (Hellbender) diet samples collected in Little River, TN. Percentages represent the number of identified food items from each category in relation to the total number of identified food items from all samples.

Table 1. Contents of diet samples collected from larval *Cryptobranchus alleganiensis* (Hellbender) in Little River, TN ($n = 23$).

Diet item	Number of stomachs containing item	Percentage of stomachs containing item
Insecta		
Coleoptera (adult)	1	4.3%
Diptera	3	13.0%
Ephemeroptera	10	43.5%
Plecoptera	4	17.4%
Trichoptera	6	26.1%
Malacostraca		
Decapoda	5	21.7%
Amphibia		
Caudata	1	4.3%
Miscellaneous		
Gravel	4	17.4%
Plant matter	3	13.0%

comprise the majority of adult Hellbender diets (Green 1933, 1935; Netting 1929; Nickerson and Mays 1973; Peterson et al. 1989), were the third most numerous prey group found in larval diet samples. Unger and Mathis (2013) noted that captive Hellbender larvae readily consumed small crayfish when they attained 60 mm TL. Decapods, however, only represented 13% of identified diet items in our study, and consumed individuals were very small. While past studies of Hellbender prey availability focused primarily on the abundance of crayfish populations (Bodinof et al. 2012, Nickerson et al. 2003), aquatic insects and their habitat may be important for the survival of larval Hellbenders, and should be considered in future management and conservation actions.

Young larvae may have similar feeding mechanisms as adults, allowing them to capture and swallow large prey items. While most prey items identified were small, the consumption of a 40-mm TL *Eurcyea* sp. salamander by a 50-mm TL individual suggests that Hellbender larvae are able to consume relatively large prey items. Like adult Hellbenders that utilize suction-feeding strategies (Elwood and Cundall 1994), larvae lack vomerine tooth patches, which may be advantageous for swallowing whole food items quickly via suction feeding (Greven and Clemens 2009). According to Larghi (2013), immature Hellbenders exhibit a reduced bite force compared to their larger conspecifics, but estimates of suction-feeding pressure remain similar regardless of Hellbender size.

Stream macroinvertebrate communities are often dependent on water quality and stream substrate characteristics (Erman and Erman 1984). The 3 aquatic insect orders most commonly consumed by larval Hellbenders (Ephemeroptera, Trichoptera, and Plecoptera) were abundant in benthic samples and regularly seen during our surveys. Ephemeroptera and Trichoptera were also the most well-represented invertebrate groups found during index of biological integrity (IBI) surveys conducted downstream in adjacent Townsend, TN (Carter et al. 2008, 2009, 2010). As these orders are known to be associated with high water quality, the abundance of these organisms is used as a biological indicator of stream health through use of the EPT (Ephemeroptera, Plecoptera, and Trichoptera) index (Weber 1973). While it is not clear if Hellbenders require these dietary items or they were eaten more often because they were more abundant, this study prompts further research into the importance of stream quality in regards to larval Hellbender dietary requirements. As communities of macroinvertebrates commonly change throughout the year (Giller and Malmqvist 1998), diet studies on a longer time scale are necessary to determine if Hellbender larval diet remains consistent throughout the year.

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