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Repeatability of Locomotor Endurance in the Small-mouthed Salamander, *Ambystoma texanum*

Amphibian population declines have been linked to a variety of factors, including climate change and habitat destruction (Kiesecker et al. 2001), making assessment of the viability of habitat corridors and assisted dispersal a priority for conservation research (Milanovich et al. 2010). To quantify and evaluate dispersal ability, researchers commonly conduct physiological experiments involving multiple trials on wild-caught specimens. The performance of individuals during these experiments is assumed to be consistent and repeatable, but a direct assessment of whether or not this is the case is lacking. Assessing the value of laboratory experiments for describing performance in nature and for quantifying physiological measures for individuals requires knowledge of the impacts of experimental conditions on these measures (Bennett 1987).

Dispersal in *Ambystoma* salamanders is one case where repeatability of lab-based measurements of locomotor endurance is important because such experiments are used to infer how salamanders move across landscapes. Endurance measures can be combined with a measure of vagility to predict the genetic framework of amphibian metapopulations, as increased endurance and vagility have been shown to decrease genetic heterogeneity between populations (Johnson et al. 2010;

PAUL E. HUDSON
Department of Evolution, Ecology, and Organismal Biology,
300 Aronoff Laboratory, Columbus, Ohio 43210, USA
ROBERT D. DENTON
Department of Evolution, Ecology, and Organismal Biology,
300 Aronoff Laboratory, Columbus, Ohio 43210, USA;
Ohio Biodiversity Conservation Partnership,
300 Aronoff Laboratory, Columbus, Ohio 43210, USA
MATTHEW L. HOLDING
Department of Evolution, Ecology, and Organismal Biology,
300 Aronoff Laboratory, Columbus, Ohio 43210, USA
H. LISLE GIBBS
Department of Evolution, Ecology, and Organismal Biology,
300 Aronoff Laboratory, Columbus, Ohio 43210, USA;
Ohio Biodiversity Conservation Partnership,
300 Laboratory, Columbus, Ohio 43210, USA

Corresponding author; e-mail: hudson.336@osu.edu

Hillman et al. 2014). However, information pertaining to the repeatability of endurance is scarce. One of the very few studies to investigate this matter found that locomotor endurance of the endangered California Tiger Salamander (*Ambystoma californiense*) is repeatable over a period of a few days but not over a longer period of fifteen months (Austin and Shaffer 1992).

However, this estimation of repeatability may not be generalizable to other salamander species for several reasons. First, *A. californiense* is an endangered habitat specialist found only in Pacific coastal grasslands and is also one of the largest *Ambystoma* species (Trenham et al. 2000). Because surface area plays a major role in the regulation of body temperature and respiration of amphibians, it is difficult to scale the physiology of salamanders of vastly different sizes (Pincheira-Donoso et al. 2008). Second, multiple trial experiments on wild-caught and lab-raised salamanders may occur over a period that falls between just a few days and 15 months in order to measure average performance at a given time point or life-stage, but data on the repeatability of endurance in these time spans is currently lacking.

Common factors associated with the basic housing and care of animals, such as the frequency of feeding prior to trials, might also influence performance. The effects of food quantity on locomotor performance are unclear and other research has rarely taken into account unique aspects of the physiology of the species being studied, such as the remarkably slow metabolisms of salamanders compared to other vertebrates (Feder 1976). For example, feeding can decrease the burst speed of Garter Snakes (*Thamnophis elegans*; Garland and Arnold 1983) or not affect performance at all (Ford and Shuttlesworth 1986). In Trinket Snakes (*Elaphe helena*), large meals limit both burst speed and endurance (Mehta 2006). While an optimal meal size that maximizes endurance and antipredator behavior has been identified in these wild snakes, an optimal meal size or feeding schedule for captive amphibians participating in physiological trials has not been investigated (Sih and Christensen 2001). If we wish to maximize consistency in future experiments involving amphibians that will likely require feeding, we must determine how feeding affects amphibian performance and optimize our research protocol accordingly.
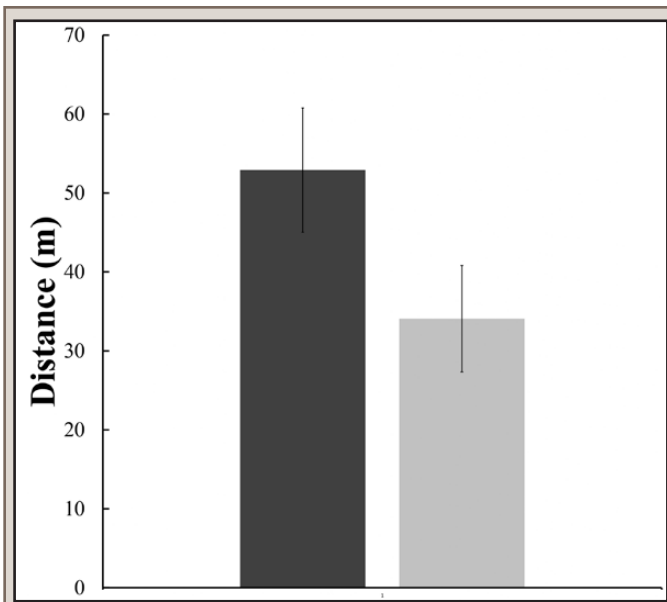
Fig.1. The average distance *Ambystoma texanum* were able to run in meters during their first (dark gray) and second (light gray) trials. Bars show mean ± SE. Individuals ran a significantly shorter distance during their second trial (P = 0.02).
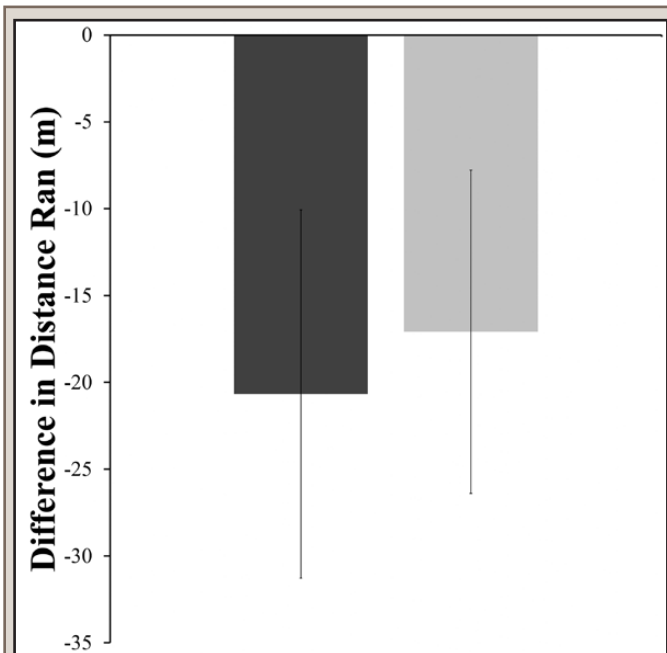

Fig. 2. The average difference in distance *Ambystoma texanum* in the Fed (dark gray) and Unfed (light gray) groups were able to run between Trial 1 and Trial 2. Bars show mean ± SE. There was not a significant difference in endurance between the Fed and Unfed groups (P = 0.49)

Here, we fill these gaps in knowledge by studying the repeatability of locomotor endurance in lab-raised, juvenile Small-mouthed Salamanders (*Ambystoma texanum*) that were maintained on two different feeding schedules. We conducted two treadmill trials with each individual to assess whether the number of trials would affect the distance they were able to travel. In addition, we selected feeding schedules that we predicted would safely warrant a change in performance and

replicate natural patterns of feeding frequency in salamanders (J. Bogart, personal communication). We chose *A. texanum* as a generalizable species in the genus *Ambystoma* because of their generalist habitat requirements and widespread range across central North America (Petranka 2010).

METHODS

We collected six *A. texanum* egg masses from two wetlands (N = 3 per site) in Crawford County, Ohio, USA, in April 2014 and allowed embryos to develop in a 14°C cold room. We separated larvae and fed them Brine Shrimp and *Daphnia magna* depending on size. After metamorphosis, we fed metamorphs three appropriately sized crickets (measured as less than the distance between the salamander's eyes) once a week. Individuals were housed under a 12 h light/dark photoperiod in 33 x 19 x 10.8 cm clear plastic shoeboxes that included two sheets of wet, non-bleached paper towel as substrate.

All locomotor endurance trials were conducted in the same room where animals were housed to maintain a consistent environment with as little disruption to photoperiod or temperature as possible. Temperature was recorded daily and never deviated from 14°C throughout the experiment. In July 2015, the surviving 28 juvenile *A. texanum* were divided into two groups of 14 with two different feeding schedules: Fed and Unfed. Both groups were fed two appropriately sized crickets before their first trial. Individuals in the Fed group were fed two appropriately sized crickets two weeks later before their second trial while those in the Unfed group were not fed before their second trial. In other words, salamanders from both groups were fed on Day 0 and then participated in their first endurance trial on Day 3. Salamanders from the Fed group were then fed on Day 14 and participated in their second trial on Day 17, while salamanders from the Unfed group were not fed any time after Day 0 and participated in their second trial on Day 17. Because we could locate no information regarding feeding frequency in wild *Ambystoma*, we chose the two week fasting period based on doubling the current feeding schedule of captive salamanders as recommended by Bogart (pers. comm.). Animals are currently fed weekly and demonstrate consistent weights for adults (unpubl. data), and we chose to double this duration. We recorded weight (g) with a scale accurate to 0.01 g and snout–vent length (mm) with a Salamander Stick (Walston and Mullin 2005) for all animals prior to each endurance trial, on Days 3 and 17. Because immediate digestion can affect locomotor endurance, individuals were fasted three days prior to every endurance trial (Garland and Arnold 1983).

Endurance trials were conducted on the custom treadmill from Johnson et al. (2010) and followed the same protocol. Briefly, we encouraged salamanders to walk on the belt by swiping their tail from side to side with a metal ruler. We measured endurance as the amount of time an individual could keep moving at a constant speed of 0.035 m/s and then transformed into distance ran. We rehydrated all *A. texanum* every three minutes and then checked for exhaustion as measured by righting response; if an individual was unable to right itself after we flipped it onto its back, we immediately ended the trial. We used a repeated-measures ANOVA in SPSS (version 22; IBM Corporation, 2013) with the averages of both SVL and weight measurements as covariates to compare the distance ran between Fed and Unfed groups. We predicted that salamanders in the Fed group would run significantly farther than those in the Unfed group because they would have more available energy for locomotion.

Results

Fed salamanders, on average, weighed 2.73 g (± 0.11 standard error [SE]) and were 41.29 mm (± 0.70 SE) in SVL. Unfed salamanders, on average, weighed 3.00 g (± 0.15 SE) and were 41.15 mm (± 0.60 SE) in SVL. The Unfed group did lose more mass over time than the Fed group, as expected. Between trials, the Fed group lost an average of 0.05 g (± 0.06 SE), while the Unfed group lost an average of 0.95 g (± 0.25 SE). The measurement variable met all assumptions of repeated measures ANOVA, including sphericity. Salamander weight and SVL were highly correlated; therefore, we used only weight as a covariate in the repeated measures ANOVA because we felt like this measurement was more accurate due to the difficulties of measuring small animals' SVL.

We found that the number of trials a juvenile *A. texanum* was subjected to did significantly reduce the distance it was able to run: movement distance on the second trial was 35.7% shorter than the first trial, independent of feeding group (repeated measures ANOVA, $P = 0.02$, $F[1,26] = 6.78$, Fig. 1). However, we could not detect any effect of feeding schedule on endurance, as there was no significant difference in endurance between the Fed and Unfed groups (repeated measures ANOVA, $P = 0.49$, $F[1,26] = 0.47$, Fig. 1). Fed animals traveled an average of 40.24 m (± 9.37 SE) compared to an average of 49.33 m (± 9.35 SE) for Unfed animals. There was no significant interaction between feeding schedule and the number of times *A. texanum* were run on the treadmill ($P = 0.82$, $F[1,26] = 0.05$, Fig. 2), and there was no significant interaction between endurance and weight for either group ($P = 0.211$, $F[1,26] = 1.62$).

Discussion

Because multiple endurance trials significantly reduced a salamander's endurance over time, ensuring that study organisms are given enough time to recover between trials may be important for making accurate endurance estimates. In fact, similar results were found in a study of the speed and endurance of Green Snakes (*Oepheodrys aestivus*), in which the crawl speed of female Green Snakes was dependent on trial number and some females would sometimes refuse to move in later trials (Plummer 1997). Even more similar results were found in a study of the terrestrial movement capability of Greater Sirens (*Siren lacertina*) and Two-toed Amphiumas (*Amphiuma means*). It was found that these two species of aquatic salamander presented, on average, a 27.8% decline in endurance between trials across multiple simulated environmental conditions when given an hour to rest (Schalk and Luhring 2010). Many papers evaluating endurance or speed, however, do not consider the effect of their repeat trials and have no standard recovery time: some trials are conducted in rapid succession while other study organisms are given anywhere from a few minutes to a day to recover (Else and Bennett 1987; Bennett et al. 1989; Plummer 1997; Adams et al. 1999). Other papers do not mention a recovery time at all (Finkler et al. 2003). It would be very useful to conduct experiments similar to ours on frequently studied organisms in order to avoid biased estimates resulting from an overtired organism that cannot perform consistently. The significant decline in our salamander's endurance as well as anecdotal observations of their stubbornness or struggle to get moving in the second trial indicate that a tired salamander will not yield repeatable results.

Contrary to our predictions, the two different feeding schedules we designed did not affect the locomotor performance of *A. texanum*, as locomotor endurance decreased between the first and second trials regardless of feeding group. These results provide insight into how salamanders and other animals being subjected to physiological experiments should be cared for. Because how much an individual was fed did not play a significant role in its performance, perhaps a dedicated and meticulously recorded feeding regimen is not critical to obtaining accurate results. Alternatively, there may be an optimal feeding schedule (nutritional content, intermediate frequency) for captive amphibians that would prevent the stark drop in endurance from trial to trial we encountered in our experiment. Research pertaining to the natural feeding frequencies of adult *Ambystoma* salamanders is lacking, but would be helpful in this pursuit.

Our results may also inform our understanding of dispersal in *Ambystoma*. Salamanders like the Small-mouthed Salamander migrate to their breeding ponds during a short, rainy period in early spring (Husting 1965; Semlitsch 1998). The movement period of *A. texanum* is likely the time period of lowest prey abundance, and they are most likely fueled by stored fat built up while living underground the rest of the year (Lindquist and Bachmann 1980; Whiteman et al. 1994). This may explain why our juvenile *A. texanum* seemed to require more time than expected to rebuild energy after exhaustion, because wild *Ambystoma* likely only take part in a maximum of two movement periods during a year that are greater than two weeks apart from one another.

As amphibians are a taxonomic group of high conservation priority and particular spatial demands for reproduction, a better understanding of their movement is critical. Reliable measurements of locomotor endurance can be valuable for inferring movement capability in wild populations, which is crucial when designing migration corridors, minimizing habitat fragmentation, and studying metapopulation mechanics (Milanovich et al. 2010; Hillman et al. 2014). However, a standardized methodology for these endurance investigations is currently lacking.

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Theory and Practice of the Hydrodynamic Redesign of Artificial Hellbender Habitat

The Hellbender (*Cryptobranchus alleganiensis*) is a cryptic, large-bodied amphibian endemic to cool Appalachian and Ozark mountain streams (Nickerson and Mays 1973; Petranka 1998). As is the case with many salamanders, a long lifespan, reliance on aquatic habitat and sensitivity to environmental change make *C. alleganiensis* an excellent indicator of ecosystem health (Olson et al. 2012; Welsh and Ollivier 1998). Unfortunately, these same

MOHAMMED G. MOHAMMED
Department of Civil and Environmental Engineering, University of Missouri, Columbia, Missouri 65201, USA
e-mail: mgm7fc@mail.missouri.edu
ARIANNE F. MESSERMAN
Division of Biological Sciences, University of Missouri, Columbia, Missouri 65201, USA
e-mail: afmxw7@mail.missouri.edu
BRYAN D. MAYHAN
Department of Civil and Environmental Engineering and Geographic Resources Center, University of Missouri, Columbia, Missouri 65201, USA
e-mail: mayhanb@missouri.edu
KATHLEEN M. TRAUTH
Department of Civil and Environmental Engineering, University of Missouri, Columbia, Missouri 65201, USA
e-mail: trauthk@missouri.edu

qualities have led to drastic declines in populations of these amphibians nearly ubiquitously across their range (Nickerson and Mays 1973; Wheeler et al. 2003). As a result, the Ozark subspieces, *C. a. bishopi*, was listed under the U.S. Endangered Species Act as Endangered in 2011 (Federal Register 2011), and has been deemed Imperiled (N2) by NatureServe (2015). The eastern subspecies, *C. a. alleganiensis*, has been listed due to varying degrees of risk in 13 of the 16 states in which it occurs (Mayasich et al. 2003; KDFWR 2013), and has been assigned a national NatureServe (2015) status of Vulnerable to Apparently Secure (N3/N4). As a species, *C. alleganiensis* is considered Near Threatened by the International Union for Conservation of Nature (IUCN; Hammerson and Phillips 2004).

Habitat loss due to increased sedimentation from development within inhabited watersheds is suspected as a leading cause of declines among *C. alleganiensis* populations (Wheeler et al. 2003). Large loads of particulate matter entering streams deplete dissolved oxygen levels and fill the concave undersides of the large, flat rocks that serve as shelter and nesting sites for *C. alleganiensis*. In combination, these effects can reduce animal fitness and lead to breeding failure (Ringler and Hall 1975; Harlan and Wilkinson 1981; Briggler and Ackerson 2012; Browne et al. 2012). To combat this landscape-level threat to *C. alleganiensis*, Briggler and Ackerson (2012) developed artificial nesting structures to increase Hellbender habitat while