

TRANSLOCATION AND CONSERVATION OF HELLBENDERS

(*CRYPTOBRANCHUS ALLEGANIENSIS*) IN MISSOURI

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by

Catherine M. Bodinof

Dr. Joshua J. Millspaugh, Thesis Supervisor

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The undersigned, appointed by the dean of the Graduate School, have examined the
thesis entitled

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presented by Catherine M. Bodinof,
a candidate for the degree of Master of Science
and hereby certify that, in their opinion, it is worthy of acceptance.

Professor Joshua J. Millspaugh

Professor Charles Rabeni

Professor Hong H. He

Doctor Jeffrey T. Briggler

Jeff Beringer

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ABSTRACT

Conservation and Translocation of Hellbenders (*Cryptobranchus alleganiensis*) in Missouri

Catherine M. Bodinof

Dr. Joshua J. Millspaugh, Thesis Advisor

ABSTRACT

Populations of eastern hellbenders (*Cryptobranchus alleganiensis alleganiensis*) and Ozark hellbenders (*C. a. bishopi*) in Missouri have declined precipitously in recent decades for unclear reasons. As a result, both subspecies are endangered in Missouri and the Ozark hellbender is a candidate for listing under the Endangered Species Act. In addition to working towards identifying the ultimate drivers of declines in Missouri, state agencies are considering release of captive-reared hellbenders as a strategy to bolster wild populations. However, few herpetofauna translocations have been successful, commonly due to long distance movements of animals away from selected release sites or the persistence of factors involved in original declines. To determine whether one suspected driver of hellbender declines, the pathogenic fungus *Batrachochytrium dendrobatis*, was present when declines occurred and may have caused declines, we used histological techniques and an immunoperoxidase stain to detect the fungus in hellbenders archived in museum collections. As an immediate attempt to improve wild populations we also released 36 captive-reared Ozark hellbenders at 2 sites on the North Fork of the White

River, Missouri, and used radiotelemetry to monitor movements, resource selection and survival post release. We detected *B. dendrobatidis* prior to or during declines in 4 of 7 rivers including the North Fork of the White (1969, 1973, 1975), Meramec (1975, 1986), Big Piney (1986) and Current (1988) rivers, which suggests the pathogen may have contributed to hellbender declines. However, the fungus occurred in the North Fork of the White River for over a decade while hellbenders were intensively studied and populations appeared to remain stable. Therefore, if *B. dendrobatidis* were responsible for declines in the North Fork, effects of the fungus there were likely indirect as opposed to the mass mortality of adult amphibians other studies have observed. We collected 3635 locations of 36 captive-reared hellbenders translocated to the North Fork of the White River between May 2008 and August 2009. At the end of our study 16 hellbenders were alive, 13 were dead and we could not confirm status of 7 animals. We observed 3 distinct types of hellbender dispersal including non-dispersal ($n = 11$), slow-and-steady dispersal ($n = 9$), and abrupt long-distance-dispersal ($n = 16$). Most hellbenders (26 of 36) dispersed downstream and mean dispersal distance at the upper site ($\bar{x} = 318.28$ m, SE = 115.39 m) was over 2 times larger than at the lower site ($\bar{x} = 121.95$ m, SE = 34.13 m). At both sites, daily movements of hellbenders were fewer and covered shorted distances, and home range sizes were reduced in the second season of monitoring, suggesting most hellbenders had settled at least semi-permanently in the wild. At the home range and reach scale, hellbender resource selection was positively associated with presence of coarse substrate relative to fine substrates; and with decreasing distance to nearest rock in all meso-habitats (i.e., pool, run, riffle). In 3 of 4 models, the negative association between increasing distance to rock and selection was

intensified as benthic water velocity increased. Annual survivorship of captive-reared hellbenders was 0.7467 (lower site) and 0.4816 (upper site). Release site was the most strongly supported factor associated with the nearly two-fold difference in annual survival rates, though site specific factors driving the difference were not obvious. Annual survival rates of captive-reared hellbenders at the lower site were similar to estimated annual survival rates (0.81) of wild hellbenders from the same river in 1978-1979; but annual survival rates observed in our study were 30-100 % lower than indicated annual survival (0.975) in a wild population from the same river consisting primarily of 12 - 20 year old hellbenders. In addition to having lower survival rates, upper site hellbenders tended to gain less weight post release, and a greater proportion carried *B. dendrobatidis*, leech parasites and accrued injuries and open sores. Our study is the first to intensively monitor captive-reared or juvenile hellbenders via radiotelemetry for over 1 year. The site differences we observed in dispersal, survival and body condition of captive-reared hellbenders following translocation emphasizes the importance of release site selection and the usefulness of pilot studies to determine suitability of release sites. Regardless of site differences, our study demonstrates that captive-reared hellbenders were capable of remaining and settling in release sites while maturing in a wild environment for over 1 year.

DESCRIPTION OF CHAPTERS

All 4 chapters of this thesis were written as independent manuscripts to be submitted for publication in peer-reviewed journals. As a result some introductory material is repeated throughout chapters and an independent literature cited section follows each chapter. I listed co-authors after each chapter title and used the plural nouns "we" and "our" rather than "I" throughout each chapter.

CHAPTER 1

Historic Occurrence of Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*) Infection in Hellbenders (*Cryptobranchus alleganiensis*) from Missouri

Catherine M. Bodinof, Jeffrey T. Briggler, Mary C. Duncan, Jeff Beringer, Joshua J. Millspaugh

ABSTRACT

The pathogenic chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) has caused mass mortality and local extinction of several amphibians. While *Bd* has been detected in several populations that have declined, it is often unclear whether the fungus is a benign co-occurrence or the driver of declines. Identifying whether the fungus was present in a population before and during historic declines can help to determine the potential impact the fungus may have had. To identify historical occurrence of *Bd* in hellbender (*Cryptobranchus alleganiensis*) populations that have declined, we collected epidermal tissue from 92 eastern hellbenders (*C. a. alleganiensis*), 117 Ozark hellbenders (*C. a. bishopi*) and 13 unknown subspecies from Missouri collected and archived in museum collections between 1896 and 1994 from 7 rivers. We used histological techniques and an immunoperoxidase stain to confirm *Bd* infection in hellbenders from the North Fork of the White (1969, 1973, 1975); Big Piney (1986); Meramec (1975, 1986) and Current rivers (1988). We did not detect *Bd* in hellbenders from the Niangua, Gasconade or Eleven Point rivers, though few samples were available from these rivers during the

period of declines. Our findings are consistent with the hypothesis that *Bd* is a non-endemic pathogen in North America that was introduced in the latter half of the 20th century. In all 4 rivers where we detected *Bd*, infection occurred before or during the period over which hellbenders are estimated to have declined, and therefore may have contributed to declines. However, contrary to studies where *Bd* is first detected immediately prior to mass die-offs, *Bd* occurred in North Fork of the White River hellbenders during a decade when hellbenders were intensely studied and before population declines were noted. While *Bd* may have indirectly contributed to declines in the North Fork of the White River, our results suggest that it is unlikely that the pathogen caused mass mortalities of adult hellbenders in the decade after we first detected its presence.

INTRODUCTION

Chytridiomycosis, a potentially lethal disease caused by the pathogenic fungus, *Batrachochytrium dendrobatidis* (hereafter *Bd*) (Longcore et al. 1999; Pessier 2007), has been implicated in rapid declines, mass mortalities and local extinctions of amphibians around the world (Skerratt et al. 2007; Stuart et al. 2004). First described in 1998 (Longcore et al. 1999), *Bd* is the only member of the family Chytridiales to infect vertebrate hosts, by colonizing keratinized mouthparts of larvae and keratinized portions of the epidermis in post metamorphic amphibians (Berger et al. 1998). In the first study to identify means by which chytridiomycosis can cause mortality, Voyles et al. (2009) observed disrupted cutaneous function followed by cardiac arrest in green tree frogs (*Litoria caerulea*) infected with *Bd*. It remains unclear whether the chytrid fungus is a

widespread endemic pathogen undergoing increasing outbreaks (Rachowicz et al. 2005) or an introduced and 'spreading pathogen' (Skerratt et al. 2007). However, recent genetic evidence supports the hypothesis that the pathogen has recently spread and is novel to most species (James et al. 2009; Fisher et al. 2009). Currently *Bd* has been identified on 6 continents in over 200 species (Skerratt et al. 2007; www.spatialepidemiology.net/bd). Importantly, while most amphibian species can become infected with *Bd* (Pessier 2008), severity of infection and lethality can vary by host species (Woodhams et al. 2007), life stage (Garner et al. 2009) habitat (Kriger and Hero 2007a) and climate (Kriger and Hero 2007b). Additionally, there is some evidence to suggest some species may be able to acquire immunity to *Bd* infection over time (Retallick et al. 2004; Richmond et al. 2009). Therefore, detection of the fungus does not always imply occurrence of chytridiomycosis or lethality for hosts.

Considering the potential virulence of chytridiomycosis (Skerratt et al. 2007) and the broad geographical range of *Bd* (Fisher et al. 2009) the disease is often implicated as a cause for historical amphibian declines when obvious causes (i.e., habitat loss) are lacking (Stuart et al. 2004). However, because the chytrid pathogen was undescribed prior to 1998, it is commonly undetermined whether the fungus was present when several amphibian declines began, as early as the 1960s (Houlihan et al. 2000; Stuart et al. 2004). Examination of archived museum specimens has revealed presence of *Bd* only immediately prior to and during some amphibian declines, supporting the theory that the disease contributed to declines. (Berger et al. 1998; Lips et al. 2006). For example, at site in Panama (Lips et al. 2006) *Bd* was not detected in 1,566 amphibians sampled over 4 years prior to the first detection in September 2004. However, mass amphibian mortality

linked to chytridiomycosis was evident within weeks of the first detection (Lips et al. 2006). In contrast, populations of green frogs (*Rana clamitans*) in Maine were recently found to carry *Bd* at relatively high prevalence but no declines or mass mortalities have been reported (Longcore et al. 2007); even though the fungus was detected in *R. clamitans* specimens from the region (i.e., Quebec, Canada) as early as 1961 (Ouellet et al. 2005). Similarly, archived frogs of the genus *Xenopus* revealed that *Bd* occurred in parts of southern Africa as early as 1938 though no declines of the species have been observed (Weldon et al. 2004). Others (Retallick et al. 2004) have reported population recoveries and stable levels of infection after populations have recovered from declines which may have initially been caused by chytridiomycosis. Based on these scenarios management strategies to conserve amphibians might vary from preventing spread of diseased individuals, to identifying other causes for the declines, to controlling environmental factors that influence outbreaks.

Missouri hellbender (*Cryptobranchus alleganiensis*) populations have undergone precipitous declines in recent decades, which may be explained by chytridiomycosis. Hellbenders are large, fully aquatic salamanders that can live over 25 years (Taber et al. 1975) and inhabit clean, swift moving and highly oxygenated streams (Smith 1907; Nickerson and Mays 1973a,b). Two subspecies occur including the eastern hellbender (*C. a. alleganiensis*) which, in Missouri, is restricted to north flowing streams in the Missouri-Mississippi drainage; and Ozark hellbenders (*C. a. bishopi*) which are endemic to south flowing streams of the Black and White river drainages of southern Missouri and northern Arkansas (Nickerson and Mays 1973a). In Missouri, both subspecies have undergone declines averaging 77% since the 1980's (Wheeler et al. 2003). The

amphibian chytrid fungus was detected in Missouri for the first time in 2006 (Briggler et al. 2007a) and has since been confirmed in *C. a. alleganiensis* from the Big Piney and Niangua rivers and in *C. a. bishopi* from the Current, Eleven Point and North Fork of the White rivers (Briggler et al. 2008). Though hellbender susceptibility to chytrid infection is poorly understood, the current presence of *Bd* in Missouri hellbender populations has led to suggestions that the fungus may have been responsible for past declines (Briggler et al. 2007a,b; Briggler et al. 2008). However, it remains unclear whether *Bd* occurred in Missouri streams during the 1980s and 1990s when most hellbender populations were estimated to have declined (Wheeler et al. 2003). The objective of our study was to document historical occurrence of chytrid infection in archived hellbenders collected from 7 Missouri streams between 1896 and 1994 and identify whether the pathogen infected hellbenders prior to and during noted declines.

METHODS

Between March 2008 and May 2009, we visited 11 museum collections and accessed 234 archived *C. alleganiensis* collected from 7 Missouri streams between 1896 and 1994. We recorded collector, date of collection, river and locality information for each specimen. We recorded gender when evident without destructive sampling. Because gender and river can influence the length at which hellbenders reach sexual maturity (Peterson et al. 1988) we recorded total length (mm) of each hellbender. We categorized hellbenders as larvae (external gills present), juveniles (total lengths < 300 mm and lacking swollen cloaca) or adults (total length > 300 mm or swollen cloaca). We were restricted from collecting tissue from 3 type specimens; 10 additional animals were

larvae that were hatched in captivity, lacked keratin or were too small to prevent destruction of skeletal tissue during sampling and therefore were not included. To minimize destruction to valuable archived specimens and because infection of *Bd* is most easily detected in the digits of amphibians (Pessier 2007; Berger et al. 1999), we collected tissue from a single digit of each hellbender, similar to methods of Ouellet et al. (2005), from each of the remaining 222 hellbenders. We collected longitudinal cross-sections of epidermis from dorsal and ventral surfaces of each digit by filleting tissue from bone and leaving skeletal tissue intact. We placed each sample into a histology cassette and labeled with unique catalog numbers and museum codes. We placed cassettes in neutral buffered 10% formalin and transferred samples to the University of Missouri Veterinary Diagnostics Lab where samples were embedded in paraffin, sectioned at 4 µm and stained with haematoxylin and eosin (H&E) for histological diagnosis following methods of Berger et al. (1999). To determine presence of *Bd*, we scanned all sections at x200 power, suspicious areas of the integument at x400 and confirmed chytrids x1000 power using oil emersion (Berger et al. 1999). We diagnosed chytridiomycosis on the basis of zoosporangia locality within the epidermis (intracellular and restricted to stratum corneum or stratum granulosum of the epidermis), zoosporangium size (:S 20 µm) and shape (spherical or collapsed sphere), zoosporangium contents and uniformity and thickness of zoosporangium wall (Berger et al. 1999) We diagnosed each sample as negative, positive, or suspicious (i.e., structures observed that may be chytrids but confirmation is needed) following recommendations by Berger et al. (1999).

To confirm positives and clarify diagnosis of suspicious samples, we obtained *Bd* antibodies developed by Berger et al. (2002) from Dr. Alex D. Hyatt (CSIRO Animal Laboratory, Australia). We provided the University of Missouri Veterinary Diagnostics lab with antibodies which were used to produce an immunoperoxidase (IPX) stain as per methods described by Berger et al. (2002) at a dilution of 1:1000. After confirming effective staining with the IPX using *Bd* positive and negative controls, blocks of positive and suspicious tissue were sectioned at 4 µm and stained with the IPX. We diagnosed chytridiomycosis according to the above described criteria in combination with staining from the IPX (Berger et al. 2002).

RESULTS

Upon examination, 10 of the 222 samples we collected lacked keratin, probably due to abrasion of epidermal tissue during storage in chemicals, and therefore were not included in this study. Of the remaining 212 hellbenders, 50 were juveniles and 162 were adults, and total lengths ranged from 87 mm - 585 mm. We confirmed gender of 91 males and 53 females.

We detected *Bd* in 6.1 % (13 of 212) of hellbenders (Table 1) and diagnosed 199 hellbender samples as negative for presence of the fungus. Despite the fact that nearly one quarter of our sample were juveniles, all hellbenders infected with *Bd* were adults and ranged in total length from 274 mm - 490 mm. More than half ($n = 8$) of the infected hellbenders were males, 1 was a female and gender was undetermined for 4 *Bd* positive hellbenders. Histology revealed that infection of *Bd* in hellbenders was restricted to discrete areas of the integument and morphology of the fungus was consistent with that described by Longcore et al. (1999) and Berger et al. (1999). Pathology of chytrid

infection in hellbenders was characterized by mild focal hyperkeratosis and occasional sloughing of the epidermis near area of infection (Fig. 1). Generally infection appeared light. The heaviest infection we observed was characterized by approximately 100 chytrids in a single frame of view (400x), but 0-25 chytrids were typically visible within a given frame of any positive sample. We observed all life stages of *Bd* including immature and mature thalli with internal zoospores (Fig. 2) and empty zoosporangia sometimes with obvious discharge papillae (Fig. 3). When using the IPX, *Bd* stained a deep red color in contrast to other artifacts, and rhizoids of *Bd* zoospores were visible, which rarely occurred with the H&E stain (Fig. 3 and 4). In 4 samples, chytrids occurred along with the Oomycete fungus *Saprolegnia* sp. which is a common, sometimes lethal, secondary infection (Saprolegniasis) to cutaneous injury or immuno-compromised individuals (Pessier 2002; Noga 1993). In contrast to *Saprolegnia* sp., *Bd* stained heavily with the IPX, occurred within epidermal cells of the outermost layer of the epidermis (stratum corneum) and lacked hyphae which, in *Saprolegnia*, extended into dermal layers and stained light blue (Fig. 5). The IPX was useful for confirming presence of the *Bd* when *Saprolegnia* sp. was present or when presence of *Bd* was limited to immature or developing life stages indistinguishable from epidermal cell nuclei when using the H&E stain alone.

We detected *Bd* hellbenders from 4 of 7 rivers sampled, including the North Fork of the White ($n = 5$, 1969; $n = 1$, 1973; $n = 1$, 1975), Meramec ($n = 3$, 1975; $n = 1$, 1986), Big Piney ($n = 1$, 1986), and Current rivers ($n = 1$, 1988) (Table 1). Very few samples existed from each of these rivers prior to the earliest occurrence of chytridiomycosis we detected (Fig. 6); therefore it is possible that *Bd* was present in these populations earlier.

Simultaneous occurrence of chytrid infection and saprolegniasis occurred in a Current River sample from 1988 and in 3 Meramec River samples from 1975. We detected no sign of *Bd* or saprolegniasis in hellbenders collected from the Niangua, Eleven Point or Gasconade rivers.

Our sample size was restricted by historical collection and deposit of hellbenders into museum collections; therefore, samples were unevenly distributed both temporally (range = 0 - 79 per decade) and spatially (range = 5 - 80 per river) (Table 2). Sixty three of 212 (29.7 %) samples were collected prior to the earliest date in which we detected *Bd* (i.e., 1969); and the Meramec was the only river not represented prior to 1969. Pooling all rivers, the number of hellbenders collected during the 1970s ($n=79$), when hellbender research was prevalent in Missouri, were at least approximately double the number from any other decade (range = 0 - 41) (Table 2). The number of samples from the North Fork of the White River ($n=80$) were 1.5 to 16-fold greater than the number of samples from any other river (range = 5 - 54), which may account for the high number of *Bd* positive samples from this river relative to others. Although there were fewer samples from the Meramec ($n=5$), Current ($n=7$) and Big Piney ($n=12$), we detect *Bd* from all 3 rivers, suggesting *Bd* may have occurred in high prevalences at the time of collection or that infected animals were more susceptible to capture by collectors. Only 1 sample existed from the Eleven Point River post-1969, and only 4 samples existed from the Gasconade post 1954; therefore detection was impossible if *Bd* had been introduced to these rivers later. In contrast, we did not detect *Bd* in the Niangua population, despite it having the second largest sample size ($n=54$) in our study.

DISCUSSION

We found no evidence for endemism of *Bd* in Missouri hellbender populations prior to 1969, despite the fact that nearly one third of our samples were collected earlier. Our documentation of chytrid infection in 1969 is the earliest reported occurrence in *Cryptobranchus alleganiensis* throughout the species' range, the earliest report of the disease from an amphibian in Missouri that we are aware of, and one of the earliest confirmed cases for any amphibian within the United States. In comparison, the earliest report of *Bd* infection in North America comes from a green frog (*Rana clamitans*) collected in 1961 from Quebec, Canada (Ouellet et al. 2005). In a retrospective study involving 3371 archived amphibians, Ouellet et al. (2005) also reported a single occurrence of chytridiomycosis from 1960 - 1969 (Wyoming), 10 occurrences from 1970 - 1979 (California and Indiana), 8 occurrences from 1980 - 1989 (Minnesota, Wisconsin and Virginia) and 1 occurrence from 1990 - 2001 (Missouri) (Ouellet et al. 2005).

Though the mode by which *B. dendrobatidis* may have been introduced to Missouri waterways can only be speculated, non-native trout stocking and the sale of trout tags increased dramatically on the North Fork of the White River after 1960 (Alsup 2005), which roughly coincides with our earliest detection. Therefore, the fungus may have been introduced anthropogenically through contaminated water sources or recreational traffic. Collectively, our results are consistent with the hypothesis that *Bd* is a recently spread and novel pathogen in North America where it was first introduced in the latter half of the twentieth century.

In the 4 rivers where we documented historical occurrence of *Bd*, we cannot disprove the hypothesis that chytrid infection contributed to hellbender declines. A lack of continuous monitoring data precludes estimation of the exact onset (e.g., year) of

hellbender declines in Missouri streams (see Wheeler et al. 2003). However, we know hellbenders from the North Fork of the White, Eleven Point, Big Piney and Gasconade rivers declined precipitously between the 1980s and 1990s; and Niangua populations plummeted between the 1970s and 1990s. The regional trend made evident by Wheeler et al. (2003) suggests that declines in the Current and Meramec rivers may have occurred over the same time period. Because our results confirm the chytrid pathogen was present in at least 4 rivers before or during declines (Fig. 6), hellbender declines in these rivers may have been the direct result of chytridiomycosis-linked mortality or an indirect result of non-lethal chytrid infection. Our inability to confirm historic occurrence of chytrid infection in Gasconade, Eleven Point and Niangua rivers does not preclude the possibility that it was present but not represented in our samples. Because very few samples were collected from any rivers during the 1980s, our probability of detecting the fungus if it were introduced post 1980 was probably very low.

Bd occurred in the North Fork of the White River for at least a decade while hellbenders there were relatively well-studied and populations appeared to remain stable. Our findings contrast sharply with other studies that have identified rapid amphibian declines and mass mortalities immediately following introduction of *Bd* (Lips et al. 2006; Berger et al. 1998). The 6 earliest cases of chytrid infection we detected in the North Fork of the White River occurred in a 4.6 km section of river recorded as "Blair Bridge/Althea Spring" (See Table 1) where hellbenders were studied in 1969-1970 by Nickerson and Mays (1973a,b) and in 1978 by Peterson (1979). Though study sites of the two parties largely did not overlap, both produced population estimates for 1 discrete riffle (i.e., riffle 2-3, [Nickerson and Mays 1973a]) within the "Blair Bridge/Althea

"Spring" section which suggest that the hellbender population there remained stable between 1969 and 1978 (Nickerson and Briggler 2007). Because precise localities of the *Bd* positive specimens from 1969 were not recorded, it is possible they were collected outside of riffle 2-3. However, characteristics of the fungus, lack of awareness of the pathogen prior to 1998, and recreation and research that took place on the North Fork of the White River as early as 1969 (Nickerson and Mays 1973a,b) suggest that presence of the pathogen in any portion of the 4.6 km section might easily imply presence along the entire stretch. For example, *Bd* can survive without a host in an aquatic environment for 3 - 6 weeks, in moist sterile media (e.g., river sand) for up to 3 months, and can withstand short term exposure to air-drying (though not complete desiccation) to allow transport via bird feathers (Johnson and Speare 2005; Johnson et al. 2003). Therefore, while chytridiomycosis is known to spread rapidly through terrestrial ecosystems (Berger et al. 1998; Lips et al. 2006), it may spread even more quickly along an aquatic longitudinal gradient. Our results suggest that though *Bd* may have contributed to hellbender declines in the North Fork of the White River indirectly, it is unlikely that the pathogen caused mass mortality of adult animals immediately following its introduction.

While chytrid infection may not have immediately caused mortality of adult hellbenders in the North Fork of the White, presence of the pathogen may have affected recruitment. Variations in susceptibility of larvae or small hellbenders, versus adults, to chytridiomycosis may explain why hellbender declines were not evident for several years after the fungus was present. For example, Garner et al. (2009) noted that exposure of larval common toads (*Bufo bufo*) to *Bd* reduced the probability of surviving through metamorphosis and reduced the size of toads at metamorphosis. Furthermore, both

Garner et al. (2009) and Carey et al (2006) found that larger post-metamorphic toadlets have a higher probability of surviving *Bd* infection than smaller toadlets. Hellbenders are long lived (> 25 years) (Taber et al. 1975) and small (< 20 cm total length) hellbenders are encountered less often than larger animals in most rivers, including the North Fork of the White (Nickerson et al. 2003). Therefore, if young hellbenders experienced reduced survivorship following *Bd* exposure, but large hellbenders did not, the effects of *Bd* exposure may have only been obvious in the form of reduced recruitment following a time lag, which is consistent with hellbender declines on the North Fork of the White River (Wheeler et al. 2003). Another explanation may be that hellbender susceptibility to chytridiomycosis in Missouri streams may have increased with environmental changes (e.g., alterations to flow, water temperature, and introduced stressors) similar to an endemic pathogen hypothesis (Rachowicz et al. 2005); or with introduction of a new, more virulent, form of *Bd* since the 1970s, as recent studies have linked genetic differences to variations in virulence of *Bd* isolates (Fischer et al. 2009).

Our findings support leading hypotheses suggesting the amphibian chytrid fungus is a novel and spreading pathogen (Skerratt et al. 2007) in North America and emphasize the importance of identifying susceptibility of unique species that are likely vulnerable to *Bd*. In Missouri, amphibian conservation efforts should consider the current distribution and hosts for *Bd*. The geographical isolation of cave obligate amphibian populations from which the fungus has been recently detected in Missouri (Rimer and Briggler 2010) suggests that susceptible species may be particularly at risk to local extinction.

Our findings do not allow us to confirm or disprove whether *Bd* played a role in Missouri hellbender declines. However, hellbender declines in Missouri were only

apparent after our earliest detection of the fungus in the state. Our result are consistent with one hypothesis that Missouri hellbender declines were the result of decreased recruitment caused by chytrid infection in adult hellbenders or decreased survival of young age classes infected with *Bd*. However, more research is needed to identify non-lethal affects of *Bd* infection for amphibians including hellbenders; and to determine whether life stage or subspecies may influence susceptibility of hellbenders to chytridiomycosis. Finally, future studies should investigate possible differences in genetics and virulence of *Bd* isolates from various hellbender populations and compare Missouri haplotypes of the fungus to those that appear unique and benign to captive and wild Japanese giant salamanders (*Andrias japonicus*) (e.g., see Goka et al. 2009), which are one of the closest extant relatives to *C. alleganiensis*.

Table 1. Archived hellbenders (*Cryptobranchus alleganiensis*) collected from 4 Missouri streams between 1969 and 1988 that were confirmed positive for *Batrachochytrium dendrobatidis* infection via histology.

Museum ^a	Catalog No.	River ^b	Locality	Date	Year	Collector	Gender
MVZ	205728	BPR	Boiling Spring access/Texas County	23 MAY	1986	E. Routman	Unk
INHS	11236	CR	Cave Spring/Shannon County	1 JAN	1988	E. Routman	F
UMMZ	139000A	NFWR	Blair Bridge/Althea Spring	13 SEP	1969	M. Nickerson and S. Arnold	Unk
UMMZ	139000B	NFWR	Blair Bridge/Althea Spring	13 SEP	1969	M. Nickerson and S. Arnold	Unk
UMMZ	139000C	NFWR	Blair Bridge/Althea Spring	13 SEP	1969	M. Nickerson and S. Arnold	M
UMMZ	139000D	NFWR	Blair Bridge/Althea Spring	13 SEP	1969	M. Nickerson and S. Arnold	M
UMMZ	139000F	NFWR	Blair Bridge/Althea Spring	13 SEP	1969	M. Nickerson and S. Arnold	M

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MPM	7237	NFWR	Blair Bridge/Althea Spring	3 OCT	1973	M. Nickerson	M
MPM	11248	NFWR	Blair Ford	2 JULY	1975	M. Nickerson	M
MPM	11309	MR	5-6 miles upstream Indian Springs campground	21 JULY	1975	M. Nickerson	M
MPM	11310	MR	5-6 miles upstream Indian Springs campground	21 JULY	1975	M. Nickerson	Unk
MPM	11311	MR	5-6 miles upstream Indian Springs campground	21 JULY	1975	M. Nickerson	M
MVZ	205737	MR	Phelps County, where Meramec Spring enters	10 SEP	1986	E. Routman	M

^a MVZ = Berkeley Museum of Vertebrate Zoology, INHS = Illinois Natural History Survey, UMMZ = University of Michigan Museum of Zoology, MPM = Milwaukee Public Museum

^b BPR = Big Piney River, CR = Current River, NFWR = North Fork of the White River, MR = Meramec River

Table 2. Prevalence of *Batrachochytrium dendrobatidis* infection over time in archived hellbenders (*Cryptobranchus alleganiensis*) collected from 7 Missouri streams between 1896 and 1994.

	Time Period								Unknown Date	Total
	Pre-1930	1930-39	1940-49	1950-59	1960-69	1970-79	1980-89	1990-94		
<i>Cryptobranchus alleganiensis alleganiensis</i>										
Big Piney River	*	*	*	0 / 7	0 / 2	*	1 / 2	0 / 1	*	1 / 12
Gasconade River	0 / 4	*	*	0 / 14	0 / 1	*	0 / 3	*	*	0 / 22
Meramec River	*	*	*	*	*	3 / 3	1 / 2	*	*	4 / 5
Niangua River	*	0 / 1	0 / 1	0 / 18	*	0 / 29	0 / 4	*	*	0 / 54
<i>Cryptobranchus alleganiensis bishopi</i>										
Current River	*	0 / 4	*	*	*	0 / 2	1 / 1	*	*	1 / 7
Eleven Point River	0 / 4	*	*	0 / 2	0 / 12	*	*	0 / 1	0 / 1	0 / 20
North Fork White River	*	*	0 / 1	*	5 / 25	2 / 45	0 / 3	0 / 1	0 / 5	7 / 80

<i>Unknown location</i>	*	*	*	*	*	*	*	*	0 / 12	0 / 12
Total	0 / 8	0 / 5	0 / 2	0 / 41	5 / 40	5 / 79	3 / 15	0 / 3	0 / 18	13 / 212

Prevalence is noted as "number of chytrid infected samples / number of total samples"

* No samples were available

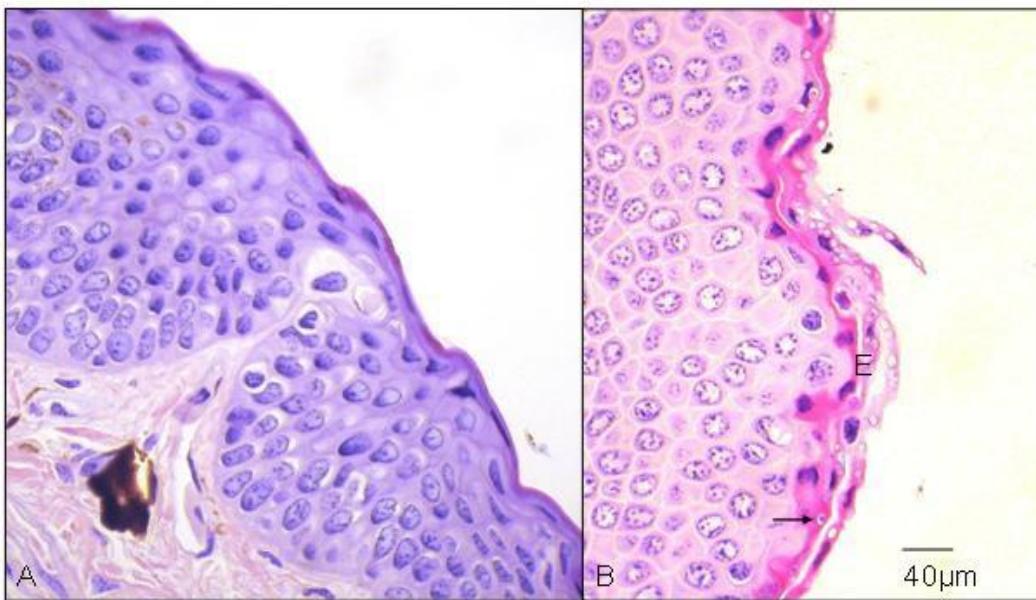


Figure 1. Normal (A) and infected (B) sections of hellbender (*Cryptobranchus alleganiensis*) skin stained with haematoxylin and eosin. Note that infection is restricted to the epidermis (E) which is thickened and beginning to slough. Empty chytrid zoosporangia appear as clear circular structures and immature chytrid thalli (arrow) are characterized by a dark central mass.

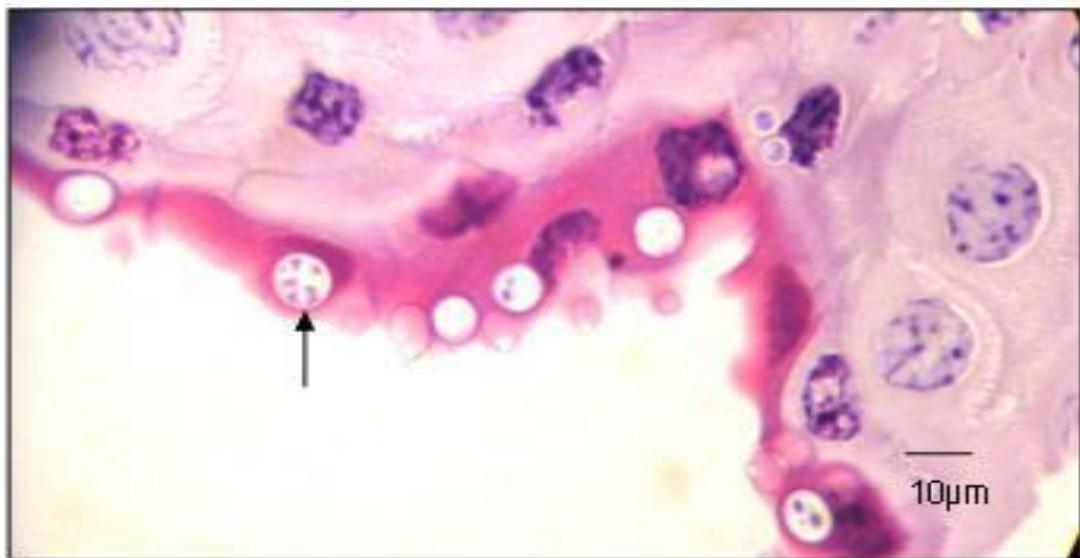


Figure 2. A section of skin from a hellbender (*Cryptobranchus alleganiensis*) collected from the North Fork of the White River in 1969 that has been stained with haematoxylin and eosin. Mature (arrow) and empty *Batrachochytrium dendrobatidis* zoosporangia are visible.

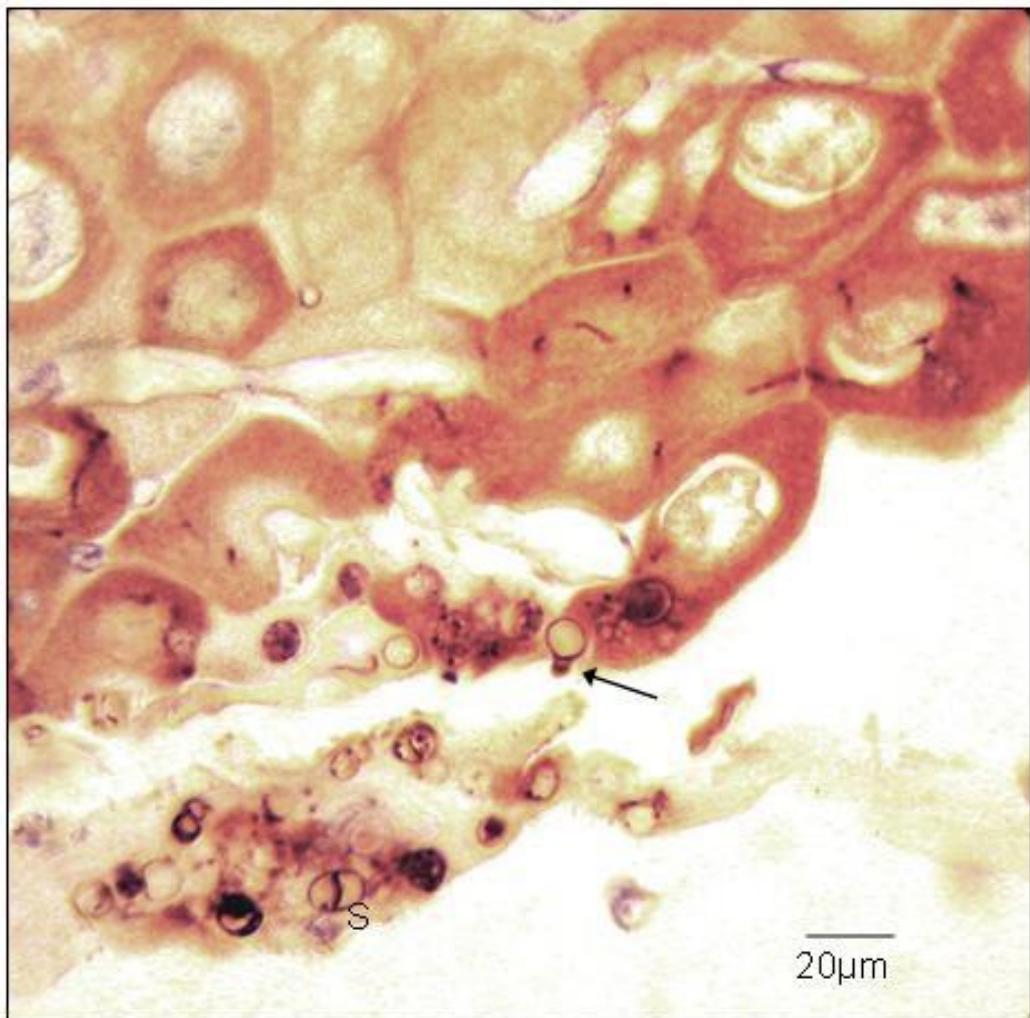


Figure 3. A section of skin from a hellbender (*Cryptobranchus alleganiensis*) collected from the Meramec River in 1975 stained with the immunoperoxidase stain. An empty zoosporangia with a discharge tube (arrow) is clearly visible as are empty zoosporangia with internal septae (S).

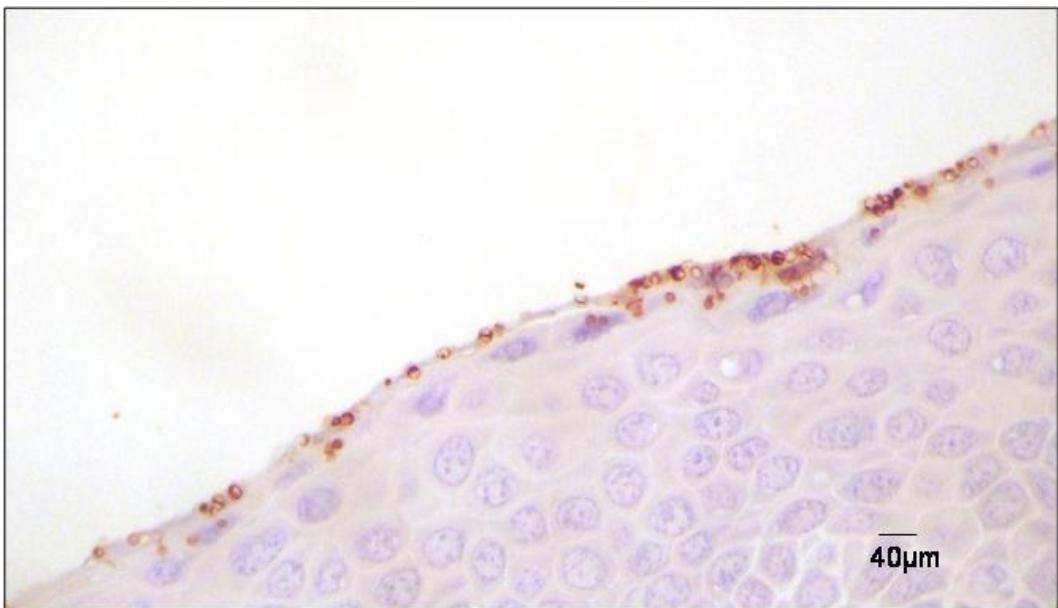


Figure 4. A section of hellbender (*Cryptobranchus alleganiensis*) skin stained with the immunoperoxidase stain, showing deep red staining of *Batrachochytrium dendrobatidis* in contrast to surrounding tissues.

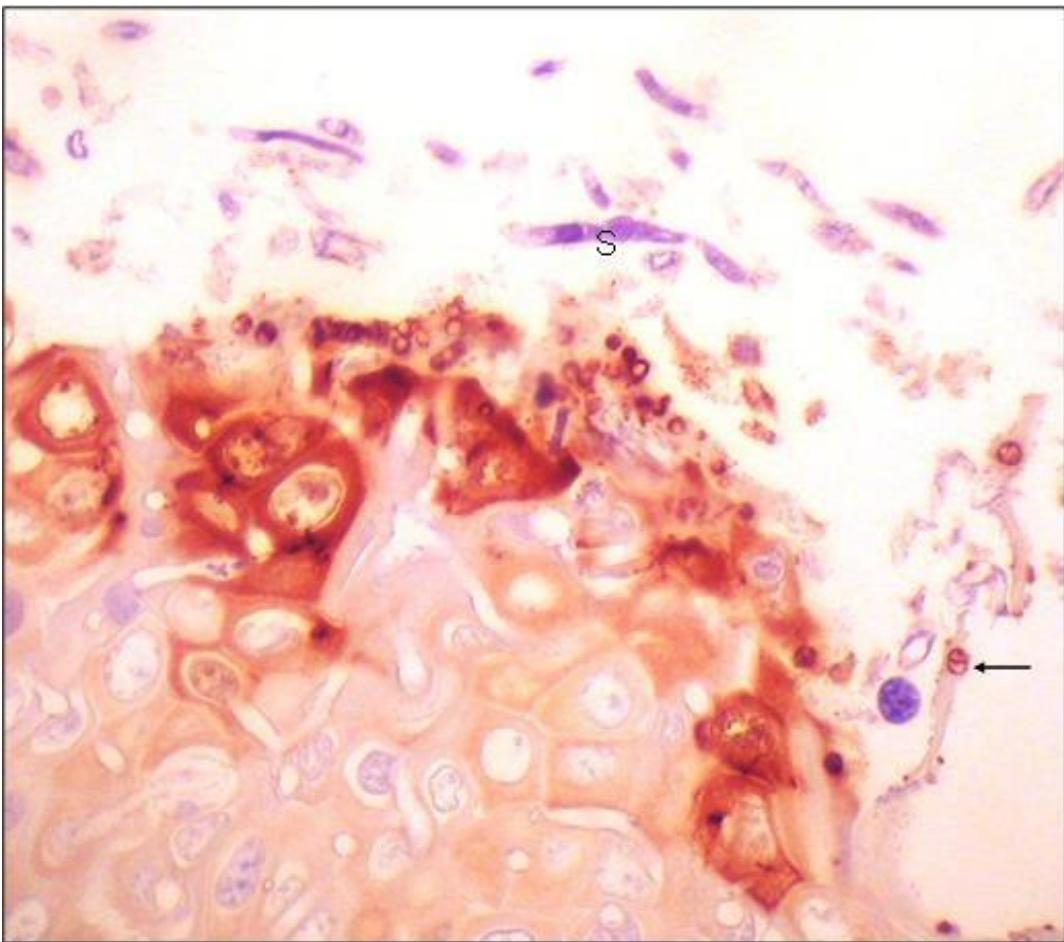


Figure 5. A section of hellbender (*Cryptobranchus alleganiensis*) skin infected with *Batrachochytrium dendrobatidis* and *Saprolegnia* sp. The immunoperoxidase stains *Saprolegnia* hyphae (S) light purple and stains chytrid zoosporangia (arrow) deep red.

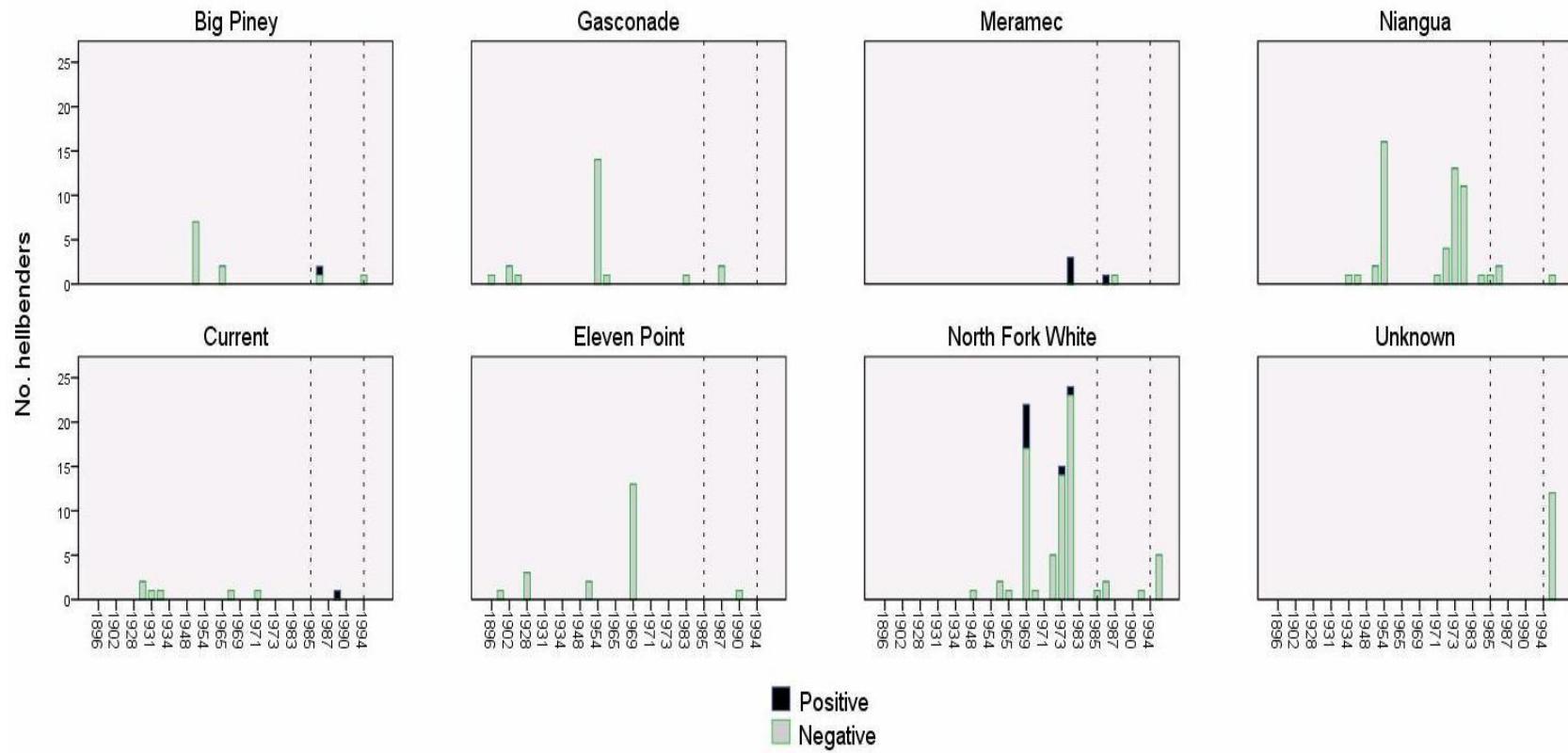


Figure 6. Distribution of hellbender (*Cryptobranchus alleganiensis*) samples by river and time showing detection of *Batrachochytrium dendrobatidis* infection (black) prior to or during the approximate period over which hellbender populations declined (area between dotted lines) as per Wheeler et al. (2003).

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CHAPTER 2

Movements and Space Use of Captive-Reared, Juvenile, Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*) Translocated to the Wild

Catherine M. Bodinof, Jeffrey T. Briggler, Randall E. Junge, Jeff Beringer, Mark Wanner, Chawna Schuette, Jeff Ettling and Joshua J. Millspaugh

ABSTRACT

Although translocations are often the only method for restoring extirpated species to their native range, they are frequently unsuccessful for herpetofauna due to long distance movements of subjects away from release sites. Translocation success can be improved by understanding the natural dispersal tendencies of a species. In an effort to bolster current populations of rapidly declining Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) and inform future hellbender translocations, we reared 36 hellbenders for 5.5 years in captivity and translocated them to 2 sites on the North Fork of the White River, Missouri. We monitored hellbender movements via radiotelemetry from May 2008 - August 2009 and collected 3,635 locations. We observed 3 distinct types of post-release movement patterns away from release points prior to settlement, including non-dispersal ($n = 11$), slow-and-steady dispersal ($n = 9$), and abrupt-long-distance dispersal ($n = 16$). At the end of our study most hellbenders were located downstream from the point of release, though mean distance between release and last observed location in the study (i.e., dispersal) was more variable and twice as long at the

upper site ($\bar{x} = 318.28 \pm 115.39$ m) than the lower ($\bar{x} = 121.95 \pm 34.13$ m). During the second season of monitoring, daily hellbender movements (i.e., occurring over a 24 - 36 hour period) were reduced and covered shorter distances. Home ranges were also smaller in the second season of monitoring. Contrary to herpetofauna translocations resulting in overwhelming dispersal away from release sites, juvenile hellbenders raised in captivity showed high site fidelity to release sites and moved similarly to wild hellbenders. More research is needed to identify factors that influence variations in hellbender dispersal.

INTRODUCTION

In response to global amphibian declines (Houlahan et al. 2000; Stuart et al. 2004), translocations are increasingly used to augment or replace populations of declining or extirpated species (Gascon et al. 2007; Griffiths and Pavajeau 2008; Bowkett 2009). However, the suitability of amphibians for translocation programs has been heavily debated (Dodd and Seigel 1991; Reinert 1991; Seigel and Dodd 2002; Trenham and Marsh 2002; Kraaijeveld-Smit et al. 2006), primarily due to limited success in herpetofauna translocation programs relative to other taxa (Dodd and Seigel 1991; IUCN 1998). Despite these concerns, translocation of captive populations remains the only method to restore some amphibian species to their native ecosystems. Identifying reasons for translocation failures would improve success of future attempts.

Germano and Bishop (2009) found that 10 of 25 failed amphibian and reptile translocations reported unanticipated long distance movements away from release sites as a reason for failure. Because habitat for many declining species is often limited, translocation sites may be characterized by hard boundaries outside of which suitable

habitat and the existence of conspecifics is limited or nonexistent (e.g., isolated breeding ponds). As a result, long distance movements from release sites can result in increased mortality or predation (Matthews 2003; Reinert and Rupert 1999; Sullivan et al. 2004) or decreased probability of encountering conspecifics and contributing to a self-sustaining population. High site fidelity to their native range and homing ability in translocated wild adult mountain yellow-legged frogs (*Rana muscosa*) in California (Matthews 2003), natterjack toads (*Bufo calamita*) in France (Huste et al. 2006) and Gila monsters (*Heloderma suspectum*) in Arizona (Sullivan et al. 2004) resulted in a large proportion of individuals abandoning release sites, often in an attempt to return to breeding ponds (Matthews 2003; Huste et al. 2006) or sites of original capture (Sullivan et al. 2004). In contrast, a study of translocated common geckos (*Hoplodactylus maculatus*) in New Zealand found that 10 of 11 geckos translocated approximately 1 km from site of capture remained at the release site after 1 year of monitoring and moved similar distances as resident geckos during that time (Lettink 2007). Given the vast diversity in species response to translocation, reintroduction programs aimed at restoring populations at a discrete site should consider the natural dispersal tendencies of a species to determine whether translocated individuals are likely to remain at the release site (Dodd and Seigel 1991; Reinert 1991).

Monitoring movements of translocated animals at various spatiotemporal scales can identify the potential distance an individual can travel in a give time period, identify whether or not animals eventually settle within release sites and identify the home range size of translocated animals. Motivations for animal movements may include seeking refuge, defending a territory, gaining information on surroundings, gaining energy or

reproducing (Nathan et al. 2008). Though motivations for movement themselves are often difficult to discern, collection of sequential locations of individuals (hereafter 'steps') can reveal movement 'phases' (e.g., dispersal, foraging, settlement) "associated with the fulfillment of a particular goal(s)" (Nathan et al. 2008, pp. 19053). Because motivations for movement can vary in space and time, it is useful to consider animal movements at various spatiotemporal scales. For example, the average distance between steps provides information on the tendency of a species to move and potential distance a species can traverse in a given time period; whereas a sequence of steps (i.e., movement path) (Nathan et al. 2008) can identify whether an individual is dispersing (e.g., relatively continuous movement to new areas with little time spent in any one place), exploring (e.g., makes 'excursions' to new areas before returning to a more heavily used area) or has settled into a more permanent home range (e.g., there is a estimable probability of an animal occurring in a defined area during a season, year or lifetime [Kernohan et al. 2001]). Understanding the fine and broad scale movement tendencies of a species as well as the spatial extent required by an animal to meet its needs improves our ability to select release sites of a suitable extent and thereby increase translocation success.

The hellbender (*Cryptobranchus alleganiensis*) is a rapidly declining amphibian, for which captive breeding and translocation is being considered as a potential management strategy. Hellbenders are large, fully aquatic salamanders that require clean, swift moving, and highly oxygenated streams with abundant rock cover, and can live over 25 years (Taber et al. 1975; Nickerson and Mays 1973a). The eastern subspecies (*C. a. alleganiensis*) originally occupied streams from New York south to Georgia and west into Missouri while the Ozark subspecies (*C. a. bishopi*) is endemic to southern Missouri

and northern Arkansas. Declines of both subspecies have been noted throughout the range (Furniss et al. 2003; Mayasich et al. 2003), however historical data from 5 Missouri streams reveals declines in both subspecies averaging 77 % over 20 + years (Wheeler et al. 2003). Proximate causes for declines remain unclear, however decreased water quality, over harvesting (Nickerson and Briggler 2007), introduced predators (Gall and Mathis 2010) and disease (e.g., chytridiomycosis) (Briggler et al. 2008; Briggler et al. 2007a,b) are some suspected factors. In light of rapid declines in the Ozarks, state and federal agencies are interested in applying captive-rearing and translocation to bolster hellbender populations throughout the region. However, a dearth of information exists concerning hellbender movements, especially in response to translocation, making it difficult to predict the utility of captive-rearing and translocation as a conservation strategy.

Previous studies have revealed that hellbenders require small home ranges (28 m^2 for females and 81 m^2 for males) (Peterson and Wilkinson 1996), rarely move long distances (i.e., up to 990 m) (Nickerson and Mays 1973a,b; Ball 2001) and show high site fidelity (Nickerson and Mays 1973a,b; Hillis and Bellis 1971; Blais 1989). These life history characteristics suggest that if translocated hellbenders settle in release sites, they will likely remain as a long-term investment in the population. However, the few studies that have documented adult hellbender movement following translocation reported "homing" to original capture sites (Blais 1989) or activity areas (Hillis and Bellis 1971) or long distance dispersal from release sites averaging 1026 m (Gates et al. 1985) similar to other herpetofauna (Matthews 2003; Huste et al. 2006, Sullivan et al. 2004). While hellbenders tend to settle in small areas, wild adults may be unlikely to remain and thus

establish a new population at a new discrete site. A few translocations involving taxa that exhibit problematic dispersal of wild or adult subjects have reported higher release site fidelity for immature age classes (Tuberville et al. 2005; Cooke and Oldham 1995) or captive-reared animals (King et al. 2004). Therefore, further research is needed to determine whether captive-reared or juvenile hellbenders will accept release sites more readily than wild adults.

In an effort to evaluate the potential of captive-rearing and translocation as a management strategy for hellbenders, we monitored movements of captive-reared juvenile *C. a. bishopi* released at 2 locations in the North Fork of the White River (hereafter NFWR), Missouri. Our objectives were to 1) document hellbender movement over a broad spatiotemporal scale as the direction of movement and distance (m) between release to last observed location in the study (i.e., dispersal); 2) document movement at a fine spatiotemporal scale as approximate daily steps, and calculate the proportion of steps revealing no movement and the mean distance (m) between steps when hellbenders did move; and 3) calculate home range of hellbenders during 2 seasons of monitoring. We hypothesized that hellbenders would disperse primarily downstream from release locations similar translocated hellbenders in Maryland (Gates et al. 1985), but would remain within the core habitat (defined below) of release sites to avoid leaving cover over long distances. We predicted that because hellbenders had no knowledge of release sites, the proportion of steps revealing no movement would be reduced in the first season and the average distance covered by steps in the first season would be greater as animals explored and moved to new areas of the stream. We predicted that home range size of

hellbenders would be larger in the first season of monitoring, as a result of longer steps involved in dispersal.

METHODS

Study Site Description

The NFWR is a 7th order stream originating in Wright County, Missouri that flows south to join Bryant Creek before entering Norfork Reservoir near Tecumseh. The NFWR is primarily spring fed with a watershed encompassing 3597 km² of southern Missouri. Landscape use primarily consists of forested woodlands (61.9%) and crop and grassland (37.5%) (<http://mdc.mo.gov/fish/watershed/northfrk/>). Although detailed information concerning recreational use of the NFWR is lacking, surveys revealed an average of 163 canoe put-ins occurred prior to 1300 hrs on weekends between 29 May and 8 August (<http://mdc.mo.gov/fish/watershed/northfrk/watqual/>) and that 12,437 days were spent angling on the NFWR and its tributaries from 1983-1986 (<http://mdc.mo.gov/fish/watershed/northfrk/watqual/>). Average gradient of the river is 2.42 m/km and flow near Tecumseh, Missouri averages 21.4 m³/sec (<http://waterdata.usgs.gov/mo/nwis/rt>). Substrate along the river includes long stretches of pebble and gravel beds interspersed with beds of large dolomite and limestone slabs and bedrock. Habitat associated with the occurrence of hellbenders is characterized by the presence of relatively large boulders with low embeddedness (Fobes 1995; Nickerson and Mays 1973a,b) which, in the NFWR, occur in discrete patches sometimes spaced several kilometers apart. Crayfish are the main food source of hellbenders (Nickerson and Mays 1973a) and 3 species, including *Orconectes neglectus*, *O. longidigitus* and *O. punctimanus*, are prevalent throughout the NFWR. Ozark hellbenders were historically

abundant in the NFWR (Nickerson and Mays 1973a,b); however the hellbender population in this river declined by approximately 70% between the early 1980s and late 1990s (Wheeler et al. 2003).

We selected 2 release sites along the NFWR with few resident hellbenders (based on prior surveys), an abundance of crayfish and boulder cover and a variety of mesohabitat (pools, runs, riffles) (Appendix 1-2). Our study sites were separated by approximately 17 km of river and we choose not to reveal their discrete locations due to threats of illegal collecting and the current status of hellbenders in Missouri. Our upper site spanned 1,026 m in length and approximately 36,115 m². Wetted width of the river ranged from 15 - 50 m and mean monthly water depth during our study ranged from 51.54 cm in August 2008 to 75.41 cm in May 2008 (Appendix 3). We defined core habitat at the upper site as a relatively isolated patch of boulder and bedrock confined to a narrow patch along 350 m of the western shore in the upper portion of the site (Fig.1). An intensive effort to map proportion of each substrate type present throughout the entire release site between July - August 2008 revealed that the extent of core habitat where the proportion of boulder substrate was approximately 2' 50 % was about 3,300 m² (Fig.1). Clusters of boulder at the upper site could be found in deep (> 1 m) pools and runs and shallow (< 1 m) riffles. Water temperature at the upper site reached greater extremes than the lower site, probably due to extensive shallow habitat upstream of the site. Mean monthly water temperature during our study ranged from 5.03° C in January 2009 to 23.55° C in August 2009 (Appendix 4). Crayfish density, estimated from random sampling of runs and riffles (< 1 m deep), using a 1 m kick-seine technique (Mather and Stein 1993) in August 2008, was 12.16 crayfish/m² (SE = 2.4).

Our lower site was larger and deeper than the upper site, spanning 960 m in length and approximately 52,945 m². Wetted width at the lower site ranged from 48 - 88 m and mean monthly water depth during our study ranged from 62.75 cm in November 2008 to 95.48 cm in May 2008 (Appendix 3). We defined core habitat at the lower site as a relatively isolated patch of boulder oriented along 320 m of the western shore in the center of the site (Fig. 2). Mapping the proportion of each substrate type present throughout the release site between July - August 2008 revealed the extent of core habitat where the proportion of boulder substrate was approximately 2' 50 % was about 7,700 m² (Fig.2). The area we defined as core habitat occurred throughout a deep (> 1 m) slow moving run and the adjacent downstream shallower (< 1 m) riffle. Mean monthly water temperatures at the lower site were probably buffered by the input from at least 1 large spring entering the NFWR between sites and greater depths, and ranged from 8.61° C in January 2009 to 20.96° C in August 2008 during our study (Appendix 4). The lower site occurred within a portion of the NFWR designated as a Blue Ribbon Trout Zone (daily limit is 1 trout > 18 inches) that is periodically stocked with rainbow (*Oncorhynchus mykiss*) and brown (*Salmo trutta*) trout. Crayfish density in runs and riffles at the lower site, estimated from random sampling of runs and riffles (< 1 m deep) using a 1 m kick-seine technique (Mather and Stein 1993) was 12.61 crayfish/m² (SE = 1.52).

Study Animals

We released 36 juvenile *C. a. bishopi* during our study. Juveniles originated from eggs collected from the NFWR in 2002 by Unger (2003) and were reared in captivity between 2003 and 2008 at the Saint Louis Zoo's Ron Goellner Center for Hellbender Conservation. Small groups of captive hellbenders were maintained in aquaria with rock

cover similar to native habitat, tong-fed and allowed to forage at will on a diet of crayfish and small fish. Water in captive enclosures was continuously aerated and circulated and was maintained to mimic approximate seasonal temperatures of southern Missouri streams. To prevent possible disease transmission from captive conditions, hellbenders received regular health exams prior to release. Though the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) has been detected in wild hellbenders the NFWR (Briggler et al. 2008), we wanted to ensure that translocated hellbenders were not carrying the fungus prior to release. We only released hellbenders after receiving 3 sequential negative results from PCR swab and assays used to test the release cohort for presence of the fungus in the weeks preceding release. Prior to release, each hellbender was implanted with an AVID passive integrated transponder used for individual identification.

Radio Transmitters

We implanted hellbenders with Sirtrack Limited model RVI 118 ($n = 9$) or RVI 218 ($n = 18$) radio transmitters tuned to a unique frequency between 150.000 and 151.999 MHz prior to release. Both transmitter models were completely encapsulated, included an internal helix antenna and had an estimated range of 100-300 m. Model RVI 118 measured approximately 30 x 13 x 8 mm, weighed 5-6 g and had an estimated battery life of 7.5 months. Model RVI 218 measured approximately 35 x 15 x 15 mm, weighed 9-10 grams and had an estimated battery life of 15 months. We conducted surgeries at the Saint Louis Zoo on 3 - 4 May ($n = 20$), 7 July ($n = 7$) and 3 September ($n = 9$) 2008. We implanted hellbenders in a staggered fashion to allow time for smaller hellbenders to reach weight sufficient for transmitter implant. We implanted hellbenders with the

largest model possible permitting the weight of the transmitter was :S 5 % hellbender mass. At surgery, juvenile hellbenders weighed 142 - 334 g, measured 29 - 36 cm total length and none had displayed morphological sex characteristics (cloacal swelling or gravid appearance). We confirmed gender of hellbenders if evident during subsequent recaptures.

We anesthetized hellbenders individually by placing them in a 250 mg/L solution of tricaine methanesulfonate (MS222) buffered with sodium bicarbonate (baking soda) added to saturation. We removed hellbenders from the solution once immobile and their righting reflex was lost. We swabbed a 2 cm² area of the ventral body wall with dilute chlorhexidine solution before making a 2 cm incision opening to the body cavity, left of midline. We rinsed transmitters that had been cold-sterilized in nolvasan for 2' 24 hrs with sterile saline and placed them into the coelom. We closed the body wall muscle layers with 3 sutures and closed the skin with 3 (3/0 polydioxane or nylon) sutures. Finally, we injected each hellbender with 10 mg/kg of enrofloxacin (antibiotic) and returned them to holding water where they were monitored until voluntary swimming occurred (within 30 minutes). To monitor healing we maintained hellbenders in captivity for 2-4 weeks before release. If dehiscing of the sutures was observed in captivity we repaired or replaced sutures and held animals for an additional 2-4 weeks prior to release.

Initial Release

We released 18 *C. a. bishopi* at each study site over 4 discrete events between May and October 2008. We released hellbenders at the upper site on 29 May ($n = 7$), 11 August ($n = 5$) and 3 October ($n = 6$) and at the lower site on 19 May ($n = 10$), 11 August ($n = 4$) and 3 October ($n = 4$). On release dates, we transported hellbenders from the

Saint Louis Zoo to study sites by vehicle. During transport hellbenders were maintained in coolers of continuously oxygenated water maintained at the approximate temperature of release locations. We randomly assigned hellbenders from each surgery cohort to study sites. We pre-selected discrete release rocks spaced 2' 5 m from the nearest known hellbender and staggered along a longitudinal gradient within core habitat of each site. We lowered hellbenders by hand to the river bottom near an opening on the downstream side of release rocks and allowed them to move independently under cover. We visually monitored hellbenders until they ceased to leave cover (within 5 minutes) and recorded UTM (Universal Transverse Mercator) coordinates of hellbender locations with a Trimble® GeoXT (Trimble Navigation Ltd., Sunnyvale, CA) handheld unit accurate to :S 1.5 m.

Monitoring

To avoid loss of contact with hellbenders due to immediate long distance movement away from release location, we located hellbenders every hour from 1 hour before sunset to 1 hour after sunrise during the 48 hours following release. Subsequently, we located hellbenders approximately every 32 ± 4 hours from 2 days post-release through 14 November 2008 and from 22 March through 21 August 2009. When heavy rain threatened or precluded sampling we located animals immediately before the disturbance and as soon as feasible following the disturbance. During winter (15 November 2008 - 21 March 2009) we located hellbenders approximately 1 time each week. We hereafter refer to our first season of monitoring (release - 31 December 2008) as 2008 and our second season of monitoring (1 January - 20 August 2009) as 2009.

We located hellbenders by wading or canoeing (if water depth 2' 1.5 m) and homing procedures (White and Garrott 1990) using a 3 element Yagi antenna and an ATS (Advanced Telemetry Systems, Isanti, MN) receiver (model R2000, R4000 or R410). Homing facilitated identification of discrete cover objects (rocks, bank crevices, root masses) used by hellbenders and enabled us to document fine scale movements (2' 0.20 m). When we could not distinguish which object a hellbender was under, we confirmed locations visually by snorkeling or with an Aqua-Vu® SV 100 (Outdoor Insights, Inc., Crosslake, MN) camera used to view into rock crevices and bank openings. We recorded each location as "same" (if the current cover object used was the same as that last observed) or "new" (if hellbenders were exposed or had moved to a new cover object from the last observed).

At each new location we recorded the azimuth and distance (m) from the center of the previously used cover object (e.g. rock) to the center of the new cover object using a hand held compass and measuring tape (when :S 15 m) or a Nikon® Prostaff® 550 (Nikon Inc., Mellville, NY) laser range finder (when > 15 m). We calculated new UTM coordinates of hellbender locations by using the formulas:

$$\text{Easting UTM} = x_1 + \sin \frac{(cp)}{180} \cdot d \quad \text{Northing UTM} = y_1 + \cos \frac{(cp)}{180} \cdot d$$

where x_1 and y_1 are the easting and northing (respectively) UTM coordinates for the prior location, cp is the azimuth from the last to the new location and d is the distance in meters between the center of cover objects used.

Recaptures

We recognized that recapturing hellbenders may have influenced their movements; however we viewed translocated hellbenders as an experimental population and therefore had an interest in monitoring weight gain and physical condition of the animals during the study. We were not able to capture all individuals at regular intervals due to their use of bedrock ledges or immovable rocks. We recaptured each hellbenders a mean of 1.25 times ($n = 36$; SE = 0.19, range = 0 - 3 recaptures) during our study, making an effort to disturb the habitat as little as possible.

Recaptured hellbenders were typically held for < 1 hour; however 7 hellbenders (August 2008, $n = 1$; March - May 2008, $n = 6$) were held longer to replace transmitters that failed prematurely or were scheduled to expire > 4 months before the end of our study (Appendix 5). We quarantined these hellbenders in 5 gallon buckets to prevent possible disease transmission between individuals and transported them to the Saint Louis Zoo, where they remained quarantined, for surgery. Methods for replacement surgery were identical to that previously described, with the exception that we removed expired transmitters from the body cavity before implanting a new unit and sterilized the container used in anesthetizing hellbenders between each surgery. Following transmitter replacement surgery we held hellbenders for 6 - 8 weeks, with the exception of 1 hellbender recaptured in August 2008 that we retained through winter due to dehiscing sutures. All 7 hellbenders were released 12 May 2009, which ensured healing and avoided release during high water when visibility was poor, as was common in early 2009. We released all hellbenders at the point (under the last used rock) of recapture.

Data Analysis

As a measure of hellbender fidelity to release sites and to quantify hellbender movement over a broad spatiotemporal scale, we calculated dispersal distance and direction. We defined dispersal as the straight line distance between the release and last observed location in the study for each hellbender. To calculate dispersal distance we used the 'calculate movement parameters' function in the Hawth's Tools Extension (Beyer 2004). When straight line paths between release and last observed locations crossed land outside the river channel (e.g. due to a bend in the river), we calculated dispersal by summing straight lines following the shortest path between release and final locations while restricted to the river corridor using aerial images of the river in ArcGIS. We defined the direction of dispersal based on the final location relative to the release location for each hellbender, regardless of movement steps directed in other directions prior to the end of the study.

As a metric to describe hellbender movements at a fine spatiotemporal scale, we calculated daily step-length which we defined as the straight line distance (m) between sequential locations collected 24 - 36 hours apart. We report observations of no movement as step-lengths of 0 m. We calculated step-lengths using the 'calculate movement parameters' function in the Hawth's Tools Extension (Beyer 2004). We calculated the proportion of 0 m step-lengths (i.e., no movement) for each hellbender with 2' 20 observations during 2008 and 2009, separately. Because we were interested in distance (m) of step-lengths when hellbenders moved, we calculated mean step-length for 2008 and 2009 using all hellbender movements within each site after removing step-lengths of 0 m.

To describe the area used by a hellbender during each season we used 2 metrics to calculate home range including minimum convex polygons (MCP) and fixed kernel (FK) utilization distributions. For both methods, we used only 1 hellbender location for each 24 hour period. When multiple locations were collected for a hellbender within a 24 hour period, we used only the first point collected 2' 24 hours since the previous observation. We calculated home range for each hellbender with 2' 20 locations within a season of interest. We estimated home range using MCP and FK methods for hellbenders in 2008 and 2009, separately. We calculated 95% MCP home range using the package 'adehabitat' (Calenge 2006) in program R (R Development Core Team 2006). While MCPs are commonly used to define the minimum extent of area encompassing all recorded locations for herpetofauna (Humphries and Pauley 2005; Row and Blouin-Demers 2006; Peterson and Wilkinson 1996), they do not reveal the intensity of use throughout an area. Because other studies have used MCPs to estimate hellbender home range (Humphries and Pauley 2005; Peterson and Wilkinson 1996), we included this method. To identify use intensity as well as spatial extent of home range, we calculated 95% FK home range using a plug-in method for bandwidth selection using the package 'ks' in program R (R Development Core Team 2006). Hellbenders, similar to many herpetofauna, have the tendency to remain in the same location, which can cause problems with utilization distribution estimation (Row and Blouin-Demers 2006). Duplicate locations are sometimes removed to avoid such complications but their inclusion may reveal biologically relevant information on animal space use within a home range (DeSolla et al. 1999). Getz and Wilmers (2004) and Getz et al. (2007) redistributed duplicate locations within a defined radius as an alternative to deletion in

construction of local convex-hull (LoCoH) polygons. Following their approach, we randomly distributed duplicate locations within a radius of 40 cm (approximately one-half the mean length of rocks used by hellbenders in our study) after Skalski et al. (1987). For all analyses, mean values are reported \pm 1 standard error.

RESULTS

We collected 3,635 locations of translocated hellbenders between 19 May 2008 and 19 August 2009. We made no attempt to survey for wild hellbenders but encountered 9 within the upper study site and 6 within the lower site during our study. At least 10 of these sightings occurred < 20 m from known locations of translocated hellbenders, though we never observed physical interactions between study animals and conspecifics.

We observed 13 mortalities, including 5 that occurred :S 30 days post-release and 8 occurring 53 - 370 days post-release. Causes for mortality included dehiscing sutures following release ($n=3$), mortality in captivity following surgery ($n= 2$), disease ($n= 1$), disturbance during a high water event ($n = 1$), and unknown causes ($n = 6$). We confirmed that 16 hellbenders were alive at the end of the study and we were unable to confirm status (alive or dead) of 7 animals. Recaptures confirmed gender of 21 translocated hellbenders including 13 females and 9 males (Appendix 6-7).

We observed 3 distinct types of hellbender movement patterns following release in our study. At one extreme, 11 hellbenders (upper site, $n = 8$; lower site, $n = 3$) were non-dispersers and were located < 20 m from the point of release at the end of our study. Nine hellbenders (upper site, $n = 2$; lower site, $n = 7$) were slow-and-steady dispersers

and typically made numerous incremental short distance (< 10 m) unidirectional steps over weeks or months before settling in an area < 20 m from where they were observed at the end of the study. Nearly half (upper site, $n = 8$; lower site, $n = 8$) of the translocated hellbenders were abrupt-long-distance dispersers. Abrupt-long-distance dispersers each made at least 1 long distance (> 50 m) step over a short period (4.5 - 38.5 hours), often but not always immediately following translocation. After long distance steps, abrupt-long-distance dispersers settled around 1 or more boulders or a bedrock slab for the remainder of the study ($n = 11$) or appeared lethargic and were observed exposed for multiple days before dying ($n = 5$). Two abrupt-long-distance dispersers made long distance steps in more than one direction, suggesting exploration, including 1 hellbender that traveled 235 m upstream but returned to a point within 25 m of release < 2 days later and another that traveled 93 m downstream then 623 m upstream within 3 days of release. At least 10 abrupt-long-distance dispersers traveled through dense boulder cover and traversed shallow pebble and gravel beds or bedrock slab to make these lengthy movements.

Our hypotheses that most hellbenders would disperse downstream and remain within core habitat of release sites were supported. In total, 26 of 36 (72.2 %) hellbenders dispersed downstream; 9 dispersed upstream and 1 hellbender did not disperse (Fig. 3). Only 7 hellbenders ultimately dispersed outside of core habitat (all 2' 500 m). Mean dispersal distance at the upper site ($\bar{x} = 318.28 \pm 115.39$ m) was much more variable and more than double that of the lower site ($\bar{x} = 121.95 \pm 34.13$ m) (Appendix 6-7, Fig. 4).

Except for some animals that made frequent or long distance movements early in the study, hellbenders appeared highly sedentary. The mean proportion of step lengths equal to 0 m revealed that hellbenders moved rarely in 2008 (upper site: $\bar{x} = 0.74 \pm 0.04$; lower site: $\bar{x} = 0.66 \pm 0.07$) but movements were even less common in 2009 (upper site: $\bar{x} = 0.83 \pm 0.03$; lower site: $\bar{x} = 0.79 \pm 0.04$) as predicted (Appendix 6-7, Fig. 5). In 2008 mean step-length at the upper site ($\bar{x} = 35.55 \pm 11.19$ m), where most hellbenders were non-dispersers or abrupt-long distance dispersers, was more variable and over 4-times larger than the lower site ($\bar{x} = 7.6 \pm 2.06$ m) where there were more slow and steady dispersers (Fig. 6). Mean step-lengths in 2009 were similar between sites and shorter by 90 % at the upper site ($\bar{x} = 3.53 \pm 0.63$ m) and 60 % at the lower site ($\bar{x} = 2.97 \pm 0.29$ m) compared to the previous season (Fig. 6).

In 2008, when step-lengths were more variable, hellbender home ranges were also more variable at both the upper (MCP: $\bar{x} = 200.59 \pm 172.44$ m²; FK: $\bar{x} = 49.43 \pm 25.60$ m²) and lower sites (MCP: $\bar{x} = 235.78 \pm 96.61$ m²; FK: $\bar{x} = 214.37 \pm 10.31$ m²) (Fig. 7). In 2009, at the upper site, mean hellbender MCP home ranges had decreased by 75 % ($\bar{x} = 51.10 \pm 26.43$ m²) and FK by 40% ($\bar{x} = 28.38 \pm 10.82$ m²) (Fig. 7). Similarly, at the lower site in 2009, MCP estimates decreased by 78 % (MCP: $\bar{x} = 50.36 \pm 27.96$ m²) and FK by 72 % ($\bar{x} = 59.74 \pm 33.99$ m²) (Fig. 7). Contrary to our hypothesis, only 5 of 9 hellbenders at the upper site and 6 of 10 hellbenders at the lower site decreased their home range size from 2008 to 2009 (Appendix 8-9). Four of the 8 hellbenders with larger home ranges in 2009 were animals we released in the final release

cohort (i.e., in October), suggesting that cooling water temperatures in autumn may postpone exploratory movements and dispersal.

DISCUSSION

In contrast to other herpetofauna translocations that have observed a large proportion of animals moving long distances away from release sites (Matthews 2003; Tuberville et al. 2005), most translocated hellbenders remained in core habitat. Additionally, captive-reared juvenile hellbenders showed dramatically higher release site fidelity than wild adult hellbenders following translocation (Gates et al. 1985). For example, 80 % of captive-reared juvenile hellbenders remained at the release site, compared to only 50 % of wild hellbenders translocated to a new stream in Maryland; and only 2 captive-reared juvenile hellbenders dispersed 2' the mean dispersal distance ($\bar{x} = 1026 \pm 289$ m) of translocated wild hellbenders (Gates et al. 1985). The high release site fidelity we observed in captive-reared hellbenders from our study may have been attributable to their immature life stage, conditions of captive-rearing, or the habitat quality of release sites. Other taxa display lower release site fidelity as adults than when immature or captive-reared, though few explanations for the difference have been offered. In a study of translocated gopher tortoises (*Gopherus polyphemus*), the proportion of tortoises that dispersed away from release sites was as high as 76.9 %, yet sub adults were consistently less likely to disperse outside of core habitat than mature adults (Tuberville et al. 2005). And while several pond breeding amphibians tend to abandon release sites (i.e., ponds) when translocated as adults (e.g., Matthews 2003; Huste et al. 2006; Cooke and Oldham 1995), translocating eggs or captive-reared larvae

or juveniles can result in relatively high release site fidelity (Kinne 2004; Cooke and Oldham 1995). One reason why captive-rearing or immature life stage might increase release site fidelity is because that animals have not yet been imprinted with a habitat template (e.g., Rittenhouse et al. 2007) or developed high site fidelity to specific resources (e.g. dens or ponds) (Semlitsch 2002; Germano and Bishop 2009). Some hypothesize that long distance movements of translocated animals away from release sites are attempts to seek out familiar resources (Stamps and Swaisgood 2007, pp. 393); at an extreme resulting in "homing" to original capture sites. Therefore, rearing hellbenders in conditions (e.g., water temperature, rock cover, and live prey) similar to the wild may have increased the probability that they would identify release sites as suitable. Physical habitat throughout both study sites appeared consistent with descriptions of habitat where high densities of hellbenders were found in the 1970s (Nickerson and Mays 1973a,b) and mid 1990s (Fobes 1995). Therefore, habitat quality at release sites used in our study may have been of high quality relative to release sites where mature wild hellbenders have been translocated (e.g., Gates et al. 1985) and thereby better facilitated settlement of hellbenders. Our findings suggest that captive-reared hellbenders may accept discrete release sites more readily than wild adult hellbenders, though more research is needed to identify factors that may motivate hellbenders to travel long distances post release.

In addition to showing high release site fidelity following translocation, changes in movements collected at a fine spatiotemporal scale suggested that captive-reared hellbenders eventually settled and began moving similar to wild hellbenders (Gates et al. 1985). Our research is the first account of remote (i.e., without lifting rocks for

recapture) monitoring of > 20 hellbenders at high resolution (i.e., 1-5 locations per week) over a long period (> 1 year); therefore it is difficult to compare our results with others. However, in the only other 15-month long radiotelemetry study of hellbenders, Ball (2001) observed a mean proportion of 0 m step-lengths ($\bar{x} = 0.89 \pm 0.01$) in 10 wild hellbenders from Tennessee, similar to what we observed during 2009. While the mean step-length of hellbenders in the Tennessee study was 2 to 3-times greater ($\bar{x} = 14.67$ m for females, $\bar{x} = 10.14$ m for males) (Ball 2001) than for translocated hellbenders, both suggest use of small areas and relatively short distance movements. The order of difference between mean step-length in the Tennessee study and ours is slight enough that it could be due to variation in sampling design, arrangement of substrate or prey availability. Also similar to captive-reared translocated hellbenders, wild hellbenders use small areas of stream, as demonstrated by the short inter-capture distance (80% < 30 m) for hellbenders in the NFWR (Nickerson and Mays 1973a,b) and MCP home range estimates of hellbenders from the Niangua river in Missouri (females = 28 m²; males = 81 m²) (Peterson and Wilkinson 1996) that were highly similar to home ranges we observed in 2009. The fact that translocated hellbenders established small home ranges and began moving like wild animals implies more permanent establishment within release sites than simply remaining in core habitat.

Despite the fact that most hellbenders remained and settled in small areas of core habitat, we observed contrasting types of hellbender dispersal patterns and the potential for long distance dispersal away from release sites. The variations we observed in hellbender response to translocation emphasize the importance of understanding factors that drive animal movement to further increase translocation success. Though we did not

quantify factors that might have influenced movement in this study, we speculate that variation in substrate between sites may explain why more abrupt-long-distance dispersers left core habitat at the upper site and more slow-and-steady dispersers occurred at the lower site. Variations that may have been important include the extent of boulder cover (m^2), the density of boulders and the substrate composition in riffles at the lower end of core habitats (Fig.1, 2) which might have influenced carrying capacity as well as hellbender step-lengths. In lotic systems, substrate provides habitat directly by providing interstitial spaces for cover; and indirectly by influencing near-bed hydraulics, thereby influencing the distribution and movements of benthic species (Lancaster et al. 2006; Wellnitz et al. 2001; Barr and Babbit 2002; Hoffman et al. 2006). Resource selection analysis of hellbenders in our study indicated that selection at both study sites was positively associated with decreasing distance to the nearest substrate particle with at least one axis 2' 15 cm (i.e. rock); and that in some instances probability of selection decreased exponentially as distance to rock exceeded even 0.5 m (See Chapter 3). Therefore, it is plausible that availability and distribution of physical habitat may explain movement patterns and distribution of hellbenders in our study. It is also possible that conspecific interactions, though undetected by our methods, may also explain motivations for some hellbender movements. We encourage future studies to consider potential impacts of substrate arrangement and wild conspecifics on step-lengths and dispersal tendencies of translocated hellbenders.

Because long distance movement away from release sites is a commonly cited cause of failure in animal translocations (Germano and Bishop 2009; Stamps and Swaisgood 2007), reintroduction programs should consider the dispersal tendency for

translocation subjects and factors that influence dispersal (Dodd and Seigel 1991; Reinert 1991). We are the first to report broad and fine scale post-translocation movements of juvenile or captive-reared hellbenders as a means to inform future translocation efforts as a management strategy for declining hellbender populations. Our findings suggest hellbenders rarely disperse long distances from release sites and adopt daily movements similar to wild animals if suitable habitat exists, emphasizing the importance of correctly selecting suitable release sites. When selecting future translocation sites for hellbenders, we recommend that researchers consider the availability and arrangement of resources at a fine scale in addition to a larger extent throughout the stream, because the sedentary behavior and small home range of hellbenders suggest the importance of fine scale habitat attributes to meet daily needs. Furthermore, we recommend rearing hellbenders in conditions as similar as possible to future translocation sites, which may increase the likelihood of identifying release habitat as suitable once released (Stamps and Swaisgood 2007). Our findings suggest that captive-rearing could be a valuable tool for improving release site fidelity in future hellbender translocations. However, we also recognize that the ultimate success of translocation depends on long-term survival and reproduction of individuals which was not addressed in our study.

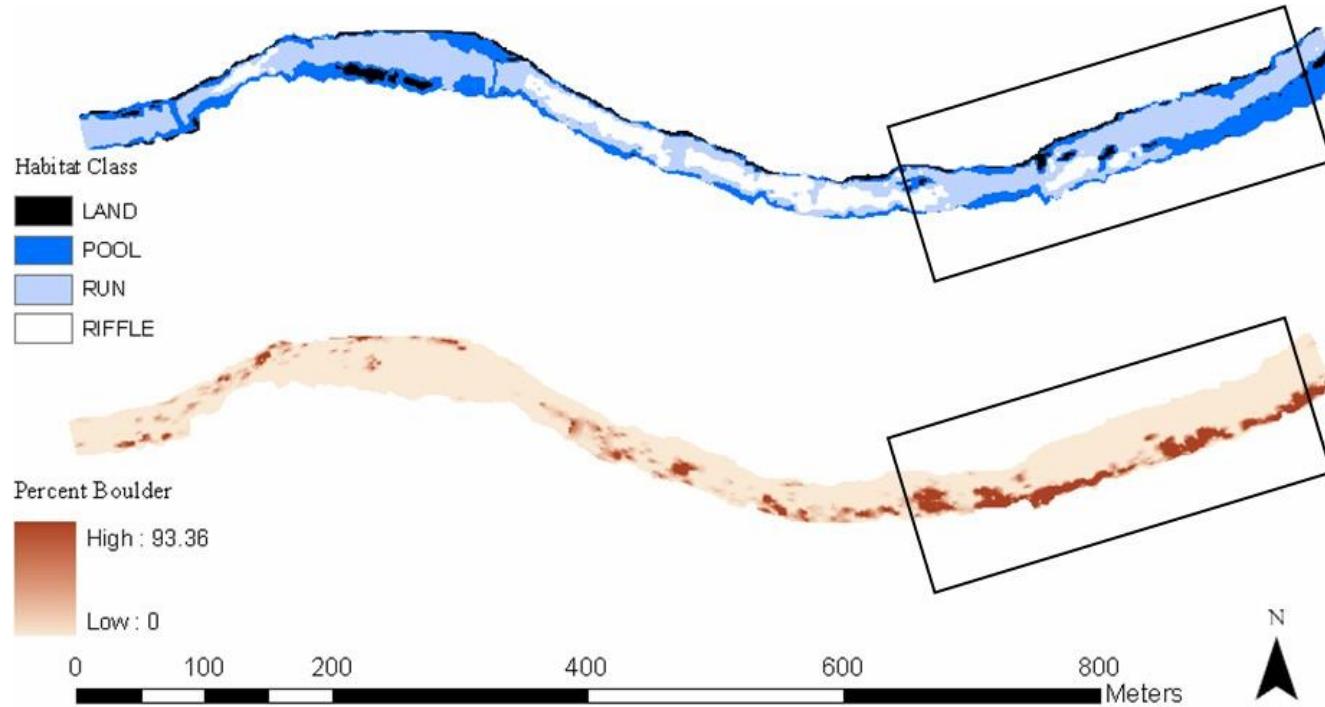


Figure 1. The upper study site on the North Fork of the White River, Missouri, USA, where captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) were translocated, 2008-2009. Core habitat (box) indicates areas where animals were released. Rasters represent the arrangement of pools, riffles and runs (upper image) and percentage of boulder cover throughout the site (lower image).

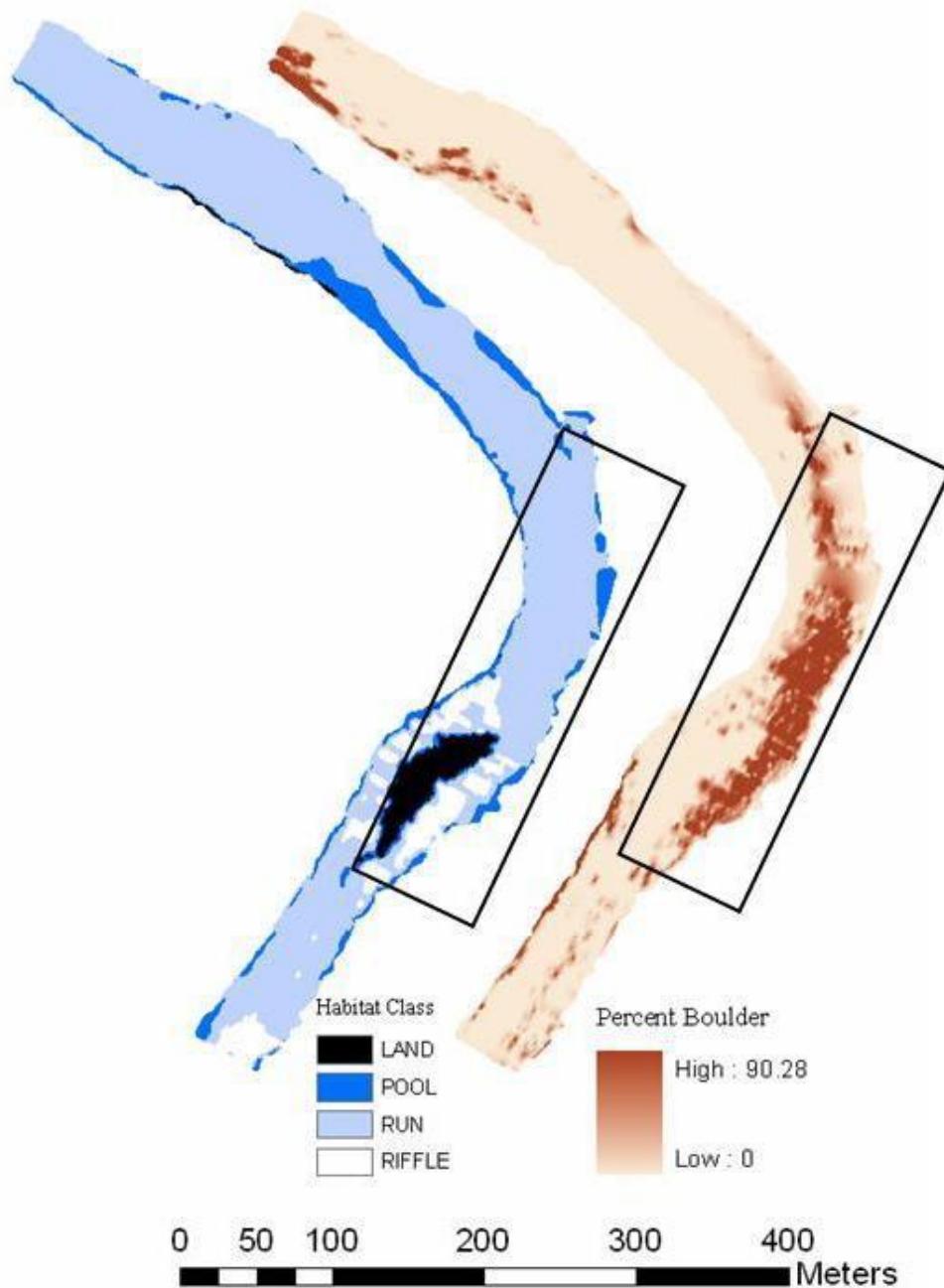


Figure 2. The lower study site on the North Fork of the White River, Missouri, USA, where captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) were translocated, 2008-2009. Core habitat (box) indicates areas where animals were released. Rasters represent the arrangement of pools, riffles and runs (left image) and percentage of boulder cover throughout the site (right image).

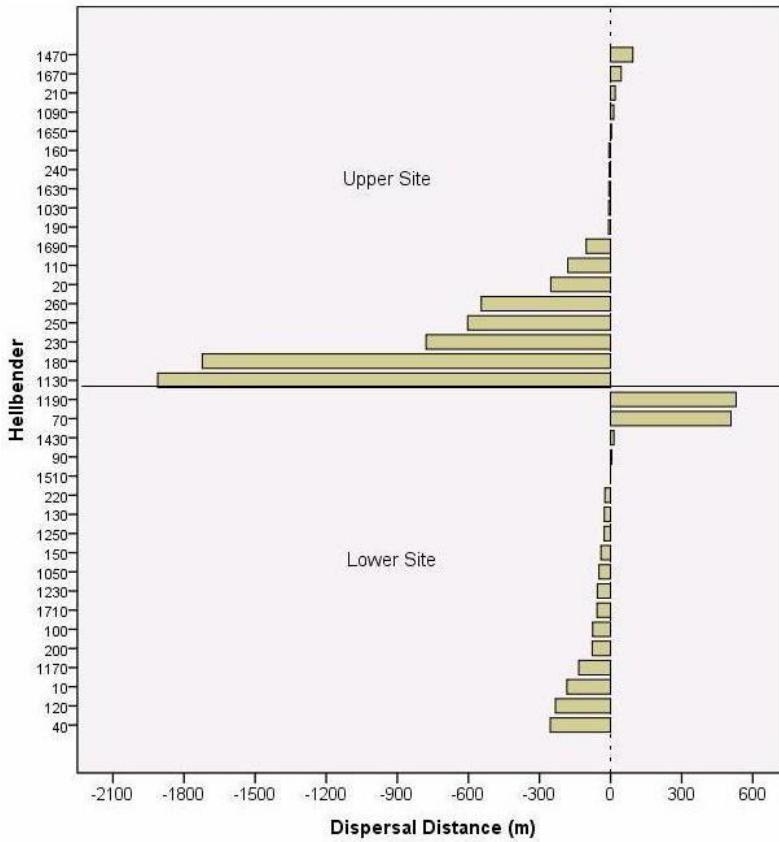


Figure 3. Direction and distance (m) of dispersal for captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*)

translocated to the North Fork of the White River, Missouri, USA, 2008-2009. Positive values indicate net upstream dispersal and negative values indicate net downstream dispersal from the point of release to the last observed location in the study.

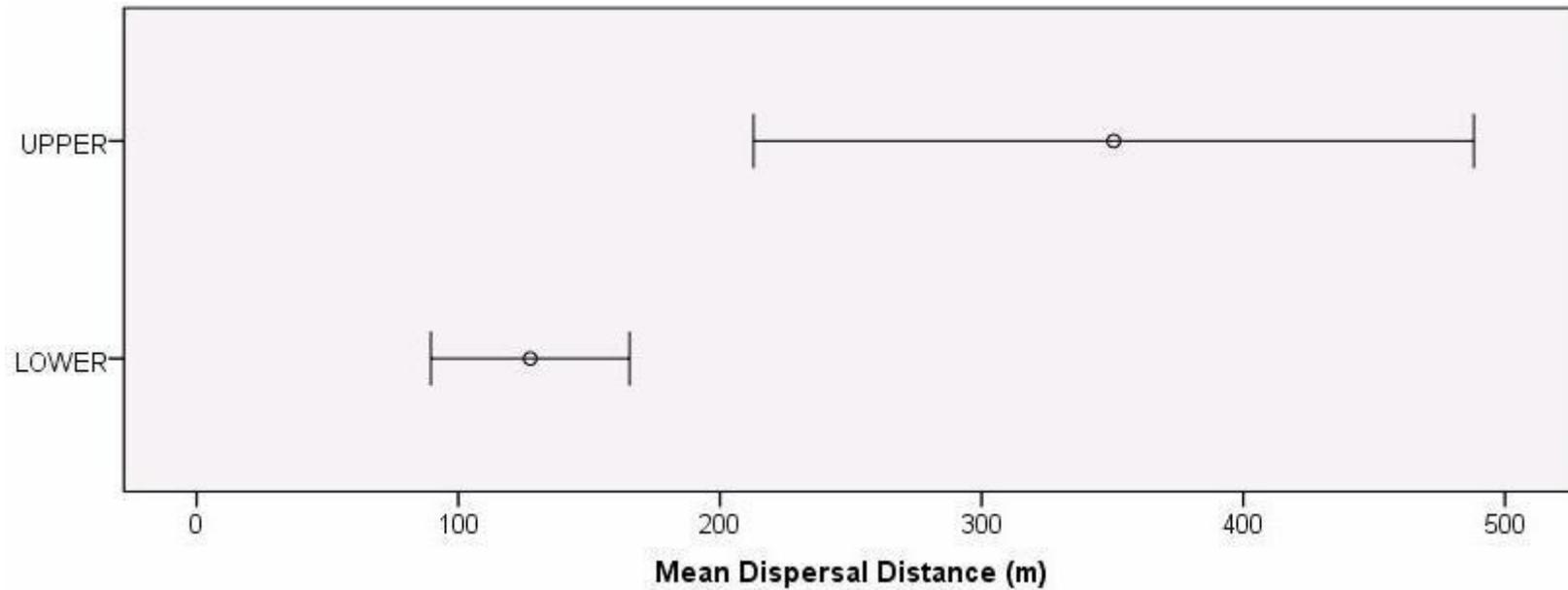


Figure 4. Mean dispersal distance (m) for captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the upper and lower site on the North Fork of the White River, Missouri, USA, 2008-2009. Error bars represent \pm 1 standard error.

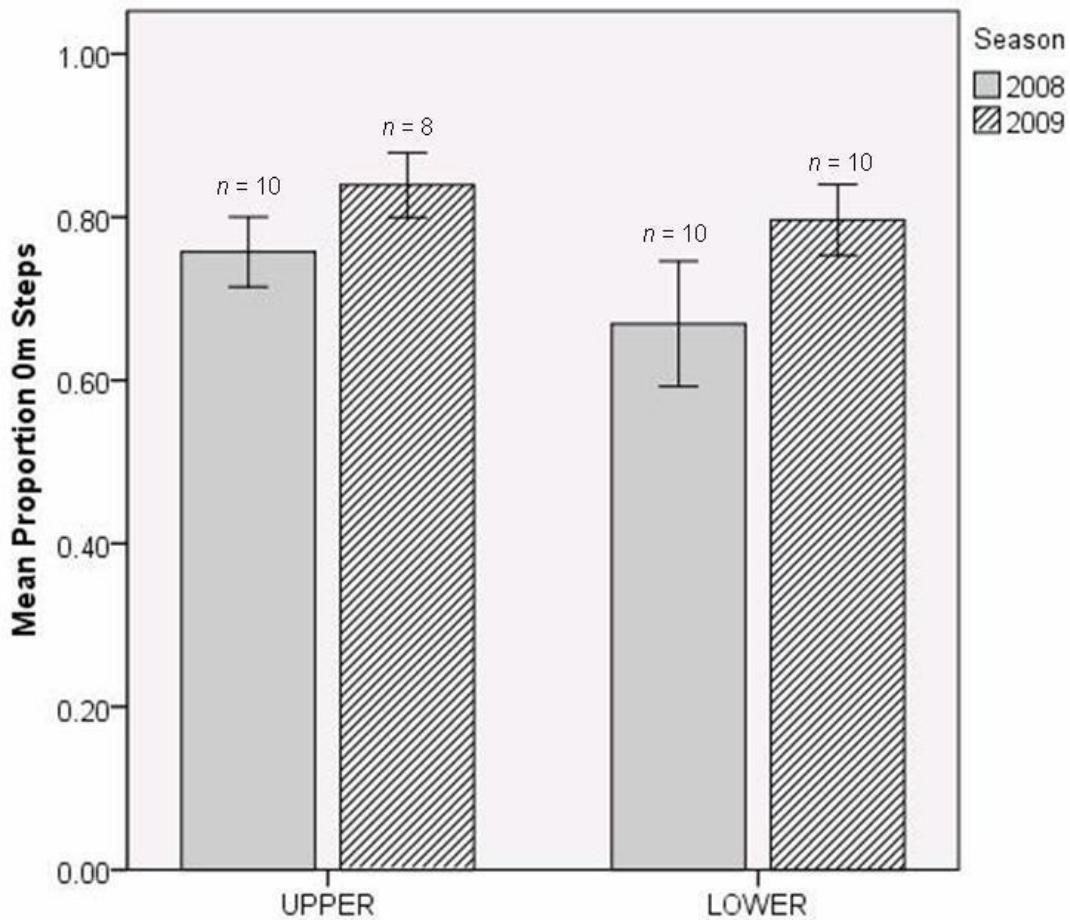


Figure 5. Proportion of captive-reared Ozark hellbender (*Cryptobranchus alleganiensis bishopi*) daily step-lengths revealing no movement (0 m step-length) during 2008 (solid) and 2009 (striped) following translocation to the upper and lower site on the North Fork of the White River, Missouri, USA. Error bars represent +/- 1 standard error and *n* is the number of hellbenders used in each estimate.

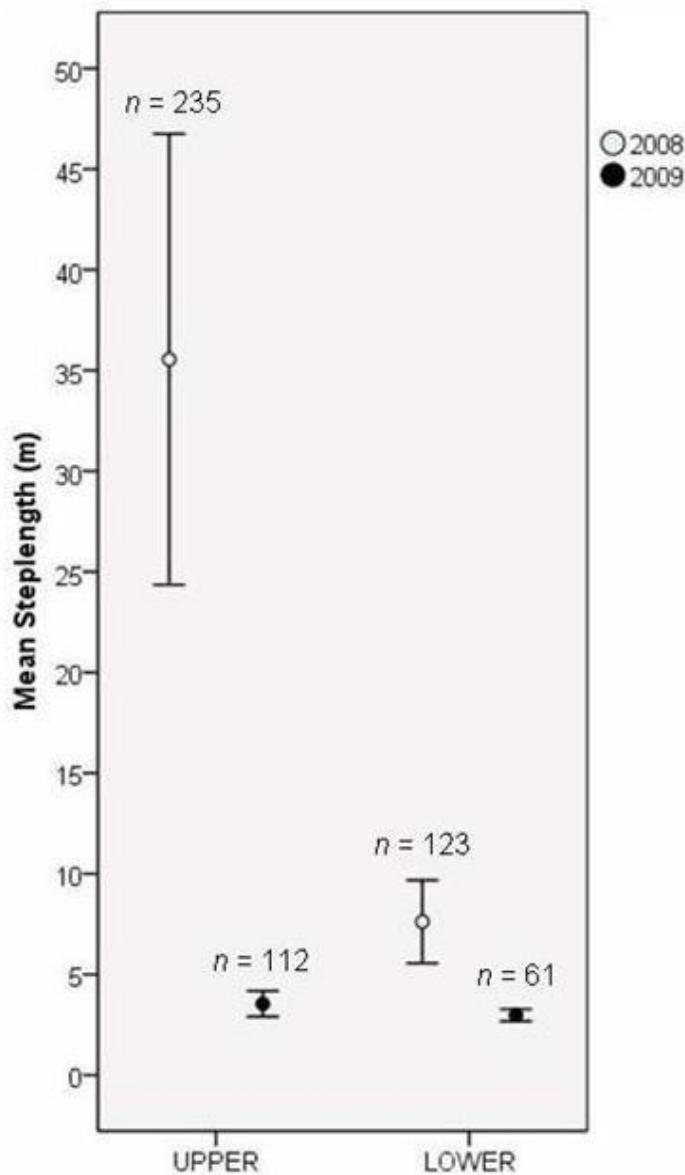


Figure 6. Mean daily step-length (m) of captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the upper and lower sites on the North Fork of the White River, Missouri, USA, during 2008 (open circles) and 2009 (closed circles) after removing 0 m step-lengths. Error bars represent ± 1 standard error and n is the number of steps used in each estimate.

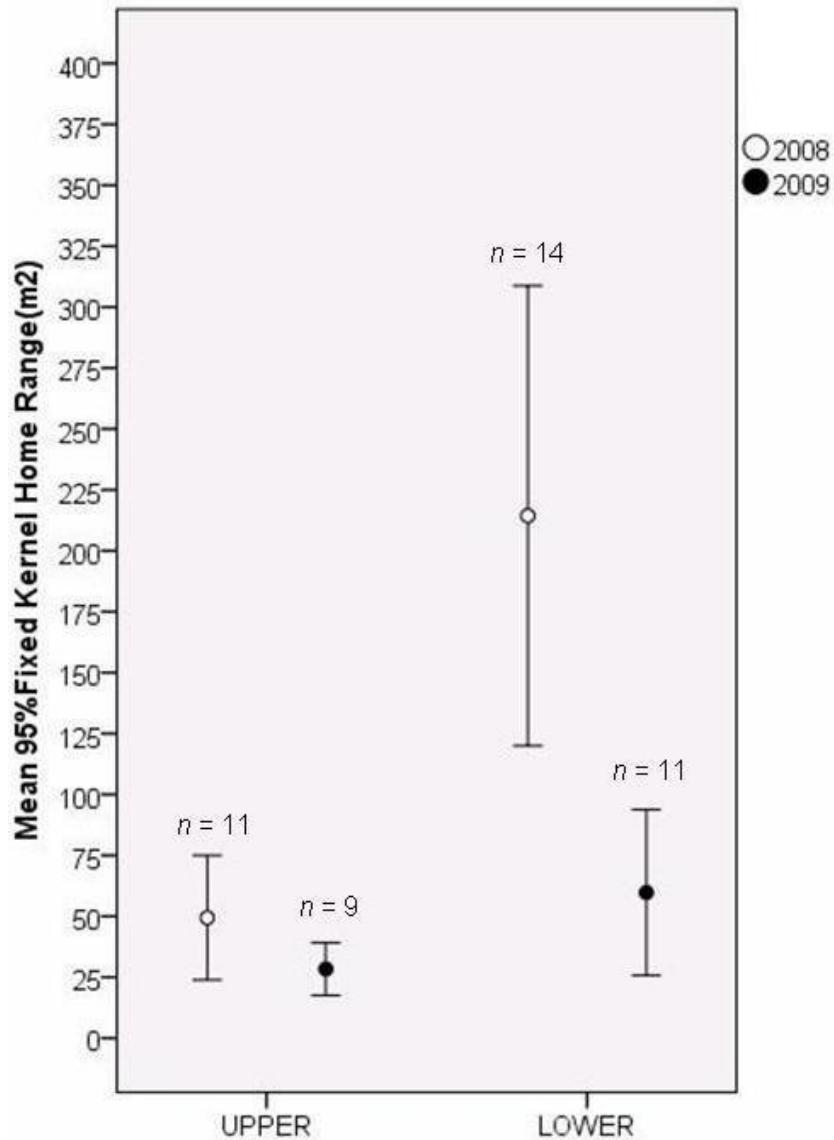


Figure 7. Mean 95% fixed kernel home range size (m^2) of captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the upper and lower sites on the North Fork of the White River, Missouri, USA, during 2008 (open circles) and 2009 (solid circles). Error bars represent ± 1 standard error and n is the number of hellbender home ranges used in the estimation.

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CHAPTER 3

Resource Selection of Captive-Reared Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*) Following Translocation to the Wild

Catherine M. Bodinof, Jeffrey T. Briggler, Randall E. Junge, Jeff Beringer, Mark Wanner, Chawna Schuette, Jeff Ettling and Joshua J. Millspaugh

ABSTRACT

When translocation is used to restore native species to the wild, it is useful to understand how habitat attributes may influence use and therefore settlement of a species. We compared abiotic habitat characteristics at used and available locations within a reach scale (i.e., 50 - 100 m radius) and a home range scale (i.e., 5 m radius) for 36 captive-reared juvenile Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) that were released at 2 sites on the North Fork of the White River, Missouri and monitored via radiotelemetry between May 2008 and August 2009. We fit discrete choice models and compared a priori models representing hypothesis about hellbender resource selection, using Akaike's Information Criterion. At both sites and spatial scales, there was no model uncertainty and hellbender resource selection was positively associated with coarse substrate and bedrock and negatively associated with increasing distance to the nearest substrate particle with at least one axis 2' 15 cm in length (i.e., rock). At the home range scale for both sites and in 1 reach scale model, the negative association between selection and increasing distance to rock intensified as benthic water velocity increased.

Though our models support the hypothesis that coarse substrate is an important habitat component for hellbenders, we observed 10 hellbenders using crevices along the bank or within root masses for 1 day to over 1 year; however logistics prevented us from comparing use and availability of these habitat types. Selection of future hellbender translocation sites on the North Fork of the White River should consider spacing of coarse substrate over meso-habitat types (pool, run, riffle) present, and coarse substrate within translocation sites should be spaced < 1 m apart on average and < 0.5 m on average where benthic water velocity averages $2' 0.34 \pm 0.10$ m/sec.

INTRODUCTION

In addition to identifying reasons for decline, recovery of declining species requires that managers understand habitat attributes necessary to ensure survival once causes for the decline have been ameliorated. In response to rapid loss of amphibian biodiversity (Houlahan et al. 2000) much research has been focused on identifying reasons for amphibian declines (Alford and Richards 1999; Collins and Storfer 2003; Stuart et al. 2004). However, even when causes for decline are obvious, they may be difficult to reverse *in situ* quickly enough to ensure survival of some species. In some cases, translocation of captive populations may be the only method for restoring some amphibians to their native range (Gascon et al. 2007). If translocation is used, selection of future release sites will be an important determinant of reintroduction success (Stamps and Swaisgood 2007). Therefore, while conservation and management of founder amphibian populations is important for species recovery, it is equally important for

recovery programs to identify habitat attributes that would promote use and settlement of translocated animals once released back to the wild.

Wildlife resource selection studies assume that animals select resources over other available ones (Cooper and Millspaugh 1999), presumably due to increased fitness associated with use of one resource relative to another. Such selection may be particularly evident in herpetofauna which are often habitat specialists (Moore and Gillingham 2006). Comparisons of used and available resources has been used to identify factors that influence occurrence (Waldron and Humphries 2005; Moore and Gillingham 2006) or behaviors such as spawning (Wang et al. 2008) for various herpetofauna. Resource selection studies can also reveal novel information concerning rare or difficult-to-detect species. For example, green salamanders (*Aneides aeneus*) were typically regarded as an amphibian occurring largely in crevices of rock outcrops until a study in South Carolina revealed that salamanders seasonally selected hardwood arboreal habitat; suggesting forests were an overlooked but important resources for the imperiled amphibian (Waldron and Humphries 2005). By identifying resources that influence patterns of occurrence of a species across a landscape, resource selection studies can inform habitat restoration efforts and selection of translocation sites.

Translocated animals are unique because they typically have no a priori knowledge of release sites. Selection of resources following translocation may be strongly influenced by habitat where animals matured or driven by primitive instincts (Stamps and Swaisgood 2007). For example, tadpoles that were reared for only 17 days in artificial enclosures with various substrate patterns (striped or checkered) sometimes showed strong selection for substrate patterns similar to where they were raised (Wiens

1970, 1972). However, the relationship was strongest in Red-legged frogs (*Rana aurora*) exposed to striped patterns but in Cascade frogs (*Rana cascadae*) exposed to checkered patterns, possibly due to differences in habitat where each species would naturally mature (Wiens 1970, 1972). Similarly, translocated three-toed box turtles (*Terrapene carolina triunguis*) in Missouri selected habitat differently than resident turtles at a translocation site, but similar to turtles that remained at the capture site suggesting an imprinted habitat template (Rittenhouse et al. 2007). Although the mechanisms by which animals identify suitable habitat may be difficult to discern, monitoring recently translocated animals can identify habitat attributes that influence settlement of animals. Quantification of habitat attributes that influence utility and promote settlement, allows application of useful criteria when selecting future release sites.

Our ability to correctly identify variables influencing selection by a species depends on our ability to detect habitat variability at a scale at which an organism perceives habitat differences (Wiens 1989). Much emphasis has been placed on the importance of considering scale in resource selection studies, because factors that influence species occurrence at a relatively small scale (e.g., within a stream reach) may be very different from factors influencing a species occurrence at a larger spatial scale (e.g., within a watershed) (Rabeni and Sowa 2002). For example, a study of common toads (*Bufo bufo*) and green toads (*B. viridis*) in Italy revealed that habitat type largely dictated establishment of home range but that occurrence of toads within home ranges was better predicted by prey density and temperature (Indermaur et al. 2009). Similarly, Moore and Gillingham (2006) found that factors influencing placement of a home range were different from factors influencing microhabitat site selection of Massasauga

rattlesnakes (*Sistrurus catenatus catenatus*) in Michigan within their home range.

Therefore, quantifying resource selection at various scales can improve our ability to identify suitable habitat at the scale appropriate for a particular species.

The objective of our study was to identify abiotic resources that best identify use within the home range and use within a larger stream reach for 2 populations of captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the North Fork of the White River, Missouri. Though biotic resources such as prey (crayfish) or conspecifics may also be important predictors of hellbender resource selection, measuring biotic variables would have required disturbing substrate near hellbender locations. Because disturbing substrate may have influenced movements that were being monitored as part of a separate study (see Chapter 2), and possibly abiotic factors (e.g., substrate arrangement) that may have been influencing hellbender selection, we focused our study on only abiotic habitat attributes. The Ozark hellbender (*C. a. bishopi*) is endemic to the Black and White river drainages of southern Missouri and northern Arkansas and populations have declined by 80 % in the last 2 decades (Wheeler et al. 2003). In an effort to bolster wild populations, captive-rearing and translocation is being considered as a management strategy for Ozark hellbenders. However, it is unclear what abiotic habitat conditions might be important for increasing utility and thus encouraging settlement of captive-reared hellbenders following translocation. Hellbenders are benthic and lotic salamanders typically occurring in cool, clean, swift moving water where there is a prevalence of large rocks for cover (Bishop 1941; Nickerson and Mays 1973a,b; Williams et al. 1981; Fobes 1995; Hillis and Bellis 1971). Therefore, we hypothesized that cooler water temperatures, presence of coarse substrate (cobbles and

boulders [Wentworth 1922]), riffles and increasing benthic water velocity would positively influence selection by hellbenders. Hellbenders rarely are observed away from cover, are benthic and often found in swift moving water (Ball 2001; Humphries and Pauley 2005; Nickerson and Mays 1973a,b; Blais 1989; Smith 1907). Therefore, we hypothesized that selection would be positively associated with decreasing distance to rock which would indicate more continuous cover, provide more interstitial spaces for hellbenders and their prey (crayfish), and reduce near bed turbulence (i.e., Reynolds number) which may limit mobility of hellbenders.

METHODS

Study Site Description

Our study was conducted along the North Fork of the White River (hereafter NFWR) which is a 7th order stream originating in Wright County, Missouri that flows south to join Bryant Creek before entering Norfork Reservoir near Tecumseh, Missouri. The NFWR is primarily spring fed with a watershed encompassing 3597 km² of southern Missouri. Land use primarily consists of forested woodlands (61.9%) and crop and grassland (37.5%) (<http://mdc.mo.gov/fish/watershed/northfrk/>). Average gradient of the river is 2.42 m/km and flow near Tecumseh, Missouri averages 21.4 m³/sec (<http://waterdata.usgs.gov/mo/nwis/rt>). Substrate along the river includes long stretches of pebble and gravel beds interspersed with beds of large dolomite and limestone slabs and bedrock that historically provided (Nickerson and Mays 1973a,b; Peterson et al. 1983) and still appears to provide suitable habitat for *C. a. bishopi*. Hellbenders were historically abundant in the river and reached densities of 341 - 573 hellbenders per km

of streambed (Nickerson and Mays 1973a,b), but declined dramatically between the 1980s - 1990s for unknown reasons (Wheeler et al. 2003). Crayfish are the main food source of hellbenders (Nickerson and Mays 1973a) and 3 species, including *Orconectes neglectus*, *O. longidigitus* and *O. punctimanus*, are prevalent throughout the NFWR.

Our study occurred at 2 sites (hereafter upper and lower) along the NFWR separated by approximately 17 km of river. We choose not to reveal the discrete location of study sites due to threats of illegal collecting and the current status of hellbenders in Missouri. We selected release sites with an abundance of coarse substrate and lack of heavily silted areas, since similar habitat has been associated with use by hellbenders in other studies conducted in the NFWR (Nickerson and Mays 1973a,b; Fobes 1995). Prior surveys conducted by lifting boulders and snorkeling to locate hellbenders had yielded fewer than 10 wild hellbender residents at each site. The upper site spanned 1026 m in length and approximately 36,115 m². Wetted width of the river ranged from 15-50 m and mean monthly water depth during our study ranged from 51.54 cm in August 2008 to 75.41 cm in May 2008 (Appendix 3). Water temperature at the upper site reached greater extremes than the lower site, probably due to extensive shallow habitat upstream of the site. Mean monthly water temperature during our study ranged from 5.03° C in January 2009 to 23.55° C in August 2009 (Appendix 4). Crayfish density, estimated from random sampling of runs and riffles (< 1 m deep), using a 1 m kick-seine technique (Mather and Stein 1993) in August 2008, was 12.16 crayfish/m² (SE = 2.4).

Our lower site was larger and deeper than the upper site, spanning 960 m in length and approximately 52,945 m². Wetted width at the lower site ranged from 48 - 88 m and mean monthly water depth during our study ranged from 62.75 cm in November 2008 to

95.48 cm in May 2008 (Appendix 3). Mean monthly water temperatures at the lower site were probably buffered by deeper water and the input from at least 1 large spring entering the NFWR between sites, and ranged from 8.61° C in January 2009 to 20.96° C in August 2008 during our study (Appendix 4). The lower site occurred within a portion of the NFWR designated as a Blue Ribbon Trout Zone (daily limit is 1 trout > 18 inches) that is periodically stocked with rainbow (*Oncorhynchus mykiss*) and brown (*Salmo trutta*) trout. Crayfish density in runs and riffles at the lower site, estimated from random sampling of runs and riffles (< 1m deep) using a 1 m kick-seine technique (Mather and Stein 1993) was 12.61 crayfish/m² (SE = 1.52).

Study Animals

We monitored 36 juvenile *C. a. bishopi* that were released in an effort to bolster small remnant populations of hellbenders. Juveniles originated from eggs collected from the NFWR in 2002 by Unger (2003) and were reared in captivity between 2003 and 2008 at the Saint Louis Zoo's Ron Goellner Center for Hellbender Conservation. Captive hellbenders were maintained in aquaria, provided with rocks to use as cover and fed a diet of crayfish and small fish. Water in captive enclosures was continuously aerated and circulated and was maintained to mimic approximate seasonal temperatures of southern Missouri streams. Before release Saint Louis Zoo staff surgically implanted each hellbender with Sirtrack Limited model RVI 118 or RVI 218 radio transmitters to allow continuous monitoring of individuals (See Chapter 2). To reduce potential transmitter effects, zoo staff only implanted hellbenders if the transmitter weight was :S 5 % hellbender mass. The hellbenders were implanted over 4 events between May and July 2008 to allow individuals to reach a weight sufficient for transmitter implantation (140 g

for RVI 118 and 180 g for RVI 218). At surgery, hellbenders showed no signs of sexual maturity and therefore were considered juveniles, weighing 142 - 334 g with total lengths ranging from 29 - 36 cm. We released hellbenders 2 - 4 weeks following transmitter implant to allow healing unless we observed dehiscing of the sutures, in which case we held animals for an additional 2-4 weeks prior to release.

We randomly selected 18 hellbenders to be released at each study site over 4 discrete events (following the staggered fashion of surgeries) between May and October 2008 (See Chapter 2). We transported hellbenders from the Saint Louis Zoo to study sites by vehicle and released hellbender by hand at pre-selected boulders spaced 2' 5m from the nearest known hellbender and staggered along a longitudinal gradient within release site.

Sampling of Used and Available Resources

To account for variation in resource selection across all times of day, we systematically located each hellbender every 32 ± 4 hours (approximately 5 locations per week) between 19 May 2008 and 14 November 2008 (hereafter 2008) and between 26 March 2009 and 18 August 2009 (hereafter 2009). Between 14 November 2008 and 25 March 2009 (hereafter winter) we located hellbenders approximately once per week to monitor survival, but did not include winter observations in our resource selection analyses. We identified hellbender locations by wading or canoeing (if water depth 2' 1.5 m) and homing procedures (White and Garrott 1990) using a 3 element Yagi antenna and an ATS (Advanced Telemetry Systems, Isanti, MN) receiver (model R2000, R4000 or R410). Homing allowed us to identify discrete resources (rocks, bank crevices, root masses) used by hellbenders and allowed us to document fine scale (2' 0.30 m) movement

between cover objects. When we could not distinguish between overlapping cover objects by homing, we confirmed locations visually by snorkeling or with an Aqua-Vu® SV 100 (Outdoor Insights, Inc., Crosslake, MN) camera used to view into crevices.

Each time we located a hellbender (except during winter sampling) we identified 2 associated random 'available' locations within the river. One location occurred in the stream reach, which we defined as a section of stream ranging from 100 m upstream to 100 m downstream of a used location. We identified available reach locations based on a random azimuth and random distance between 50 and 100 m to represent an area within the stream that hellbenders could have traveled to between relocations (i.e., it was available) but likely occurred outside of the home range. Movement analysis of translocated hellbenders confirmed that translocated hellbender were capable of traveling 50 m to > 550 m in a 34 hour period, though this occurred rarely (see Chapter 2). If the available reach scale point fell outside of the river corridor (e.g., on dry land) we selected a new random azimuth until the location fell within the wetted river channel. We selected a second random location within the home range, which we defined as within a radius of 5 m (i.e., 78 m²), using a random azimuth and random distance between 0.5 m and 5.0 m. Our decision to define home range using a 5 m radius was based on previous mean home range estimates for hellbenders in the Niangua River, Missouri which were 28 m² for females and 81 m² for males (Peterson and Wilkinson 1996). Space use analyses of translocated hellbenders suggest our definition of home range may have been conservative during 2008 but more accurate during 2009 (See Chapter 2).

At each used and available location we measured 8 abiotic habitat variables that we hypothesized might influence hellbender use (Table 1). First, we recorded the size

class (Wentworth 1922) of the particle first encountered by a meter-stick placed vertically over the transmitter signal or placed blindly at randomly selected available locations. If hellbenders were not using cover, we recorded the size class of substrate the majority of animal was resting on. When hellbenders were using cover objects other than rocks (e.g., undercut bank crevices and root masses), we defined substrate size class of the first particle encountered within the den used by the animal. When the size class of substrate was cobble or boulder, we recorded length (longest axis), width (longest axis perpendicular to length axis) and depth (cm) of the object. The smallest particle under which hellbenders were discovered during prior surveys in the NFWR had a length axis of 15 cm (J. Briggler pers. comm.). Therefore, we defined a 'rock' to be any substrate particle with at least one axis \geq 15 cm and measured 'distance to rock' as the shortest distance (m) between the edge of substrate particle at each location and the edge of the nearest rock using a tape measure (when \leq 15 m) or handheld Nikon Prostaff laser rangefinder (when $>$ 15 m). When hellbenders were using a boulder that touched a rock, distance to rock was recorded as 0 m. We measured maximum and average water velocity (m/sec) over approximately 10 seconds at the benthic level immediately upstream of the substrate particle encountered at each location using a hand held flow meter (Global Water Instrumentation, Inc, Gold River, CA). We recorded meso-habitat at each location, including pool (little to no or circulating water movement), riffle (swift moving water with a disrupted surface) or run (slow to swift moving water with a smooth surface). Because definitions of pool, riffle and run are somewhat subjective, we quantified mean benthic water velocity within each meso-habitat for each site, separately, using data collected at random available locations. We measured water depth (cm)

immediately downstream of the substrate particle at each location using the same flow probe device which had depth increments noted along its length. Finally, we measured water temperature at the benthic level to the nearest 0.1° Celsius using a ClineFinder™ digital thermometer (Catlina Technologies Inc., Tuscon, AZ).

Model Development

We developed 15 a priori models representing our hypotheses about resource selection of translocated hellbenders (Table 2). We generated models using the 2 categorical and 6 continuous variables as well as a random effect to account for variability in resource selection of individual hellbenders. To reduce the number of parameters in our models we collapsed substrate particle size into 3 categories, including coarse substrate (particles 2' cobble), fine substrate (particles < cobble) and bedrock. Dummy variable coding for categorical variables required that one category be removed from each model as a reference category for comparison. We removed the fine substrate category as reference for substrate type, and removed the riffle category as reference categories for habitat class. For covariates that were often represented by values of 0 (e.g., distance to nearest rock and average benthic water velocity), we added a constant of 0.01 to each value used in the analysis (See table 1). Rather than assuming linear relationships between hellbender use and each continuous variable, we plotted used data prior to model fitting and model selection to determine whether evidence existed to support non-linear (e.g., quadratic or negative exponential) distribution of data. We considered a quadratic form ($\beta_1 x_1 + \beta_2 (x_1^2)$) of width of coarse substrate, water temperature, water depth and a negative exponential form ($\beta_1 x_1 + \beta_2 (\exp[-x_2])$) of

distance to nearest rock, average benthic water velocity and maximum benthic water velocity (See table 2).

Model Fitting and Selection

Before model fitting, we pooled data from 2008 and 2009 and removed data from individuals with :S 20 used observations over the course of the study. We fit discrete choice models (Cooper and Millspaugh 1999, 2001) to our data, with each choice set consisting of 1 used and 1 corresponding available observation. Discrete choice modeling in wildlife studies assumes that animals perceive utility of a particular resource due to attributes offered by that resource. The utility of resource i to individual j takes the form:

$$U_{ij} = B X_{ij} + e_{ij} = \beta_1 x_1 + \beta_2 x_2 - \beta_m x_m$$

where B is a vector length of m estimable parameters and X is a vector of m measurable attributes of resource i (Cooper and Millspaugh 1999, 2001). We fit models separately for each study site and each spatial scale using PROC MDC in SAS[®] (SAS Institute, Cary, NC). We used Akaike's Information Criterion adjusted for small sample size ($AICc$, Burnham and Anderson 2002) to rank each model in the candidate set. We defined the top model as the model assigned 2' 90 % of the $AICc$ model weight (w_i , Burnham and Anderson 2002). We report all mean values and model parameter coefficients ± 1 standard error.

Model Validation

Model ranking within an AIC framework identifies the most supported models in the candidate set, but provides no measure of model accuracy (Burnham and Anderson 2002). To assess predictive ability of each of our top ranked models, we used k-fold

cross validation, (Boyce et al. 2002). To apply k-fold methods we first randomly selected approximately 80% of our observations (maintaining the 1:1 ratio of used to available locations) as 'training data' and designated the remaining 20 % of observations as a 'test set'. For each data set (both scales at each site) we replicated the random separation 5 times. After refitting top ranked models to each replicate of training data, we applied the newly fitted models to estimate utility of each observation within their complementary test set. We calculated the relative probability of selecting each resource within a choice set, using:

$$P_j(A) = \frac{\exp(U_{Aj})}{\sum_{Ai} \exp(U_{ij})}$$

where j is the individual and A is one of i total resources available (Cooper and Millspaugh 1999). We assessed model performance by identifying the percentage of used observations that were correctly predicted (i.e., had a greater relative probability of selection) after pooling all test sets for each model.

RESULTS

During 2008 and 2009 we collected 1387 reach scale and 1416 home range scale choice sets for 15 hellbenders at the upper site. We collected 1761 reach scale and 1765 home range scale choice sets for 14 hellbenders at the lower site. The mean number of reach scale choice sets collected per hellbender was 92.46 ± 15.88 at the upper site and 125.85 ± 14.22 at the lower site. Though we did not measure use and availability of abiotic habitat attributes during winter, hellbender movements were extremely rare

during this period and hellbenders did not move to new areas or appear to be selecting resources differently than in other seasons.

Data collected at random available locations throughout our study revealed that mean benthic water velocity varied among meso-habitats at both sites, but mean velocities for each meso-habitat were similar between sites (Table 3).

We made 262 observations (8.2 % of hellbender locations) of 10 hellbenders (upper site: $n = 6$, lower site: $n = 4$) using unexpected cover objects. These observations included use of bank crevices along the shore (upper site: $n = 20$ observations, lower site: $n = 5$ observations), root mass of water willow (*Justicia americana*) (upper site: $n = 9$ observations, lower site: $n = 140$ observations) and woody root wads of mature or fallen trees along the bank (upper site: $n = 2$ observations, lower site: $n = 86$ observations).

While most hellbenders used these habitats for :S 14 days, 1 male hellbender at the lower site used a deep tunnel in *J. americana* root mass lined with sand and silt for over a year. Additionally, a female hellbender at the lower site used a large cavity filled with cobble and sand between the roots of a living Sycamore (*Platanus occidentalis*) for 2' 7 months. Because bank crevices and root masses with openings large enough for hellbenders typically were difficult to detect and extended outside of the main river channel they were not encountered at our randomly selected available locations. Therefore we were unable to sample availability of these of resources and thus could not include these types of habitats as covariates in our models.

Within Reach Selection

At the reach scale, a single model incorporating substrate type and an interaction between distance to rock and habitat class (Bedrock + Coarse + Pool + Run + Distance to

rock + (Pool x Distance to Rock) + (Run x Distance to rock)) was ranked first with an AICc model weight of 1 at both sites.

Similar to our predictions, reach scale models for both sites indicated that coarse substrate was positively associated with hellbender selection; however bedrock was also positively associated with selection to a lesser degree. Odds ratios (e^{β}) revealed that when compared to fine substrate, coarse substrate increased the odds of hellbender selection by 18.76 % at the upper site and by 20.00 % at the lower site (Table 4). When compared to fine substrate, bedrock increased the odds of hellbender selection by 8.07 % at the upper site but by only 2.32 % at the lower site (Table 4), suggesting bedrock may have been a more important habitat attribute for hellbenders at the upper site.

At both sites, hellbender resource selection at the reach scale was negatively associated with increasing distance to rock in every meso-habitat class (Fig. 1), similar to our hypotheses. However, at the upper site the negative relationship between selection and increasing distance to rock was most intense in runs, then pools, and least intense in riffles (Fig.1); while at the lower site the relationship was extreme in riffles, and less intense in runs and then pools (Fig. 1). At the upper site, resources in runs were 1.5 times more likely to be used when distance to rock was 0 m (i.e., rocks were touching), than when distance to rock was just 1 m. Much more extreme, at the lower site resources in riffles where distance to rock was 0 m were 2 times more likely to be used than when distance to rock was only 0.1 m; and 6 to 10 times more likely to be used than when distance to rock reached just 0.5 m. At both sites reach scale models suggested that meso-habitat specific thresholds of distance to rock may have existed. For example, as distance to rock exceeded 0.5 to 2.0 m at the upper site, the relative probably of selecting

riffles exceeded that for either runs or pools; and at the lower site as distance to rock exceeded 0.1 to 0.5 m, the relative probability of selecting resources in runs and pools exceeded that for riffles.

Within Home Range Selection

The top ranked model explaining hellbender resource selection within the home range at the upper site (Bedrock + Coarse + Maximum benthic velocity + Distance to rock + (Maximum benthic velocity x Distance to rock)) carried an *AICc* model weight of 0.96. The top ranked model explaining hellbender resources selection within the home range at the lower site (Bedrock + Coarse + Pool + Run + Distance to rock + (Pool x Distance to Rock) + (Run x Distance to rock)) was the same model ranked first in our reach scale analysis at the site and carried a model weight of 1.

Similar to our reach scale analysis, top ranked home range scale models at both sites indicated that coarse substrate was positively associated with selection, followed by bedrock and then fine substrate (Table 5). Odds ratios indicated that coarse substrate increased the odds of hellbenders selecting a location within the home range by approximately 29 % at both sites. Compared to fine substrate, bedrock increased the odds of selection by 6.45% at the upper site but by only 3.19% at the lower site, again emphasizing the potential increased importance of bedrock for hellbenders at the upper site (Table 5).

Also similar to our reach scale analysis, the 'distance to nearest rock' covariate appeared in top ranked home range scale models at both sites. Despite the fact that mean 'distance to rock' at random locations within hellbender home ranges was 2.5 to > 30 times smaller than mean 'distance to rock' at random locations in similar meso-habitats

occurring throughout the reach (Fig. 2), selection within the home range was negatively associated with increasing distance to rock at both sites (Fig. 3-4). However, model ranking indicated that the negative relationship between selection and increasing distance to rock was modified by benthic water velocity at the upper site but by meso-habitat at the lower site.

In contrast to every other top ranked model in our analysis, the top ranked home range scale model at the upper site included a covariate representing maximum benthic water velocity, rather than meso-habitat class (Table 5). The upper site model suggested that though selection was positively associated with decreasing distance to nearest rock, water velocity may have been more of a limiting factor to utility of a resource for hellbenders. For example, when water velocity remained 0 m/sec hellbenders were 5 to 8 times more likely to select areas where distance to rock was 0 m, than when distance to rock increased to 5.0 m (Fig. 3), similar to what other scale and site models indicated. However, even when rocks were touching (i.e., distance to rock remained 0 m) the relative probability of hellbenders selecting a location was 3 times greater when water velocity was 0 m/sec than when velocity reached just 0.4 m/sec, and 6 to 12 times more likely than when velocity exceed 0.7 m/sec (Fig.3).

The interaction between habitat class and distance to nearest rock in the lower site home range scale model (Fig. 4) behaved similarly to the same interaction in our top reach scale model at the site (See fig. 1). When distance to rock was 0 m, the probability of hellbenders selecting riffles was approximately 6-8 times greater than when distance to rock approached just 0.5 m (Fig. 4). Also similar to our reach scale analysis, the lower site home range scale model indicated that a meso-habitat specific distance to rock

threshold may have existed, but was slightly lowered, where the probability of selecting pools and runs exceed that for riffles as distance to rock exceeded 0.1 to 0.3 m (Fig. 4).

Model Validation

K-fold cross validation of top ranked models suggested that reach scale models were effective predictors of hellbender resource selection. The reach scale top model accurately predicted use in 89.2 % of cases at the upper site and in 91.1 % of cases at the lower site. Predictive ability was lower at the home range scale at both sites, suggesting that factors we did not consider (e.g., embeddedness of coarse substrate or prey availability) may have also been important in determining fine scale resource selection. Top ranked models accurately predicted 77% of cases at the upper site and 74 % of cases at the lower site.

DISCUSSION

Hellbender resource selection at the reach and home range scale was positively associated with decreasing distance to nearest rock, regardless of meso-habitat class. Several studies have noted the importance of large rocks for hellbenders (Nickerson and Mays 1973a,b; Hillis and Bellis 1971; Williams et al. 1981; Fobes 1995), but our study is the first to provide quantitative evidence suggesting that even slight differences (i.e., 0.1 m) in spacing amongst coarse substrate may influence hellbender selection and thus settlement. At both sites and both spatial scales, our models indicate that utility of a location for hellbenders improved when distance to rock was minimized; though we cannot confirm the mechanism by which this habitat attribute may increase fitness for hellbenders (Cooper and Millspaugh 1999). As distance between coarse substrate is

minimized, the extent of continuous protective cover is expanded, shear stress and turbulence is reduced to facilitate movement for benthic fauna (Lancaster et al. 2006, Hoffman et al. 2006), and interstitial space increases available refugia from disturbance for benthic species like hellbenders and their prey (Scarsbrook 1995; Townsend et al. 1997). One or all of these factors may have contributed to the increased selection noted as distance to rock was reduced in our study. Hellbenders are sensitive to and avoid light (Nickerson and Mays 1973a), suggesting closely arranged coarse substrate may have been important for facilitating short distance movements while avoiding prolonged exposure. Additionally, it is important to consider that our study took place over a relatively short temporal scale (i.e., 15 months) though the major benefit of closely arranged coarse substrate may be linked to stability of benthic habitat over a much longer time period. For example, as substrate size increases, critical shear stress (the amount of energy required to move a shift a particle) also increases but shear stress in the benthos decreases. As a result, patches of closely arranged coarse substrate probably improves long term stability of benthic microhabitat and thus resistance of hellbender populations to major losses of individuals during disturbance events, similar to patterns noted in macro invertebrate stream fauna (Townsend et al. 1997).

While hellbenders selection was consistently positively associated with decreasing distance to rock, the 'distance to rock thresholds' indicated by our models in relation to the probability of selecting upper site runs and lower site riffles suggest that the utility of some meso-habitats, for hellbenders, may be limited by coarse substrate spacing. In other words, the habitat class most likely to be selected when distance to rock was 0 m was not always the most likely to be selected once distance to rock increased

even a minuscule amount (e.g., 0.1 m). The hypothesis represented by the top ranked reach scale model considered the fact that hellbenders are benthic organisms and that shear stress (assumed to decrease as distance to rock decreased) was an important determinant of hellbender resource selection. According to our hypothesis, we expected that utility of riffles would be most influenced by increasing distance to rock, followed by runs and pools; though this was only supported at the lower site. It is not entirely clear why the utility of runs were most affected by distance to rock at the upper site, though one explanation may lie in obvious differences in the substrate composing riffle habitat at the 2 study sites. At the upper site the only riffle used by hellbenders carried a smaller volume of water than the lower site riffle, contained very few boulders and was largely composed of bedrock (See Appendix 1) with prevalent crevices that several hellbenders were observed using. The lower site riffle carried a larger volume of water and was composed primarily of (apparent) densely arranged coarse substrate (See Appendix 2). Our decision to quantify 'distance to rock' was an attempt to quantify distance to the nearest cover object and a shear stress reducing structure. However, prior to this study we did not consider the potential importance of bedrock ledges or crevices in their ability to serve the same benefits as coarse substrate (i.e., provide cover and reducing shear stress). Thus, we did not quantify 'distance to ledge or crevice'. Because coarse substrate was far less prevalent in the upper site riffle, it is possible that though hellbenders were using resources relatively distant from other rocks, they were still selecting resources very close to bedrock crevices and ledges.

Though many studies have noted the importance of large rocks for hellbenders, we saw little evidence to suggest size of coarse substrate was important (e.g., model 15

[Table 2] was not supported) and observed hellbenders using a much wider range of habitats than is generally described for the species (Bishop 1941; Hillis and Bellis 1971; Nickerson and Mays 1973a,b; Williams et al. 1981; Fobes 1995). In particular, reach scale models at the upper site provide evidence that bedrock crevices can provide potentially important habitat for hellbenders. This may be the case when apparent density of coarse substrate is minimal and bedrock is riddled with deep crevices as was the case at the upper site. In addition to common use of bedrock, approximately 8 % of our observations documented hellbenders using root masses and bank crevices that were dominated by fine substrates which are generally described as avoided by hellbenders (Fobes 1995). Captive-reared hellbenders may have selected resources differently than wild hellbenders, or radiotelemetry may have improved our ability to detect use of these habitats. The only other resource selection study of hellbenders (Fobes 1995), and general descriptions of hellbender habitat have been based on data collected via search and capture methods (Nickerson and Krysko 2003) that are limited to lifting rocks and visually searching for animals. In contrast, one of the few radiotelemetry studies of wild hellbenders also reported hellbenders use of undercut banks and sunken logs in a New York stream (Blais 1989). Therefore, it is possible that wild hellbenders also utilize habitat that differs dramatically from that typically described for the species; which should be considered when accounting detectability in survey efforts.

Though we considered spatial scale in our study, our results revealed minimal differences in the abiotic habitat attributes that best explain resource selection by hellbenders within a reach versus within a home range. At both scales, top models included a covariate for substrate type and an interaction between distance to rock and a

covariate corresponding to water velocity (i.e., meso-habitat or maximum benthic water velocity). However, home range scale models were less accurate than reach scale models at predicting used locations when presented with used and available data points, suggesting that factors we did not consider, possibly including prey abundance, presence of conspecifics, or embeddedness of cover objects may have been important drivers of selection at the finest spatial scale.

Collection and preservation of rapidly declining amphibians in ex-situ conditions under the amphibian ark concept (Gascon et al. 2007) is increasingly used as a conservation strategy. However, attempts to translocate amphibians to wild conditions have experienced limited success (Griffiths and Pavajeau 2008; Germano and Bishop 2009; Dodd and Seigel 1991). When re-establishment of captive populations back to the wild is the ultimate goal for species, understanding the factors that influence survival, settlement and reproduction of the species across a landscape is imminent for achieving success. Our study is the first to quantify resources selection of hellbenders based on data collected from radiotelemetry rather than common search and capture techniques. We recognize that selection observed in our study may represent selection of the 'best available' resources but not necessarily ideal resources (e.g., if 'ideal' resources were not present or available). However, we observed no obvious evidence that abiotic habitat within either study site was degraded, based on physical habitat descriptions of sites where healthy and high density hellbender populations were found in the same river during the early 1970s (Nickerson and Mays 1973a). Our findings have important implications for conservation of Ozark hellbenders in the NFWR and possibly in other streams. Collectively, our reach scale results suggest that hellbenders settled in areas of a

reach where coarse substrate was relatively closely arranged, regardless of meso-habitat class. Additionally, our results provide evidence that hellbenders responded to even slight variability in the spacing of rocks as well as benthic water velocity at the home range scale, emphasizing the importance of considering fine scale arrangement of resources in habitat restoration efforts and selection of future translocation sites for hellbenders. Future hellbender translocation sites in the NFWR should contain coarse substrate that is as densely arranged as possible, even at the home range scale (e.g., < 20 m²) (See chapter 2). In habitats where mean benthic water velocity equals or exceeds 0.34 ± 0.1 m/sec (i.e., mean benthic water velocity of lower site riffles), we recommend that coarse substrate should be spaced on average < 1 m, and preferably < 0.5 m, apart. Where mean benthic velocity equals or exceeds 0.14 ± 0.01 m/sec (mean benthic water velocity in upper site runs) we recommend that coarse substrate be spaced on average < 2 m apart. We recommend that when mean distance to rock in potential translocation sites exceeds that in our above recommendations release sites should include pools or deep runs where coarse substrate is present but may be more moderately spaced (e.g., 1 - 3 m) over sites where rocks are spaced moderately but restricted to riffles swift runs. We also encourage future translocation attempts to consider the potential importance of bedrock crevices, root masses and bank crevices for hellbenders, especially when coarse substrate is less abundant or not densely arranged. Finally, selecting future translocation sites that provide a diversity of pool, run, and riffle habitat ensures refugia during high flow events and potentially a larger carrying capacity for a future population.

Table 1. Covariates used in candidate resource selection models generated for captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the North Fork of the White River, Missouri, USA, 2008 - 2009.

Variable	Description	Adjusted value
S_b	Bedrock	
S_c	Coarse substrate	
W	Width of coarse substrate, other substrates = 0	$W + 0.01$
D_r	Distance to rock (m)	$D_r + 0.01$
H_p	Pool	
H_{run}	Run	
F_{ave}	Average benthic water velocity (m/sec)	$F_{ave} + 0.01$
F_{max}	Maximum benthic water velocity (m/sec)	$F_{max} + 0.01$
D	Water depth (cm)	
T	Benthic temperature ($^{\circ}$ C)	
I	Random effect for individual	

Table 2. A priori models representing hypotheses concerning resource selection of captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the North Fork of the White River, Missouri, USA, 2008-2009.

Hypothesis	Model structure	Expected results
1). Positive effects of S_c	$= \beta_1(S_b) + \beta_2(S_c) + \beta_3(I)$	$\beta_1 > 0, \beta_2 > \beta_1$
2). Positive effects of W and negative effects of increasing D_r	$= \beta_1(W) + B_2(D_r) + B_3(I)$	$\beta_1 > 0, \beta_2 < 0$
3). Positive effects of a mid range of W	$= \beta_1(W) + \beta_2(W^2) + \beta_3(I)$	$\beta_1 > 0, \beta_2 < 0$
4). Positive effects of S_c and exponential negative effects of increasing D_r	$= \beta_1(S_b) + \beta_2(S_c) + \beta_3(\exp(-D_r)) + \beta_4(I)$	$\beta_1 > 0, \beta_2 > \beta_1$
5). Influence of S_c and S_b on D_r	$= \beta_1(S_b) + \beta_2(S_c) + \beta_3(D_r) + \beta_4(S_c * D_b) - r + \beta_5(S_c * D_r) + \beta_6(I)$	$\beta_1 < 0, \beta_2 > 0$
6). Positive effects of riffle and H_{run}	$= \beta_1(H_p) + B_2(H_{run}) + B_3(I)$	$\beta_1 < 0, \beta_2 < 0$
7). Positive effects of riffle and H_{run} , negative effects of increasing T	$= \beta_1(H_p) + B_2(H_{run}) + B_3(T) + \beta_4(I)$	$\beta_1 < 0, \beta_2 < 0, \beta_3 < 0$
8). Positive effects of a mid range of D	$= \beta_1(D) + B_2(D^2) + B_3(I)$	$\beta_1 > 0, \beta_2 < 0$

9). Negative effects of increasing T and positive effects of increasing F_{ave}

$$= \beta_1(T) + B_2(\exp(-F_{ave})) + B_3(I) \quad \beta_1 < 0, \beta_2 > 0$$

10). Negative effects of increasing T and positive effects of increasing F_{max}

$$= \beta_1(T) + B_2(\exp(-F_{max})) + B_3(I) \quad \beta_1 < 0, \beta_2 > 0$$

11). Positive effects of S_c , increasing negative effect of F_{max} on D_r

$$\begin{aligned} &= \beta_1(S_b) + \beta_2(S_c) + \beta_3(F_{max}) + \beta_4(D_r) + \beta_5(F_{max} * D_r) \quad \beta_1 > 0, \beta_2 > \beta_1 \\ &\quad + \beta_6(I) \end{aligned}$$

12). Positive effects of S_c , increasing negative effects of F_{ave} on D_r

$$\begin{aligned} &= \frac{\beta_1(S_b)}{b} + \frac{\beta_2(S_c)}{c} + \frac{\beta_3(F_{ave})}{3} + \frac{\beta_4(D_r)}{4} + \frac{\beta_5(I)}{5} \quad \beta_1 > 0, \beta_2 > \beta_1, \\ &\quad \beta_3 > 0, \beta_4 < 0 \end{aligned}$$

16
13). Positive effects of S_c , negative influence of D_r

$$\begin{aligned} &= \beta_1(S_b) + \beta_2(S_c) + \beta_3(H_p) + \beta_4(H_{run}) + \beta_5(D_r) \quad \beta_1 > 0, \beta_2 > \beta_1 \\ &\quad + \beta_6(H_p * D_r) + \beta_7(H_{run} * D_r) + \beta_8(I) \end{aligned}$$

14). Increasingly negative influence of F_{max} on D_r

$$= \beta_1(D_r) + B_2(F_{max}) + B_3(D_r * F_{max}) + \beta_4(I) \quad \beta_1 > 0, \beta_2 > 0$$

15). Positive effects of increasing W , negative effect of increasing F_{max} on D_r

$$\begin{aligned} &= \beta_1(W) + \beta_2(D_r) + \beta_3(F_{max}) + \beta_4(D_r * F_{max}) + \beta_5(I) \quad \beta_1 > 0, \beta_2 > 0 \\ &\quad \beta_3 < 0, \beta_4 < 0 \end{aligned}$$

Table 3. Mean benthic water velocity (m/sec) of pools, runs and riffles at the upper and lower Ozark hellbender (*Cryptobranchus alleganiensis bishopi*) translocation sites on the North Fork of the White River, Missouri, USA, 2008-2009, based on data collected at random available locations within each site.

	Upper Site		Lower Site	
	\bar{x}^a	SE ^b	\bar{x}^a	SE ^b
Pool	0.03	0.06	0.00	0.00
Run	0.14	0.01	0.11	0.01
Riffle	0.36	0.08	0.34	0.10

^amean benthic water velocity

^b1 standard error

Table 4. Parameter estimates for variables in top ranked models explaining reach scale resource selection by translocated Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) released at the upper and lower sites on the North Fork of the White River, Missouri, USA, 2008-2009.

β^a	Upper Site			Lower Site		
	Estimate	SE ^b	e^{x^c}	Estimate	SE ^b	e^{x^c}
S_b	2.0884	0.2217	8.0719	S_b	0.8793	0.3786
S_c	2.9319	0.2587	18.7630	S_c	2.9465	0.2414
H_p	0.5494	0.2841	1.7322	H_p	1.3352	0.3274
H_{run}	0.1890	0.2467	1.2080	H_{run}	-0.9353	0.2084
D_r	-0.2262	0.0798	0.7975	D_r	-5.6258	1.4903
$H_p * D_r$	-0.5910	0.2300	0.5537	$H_p * D_r$	4.6334	1.5039
$H_{run} * D_r$	0.4634	0.1157	1.5894	$H_{run} * D_r$	4.7622	1.4908
ID_STD	1.0000			ID_STD	1.0000	

^a Model parameter

^b 1 standard error

^c Odds ratio

Table 5. Parameter estimates for variables in top ranked models estimating home range scale resource selection of translocated Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) released at the upper and lower sites on the North Fork of the White River, Missouri, USA, 2008-2009.

Upper Site				Lower Site			
β^a	Estimate	SE ^b	e^{x^c}	β^a	Estimate	SE ^b	e^{x^c}
S_c	3.3986	0.2193	29.9221	S_t	1.1601	0.3703	3.1902
S_b	1.8649	0.2075	6.4552	S_c	3.3937	0.2061	29.7750
F_{\max}	-2.9636	0.5107	0.0516	H_p	1.9442	0.3517	6.9880
D_r	-0.2892	0.0750	0.7488	H_{run}	0.4074	0.3209	1.5029
$D_r * F_{\max}$	0.5069	0.1492	1.6601	D_r	-4.1448	1.0851	0.0158
ID_STD	1.0000			$H_p * D_r$	3.5441	1.0933	34.6080
				$H_{run} * D_r$	3.7782	1.0913	43.7370
				ID_STD	1.0000		

^a Model parameter

^b 1 standard error

^c Odds ratio

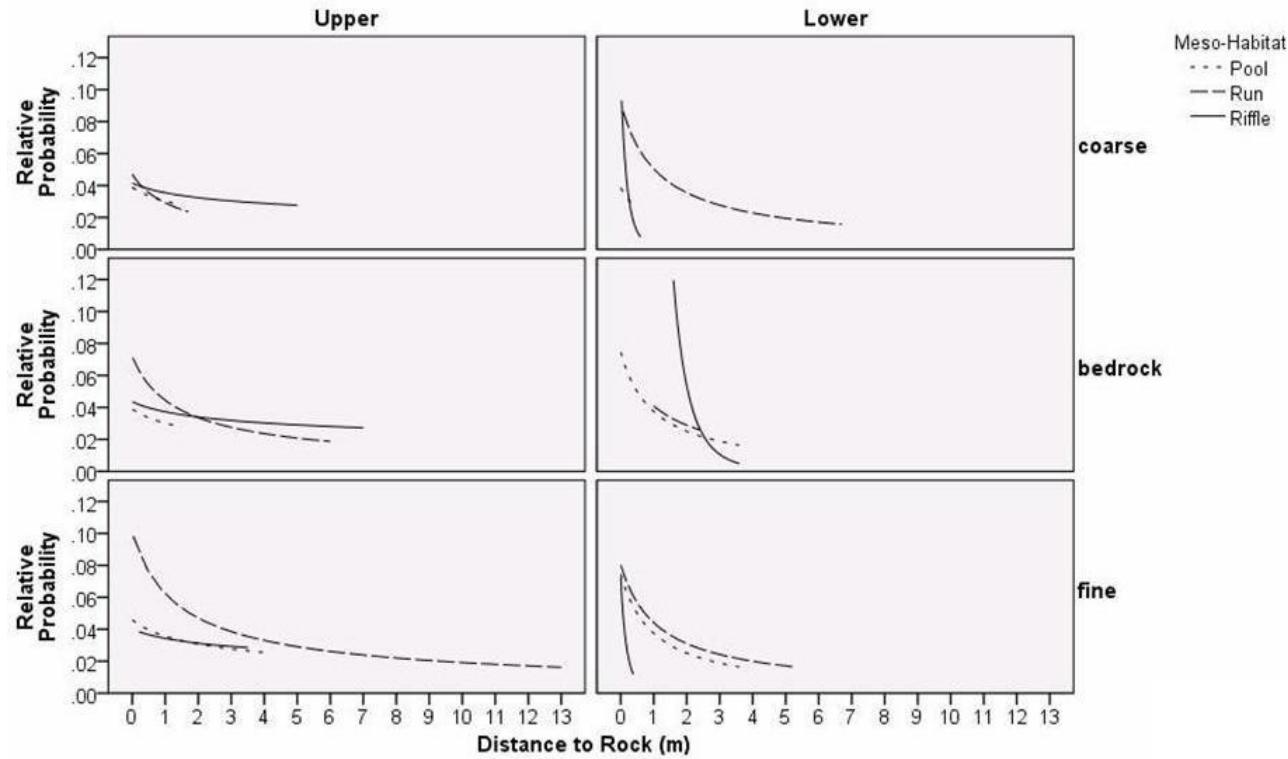


Figure 1. Interaction between distance to rock (m) and meso-habitat (pool, run, riffle) and the associated change in relative probability of Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) selecting a resource within the lower site at the home range scale when various substrates are present. Resource selection functions are based on parameter estimates of top ranked reach scale resource selection models for captive-reared hellbenders released at the lower site in the North Fork of the White River, Missouri, USA, 2008-2009.

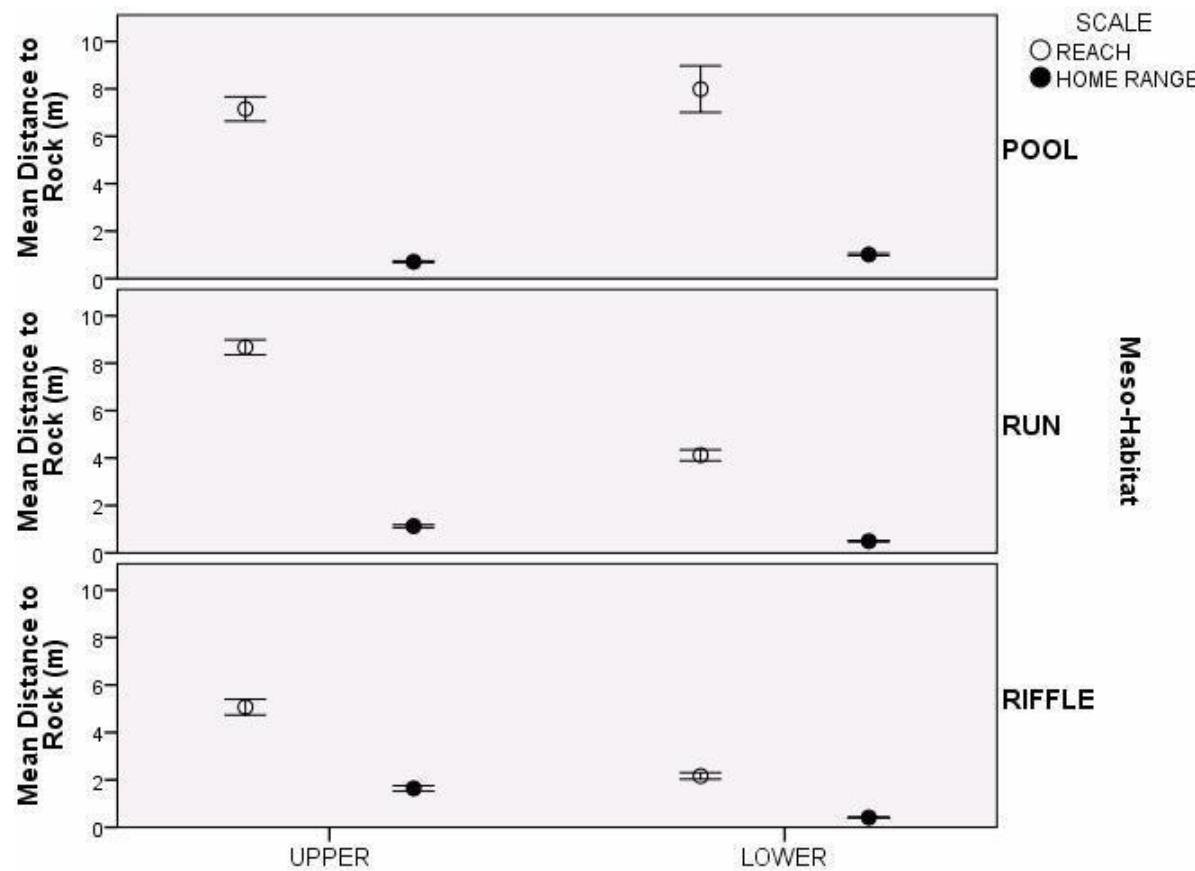


Figure 2. Comparison between mean distance to rock (m) in pools, runs, and riffles based random locations occurring within the 50 - 100 m reach (open circles) and within a 5 m radius home range (closed circles) of Ozark hellbender (*Cryptobranchus alleganiensis bishopi*) locations within the upper and lower sites on the North Fork of the White River, Missouri, USA, 2008-2009.

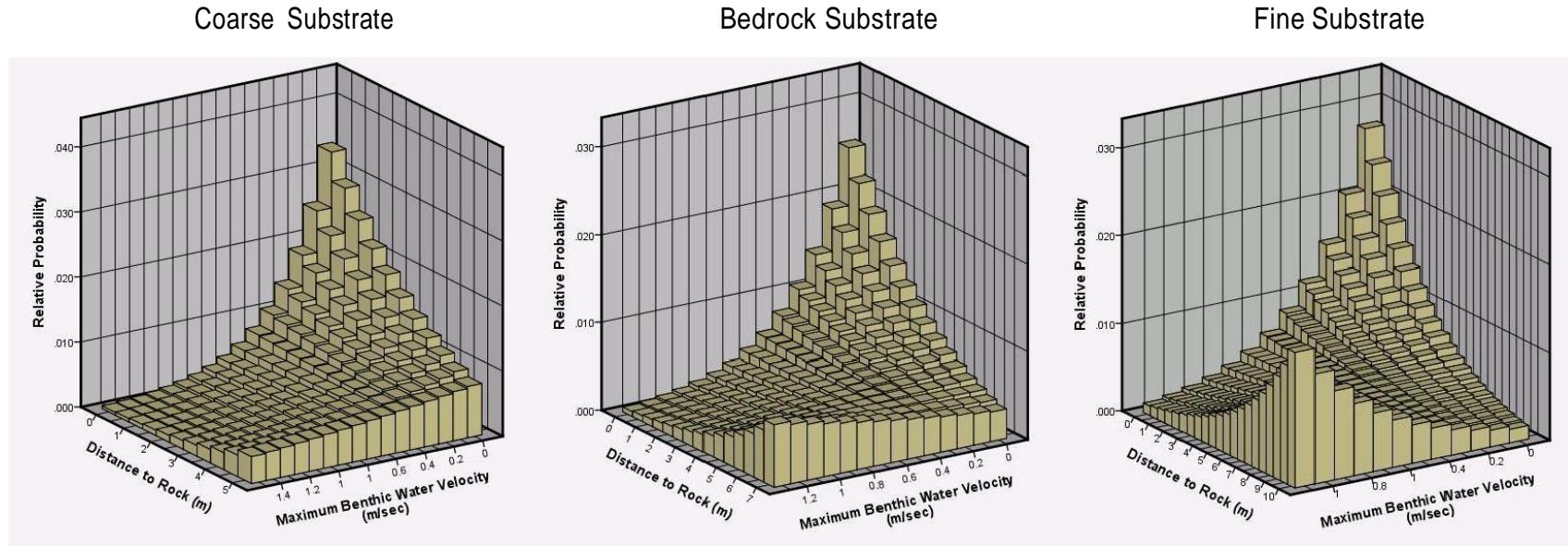


Figure 3. Interaction between maximum benthic water velocity (m/sec) and distance to nearest rock (m) and the associated change in relative probability of Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) selecting a given resource within the upper site, at the home range scale when various substrates are present. Resource selection functions are based on the top ranked home range scale resource selection models generated for captive-reared hellbenders released at the upper site in the North Fork of the White River, Missouri, USA, 2008-2009.

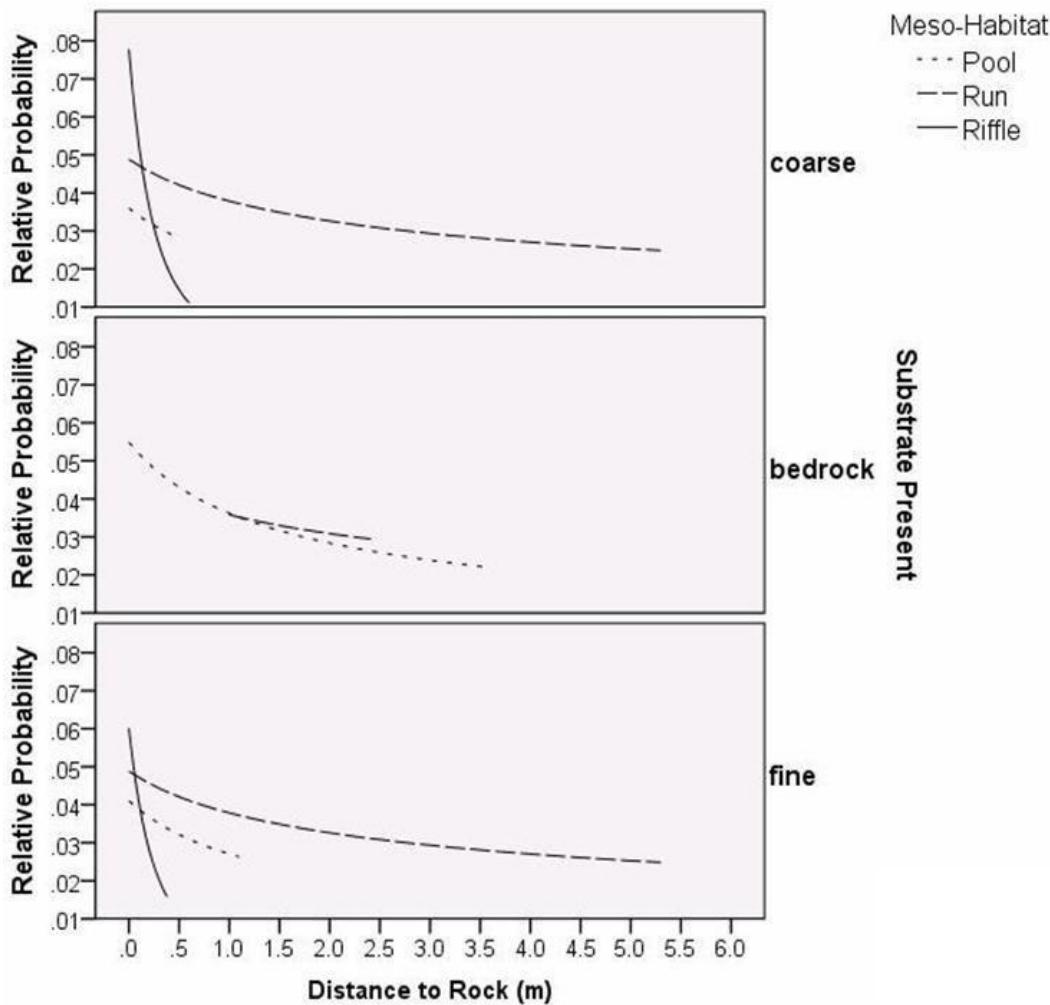


Figure 4. Interaction between distance to rock (m) and meso-habitat (pool, run, riffle) and the associated change in relative probability of Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) selecting a resource when various substrates are present. Resource selection functions are based on top ranked home range scale resource selection models for captive-reared hellbenders released at the lower site in the North Fork of the White River, Missouri, USA, 2008-2009.

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CHAPTER 4

Survival and Body Condition of Captive-Reared Juvenile Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*) Following Translocation to the Wild

Catherine M. Bodinof, Jeffrey T. Briggler, Randall E. Junge, Jeff Beringer, Tony Mong,

Mark Wanner, Chawna Schuette, Jeff Ettling and Joshua J. Millspaugh

ABSTRACT

Captive-rearing and translocation are being considered as potential management strategies to improve Missouri hellbender populations that have declined in recent decades. Knowledge of survival rates for captive-reared hellbenders is critical to understanding the viability of this management option. Between May 2008 and August 2009 we used radiotelemetry to monitor survival and body condition of 36 Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) that had been reared in captivity for 5.5 years before being translocated to 2 sites on the North Fork of the White River, Missouri. We used the known-fate model in Program MARK to estimate daily survival of hellbenders and evaluate factors correlated with survival. At the end of our study, 16 hellbenders were living, 13 had died and the status of 7 hellbenders could not be

determined. Daily survival was higher at the lower site (0.9992 ± 0.0004 SE) compared to the upper site (0.9980 ± 0.0007 SE), resulting in annual survival at the lower site (0.7467) being nearly double that of the upper site (0.4816). A site only model was most supported, though additional supported models suggested increased mass at release may have positively influenced survival. Along with having lower survival, hellbenders at the upper site gained less body mass and a greater proportion accrued scratches and flesh wounds, leech parasites and carried *Batrachochytrium dendrobatidis*. Annual survival rates of captive-reared hellbenders at the lower site were similar to estimated annual survival rates (0.81) of wild hellbenders from the same river in 1978-1979; but annual survival of translocated hellbenders was 30 - 100 % lower than annual survival (0.975) indicated for a wild population consisting primarily of 12 - 20 year old hellbenders. Time since release was not related to survival suggesting captive-rearing and stress of translocation did not affect hellbender survival. However, because mortality of at least 3 hellbenders in our study was associated with dehisced sutures following transmitter implant our survival estimates at both sites are likely conservative. In contrast to studies that observe an inability of translocated populations to persist longer than a few months, our findings suggest captive-rearing can produce hellbenders capable of surviving and maturing in the wild for over 1 year, given release sites are well selected.

INTRODUCTION

While head starting and translocation are increasingly being used to bolster or restore wild populations of herpetofauna (Gascon et al. 2007), the effectiveness of these programs has been debated because few attempts have been successful (Dodd and Seigel

1991; Seigel and Dodd 2002; Germano and Bishop 2009; Griffiths and Pavajeau 2008).

Amphibians are one of the most threatened taxa, with over 40% of species currently in decline (Stuart et al. 2004; Houlahan et al. 2000). Regardless of the ultimate drivers of decline, translocation may be the only method for restoring some species to their native range (Gascon et al. 2007). However, a recent review reported that only 18 out of 58 reintroduced amphibian species have successfully bred in the wild, and only 13 reintroduced species have established self sustaining populations (Griffiths and Pavajeau 2008). Reasons for the lack of success of amphibian translocations include emigration from release sites and poaching of translocated animals, but in several cases the reasons for failure are undetermined (Germano and Bishop 2009).

Close monitoring of translocated animals can identify factors influencing survival and help determine whether translocation may be an effective long-term conservation strategy. For example, a pilot attempt to reintroduce Mountain yellow-legged frogs (*Rana muscosa*) to sites where they had been extirpated for unknown reasons in Sequoia National Park, California found that translocated populations did not persist for more than 12 months because drivers of decline, possibly disease, remained (Fellers et al. 2007). In contrast, a radiotelemetry study of translocated Massasauga rattlesnakes (*Sistrurus catenatus catenatus*) to a site in Wisconsin suggested season of release strongly influenced survival of translocated snakes (King et al. 2004). In these examples, knowledge gained from monitoring translocated animals provided valuable information which in one case redirected conservation strategies and in another increased the potential for success in future translocation attempts.

Captive-rearing and translocation are being considered as potential strategies to improve wild populations of hellbenders (*Cryptobranchus alleganiensis*) in Missouri. Hellbenders are lotic salamanders requiring relatively cool, clean water with abundant rock cover for hiding and crayfish as prey (Williams et al. 1981; Nickerson and Mays 1973a,b). Both the eastern hellbender (*C. a. alleganiensis*) and the Ozark subspecies (*C. a. bishopi*) in Missouri have undergone declines of around 80% over recent decades (Wheeler et al. 2003). Reasons for declines in Missouri are poorly understood but may include alterations to water quality via increased siltation or contaminants (Huang et al. 2010), illegal harvest (Nickerson and Briggler 2007), introduced predators (Gall and Mathis 2010), and chytridiomycosis caused by infection of the chytrid fungus, *Batrachochytrium dendrobatidis* (hereafter *Bd*) (Briggler et al. 2007; Briggler et al. 2008). Both subspecies are endangered (S1 rank) in Missouri and the Ozark hellbender, which is endemic to the Black and White river drainages in southern Missouri and northern Arkansas, is a candidate for federal listing under the Endangered Species Act. Captive-rearing and translocation may be one way to increase the number of juveniles surviving to adulthood as well as the number of young females (7-16 years), which have been suggested to have greatest reproductive potential in a population (Peterson et al. 1988). However, the cost and risks associated with captive-rearing hellbenders are considerable and we are unaware of any studies documenting the survival of captive-reared hellbenders once they have been translocated to the wild.

The objectives of our study were to estimate daily survival rates of captive-reared juvenile Ozark hellbenders released at 2 study sites on the North Fork of the White River Missouri and identify factors that may explain their survival. Additionally, we wanted to

monitor changes in body mass of captive-reared hellbenders, as a more subtle indicator of translocation success. Finally, we monitored rate of leech parasitism, accrual of physical injuries and frequency of detecting *Batrachochytrium dendrobatidis* on newly released hellbenders, as a measure of potential sub-lethal stressors that may influence translocation success.

METHODS

Study Site Description

The North Fork of the White River (hereafter NFWR) is a largely spring fed, 7th order stream flowing south into Arkansas from south-central Missouri. The landscape within the NFWR watershed consists of forested woodland (61.9%) and crop and grassland (37.5%) (<http://mdc.mo.gov/fish/watershed/northfrk/>), and the river is a popular canoeing and fishing destination, especially from late May through August (<http://mdc.mo.gov/fish/watershed/northfrk/watqual/>). Substrate along the NFWR includes long stretches of pebble and gravel beds interspersed with beds of large dolomite and limestone slabs and bedrock which historically provided (Nickerson and Mays 1973a,b; Peterson et al. 1983), and still appear to provide, suitable habitat for *C. a. bishopi*. Three species of crayfish, the main food source of hellbenders (Nickerson and Mays 1973a), including *Orconectes neglectus*, *O. longidigitus* and *O. punctimanus*, are prevalent throughout the river. Ozark hellbenders were historically abundant in the NFWR (Nickerson and Mays 1973a,b) though the population declined by approximately 70% between the early 1980s and late 1990s for unknown reasons (Wheeler et al. 2003). Few predators of hellbenders are known, however raccoon (*Procyon lotor*), otter (*Lontra canadensis*) and mink (*Mustela vision*), are common along the NFWR and pose as

potential terrestrial predators. The disease causing fungus, *Batrachochytrium dendrobatidis* (*Bd*) is another potential threat to hellbender survival that has been known to occur in wild hellbenders captured from the NFWR since at least 1969 (See Chapter 1; Briggler et al. 2007; Briggler et al. 2008). Fungal infection of *Bd* has caused lethality in some amphibians (Berger et al. 1998) but its lethality for hellbenders remains poorly understood.

We selected 2 release sites along the river, hereafter the upper and lower site, separated by approximately 17 km of river. Prior surveys suggested both sites had few resident hellbenders, an abundance of crayfish and boulder cover and a variety of pools, riffles and runs (Appendix 1-2). We choose not to reveal the discrete location of our study sites due to the endangered status of hellbenders in Missouri and threats of illegal collecting. Our upper site spanned approximately 1 km in length and covered approximately 36,115 m². Wetted width of the river ranged from 15 - 50 m and mean monthly water depth during our study ranged from 51.54 cm in August 2008 to 75.41 cm in May 2008 (Appendix 3). Suitable cover for hellbenders including large cobble, boulder and bedrock with deep crevices, appeared most dense within a 350 m reach (area = 3,300 m²) which we considered core habitat (Fig. 1). Water temperature at the upper site reached greater extremes than the lower site, probably due to extensive shallow habitat upstream of the site. Mean monthly water temperature during our study ranged from 5.03° C in January 2009 to 23.55° C in August 2009 (Appendix 4). Crayfish density in core habitat, estimated from random sampling of runs and riffles (< 1 m deep), using a 1 m kick-seine technique (Mather and Stein 1993) in August 2008, was 12.16 crayfish/m² (SE = 2.4).

Our lower site also spanned approximately 1 km of stream and covered 52,945 m². Wetted width at the lower site ranged from 48 - 88 m and mean monthly water depth during our study ranged from 62.75 cm in November 2008 to 95.48 cm in May 2008 (Appendix 3). Suitable cover for hellbenders, including large cobble and boulder appeared most dense within a 320 m reach (area = 7,700 m²) which we considered core habitat (Fig. 2). In general density of cobble and boulder appeared greater (more cover rocks per m²), and bedrock with ledges and crevices were virtually absent, within lower site core habitat relative to upper site core habitat, though this was not quantified. Mean monthly water temperatures at the lower site were probably buffered by the input from at least 1 large spring entering the river between sites and deeper water throughout the site, and ranged from 8.61° C in January 2009 to 20.96° C in August 2008 during our study (Appendix 4). The lower site occurred within a portion of the NFWR designated as a Blue Ribbon Trout Zone (daily limit is 1 trout > 18 inches) that is periodically stocked with rainbow (*Oncorhynchus mykiss*) and brown (*Salmo trutta*) trout. Crayfish density in runs and riffles at the lower site, estimated from random sampling of runs and riffles (< 1m deep) using a 1 m kick-seine technique (Mather and Stein 1993) was 12.61 crayfish/m² (SE = 1.52).

Study Animals

We monitored 36 juvenile captive-reared *C. a. bishopi* that were released as part of a larger study to monitor movements and resource selection of captive-reared hellbenders following translocation to the wild. All of the juveniles originated from eggs collected from the NFWR in 2002 by Unger (2003) and were reared in captivity between 2003 and 2008 at the Saint Louis Zoo's Ron Goellner Center for Hellbender

Conservation. In captivity, hellbenders were maintained in groups of 1- 5 per holding tank. A single source of chilled and well oxygenated water was circulated through the entire enclosure. Each tank was lined with pebble gravel substrate and large flat rocks for hellbenders to use as cover, similar to what animals might encounter in the wild. Hellbenders were tong-fed and allowed to forage at will on a diet of crayfish and small fish.

In 2006, several juvenile hellbenders intended for release tested positive for exposure to *Bd* which can lead to lethal chytridiomycosis in amphibians (Berger et al. 1998). To avoid releasing hellbenders with compromised health from infection and reduce spread of *Bd* to non-diseased wild hellbenders, zoo staff cleared the release cohort via heat treatments (Mark Wanner, Saint Louis Zoo, personal communication) of the entire holding system in early 2007. Following heat treatments, the entire cohort of hellbenders intended for released consistently tested negative for presence of *Bd* via PCR (polymerase chain reaction) assay of skin swabs collected weekly during April 2008.

Radio Tagging

Prior to release, each hellbender was implanted with an AVID passive integrated transponder used for individual identification. Saint Louis Zoo veterinarians surgically implanted hellbenders with Sirtrack Limited model RVI 118 ($n = 9$) or RVI 218 ($n = 18$) radio transmitters prior to release (see chapter 2). Model RVI 118 measured approximately 30 x 13 x 8 mm, weighed 5-6 g and had an estimated battery life of 7.5 months. Model RVI 218 measured approximately 35 x 15 x 15 mm, weighed 9-10 grams and had an estimated battery life of 15 months. To reduce possible effects of transmitter attachment we implanted hellbenders with the largest model permitting the weight of the

transmitter was :S 5 % hellbender mass. We implanted hellbenders in 3 cohorts to allow smaller animals to reach sufficient weights for transmitter attachment (140 g for RVI 118 and 180 g for RVI 218). Surgeries were conducted at the Saint Louis Zoo on 3-4 May ($n = 20$), 7 July ($n = 7$) and 3 September ($n = 9$) 2008. At surgery, juvenile hellbenders weighed 142 - 334 g, measured 29 - 36 cm total length and gender was unknown as none had displayed morphological sex characteristics (i.e., cloacal swelling or gravid appearance).

At surgery, zoo staff anesthetized hellbenders individually by placing them in a 250 mg/L solution of tricaine methanesulfonate (MS222) buffered with sodium bicarbonate (baking soda) added to saturation. Hellbenders were removed from the solution as soon as they became immobile and their righting reflex was lost. After swabbing a 2 cm² area of the ventral body wall with dilute chlorhexidine solution a 2 cm incision opening was made into the body cavity, left of midline. The transmitters which had been cold-sterilized in nolvasan for 2' 24 hrs were rinsed with sterile saline and placed them into the coelom. The body wall and muscle layers were closed with 3 sutures and closed the skin with 3 (3/0 polydioxane or nylon) sutures. After surgery, zoo hospitals staff injected each hellbender with 10 mg/kg of enrofloxacin (antibiotic) and returned them to holding water where they were monitored until voluntary swimming occurred (within 30 minutes). To monitor healing we maintained hellbenders in captivity for approximately 2-4 weeks before release. If dehiscing of the sutures was observed in captivity zoo veterinarians repaired or replaced sutures and held animals until healing was evident.

In addition to initial transmitter implant surgeries 7 hellbenders were recaptured by hand between 18 August 2008 and 10 May 2009 to replace transmitters that had failed prematurely or were schedule to expire 2' 4 months prior to the end of our study (See Appendix 5). We quarantined recaptured hellbenders in 5 gallon buckets to prevent possible disease transmission and transported the individuals to the Saint Louis Zoo for surgery. During transportation we placed ice packs in each bucket to maintain cool water temperatures (:S 20° C) and kept water well oxygenated using an aquarium pump. At the zoo hellbenders remained isolated in separate 40 gallon aquaria prior to surgery. Methods for replacement surgery were identical to that previously described, with the exception that zoo veterinarians removed expired transmitters from the body cavity before implanting a new unit and sterilized the container used in anesthetizing hellbenders between each surgery. Following transmitter replacement surgery hellbenders were held at the zoo for 6 - 8 weeks, with the exception of 1 hellbender recaptured in August 2008 that was retained through winter due to dehiscing sutures (See Appendix 5). All 7 hellbenders were released 12 May 2009, which ensured healing and avoided release during high water when visibility was poor, as was common in early 2009.

Release

Following the staggered fashion of surgeries, we released 18 hellbenders within core habitat of each study site over 4 events, each time randomly dividing hellbenders from the cohort to either the upper or lower release site. We released hellbenders at the upper site on 29 May ($n = 7$), 11 August ($n = 5$) and 3 October ($n = 6$) and at the lower site on 19 May ($n = 10$), 11 August ($n = 4$) and 3 October ($n = 4$). On release dates, we

transported hellbenders from the Saint Louis Zoo to study sites by vehicle. During transport hellbenders were maintained in coolers of continuously oxygenated water maintained at the approximate temperature of release locations. We released each hellbender at pre-selected boulders spaced 2' 5m from the nearest known hellbender. We lowered hellbenders by hand to the river bottom near an opening on the downstream side of release rocks and allowed them to move independently under cover. We visually monitored hellbenders until they ceased to leave cover (within 5 minutes) attempting to disturb the animals as little as possible.

Monitoring Survival

We monitored survival of translocated hellbenders by wading or canoeing (if water depth 2' 1.5 m) and using homing procedures (White and Garrott 1990) with a 3 element Yagi antenna and an ATS (Advanced Telemetry Systems, Isanti, MN) receiver (model R2000, R4000 or R410). We located hellbenders approximately every 32 ± 4 hours from 2 days post-release through 14 November 2008 and from 22 March through 21 August 2009. During winter (15 November 2008 - 21 March 2009) we located hellbenders approximately 1 time each week. Throughout the study, we attempted to visually confirm status (alive or dead) of each hellbender at least once per week by snorkeling or with an Aqua-Vu® SV 100 (Outdoor Insights, Inc., Crosslake, MN) camera used to view into rock crevices and bank openings.

Monitoring Body Condition

To monitor body condition, we recaptured 19 hellbenders (upper site: $n = 7$; lower site: $n = 12$) during fall 2008 (29 September - 6 December), 12 hellbenders (upper site: $n = 4$; lower site: $n = 8$) during Spring 2009 (6 March - 1 June) and 15 hellbenders (upper

site: $n = 7$; lower site: $n = 8$) at the end of our study (14 July - 29 August) (Appendix 5). We recaptured hellbenders by hand, lifting cover rocks only when necessary, and making an effort to disturb the habitat as little as possible. Because some hellbenders used bedrock crevices and immovable rocks, catchability was unequal. On average each hellbender was captured 1.25 times ($n = 36$; SE = 0.19, range = 0 - 3 recaptures) during our study.

We identified gender of hellbenders based on the presence of testes or egg follicles via a portable ultra-sound, or when hellbenders exhibited a swollen cloaca (male) or appeared gravid (female). At each recapture, we swabbed hellbenders to detect the presence of *Bd* via PCR assay following methods of Briggler et al. (2008). We measured total length and snout-to-vent length to the nearest mm, and weighed each animal to the nearest gram using an Ohaus® (Ohaus Corporation, Pine Brook, NJ) digital balance. We counted the number of external parasites (leeches) and recorded tail abnormalities (notches or tears), abnormalities of the digits (missing, supernumerary, fused or reduced), abrasions or scars (including scrapes, bite marks or scratches) and open sores (necrotic sores or open flesh wounds) on diagrams representing the dorsal and ventral surface of each hellbender. When hellbenders were visually encountered while being radio-tracked, we recorded visible physical abnormalities and external parasites without disturbing the animal. For recaptures that occurred on 20 May - 29 August 2009, we collected blood (:S than 1 mL blood per 100 g body mass) to be analyzed for heavy metals, health parameters and hormones as part of a separate study. To prevent disease transmission among hellbenders, we sterilized the surfaces of measuring boards, holding buckets and any other surface expected to come in contact with hellbenders using a 10%

chlorine bleach solution between each recapture. In addition, we rinsed and thoroughly dried hands between capturing each hellbender and handled animals with ungloved hands or neoprene gloves that had not been in contact with another animal. After recapture, any cover rocks that had been disturbed were replaced and hellbenders were released at the site of capture.

We calculated the proportion change in body mass of hellbenders at each recapture as,

$$\text{Proportion i1 mass} = \frac{\text{current mass} - \text{release mass}}{\text{release mass}}$$

All means are reported \pm 1 standard error unless otherwise noted.

Survival Analysis

We completed one preliminary analysis to determine if there were differences in survival rates by gender (males and females), dispersal type (Abrupt-Long-Distance, Non-Disperser, and Slow-and-Steady Disperser) (See Chapter 2) or site (Upper or Lower). Given the low number of hellbenders in this analysis, we wanted to increase the viability of a single analysis by including the least number of group variables. We compared a gender model, a dispersal type model and a site model to a constant model using the known-fate model with a logit link function within Program MARK (White and Burnham 1999) and Akaike's Information Criterion for small sample sizes (AIC_c ; Burnham and Anderson 2002). We left-censored individuals until the day they were released after transmitter attachment and we right-censored data if the transmitter failed or if an individual was not relocated on the last day of the study. Results of this preliminary analysis demonstrated support for the site model only. The constant only model was supported over gender or dispersal type (see chapter 2) models indicating we

could not differentiate survival rates by gender or dispersal type. Consequently, we pooled data across gender and dispersal type and used site as a grouping variable in the next stage of analysis.

After pooling data by gender and dispersal type, we developed 8 models to assess the relative importance of factors in explaining hellbender survival rates. We used site and time covariates that considered month (m), sampling season (19 May - 14 November, 15 November - 25 March, 26 March -21 August), and time from initial release (time). Our saturated model included an interaction term between t and site (time \times site). We fit reduced models for site, the biologically relevant time scale (month and season) and interaction terms between site, month and season (site \times month; season \times site). Also, we modeled survival based on the interaction between initial mass (mass) and the number of days held after surgery before release (dtr) and site (mass \times site and dtr \times site). We used the known-fate model with a logit link function within Program MARK (White and Burnham 1999) to estimate survival rates of hellbenders. We used Akaike's Information Criterion for small sample sizes (AIC_c ; Burnham and Anderson 2002) to assess the relative support among candidate models. We report the logit-transformed 95% confidence intervals for survival estimates.

RESULTS

At the end of our study, 16 hellbenders were alive, 13 had died and status of 7 hellbenders was undetermined (Appendix 10). Five mortalities occurred within 30 days post release, including 3 hellbenders whose sutures had dehisced post-release and 2 cases where only the transmitter was recovered near or on bank (Appendix 10). Eight

mortalities occurred 53 - 370 days post release, including 2 cases in captivity following transmitter replacement surgery, 1 likely due to chytridiomycosis, 1 instance where a hellbender was buried alive by bed load during a high water event, and 6 mortalities due to unknown causes (i.e., when only the transmitter was recovered) (Appendix 10). Saint Louis Zoo veterinarians suspected one of the 2 upper site hellbenders that died in captivity following transmitter replacement surgery may have been compromised upon return to the zoo which prevented proper healing; and death of the other was attributed to septicemia despite use of antibiotics. The mortality attributed to chytridiomycosis was characterized by lethargic behavior prior to death, and histological examination of epidermis from a digit (Fig. 3) collected post-mortem confirmed presence of heavy chytrid infection at the time of death (Appendix 10).

We made no attempt to survey for wild hellbenders but encountered 9 within the upper study site and 6 within the lower site during our study. At least 10 of these sightings occurred < 20 m from known locations of translocated hellbenders, though we never observed physical interactions between study animals and conspecifics.

Detection of Amphibian Chytrid Fungus

Four (upper site, $n=3$; lower site, $n=1$) of 24 hellbenders that were recaptured at least once and swabbed to detect presence of *Bd* via PCR assay tested positive for the fungus. One upper site hellbender (unknown gender) had contracted the fungus and died within 70 days post release (hellbender 20, Appendix 10). An upper site female was blue-grey in color, was shedding heavily and tested positive at 73 days post release, however swabs collected at 337 and 372 days post release were negative for *Bd* and the animal was surviving at the end of the study. A lower site female was negative at 157

days post release but positive at 454 days post release, though she appeared healthy and gravid; and 1 upper site hellbender (unknown gender) was first recaptured and tested positive on day 446 post release.

Leech Parasites and Abnormalities

Thirteen of 30 translocated hellbenders that were recaptured, recovered after death or visually encountered were observed carrying the leech *Placobdella cryptobranchii*, thought to be an endemic external parasite of *C. a. bishopi* (Moser et al. 2008; Huang et al. 2010). Twice as many hellbenders carried the leech at the upper site (9 of 16) as at the lower site (4 of 14). Over the 16 occasions where we observed hellbenders with leeches, the mean parasite load was 5.3 ± 1.8 leeches per hellbender (range = 1- 20 leeches per hellbender).

Three times as many hellbenders accrued physical abnormalities at the upper site (6 of 13) as at the lower site (2 of 14). Following translocation, almost half of the hellbenders recaptured, recovered or visually encountered at the upper site had accrued scars or abrasions ($n = 6$) or open sores or flesh wounds ($n = 3$). In comparison, hellbenders recaptured or recovered at the lower site had accrued tail notches ($n = 1$) or open sores ($n = 1$). Three of the 6 hellbenders that had accrued scars or abrasions at the upper site simultaneously tested positive for *Bd*. We observed no accrual of digit or tail abnormalities in hellbenders post release.

Change in Body Mass

Change in body mass was more variable in hellbenders translocated to the upper site than at the lower site where hellbenders tended to gain mass consistently over the course of the study (Fig. 4). Of the 7 upper site hellbenders alive at the end of our study,

2 had lost body mass (10 - 33 %), 3 were within 5% of release mass and 2 had gained mass (25 - 41 %) since release (Fig. 4). The upper site hellbender that lost 33 % of her body mass was the largest hellbender released (357 g) and the mass loss was evident within 73 days post release, simultaneous with testing positive for *Bd* and displaying injuries (Fig. 4). Prior to our first recapture of the hellbender, she was located under a rock where eggs were seen trailing from a small opening, therefore her weight loss may be attributable to dropping eggs. All 8 of the 9 surviving hellbenders that could be recaptured from the lower site at the end of our study had increased in mass by a minimum of around 40 % (range = 38 - 114%) (Fig. 4). Three of the 4 lower site hellbenders that had increased in mass by 2' 70 % since release were females that appeared to be gravid at the end of our study.

Survival Estimates

The site model was most supported (Table 1); however, there was some model uncertainty and we considered those models < 3 AIC units of the top model. Survival was higher at the lower site (0.9992 ± 0.0004 SE) compared to the upper site (0.9980 ± 0.0007 SE, Fig. 5). There was little support for season or month, therefore, we assumed this constant daily survival rate over time (Fig. 5). The annual survival rate of hellbenders at the lower site (0.7467) was nearly double that at the upper site (0.4816) (Fig. 5). The second most supported model indicated an interaction between site and the number of days from time of surgery to release. Based on that model, we noted survival decreased as individuals were held longer (Fig. 6), but there was a lot of variability in survival estimates as noted by the confidence intervals (Fig. 6). The only other model

within 3 AIC units of the top model indicated that as weight increased, survival increased (Fig. 7).

DISCUSSION

Few studies have reported survival rates for wild hellbender populations (Peterson et al. 1983, 1988; Wheeler 2007); however, evidence suggests survival rates at the lower study site were similar to those for wild hellbenders. Survivorship curves calculated for post metamorphic NFWR hellbenders using mark-recapture data collected in 1977 and 1978, when populations appeared robust, indicated that survivorship followed a Deevey's (1947) Type II curve where mortality rates were constant across age classes (Peterson et al. 1983). Though survival rates were not clearly reported by Peterson et al. (1983), figures presented indicated a relatively constant annual survival rate of around 0.81 for males and lumped sexed-and-unsexed individuals. Given an annual and constant survival rate of 0.81, a cohort of hellbenders numbering 1000 at age 5 would decline to around 40 - 50 individuals over about 15 years (i.e., $0.81^{15} = 0.046$). In contrast, a mark-recapture study of a single hellbender population in the NFWR conducted between 1999 and 2005 reported average Jolly-Seber survival to be 0.86 over a 6-year period, indicated annual survival rates of around 0.975 (i.e., $0.975^6 = 0.86$) (Wheeler 2007). While the 6-year survival estimate produced by Wheeler (2007) is 30 % to 100 % higher than annual estimates for our populations, Jolly-Seber survival estimation assumes equal catchability which Wheeler (2007) admits was unlikely. Additionally, it is important to consider that hellbenders captured by Wheeler (2007) averaged 419 mm total length, whereas hellbenders in our study averaged 321 mm. Age-total length relationships calculated for

NFWR hellbenders (Peterson et al. 1983) indicate that the average hellbender captured by Wheeler (2007) was 12 - 22 years old while hellbenders in our study were only 7 years old. If survivorship of females more closely follows a Deevey's (1947) Type III curve where probability of survival gradually increases with age, as reported for hellbenders on the Niangua River (Taber et al. 1975), it is plausible that annual survivorship of captive-reared hellbenders could increase in subsequent years and reach similar survival rates as those observed by Wheeler (2007). Additionally, because we observed at least 3 mortalities apparently due to dehisced sutures, we consider our survival estimates at both sites to be somewhat conservative. Assuming that censored hellbenders were alive at the end of the study, along with hellbenders that experienced mortality within 30 days post release, likely due to transmitter implant, 66 % of upper site and 83 % of lower site hellbenders would have been alive at the end of our study.

Release site caused nearly a two-fold difference in annual survivorship of captive-reared hellbenders. Because we could not recapture or recover bodies of nearly half the mortalities we observed, the factors driving the site difference in hellbender survival were not obvious. We speculate that habitat quality, density of conspecifics, density or abundance of predators, and the prevalence of sub-lethal stressors may have been involved. Regardless of the ultimate cause of mortality, the efficacy of translocation as a conservation strategy for hellbenders may vary by site, even within the same stream. Similar to the site effect we noted, early attempts to establish new populations of natterjack toads (*Bufo calamita*) identified the importance of water quality at release sites and applied their findings to improve future attempts to establish new populations (Denton et al. 1997). Our findings emphasize the importance of selecting appropriate

translocation sites for hellbenders and conducting pilot translocations to determine suitability of release sites prior to releasing large numbers of individuals; especially since increasing numbers of translocated animals has been suggested to positively influence translocation success (Germano and Bishop 2009).

The decreased weight gain in upper site hellbenders may have contributed to reduced survival. Several herpetofauna translocations report body mass loss correlated with reduced survival, though the cause of weight loss is usually assumed to be an abnormally high rate of movement post-release (Matthews 2003; Reinert and Rupert 1999; King et al. 2004). In contrast, while mean dispersal distance was greater at the upper site, hellbenders there moved less over the course of the study than hellbenders at the lower site (see chapter 2). Furthermore, 4 of the upper site hellbenders surviving at the end of our study had dispersed < 20 m from the point of release, but had accrued body mass similar to the 2 living hellbenders there that had dispersed 545 m and 603 m. Because crayfish densities were similar between sites, site differences in mass accrual of translocated hellbenders suggest that feeding was reduced, access to prey was limited or metabolic demands were increased at the upper site. Potential competitors for prey that were encountered at both sites include conspecifics and otters (*L. canadensis*) which, in the Missouri Ozarks, have been shown to seasonally consume primarily crayfish (Roberts 2008). The increased prevalence of leeches, injuries and *Bd* that we observed in upper site hellbender may have reduced growth and possibly compromised translocated hellbenders. Sub-lethal injury has been linked to reduced growth, survival (Semlitsch and Reichling 1989), and reproductive effort in other amphibians (Bernardo and Agosta 2005). The frequency of leech parasitism and parasite loads we observed in upper site

hellbenders were similar to frequencies (67 - 100 %) and parasite loads (4.5 ± 3.5 SD) observed in wild hellbenders from the NFWR from 2002 - 2005 (Moser et al. 2008). Therefore, while parasitism appeared more common at the upper site we see no evidence to suggest translocated hellbenders were parasitized at higher rates than wild animals. Because stress is likely to increase with parasitism (Esch et al. 1975), injury or disease, and heightened stress can inhibit feeding in amphibians (Carr 2002), the reduced weight gain and possibly reduced survival of upper site hellbenders may be explained by the differences in sub-lethal stressors. More research is needed to identify possible sub-lethal effects caused by parasites, injury and disease in hellbenders.

In addition to considering the influence of site, the mass at which to release hellbenders may be an important consideration for future hellbender translocations. The 'site x dtr' model indicated that holding hellbenders longer post surgery might negatively influence survival; however this model may have been confounded by other factors. Because we required a minimum weight for transmitter implant the largest hellbenders tended to be released in the first cohort at each site; and because we observed dehiscing sutures in some animals from the first release cohort, hellbenders in later cohorts were held longer (30 - 98 days) than the first release cohorts (13 - 24 days) (Appendix 10). As a result, the 'site x dtr' model likely reflected higher survival of larger animals, similar to the 'site x mass' model, rather than higher survivorship of animals held for fewer days post surgery. Among translocated herpetofauna, pre-release mass was not a strong predictor of survival for Massasauga rattlesnakes (*S. catenatus*) (King et al. 2004) or Egyptian tortoises (*Testudo kleinmanni*) (Attum et al. 2010), however higher survival was noted in larger captive-reared juvenile redbelly turtles (*Pseudemys rubriventris*)

(Haskell et al. 1996) and Oregon spotted frogs (*Rana pretiosa*) (Chelgren et al. 2008) and in sexually mature, rather than immature, gopher tortoises (*Gopherus polyphemus*) (Tuberville et al. 2008). Size can be an important determinant of survival because smaller herpetofauna can be more susceptible to predation (Sauer and Slade 1987) and disease such as chytridiomycosis (Garner et al. 2009). Our 'site x mass' model predicted that daily survival rates for hellbenders released at 300 g may be as much as 0.001 greater than for animals released at 120 g. Such a slight difference in daily survival rates (e.g., 0.9980 vs. 0.9990) can make a considerable difference in annual survival (e.g., 0.4815 vs. 0.6940, respectively) and longer term persistence of a population (See Fig. 8). While rearing hellbenders to 300 g prior to release may improve survival, it would also likely imply an increase in the costs of captive-rearing. Therefore, captive-rearing programs may need to carefully weigh the pros and cons of releasing various sized hellbenders.

Though our survival analysis assumed that implant of transmitters, radio-tracking and recaptures of hellbenders did not influence survival it is likely that these assumptions were not entirely met. For example, dehiscing sutures caused mortality in at least 3 hellbenders, 2 hellbenders died after transmitter replacement surgery and 1 hellbender died within 7 days of recapture and having blood drawn (See Appendix 5). Dehiscing sutures were observed in a review of transmitter attachment methods for adult hellbenders (Blais 1989) and a telemetry study of newts (Jehle and Arntzen 2000), suggesting urodeles may be particularly susceptible to problems with implantation of transmitters. For at least 2 hellbenders, dehiscing sutures occurred prior to release and holding hellbenders 2' 30 days post surgery allowed veterinarians to monitor healing and repair wounds as needed thereby reducing needless mortality. We also noted no

dehiscing sutures in hellbenders implanted with the smallest size transmitter (RVI 118), despite the fact that animals receiving the implant tended to be the smallest in the study. However, the short battery life of the small unit required a second (replacement) surgery and involved moving animals from the wild back into captivity for several weeks before re-release which likely caused considerable stress. We recommend holding implanted animals at least 30 days post surgery, selecting the smallest transmitter units possible for the study, and suggest only qualified veterinarians perform surgeries.

In contrast to studies that report an inability of translocated amphibian populations to persist at release sites for more than a few months (Fellers et al. 2007; Chelgren et al. 2008; Muths et al. 2001; White and Pyke 2008; Rickard 2006), our study suggests that captive-rearing can produce hellbenders capable of surviving and maturing in the wild for over 1 year. Stress of translocation (Tiexiera et al. 2007), lack of prior knowledge of release sites (Stamps and Swaisgood 2007) and persistence of factors driving original declines (Dodd and Seigel 1991) can reduce the probability of survival in translocated animals. For example, populations of boreal toads (*Bufo boreas*) (Muths et al. 2001), mountain yellow-legged frogs (*R. muscosa*) (Fellers et al. 2007), blanchard's cricket frogs (*Acris crepitans blanchardi*) (Rickard 2006), and autumn-released Massasauga rattlesnakes (*Sistrurus catenatus catenatus*) (King et al. 2004) did not persist more than a few weeks to months post translocation. In addition, several amphibian translocations report reduced survival immediately following release, though survival improved with time (Cooke and Oldham 1995; Tocher and Pledger 2008). In contrast, we found no influence of time since release on hellbender survival, suggesting captive-rearing and the stress of translocation did not acutely affect hellbender survival.

Furthermore, despite the fact that we released relatively few individuals, several captive-reared hellbenders were surviving at both study sites more than 1 year after release, most had gained mass and several appeared to be in breeding condition (i.e., gravid or with swollen cloaca). Though more work is needed to quantify the long term response of hellbender populations to translocation of captive-reared hellbenders our findings indicate that the management strategy has the potential to increase the number of sexually mature individuals in a population, at least for a short-term.

Table 1. Model selection results for survival analysis of captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) released at 2 sites on the North Fork of the White River, Missouri, USA, 2008-2009.

Model	k^a	AIC_c^b	AIC_c	w_i^c	Deviance ^d
S(site)	2	198.651	0.000	0.605	194.650
S(site × dtr ^e)	4	201.207	2.556	0.168	193.203
S(site × mass ^f)	4	201.621	2.970	0.137	193.617
S(season)	4	202.689	4.035	0.080	194.681
S(site × season)	8	207.287	8.636	0.008	191.272
S(month)	12	210.313	11.663	0.002	186.281
S(site × month)	24	221.598	22.947	0.000	173.471
S(site × time ^g)	920	2122.401	1923.750	0.000	83.803

^aNumber of parameters.

^bAIC_c = Akaike Information Criterion for small samples.

^cAkaike weight.

^dDifference in -2log(Likelihood) of the current model and -2log(Likelihood) of the saturated model.

^eDays from surgery to release.

^fMass at time of capture.

^gTime from release.

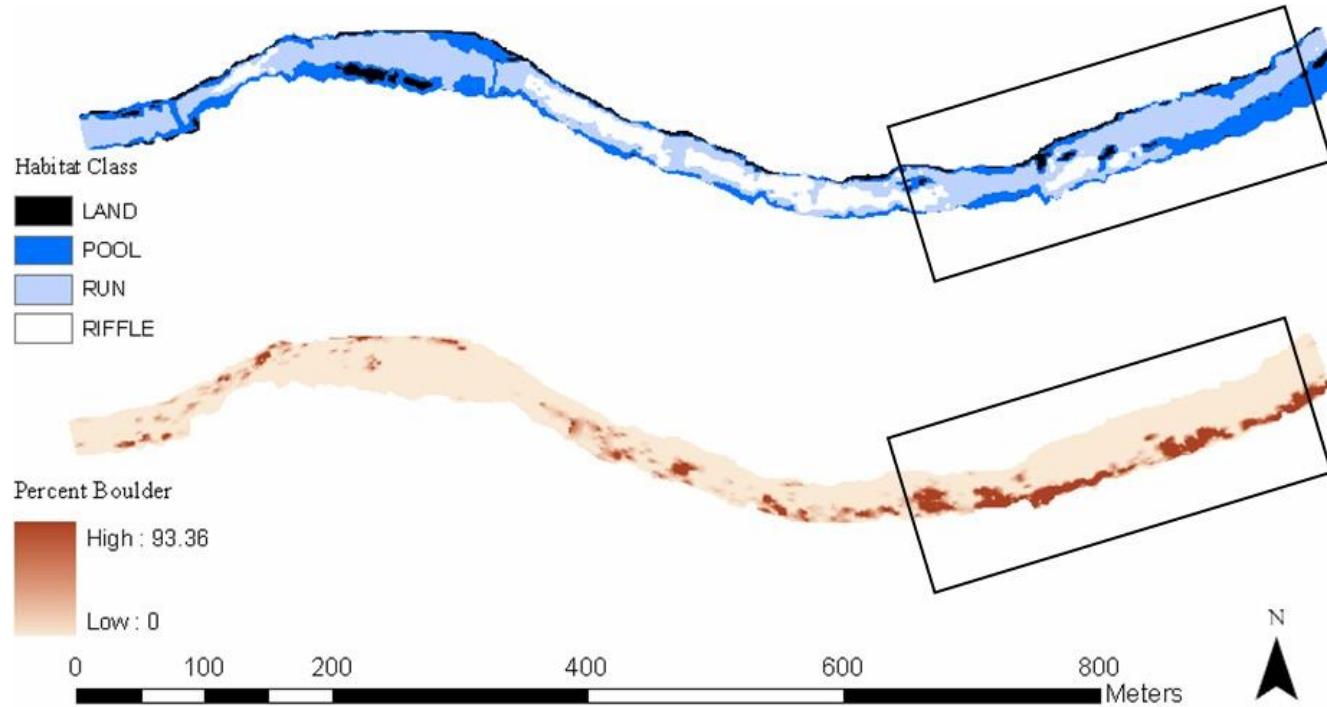


Figure 1. The upper study site on the North Fork of the White River, Missouri, USA, where captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) were translocated, 2008-2009. Core habitat (box) indicates areas where animals were released. Rasters represent the arrangement of pools, riffles and runs (upper image) and percentage of boulder cover throughout the site (lower image).

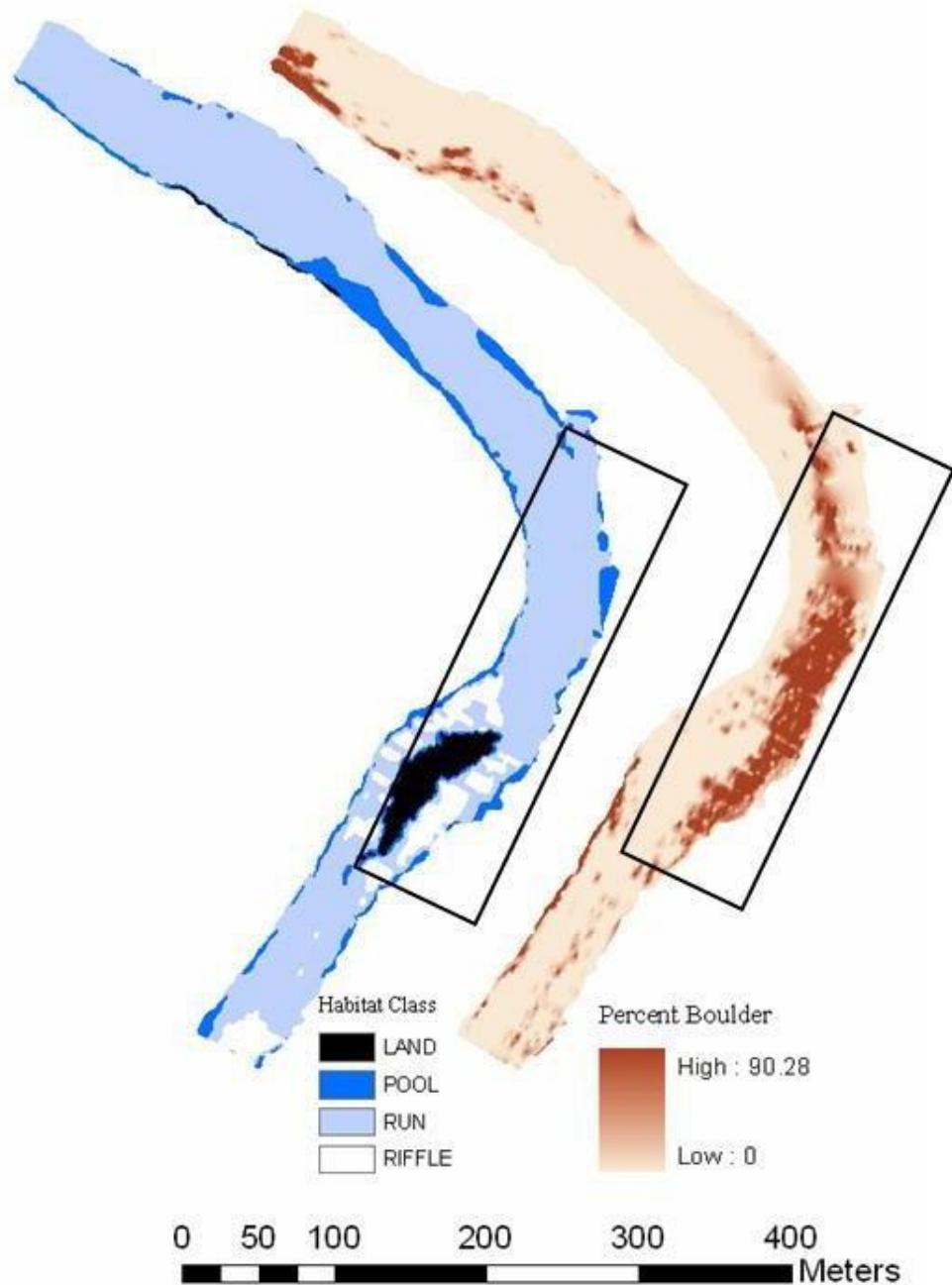


Figure 2. The lower study site on the North Fork of the White River, Missouri, USA, where captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) were translocated, 2008-2009. Core habitat (box) indicates areas where animals were released. Rasters represent the arrangement of pools, riffles and runs (left image) and percentage of boulder cover throughout the site (right image).



Figure 3. A section of epidermis from a digit collected post mortem from hellbender 20 following translocated to the North Fork of the White River, Missouri, USA in 2008 when viewed at 400x magnification. Infection of the chytrid fungus *Batrachochytrium dendrobatidis* (arrow) is characterized by clear round empty zoosporangia and mature zoosporangia with dark inner contents in the uppermost layer of the epidermis.

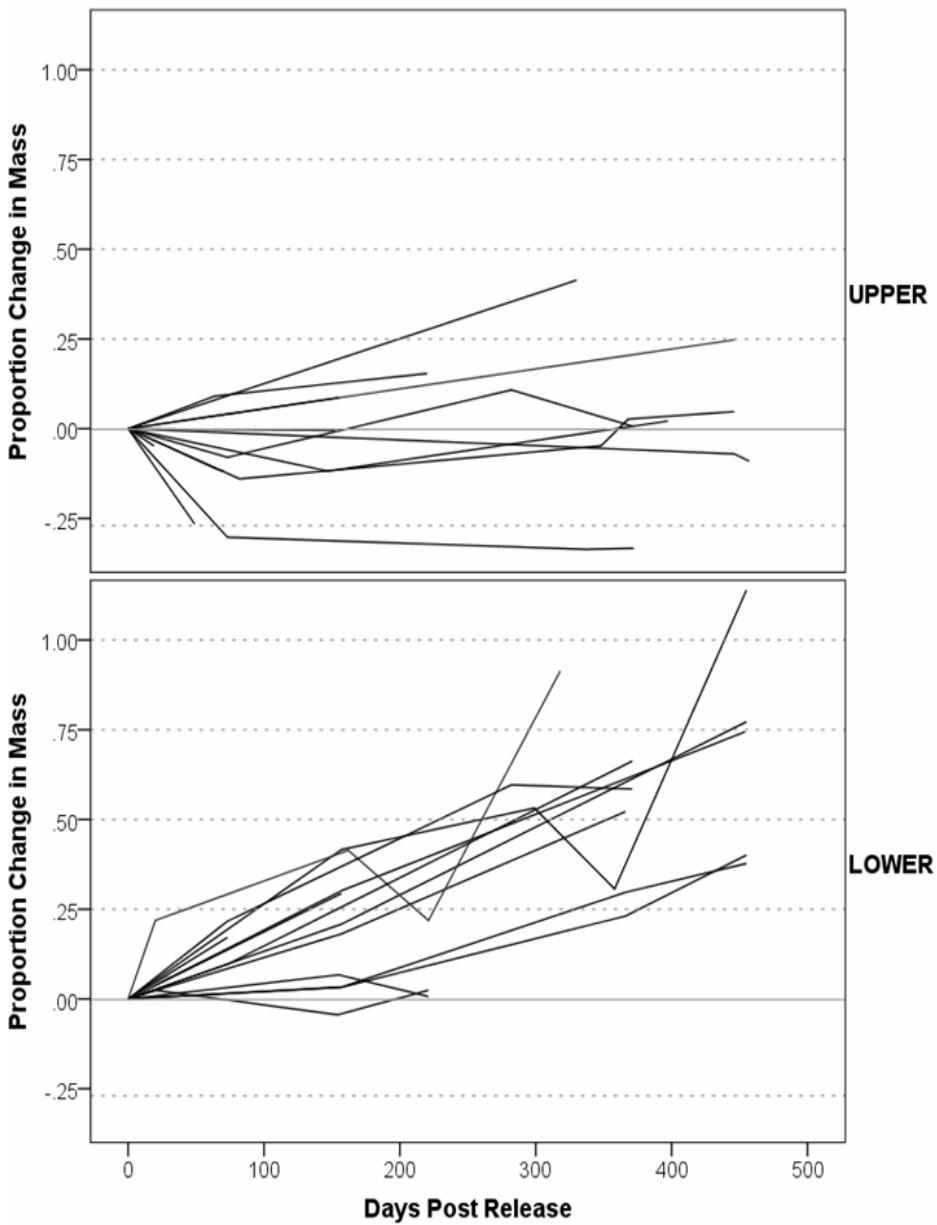
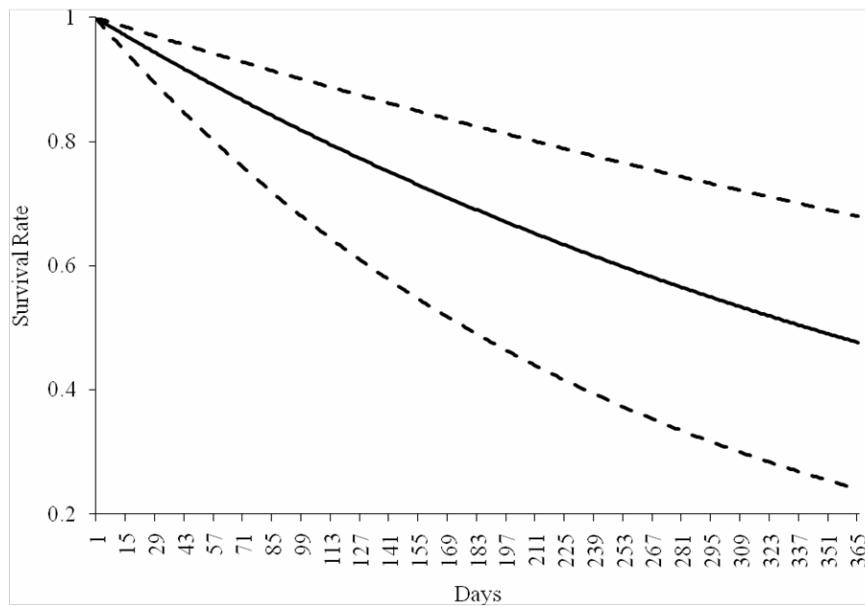
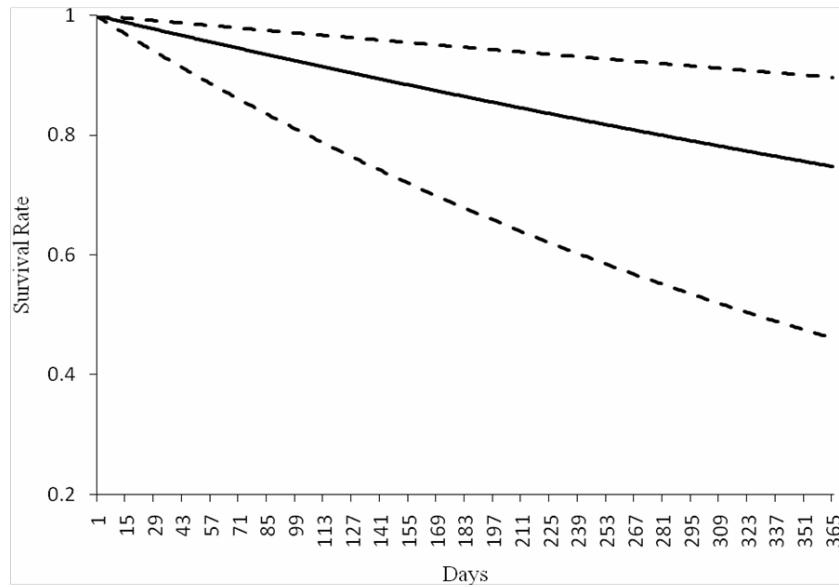


Figure 4. Proportion change in body mass of individual Ozark hellbenders

(*Cryptobranchus alleganiensis bishopi*) that were recaptured at least once following translocated to upper and lower sites on the North Fork of the White River, Missouri, USA, 2008-2009. Each line represents proportion mass change for an individual hellbender.

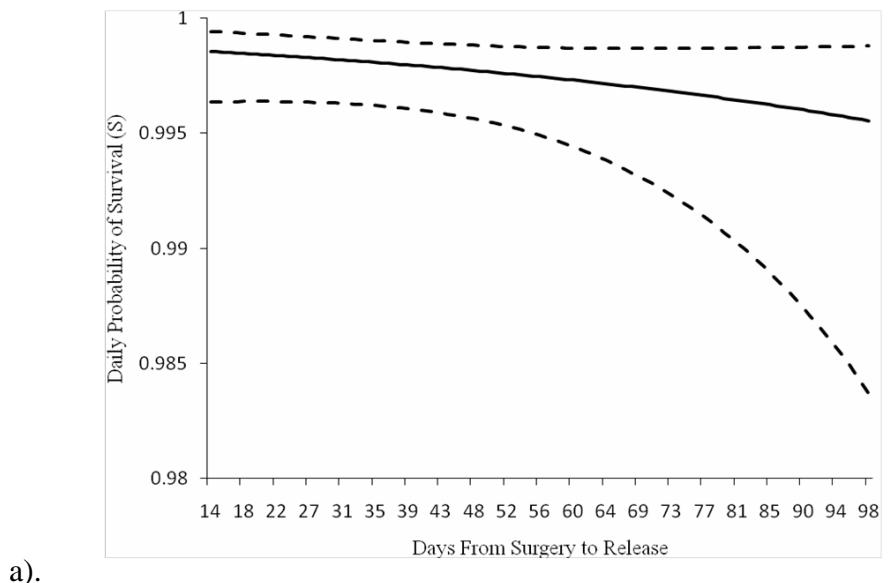


a).

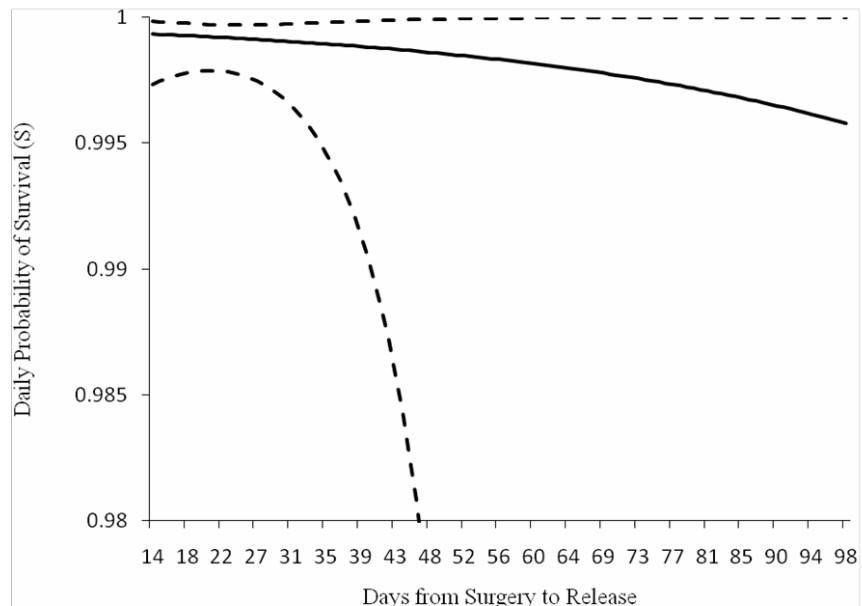


b).

Figure 5. Survival rate over 1 year for captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the upper (a) and lower (b) release site on the North Fork of the White River, Missouri, USA, 2008-2009. The solid line represents the survival estimate and the dashed lines represent the 95% confidence intervals.

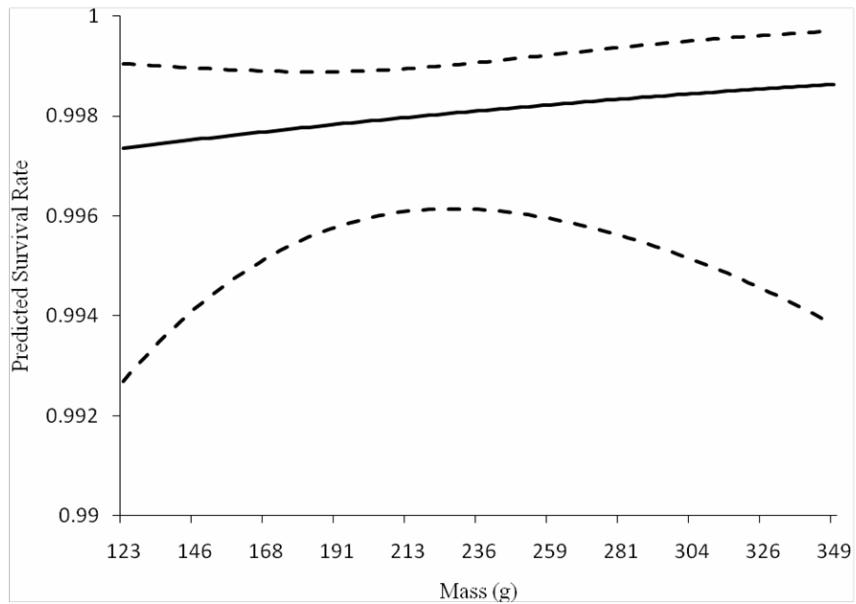


a).

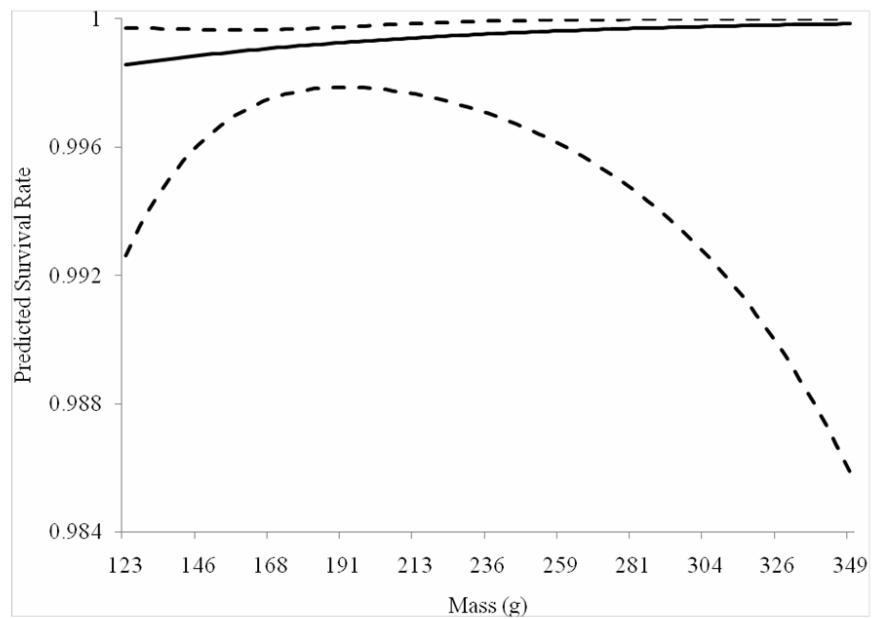


b).

Figure 6. Daily survival (survival from day to the next) of captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the upper (a) and lower (b) release site on the North Fork of the White River, Missouri, USA, 2008-2009, based on days from the time of surgery to release. The solid line represents the predicted survival estimate and the dashed lines represent the 95% confidence intervals.



a).



b).

Figure 7. Daily survival (survival from one day to the next) of captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the upper (a) and lower (b) release site on the North Fork of the White River, Missouri, USA, 2008-2009, based on mass (g) at time of release. The solid line represents the predicted survival estimate and the dashed lines represent the 95% confidence intervals.

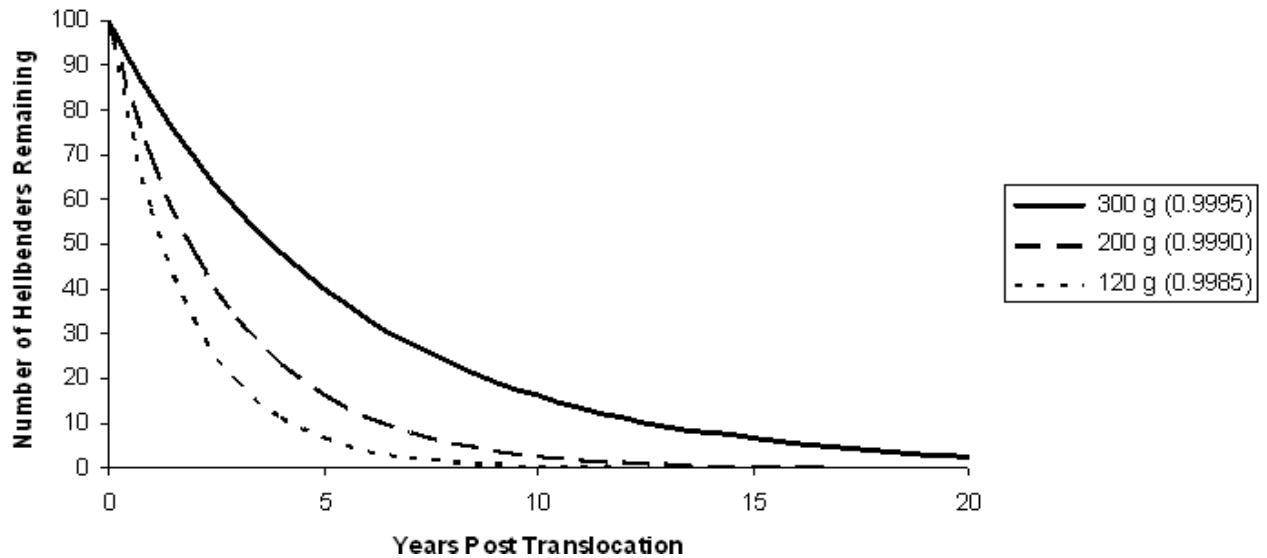


Figure 8. Predicted change in the number of captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishop*) remaining in years post release based on predicted daily survival estimates for hellbenders released at 120 g (short dash), 200 g (long dash) and 300 g (solid line). Daily survival estimates are taken from the site x mass model explaining survival of captive-reared Ozark hellbenders released at the lower release site on the North Fork of the White River, Missouri, USA, 2008-2009.

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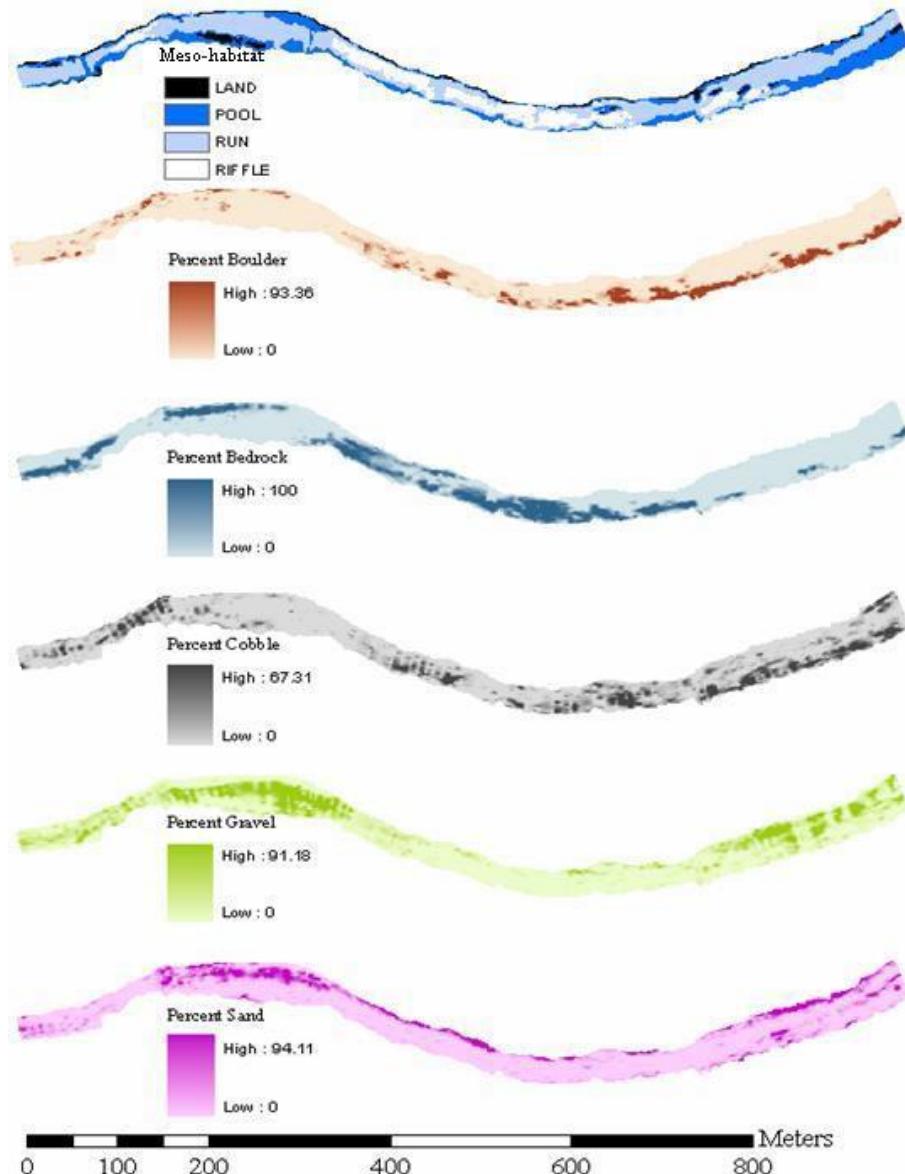
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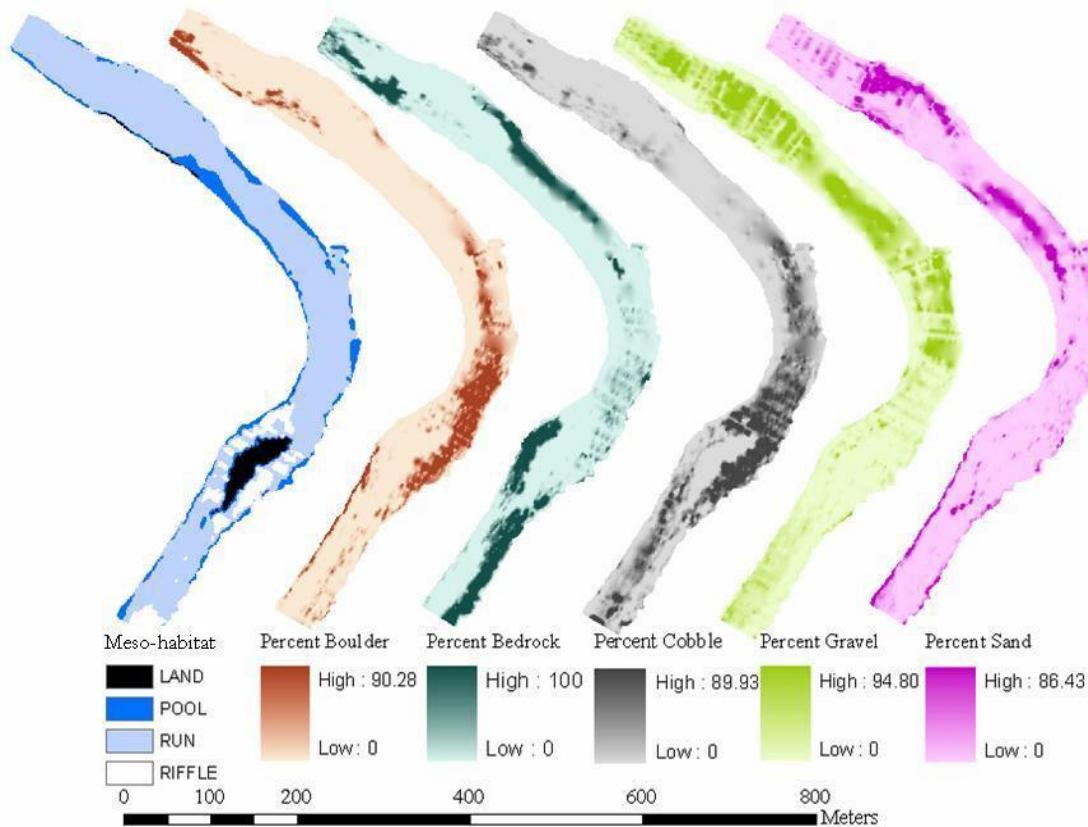
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APPENDICES



Appendix 5. Arrangement of meso-habitat and percent cover of various substrates

throughout the upper site on the North Fork of the White River, Missouri, USA, where captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) were released, 2008-2009. Rasters were generated using nearest neighbor interpolation in ArcGIS and data collected at 1 m increments along cross-sectional transects spaced approximately 5 m throughout the entire 1 km reach.



Appendix 6. Arrangement of meso-habitat and percent cover of various substrates throughout the upper site on the North Fork of the White River, Missouri, USA, where captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) were released, 2008-2009. Rasters were generated using nearest neighbor interpolation in ArcGIS and data collected at 1 m increments along cross-sectional transects spaced approximately 5 m throughout the entire 1 km reach.

Appendix 7. Mean monthly water depth (cm) at the upper and lower Ozark hellbender (*Cryptobranchus alleganiensis bishopi*) translocation sites on the North Fork of the White River, Missouri, USA, 2008-2009.

Month	Upper Site			Lower Site		
	\bar{x}^a	n ^b	SE ^c	\bar{x}^a	n ^b	SE ^c
May-08	75.41	40	5.24	95.48	147	3.22
Jun-08	73.67	283	1.81	82.12	333	2.14
Jul-08	64.65	240	1.94	70.95	262	1.87
Aug-08	51.54	338	1.46	67.64	348	1.62
Sep-08	59.27	334	1.73	71.49	382	1.50
Oct-08	51.72	52	4.01	63.50	496	1.36
Nov-08	55.12	172	2.64	62.75	223	2.10
Dec 08 - Jan 09	**	**	**	**	**	**
Mar-09	74.21	42	3.73	72.45	64	4.22
Apr-09	72.70	251	2.18	75.85	278	2.11
May-09	73.31	214	2.39	79.29	298	1.65
Jun-09	67.02	291	2.13	75.20	344	1.68
Jul-09	61.69	236	2.48	62.84	314	1.63
Aug-09	60.53	114	3.47	66.94	176	2.54

^amean monthly water depth

^bthe number of observations used to generate mean monthly water depth

^c 1 standard error

** no measurements were collected to estimate mean monthly water depth

Appendix 8. Mean monthly water temperature ($^{\circ}\text{C}$) at the upper and lower Ozark hellbender (*Cryptobranchus alleganiensis bishopi*) translocation sites on the North Fork of the White River, Missouri, USA, 2008-2009.

Month	Upper Site			Lower Site		
	\bar{x}^{a}	n ^b	SE ^c	\bar{x}^{a}	n ^b	SE ^c
May-08	20.71	30	0.06	17.56	147	0.12
Jun-08	21.16	106	0.15	19.37	329	0.10
Jul-08	22.82	228	0.08	20.73	262	0.11
Aug-08	22.47	338	0.08	20.01	348	0.10
Sep-08	19.87	334	0.08	18.58	382	0.07
Oct-08	15.91	423	0.11	15.28	496	0.09
Nov-08	13.32	172	0.14	13.71	224	0.11
Dec-08	7.88	21	0.06	9.13	28	0.15
Jan-09	5.03	43	0.07	8.61	39	0.11
Feb-09	9.80	22	0.38	11.16	16	0.20
Mar-09	13.43	42	0.24	13.43	64	0.20
Apr-09	13.92	250	0.15	14.43	277	0.12
May-09	17.79	210	0.08	17.16	298	0.09
Jun-09	22.26	290	0.13	19.45	344	0.11
Jul-09	22.33	236	0.05	19.95	313	0.08
Aug-09	23.55	114	0.13	20.96	176	0.13

^amean monthly water temperature

^bthe number of observations used to generate mean monthly water temperature

^c 1 standard error

Appendix 5. Time line of events involving captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the North Fork of the White River, Missouri, USA, 2008-2009.

ID	Site ^a	May-08	Jun-08	Jul-08	Aug-08	Sep-08	Oct-08	Nov-08	Dec-08	Jan-09	Feb-09	Mar-09	Apr-09	May-09	Jun-09	Jul-09	Aug-09	
		Events ^b																
10	L	I,R					CP											CP,BL
40	L	I,R					CP											CP,BL
90	L	I,R					CP						CP,BL,M					
100	L	I,R					CP						CP,BL					CP,BL
120	L	I,R					CP						CP,BL	CE				CP,BL
200	L	I,R					CP		CP,XMS		R							CP,BL
70	L	I,R,CE																
150	L	I,R	CE															
130	L	I,R					CP	CE										
220	L	I,R	M															
190	U	I,R					CP							CE				
210	U	I,R															CP,BL	
240	U	I,R					CP							CP,BL				

260	U	I,R												CP,BL
250	U	I,R			CP,XM					R	CE	CE		CP,BL
180	U	I,R	M											
110	U	I,R			M									
160	U	I,R				CP						CP,BL	CP,BL	
1030	U		I	R,U		CP				CP,BL			CP,BL	
1170	L		I	R,L		CP				CP,BL			CP,BL	
1510	L		I	R,L		CP							CP,BL	
1250	L		I	R,L		CP	CE							
1470	U		I	R,U,M										
1090	U		I	R,U	M									
1430	L		I	R,L	M									
230	U	I			R,U		M							
20	U	I			R,U		M							
1190	L				I	R,L								
1050	L				I	R,CP		CP,XM		R			CP,BL	
1230	L				I	R,CP		CP,XM		R,CE				
1670	U				I	R					CE			
1690	U				I	R					CE		CP,BL	

1130	U				I	R	M				
1650	U				I	R		CP,XM	M		
1630	U				I	R	CP			CP,XM,M	
1710	L				I	R		CP,XM	R		M

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^a U = upper, L = lower

^b I = transmitter implant, R = release, CE = censored, CP = individual was recaptured, XM = surgery for transmitter replacement, BL = blood drawn at recapture, M = mortality. Light shading implies animals were living in the wild and dark shading implies animals were maintained in captivity.

Appendix 6. Dispersal distance (m), direction and proportion of 0 m daily step-lengths for Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the upper site on North Fork of the White River, Missouri, USA, 2008-2009.

Gender ^a	ID	2008		2009		Distance	Direction	Type ^d
		n ^b	P _{0m} ^c	n ^b	P _{0m} ^c			
U	20	31	0.74			252.71	Down	ALD
U	110	40	0.75			180.66	Down	ALD
F	160	39	0.82	57	0.82	6.81	Down	ND
U	180	10	**			1723.13	Down	ALD
M	190	71	0.9	52	1	8.21	Down	ND
M	210	70	0.91	48	0.67	19.8	Up	ND
U	230	24	0.5			778.47	Down	ALD
M	240	72	0.72	57	0.81	4.89	Down	ND
F	250	32	0.63	9	1	602.84	Down	ALD
U	260	71	0.92	53	0.96	545.81	Down	ALD
U	1030	41	0.68	56	0.77	8.1	Down	ND
M	1090	8	**			13.48	Up	ND
U	1130	12	**	1	1	1910.95	Down	ALD
M	1470	7	**			94.33	Up	ALD
F	1630	15	**	10	0.9	7.29	Down	ND
F	1650	13	**	1	1	3.73	Up	ND
U	1670	14	**	26	0.77	44.6	Up	SSD
U	1690	13	**	24	0.92	102.69	Down	SSD

^aM = male, F = female, U = undetermined

^btotal number of observations collected 24- 36 hours apart

^cProportion of n where step-length is 0 m, ** hellbenders with < 20 locations

^dND = non-disperser, SSD = slow-and-steady disperser, ALD = abrupt-long-distance disperser

Appendix 7. Dispersal distance (m), direction and proportion of 0 m daily step-lengths for Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the lower site on the North Fork of the White River, Missouri, USA, 2008-2009.

Gender ^a	ID	2008		2009		Distance	Direction	Type ^d
		n ^b	P _{0m} ^c	n ^b	P _{0m} ^c			
F	10	74	0.88	59	0.93	184.21	Down	ALD
F	40	73	0.81	64	0.86	254.46	Down	ALD
U	70	3	**			508	Up	ALD
M	90	69	1	18	**	4.93	Up	ND
F	100	70	0.51	65	0.77	75.58	Down	SSD
F	120	71	0.28	29	1	232.38	Down	SSD
F	130	69	0.54	1	**	26.57	Down	ALD
U	150	12	**			40.36	Down	SSD
F	200	71	0.45	51	0.63	77.81	Down	SSD
F	220	13	**			24.04	Down	ALD
M	1050	14	**	49	0.59	48.94	Down	SSD
M	1170	36	0.64	62	0.73	134.4	Down	ALD
U	1190	17	**	63	0.79	530	Up	ALD
F	1230	14	**	5	**	55.39	Down	ALD
U	1250	36	0.58			26.91	Down	SSD
U	1430	10	**			13.99	Up	ND
M	1510	40	1	62	0.95	0	None	ND
F	1710	14	**	28	0.71	56.88	Down	SSD

^aM = male, F = female, U = undetermined

^btotal number of observations collected 24- 36 hours apart

^cProportion of n where step-length is 0 m, ** hellbenders with < 20 locations

^dND = non-disperser, SSD = slow-and-steady disperser, ALD = abrupt-long-distance disperser

Appendix 8. Minimum convex polygon (95%) and fixed kernel (95%) home range estimates (m^2) for captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the upper site on the North Fork of the White River Missouri, USA, 2008-2009.

ID	Release	n ^a	2008		2009		
			MCP ^b	FK ^c	n ^a	MCP ^b	FK ^c
20	11-Aug-08	33	3.85	22.52	0	**	**
110	29-May-08	45	18.74	19.41	0	**	**
160	11-Aug-08	59	101.38	114.21	98	30.36	19.98
180	29-May-08	5	**	**	0	**	**
190	29-May-08	108	35.76	15.85	93	3.26	3.26
210	29-May-08	107	54.82	19.07	85	20.76	27.01
230	11-Aug-08	28	35493.44*	23791.41*	0	**	**
240	29-May-08	108	15.51	21.4	99	4.64	12.8
250	29-May-08	50	6491.25*	7478.73*	16	**	**
260	29-May-08	109	1750.23	315.88	96	22.81	13.74
1030	11-Aug-08	59	15.34	22.3	97	35.92	27.2
1090	11-Aug-08	5	**	**	0	**	**
1130	3-Oct-08	19	**	**	6	**	**
1470	11-Aug-08	4	**	**	0	**	**
1630	3-Oct-08	22	4.67	17.22	31		4.72
1650	3-Oct-08	22		3.17	7	**	**
1670	3-Oct-08	22	5.64	7.92	60	47.17	36.79
1690	3-Oct-08	22		14.18	54	243.91	109.88

^a total number of observations used in home range calculation

^b Minimum convex polygon, = fewer than 3 locations precluded calculation

^c Fixed kernel, * Gross over estimate due to long distance movements precluded use in mean home range estimation

** Fewer than 20 observations precluded calculation of home range

Appendix 9. Minimum convex polygon (95%) and fixed kernel (95%) home range estimates (m^2) for captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the lower site on the North Fork of the White River Missouri, USA, 2008-2009.

ID	Release	2008			2009		
		n ^a	MCP ^b	FK ^c	n ^a	MCP ^b	FK ^c
10	19-May-08	111	30.36	40.88	99	2.19	3.09
40	19-May-08	112	16.62	22.14	100	3.48	9.82
70	19-May-08	0	**	**	0	**	**
90	19-May-08	125		0.66	32	**	1.16
100	19-May-08	112	725.42	951.81	98	19.48	19.07
120	19-May-08	112	875.2	986.92	54	12.22	8.15
130	19-May-08	114	404.91	408.07	0	**	**
150	19-May-08	10	**	**	0	**	**
200	19-May-08	115	421.41	457.6	71	250.45	338.24
220	19-May-08	12	**	**	0	**	**
1050	3-Oct-08	22	3.82	11.55	73	12.44	15.55
1170	11-Aug-08	57	30.36	75.8	99	13.55	10.84
1190	3-Oct-08	21	30.36	1.09	95	15.9	25.5
1230	3-Oct-08	22	**	5.6	16	**	**
1250	11-Aug-08	57	24.78	27.02	0	**	**
1430	11-Aug-08	6	**	**	0	**	**
1510	11-Aug-08	59		0.74	101		1.11

^a total number of observations used in home range calculation

^b Minimum convex polygon, = fewer than 3 locations precluded calculation

^c Fixed kernel

** fewer than 20 observations precluded calculation of home range

Appendix 10. Days to release, mass at release, gender, and fate of captive-reared Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) translocated to the North Fork of the White River, Missouri, USA, 2008-2009.

Id ^a	dtr ^b	Upper Site			Fate ^e	Id ^a	dtr ^b	Lower Site		
		Mass ^c	Gender ^d					Mass ^c	Gender ^d	Fate ^e
250	23	336	F	A		10	13	262	F	A
110	24	286	U	M ⁰		40	13	259	F	A
180	24	295	U	M*		90	13	182	M	M ⁰
190	24	195	M	C		120	13	212	F	A
210	24	198	M	A		200	13	173	F	A
240	24	237	M	A		70	14	195	U	C
260	24	299	U	A		100	14	277	F	A
1130	30	137	U	M ^f		130	14	228	F	C
1630	30	143	F	M ^t		150	14	205	U	C
1650	30	139	F	M ^t		220	14	218	F	M*♦
1670	30	152	U	C		1050	30	128	M	A
1690	30	145	U	A		1190	30	171	U	A
1030	34	176	U	A		1230	30	164	F	C
1090	34	182	M	M*♦		1710	30	149	F	M ⁰
1470	34	188	M	M*♦		1170	34	171	M	A
20	96	220	U	M		1250	34	181	U	C
160	98	357	F	A		1430	34	190	U	M ⁰
230	98	327	U	M ^{0o}		1510	34	167	M	A

^a Hellbender identity

^b Days from surgery to initial release

^c Mass at release, including the weight of the transmitter

^d F = female, M = male, U = undetermined

^e A = alive, M = mortality, U = undetermined, where * = within 30 days post release, ♦ = dehisced sutures, t = in captivity following re-implant surgery, f = used rock buried by bed load, = chytridiomycosis related, O = undetermined cause, o = signal tracked to a snapping turtle (*Chelydra serpentina*)