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Larval Growth and the Potential for Head-starting of Eastern and Ozark Hellbenders (*Cryptobranchus alleganiensis alleganiensis* and *C. a. bishopi*)

Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*) are large, paedomorphic salamanders found across the midwestern USA east to the Appalachian states of Pennsylvania and New York and south to northern Georgia (Petranka 1998), with western populations of the Ozark Hellbender subspecies (*C. a. bishopi*) confined to Missouri and Arkansas. Hellbender populations across this geographic range are experiencing significant declines largely characterized by an overall lack of recruitment, with populations composed almost entirely of older age classes (Wheeler et al. 2003). The Ozark Hellbender (*C. a. bishopi*) recently has been listed as federally endangered.

Detailed descriptions of early developmental stages (eggs and larvae) are essential for understanding life history traits for species of conservation concern. Although growth rates for adults (older age classes) have previously been reported (Horchler 2010; Peterson et al. 1988), information on growth rates at the vital gilled larval stages is surprisingly lacking in the scientific literature for this cryptic species. Hellbender larvae are rarely encountered during surveys, possibly due to high mortality rates, observer bias, or their occurrence in small interstitial spaces in gravel beds (Nickerson and Mays 1973; Williams et al. 1981). Although a select few sites within their range do harbor this early

life stage in moderate numbers (Hecht-Kardasz et al. 2012), attempts at measuring growth based on recaptured larvae have proved unsuccessful. Recently, the importance of secure larval habitat has been proposed as a major factor for maintaining large adult populations (Nickerson et al. 2003). Successful captive management strategies for this species should incorporate information concerning life-history traits to maximize head-starting recovery programs.

The primary purpose of this study was to quantify growth rates of Eastern (*Cryptobranchus alleganiensis alleganiensis*) and Ozark Hellbenders (*C. a. bishopi*) at an early life stage, six

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months post-hatching, to determine the potential for rearing larvae under laboratory conditions. A secondary goal was to compare the growth rates among clutches from several populations and two subspecies to examine potential intrinsic differences in development of gilled larvae. This information should be useful for captive management decisions. For example, if clutches differ substantially in growth, further studies should examine whether different dietary regimes might be required for salamanders from different populations.

Laboratory Rearing/Analyses.—Larvae were hatched out of egg masses collected from the field on 10, 13, and 30 September and 18 November 2002, with one clutch each from the following sites: Davidson River, North Carolina; Coopers Creek, Georgia; Gasconade River, Missouri (eastern subspecies); and the North Fork of the White River, Missouri (Ozark subspecies), respectively. Following hatching, individuals were measured (TL mm) with calipers weekly on the same day for 24 weeks (~6 months) from 5 October 2002 to 3 April 2003. Larvae were kept at 19–21°C and were housed individually in aerated plastic containers (34.5 × 21.6 × 10.6 cm; 5.4 liters) for the majority of the study with de-chlorinated tap water, which was changed three times per week. Because egg masses were collected at different stages of development, we standardized stages based on morphological features defining each stage (Smith 1912) at hatching, which occurred on the following dates: 5 and 14 October and 4 and 25 November 2002 for Eastern Hellbenders from the Davidson River, Coopers Creek, and Gasconade River, and Ozark Hellbenders from the North Fork of the White River, respectively. Hatching success was highly variable (5–95%), with mortality associated with presence of a fungus that attacked the egg mass, identified as *Saprolegnia*. Consequently, sample size for each clutch was also highly variable: Davidson River N = 111, Coopers Creek N = 9, Gasconade River N = 66, and North Fork of the White River N = 145. Growth rates among clutches (rivers) were compared using a Kruskal-Wallis test (MINITAB).

Larval diets were adjusted as the larvae changed in size. Larvae were initially fed a variety of live food items including blackworms (*Lumbriculus variegates*), mayfly nymphs (*Stenonema* sp.), cladocerans (*Ceriodaphnia* sp., *Simocephalus* sp.), and chopped earthworms (*Lumbricus terrestris*). Once larvae obtained a TL of 60–80 mm, they readily consumed small crayfish (*Orconectes* sp.) (3.5–5.1 cm). At 80–100 mm they were successfully fed small darters (*Etheostoma* sp.) (approximately 3.8 cm TL); however, blackworms made up the majority of their diet at this size.

Growth Rates.—Intra-clutch size variation during weekly measurements was so small that even slight differences among populations were statistically significant. For example, there was a statistically significant difference in average size of larvae among clutches at week 3 (Fig. 1) even though the actual difference was only about 1 mm (Kruskal-Wallis test: $H = 12.3$, d.f. = 3, $P = 0.006$). By week 13, the larvae of the Eastern Hellbenders from Missouri and Georgia were noticeably smaller than larvae from North Carolina and Ozark Hellbenders ($H = 29.1$, d.f. = 3, $P < 0.001$). However, this difference disappeared over the next two months and growth rates were strikingly similar among populations and subspecies (Fig. 1).

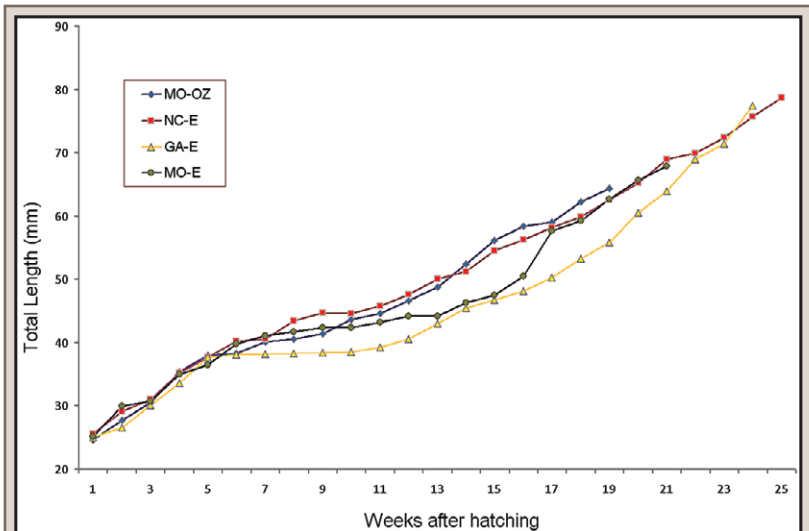


FIG. 1. Comparison of populations for weekly growth following hatching. SEs are omitted for the sake of clarity. E = eastern subspecies (*Cryptobranchus a. alleghaniensis*), OZ = Ozark subspecies (*C. a. bishopi*). MO-Ozark (N = 145–119); NC-Eastern (N = 111–47); GA-Eastern (N = 9–4); MO-Eastern (N = 66–29). Preceding numbers (in parentheses) represent the range in sample size per group measured weekly over the course of the study.



FIG. 2. Typical *Cryptobranchus* larva showing recent absorption of yolk, gill structure, and complete limb development.

We observed an overall difference in post-hatching mortality among clutches, with the Missouri Ozark clutch having the lowest post-hatching mortality (North Carolina eastern = 58%; Georgia eastern = 56%; Missouri eastern = 44%; Missouri Ozark = 18%). A critical period of development in which unusual mortality occurred is the period of change in nutrition (yolk-based to active feeding) (Smith 1912). We noted minimal mortality during the period in which the yolk is exhausted. Directly following this period, a few individuals had trouble eating and eventually starved. However, the majority of larvae did not have difficulty switching to live food and developed fully (Fig. 2).

Developmental abnormalities were seen only in larvae from the clutch of Eastern Hellbenders from Missouri. These included severely malformed (curved) spines (N = 2), tails (N = 4), and bloating (N = 15). Samples of malformed larvae were examined at the Veterinary Medical Diagnostic Laboratory in Columbia, Missouri. They reported that the larvae were infected by common bacteria (*Staphylococcus* sp. and *Aeromonas* sp.), but found no evidence of potentially harmful fungi or other parasites. The larvae were also diagnosed with hepatic lipidosis (fatty liver disease). Malformed larvae appeared to have great difficulty swimming and this disease presumably was contracted as a result of not being able to feed.

Successful Head-starting.—The similarity in larval growth rates suggests that there are no major intrinsic differences among the populations of Eastern and Ozark Hellbenders at this critical early stage. Laboratory-reared larvae had greater ranges in size in this study than those reported by Grenell (1939) and Smith (1912), who studied a combination of laboratory-reared and field-caught larvae (3–9 months old: Grenell = 35–60 mm, 6.5 months post hatching; Smith = 57 mm; this study = ~45–80 mm). However, growth rates for amphibians often are higher in the laboratory than in nature, and laboratory growth rates should be interpreted as measures of growth potential (physiological capabilities) (Jorgensen 1992). For example, laboratory-reared *Bolitoglossa subpalmata* individuals had twice the monthly growth rate as seen in nature (Houck 1982; Vial 1968). Laboratory-reared hellbender larvae begin to lose their gills at an earlier time than those in nature based on our observations. Faster growth under laboratory conditions could be the result of higher temperatures than those found in nature or could result from laboratory-reared larvae receiving more food and experiencing lower activity levels than would be required for actively foraging for food in the field.

This study demonstrates the efficacy of rearing hellbender larvae and monitoring development under relatively simple laboratory conditions. These results are encouraging since gills are typically not lost until 1.5–2 years after hatching or at ~100–130 mm TL (Grenell 1939; Smith 1912), indicating larvae reared in a laboratory setting can readily obtain this transformation size at an earlier age than observed in nature. Conservation efforts for this species can apply these accelerated growth rates and developmental ontogeny into their management programs.

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HERPETOCULTURE NOTES

TESTUDINES — TURTLES

MAUREMYS ANNAMENSIS (Vietnamese Pond Turtle). LOCALITY AND LONGEVITY. *Mauremys annamensis* is a rare geomydid turtle native to central Vietnam (Nguyen et al. 2009. *Herpetofauna of Vietnam*. Edition Chimaira, Frankfurt am Main, Germany. 768 pp.). The development of wetlands and intensive collection for the wildlife trade have resulted in the near total extirpation of *M. annamensis* from the wild (TCC [Turtle Conservation Coalition] 2011. *Turtles in trouble: the world's 25+ most endangered tortoises and freshwater turtles – 2011*. IUCN/SSC Tortoise and Freshwater Turtle Specialist Group, Turtle Conservation Fund, Turtle Survival Alliance, Turtle Conservancy, Chelonian Research Foundation, Conservation International, Wildlife Conservation Society, and San Diego Zoo Global, Lunenburg, Massachusetts. 54 pp.). Although large numbers of specimens within the wildlife trade have been documented, since 1939 only two individuals outside of captivity have been recorded in the

literature. A hatchling was recovered in 1993 from the artificial well of a village in Gia Lai Province (Parham et al. 2006. *Herpetol. Rev.* 37:239). The second, a subadult, was captured in a wetland in Quang Nam Province in 2006 (TCC 2011, *op. cit.*).

As a consequence of its scarcity, little is known about the life history of *M. annamensis*. Most aspects of the species' biology, such as life span, have only been inferred through observations of captive individuals. Snider and Bowler (1992. *Longevity of Reptiles and Amphibians in North American Collections*, 2nd ed. SSAR *Herpetol. Circ.* 21:1–40) listed an age of 23 years and 11 months for the oldest known individual, a wild-captured female residing at the Columbus (Ohio, USA) Zoo in 1990. This record has continued to be reported by subsequent authors (e.g., Slavens and Slavens 2000. *Reptiles and Amphibians in Captivity: Breeding—Longevity and Inventory* [current January 1, 1999]. Slaveware, Seattle. 400 pp.). Herein, we provide the first published details regarding the acquisition and history of this turtle, update the longevity record, and discuss the significance of this specimen.