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POPULATION ESTIMATION, SPATIAL ECOLOGY, AND HEALTH STATUS OF EASTERN HELLBENDERS IN INDIANA

A Thesis
Submitted to the Faculty
of
Purdue University
by
Nicholas G. Burgmeier

In Partial Fulfillment of the
Requirements for the Degree
of
Master of Science

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ABSTRACT

Burgmeier, Nicholas G. M.S., Purdue University, May 2010. Spatial Ecology, Population Size, and Health Status of Eastern Hellbenders in the Blue River, Indiana. Major Professor: Rod N. Williams.

The eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) is a member of an ancient salamander family comprised of three extant species. They require cool, swift flowing rivers and streams with rocky substrates and abundant large, flat rocks for shelter. Once commonly found in many rivers and streams in the eastern United States, the hellbender has experienced precipitous population declines and is now rare or extirpated throughout much of its historical distribution. While the exact cause of the declines is unknown, habitat degradation is suspected as the primary stressor. In Indiana, the hellbender's distribution has been reduced to one low density population in the Blue River in southern Indiana.

Many studies have focused on various aspects of hellbender ecology; however, most have focused on populations characterized by considerably higher density of animals. Individual behaviors may differ as a result of varying densities and habitat conditions. With this study, we intend to elucidate the behaviors of the hellbender under demographic and environmental conditions, which differ considerably from previous studies and hope to identify potential factors contributing to their decline. Our objectives were to: 1) estimate population abundance and structure, 2) investigate the home range, movement patterns, and habitat use, and 3) evaluate the physical health of the population and its surrounding environment in order to develop a better overall understanding of the problems facing the population.

Surveys were conducted in September 2007, June 2008 – October 2008, and July 2009 – September 2009. Blood was drawn from all individuals for blood chemistry analysis. Twenty-one hellbenders were implanted with radio-transmitters at eight sites and water quality was examined at a subset of seven of these locations. Eighty-eight hellbenders were captured during surveys. Using the Jolly-Seber model, we estimated a population density of 0.06 individuals/100 m^2 for our study sites. Minimum convex polygon (100%) home range estimates were larger than reported in previous studies and home ranges for males were significantly larger than for females'. Most plasma chemistry parameters were similar between sexes and comparable to other reported populations. Vitellogenin was found to be an accurate means for gender identification up to two months prior to the breeding season. Our study represents a portion of an ongoing comprehensive assessment of hellbender ecology and will provide valuable information for future management efforts.

CHAPTER 1

POPULATION STATUS OF THE EASTERN HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS ALLEGANIENSIS*) IN INDIANA

ABSTRACT

Studies that accurately assess current and historical population densities provide valuable information for management of wildlife species, particularly those in need of immediate conservation concern. The eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) has experienced drastic declines throughout its range during the past few decades. This study documents its continued decline over the previous 25 years in the last known remaining population in southern Indiana. We conducted mark-recapture surveys from June 2008 – October 2008 and July 2009 – September 2009 at 35 sites. Despite a considerable increase in effort over previous surveys, we documented fewer total captures and extremely low population densities. Density was estimated at 0.06 individuals/100 m^2 and catch per unit effort was 0.05 individuals/person hour throughout the entire study area. This represents not only a significant decline in numbers from the historical study, but is also well below that reported for populations throughout the species' range. Sex ratios were significantly skewed towards males (2.6 males: 1 female). No sub-adults or larvae were found and only two nests were located. This population consists almost exclusively of large, older-age class individuals that have reached such low densities that any natural breeding efforts would likely have a negligible effect on the long-term survival of the population.

INTRODUCTION

The giant salamanders of the family Cryptobranchidae are represented in the United States by the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) and the Ozark hellbender (*Cryptobranchus alleganiensis bishopi*). They range throughout much of the Midwest and eastern portions of the country (Petranka, 1998). Both subspecies occupy higher trophic levels and serve many important roles in lotic ecosystems (Hillis and Bellis 1971, Nickerson and Mays 1973b). They have been reported to maintain high population densities and represent a considerable amount of the biomass in a given area (Nickerson and Mays 1973b, Humphries and Pauley 2005). Hellbenders are believed to influence aquatic invertebrate community dynamics primarily through predation on crayfish (Humphries and Pauley 2005). Recently, hellbender populations have experienced precipitous declines throughout their range (Wheeler et al. 2003), including areas that historically contained large, stable populations (Mayasich and Phillips 2003). Purported reasons for these declines include habitat alteration (Nickerson and Mays 1973b), poor water quality (Briggler et al., 2007), direct persecution or illegal collection (Nickerson and Briggler, 2007), and possibly outbreaks of chytrid fungus (*Batrachochytrium dendrobatidis*; Okada et al. 2008). In the Midwest, hellbender declines have been especially severe. Populations in Ohio and Missouri continue to decline, while hellbenders have been extirpated in Illinois and reduced to a single river drainage in Indiana (Peterson 1985; Wheeler et al. 2003). Reports of these widespread population declines coupled with the species' important ecological roles within aquatic systems, has resulted in a series of new population assessments across much of the hellbender's geographic range.

Recent estimates of hellbender population density vary considerably, often by as much as an order of magnitude (Hillis and Bellis 1971, Nickerson et al. 2002, Mayasich and Phillips 2003, Wheeler et al. 2003, Humphries and Pauley 2005; Table 1.1). Many studies have reported densities ranging from 1-6 individuals/100 m^2 while some report much lower densities (Peterson et al. 1988). Considerable variation in population densities also occurs between sites. Humphries and Pauley (2005) reported an average population density of 0.8 individuals/100 m^2 in their study site, but also documented

Table 1.1: Previous studies reporting population densities and/or relative abundances for hellbender populations.

Author	Date	Density	Historic Density	Relative Abundance	Location
Bothner and Gottlieb*	1991	5.75 - 58.82/100 m^2	-	-	NY
Burgmeier et al.	2010	0.06/100 m^2	See Kern, 1984	0.05 ind/hr	IN
Foster et al. *	2009	0 - 20/100 m^2	See Bother and Gottlieb, 1991	-	NY
Hillis and Bellis*	1971	0.99/100 m^2	-	-	PN
Humphries and Pauley and	2005	0.8 - 1.2/100 m^2	-	-	WV
Humphries	2007	-	-	7.4 ind/hr	NC
Kern**	1984	20.2 ± 7.7/100 m^2	-		IN
Nickerson and Mays*	1973	10/80 - 100 m^2	-		MO
Nickerson et al.	2002	-	-	0.25 - 0.65 ind/hr	TN
Peterson et al.	1988	1 - 6/100 m^2	-	-	MO
Wheeler et al.	2003	3.3 ± 0.77 (16) - 10 ± 2.52 (4)***	6.2 ± 1.05 (35) - 54.9 ± 10.20 (17)	-	MO

* Standardized from original data to fit table. Extrapolated from reported area (m^2) to 100 m^2.
** Density estimated at only one site.
*** Expressed as the number of hellbenders captured per day. Numbers in parentheses indicates the number of sampling days.

densities as high as 1.2 individuals/100 m^2 in one section. Hillis and Bellis (1971) found similar variation in population densities within rivers in Pennsylvania. Sex ratios within populations vary, but typically can be found near parity (Smith, 1907; Kern, 1984). While estimates of current population densities are important, studies that compare current and historical population densities are particularly insightful to establish both the rate and extent of population declines. Unfortunately, only a small sub-set of studies has been able to capitalize on these data (Table 1.1). Indiana is one of only a few states in which a series of population surveys have been conducted throughout the last 15-20 years. Remarkably, many of the sample sites and sampling methods have been consistent between studies and have thus created an opportunity to empirically evaluate population declines.

Historically, hellbenders in Indiana have been found throughout both the Ohio and Wabash River drainages. Hellbender populations are now known to persist in only a small portion of the Ohio River drainage in southern Indiana. Kern (1984) surveyed the Ohio River drainages throughout southern Indiana, but found that hellbenders were restricted to the Blue River. Kern (1984) surveyed 12 sites and captured 130 individuals, including two juveniles. At one site, 68 hellbenders were captured and density was estimated to be 20.2 ± 7.7 hellbenders/100 m^2. More recent surveys (IDNR, unpubl. data) over the last decade have documented continued population declines. Herein, we used mark-recapture to develop an accurate abundance estimate for Indiana hellbenders and compare these estimates to historical studies, determine relative body size and condition, and evaluate habitat use throughout the study area.

METHODS

Study Area:

The Blue River watershed is located in southern Indiana and intersects two of Indiana's natural regions originating in the Mitchell Karst Plain Section of the Highland Rim and crossing into the Escarpment Section of the Shawnee Hills. The Blue River winds through a complex of agriculture, forest, and rocky cliffs until converging with the Ohio River. It is comprised of long stretches of deep pools with interspersed riffle and run habitats. Average summer (May 23 – Sept. 25) depths are fairly shallow (37.15 cm ±

20.53) and summer water temperatures average 21.17ºC ± 1.78. Yearly water temperatures range from 0.02ºC in the winter to 25.94ºC in the summer, but maintain relatively high dissolved oxygen levels throughout (Burgmeier et al., unpubl data). Substrate varies with some areas characterized by predominately bedrock bottom, while others consists of gravel and cobble. Regardless of substrate type, many areas maintain a thin layer of silt. Boulder sized (>256 mm diameter) limestone slabs suitable for hellbender refugia are spread throughout the river (Burgmeier et al., unpubl data).

Site Selection:

Sample sites were chosen through an iterative process. First, the entire study area was floated and characterized according to flow type, substrate composition, and boulder size (Garmin 72, Garmin, Olathe, Kansas, USA). A map was then constructed from habitat data gathered from the canoe survey using ArcGIS 9.2 (ESRI, Redlands, California). The Hawth's Tools Extension (Beyer, 2004) was used to join our data to base layer data in the United States Geological Service's National Hydrography Dataset (USGS, 2007). Sample sites were selected based on a set of criteria consisting of three categories: flow type, substrate, and boulder size. Each parameter contained subcategories, which were assigned a value based on their suitability as habitat. A selection was run to choose sites based on a set of predetermined habitat suitability characteristics (Table 1.2). Based on their potential quality as hellbender habitat, the resulting selections were divided into four categories: primary, secondary, tertiary, and unsuitable. Thirty-five sites were ultimately selected. Twenty-five sites were selected from the primary and secondary categories using the iterative process described above. The ten remaining sites were historical Indiana Department of Natural Resources sampling sites (five of which overlapped with the initial Indiana population survey conducted by Kern (1984)). IDNR sites were chosen in 1996 by IDNR personnel based on their suitability as habitat and their ease of access. Furthermore, IDNR sites are surveyed annually and are known to be areas of recent hellbender activity. An additional seven sites were surveyed multiple times as part of another study, but were not included in the mark-recapture estimates. Our sampling efforts were focused on areas with riffles and runs (although some pools were also sampled), gravel or cobble substrate containing abundant boulder-sized slab rock cover, and an average depth less than 1 m.

Table 1.2 A description and ranking of criteria used to select sample sites

	Selection Criteria		
Site Classification	Flow	Substrate	Boulders*
Primary	3	4	2
Secondary	2 or 3	4	1
Tertiary	1	3 or 4	1
Unacceptable	-	2	0

* Indicates the most frequently represented category

Flow: 1=Pool, 2=Riffle, 3=Riffle/run complex

Substrate: 1=Bedrock, 2=Silt, 3=Sand, 4=Gravel, 5=Cobble
Boulders Size: 1=Small (256-512 mm), 2=Medium (512-1024 mm), 3=Large (1024 - 4096 mm)

Capture and Handling:

Hellbenders were collected from September 2007, June – October 2008, and July – September 2009. Each of the 35 mark-recapture sites was surveyed five times. In order to obtain accurate population estimates using open population mark-recapture models, it is recommended that at least three, but preferably more sampling occasions are conducted (Donnelly and Guyer, 1994; Elzinga et al., 2001). Additional surveys not included in our mark-recapture estimate were conducted at seven other sites for exploratory purposes, bringing the total number of sites to 42. Most surveys were conducted in teams of at least four people. Hellbenders were captured by hand or net after flipping boulder sized rocks within the stream. Underwater goggles were worn to aid in visibility when needed. Stream flow conditions were evaluated before surveying to ensure conditions were within suitable ranges for effective surveys (depth <1 m). Captured individuals were placed ventral side up in a custom “bender board” squeeze box to prevent struggling (Burgmeier et al., in press). Gender, total length (cm), snout-vent length (cm), and body weight (g) were recorded for all individuals. Gender was determined via cloacal swelling during the breeding season (approximately mid-August through late September). Outside of the breeding season, individuals cannot be sexed based on morphological characters; therefore, we collected blood samples from all individuals for positive gender identification using laboratory analyses. We used a phosphoprotein assay on blood serum to identify vitellogenin (VTG) presence (female) or absence (male) to properly identify unsexed individuals (Burgmeier et al, unpubl data). All hellbenders were scanned with Biomark FS2001F-ISO and AVID Multiscan 125 PIT tag readers. If no chip was found, a Biomark 134.2 kHz tag was implanted in the tail approximately 8 cm posterior to the hind leg. Habitat measurements were taken at all capture locations and include coordinates (UTM), water temperature (^{o}C), water depth (cm), distance to shore (m), distance to nearest shelter (cm), number of shelter rocks (>20 cm diameter) within 6 m radius, weather (sunny, partly cloudy, cloudy, raining), substrate (bedrock, silt, sand, gravel, cobble), flow type (pool, riffle, run), and rock length (cm), width (cm), and depth (cm).

Data Analysis:

Absolute abundance for the Blue River watershed was calculated using the POPAN parameterization of the Jolly-Seber Model (Begon, 1979) in Program MARK.

Akaike's Information Criterion (AIC) values were used to select the best available model (Akaike, 1973). From this estimate, we calculated absolute density (the number of individuals captured per 100 m^2) as well as known density (the number of known individuals/per 100 m^2). Absolute density provides an estimate of the population density, while known density provides a known minimum density estimate for the population. The estimates should be considered applicable only to our 35 sample sites. Catch per unit effort (CPUE) was estimated as the number of hellbenders captured per sampling hour. All surveys were timed and the number of relevant surveyors recorded. We estimated biomass for both the absolute and known densities as the mean individual mass multiplied by the density. Size classes were arranged in 5 cm intervals as per Nickerson et al. (2002). A chi-square goodness of fit test was used to determine if sex ratio differed from parity. Student's t-test and non-parametric Mann-Whitney U tests were used to compare morphological and habitat use differences between genders.

RESULTS

A total of 1514 sampling hours were spent surveying approximately 203366 m^2 of river. A total of 70 individual hellbenders were captured and marked during the five mark-recapture surveys. Twenty-one individuals were recaptured at least once, but only seven were recaptured twice or more. Our recapture rate was 30% based on the 21 recaptures. When the seven exploratory sites are included (for a total of 42 sites), the total number of hellbenders captured increased from 70 to 88. Hellbenders were captured at 26 of the 42 sites (62%). The majority of sites (74%) resulted in two or fewer captured individuals, whereas the remaining 26% had three or more captured individuals per site. All individuals captured throughout the study were adults. During exploratory surveys, two nests were discovered within 25 m of each other and both were guarded by males. Eggs were fertilized and estimated to be in Harrison stage 17 (Harrison, 1969). No other nests were found throughout the study.

Using data from 35 sampling sites and five capture occasions, we produced a population estimate of 114 (95% CI = 94 & 152) hellbenders within the Blue River drainage. AIC values were lowest for the constant capture and survival with timedependent probability of entry model. The absolute density estimate was 0.06 individuals/100 m^2, while the known density for the entire study area was slightly lower at 0.04 individuals/100 m^2 (N=86). The highest known density recorded at an individual site was 0.39 individuals/100 m^2. Catch per unit effort was 0.05 individuals/sampling hour. Biomass estimated from the absolute density was 3.51 kg/ha, but decreased slightly to 2.70 kg/ha when calculated using the known density. Seventy-eight hellbenders could be accurately assigned a gender using a combination of external morphology and blood chemistry analysis. Sex ratios were 2.6:1 (56 males and 22 females) and significantly skewed towards males (x^2=14.82, p<0.05).

Size classes were skewed towards large (and presumably old) adults (Fig. 1.1). The mean total and snout-vent lengths for the population were 47.58 ± 4.10 cm and 32.01 ± 2.86 cm, respectively. Mean total weight was 627.16 ± 162.78 g. Females were significantly larger for total length, snout-vent length, and weight (Table 1.3). Most captured individuals appeared healthy with two notable exceptions. Two males, each located at different sites, appeared grossly malnourished with visible bones down their trunk and tail. Other individuals captured at these sites, however, appeared healthy (N=2). These individuals were checked for chytrid fungus for a separate study (Burgmeier et al., unpubl data).

Hellbenders were primarily found on gravel substrates (69.30%) and to a lesser extent silt (14.91%). Sand, bedrock, and cobble were rarely used. Most individuals were located in runs (72.03%), with riffles and pools being used similarly at 13.56% and 14.41%, respectively. Shelter size ranged from 1598.4 cm^2 to 26784 cm^2, with a mean size of 10303.46 ± 5662.89 cm^2. There were no significant differences in shelter use between males and females (U=-1.35, p=0.18). Mean distance to the nearest shelter was 12.00 ± 18.53 cm, while mean distance to shore was 4.53 m ± 2.54. Hellbender shelter rocks were surrounded by a mean of 57 ± 39.98 suitable alternative shelters (>20 cm) within a 6 m radius.

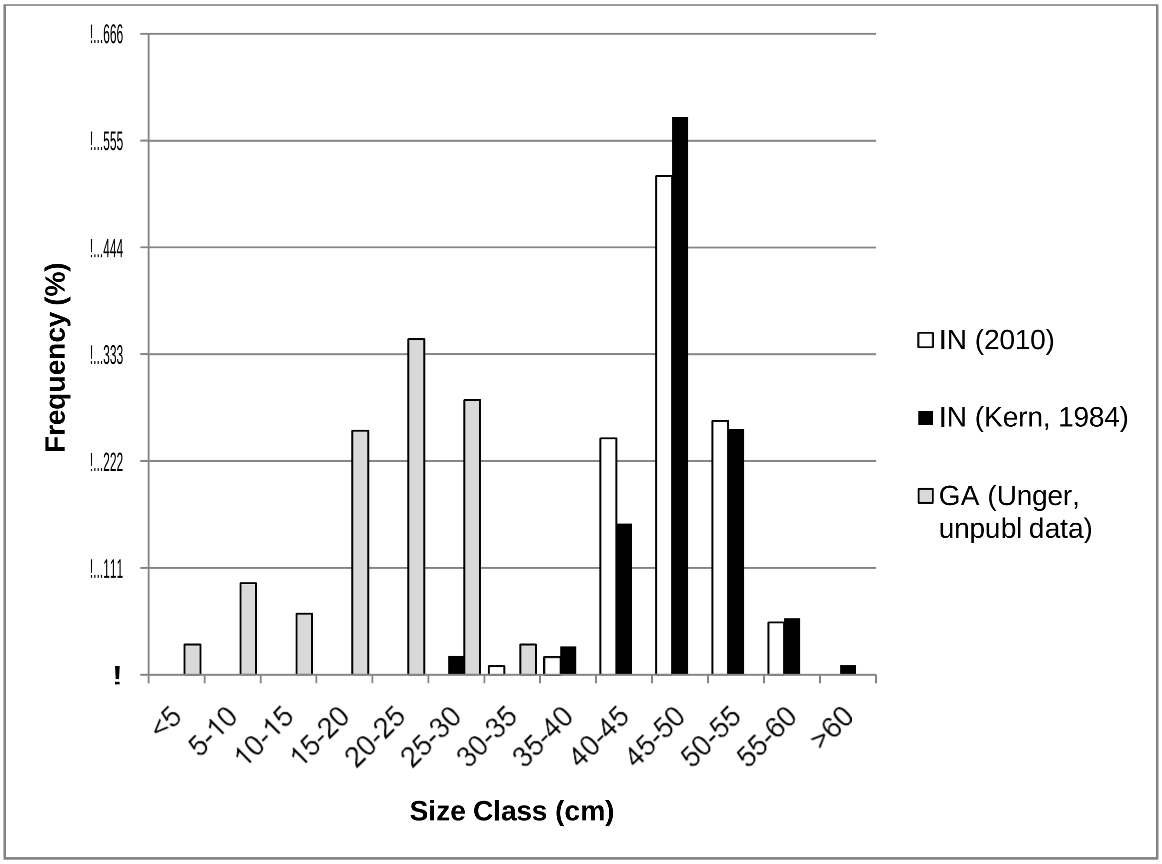

Fig 1.1. Differences in size-class distribution present between a “stable” population of hellbenders found in Georgia and a geriatric, non-productive population found in Indiana. The historic distribution for Indiana is included to illustrate the relative lack of younger age classes entering the population over the past several decades.

Table 1.3. A comparison between morphological measurements from Kern (1984) and the present study.

	Kern, 1984 Total	Total	Male	Female	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	p-value*
TL (cm)	48.18 ± 4.76	47.58 ± 4.10	46.63 ± 2.95	49.39 ± 5.75	0.017
SVL (cm)	32.00 ± 3.31	32.01 ± 2.86	31.15 ± 1.91	33.82 ± 3.92	0.001
Weight (g)	685.90 ± 216.3	627.16 ± 162.78	589.58 ± 126.76	681.26 ± 193.15	0.019

* P-value represents comparison between males and females during our study only.

DISCUSSION

Hellbenders have experienced significant declines in Indiana since the most recent extensive survey was performed in the mid 1980s (Kern, 1984). Indeed, population monitoring by the IDNR has documented noticeable declines within several commonly searched sites (Fig. 1.2) and local anglers frequently offer anecdotes concerning the perceptible lack of encounters while fishing. Despite a substantial increase in sampling effort (42 sites versus 12) over Kern's (1984) historical survey, the current surveys resulted in far fewer individual captures.

In nearly seven months of total sampling, we captured 88 individuals compared to 107 individuals just 26 years prior (Kern 1984). Eight of the 12 sites surveyed in the previous study were included, at least in part, in the current survey sites and in all but one case resulted in fewer captures (Fig. 1.3). A similar pattern was observed when comparing our survey results to more recent IDNR surveys. Two IDNR sites included in our surveys annually yielded 5-10 individuals per sampling occasion yet during our survey, these sites were intensively searched up to seven times and resulted in only 2 - 3 individuals per site. Declining numbers of hellbenders have also resulted in animals becoming patchily distributed throughout the river. Well over half of all individuals came from only seven sites. This could have tremendous implications on gene flow and resulting genetic structure within this population as an individual's ability to find a potential mate decreases.

There has been little recruitment in this population over the past two decades (Kern 1984; IDNR unpubl. data; this study). In the current study, we were unable to locate larvae or subadults at any of the 42 sites. As a result, the population was skewed toward large adults. While we recognize the sampling bias towards larger size classes when using the rock-lifting technique, a portion of the non-gilled subadults present in a stable population should have been captured using the same methods as adults (Peterson et al, 1983; Nickerson and Krysko, 2003; Foster et al., 2008; Burgmeier Pers. obs.). Indeed, as part of another study, the authors sampled hellbender populations in Georgia and found gilled larvae and non-gilled subadults using rock flipping. Adult biased populations are not uncommon, but younger age classes can still typically be

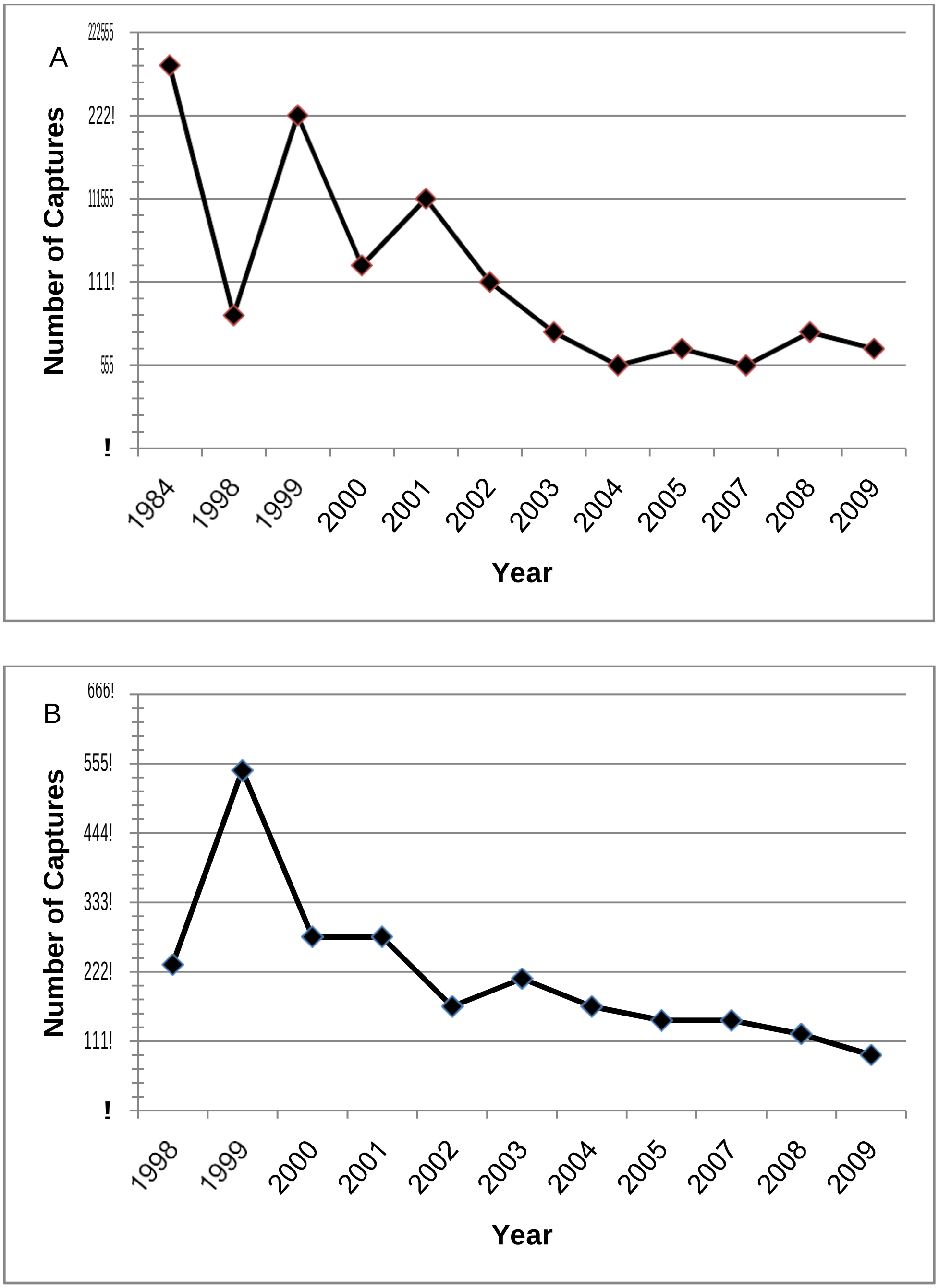

Fig. 1.2 a,b: (a) Annual captures at five sites surveyed by Kern (1984), IDNR (1998-2007), and our study (2008-2009), (b) Annual captures during the annual 11-site IDNR hellbender survey. Years 2008 and 2009 reflect the number of captures during our study at the same 11 sites. No survey was conducted in 2006 due to high water levels. The sampling effort for the final three years was approximately double that of previous years.

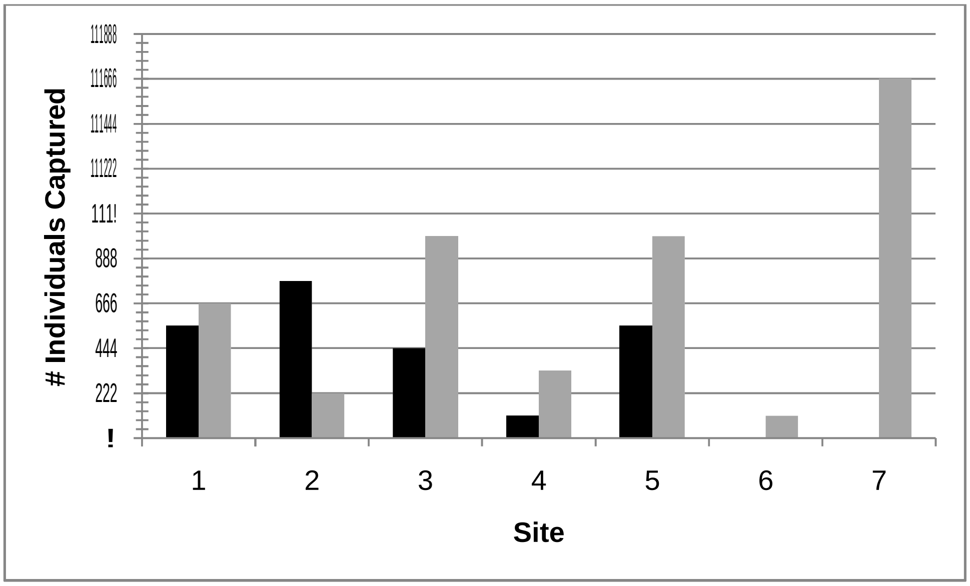

Fig. 1.3: A comparison of hellbender captures between seven sites surveyed historically by Kern (1984) and during the present study. Present study (black bars), Kern, 1984 (gray bars)

found when present, albeit in far fewer numbers than adults (Nickerson et al., 2003; Foster et al., 2009). Thus, the lack of sub-adults in this study was likely representative of their absence rather than an ascertainment bias during sampling.

Despite the absence of gilled larvae and subadults, two nests were detected in close proximity to each other during our surveys. However, no larvae were found in a monitoring survey conducted the following summer. Future larval surveys will be targeted at this area to determine reproductive success. Few other nests have been reported during the previous decade in the Blue River and those that have been documented were observed at sites known to maintain the highest densities within our study area (Walker, personal comm.). While these sites were included in our study, we detected no indication of current reproduction.

The lack of adult age classes present during surveys in Georgia is interesting considering the methods used are typically noted for capturing adults rather than subadults. Several explanations could explain this difference. It could be possible that in a population with fewer adults the amount of competition for larger, more “preferred” rocks are available for use and thus results in an ascertainment bias against the older age classes. A second explanation might be that traditional size-age class distributions are not applicable to all populations. High densities has been shown to reduce fish growth rates resulting in an overall smaller size at maturity (citation). It is possible that some hellbender populations exhibiting a skewed distribution might maintain high enough population densities to alter growth rates and reduce the size at sexual maturity. Additional research is needed to examine this relationship.

The mark-recapture estimates from this study clearly indicate that hellbender numbers are declining in Indiana. However, our population estimate is not directly comparable to most other studies because we used an open-population model. An open population model was necessary for the Blue River population because both death and emigration were documented at several locations as part of another study using radio telemetry (Burgmeier et al., in prep). The length of time between successive surveys (> three weeks) also precluded the use of a closed-population model. Stable hellbender populations have typically been reported at densities of 0.8 – 6 individuals per 100 m^2 (Hillis and Bellis, 1971; Peterson et al., 1988; Humphries and Pauley, 2005). At 0.04 individuals/100 m^2, our density estimate for the Blue River population is an order of magnitude lower than other reported populations (Table 1.1). Our estimates of catch per unit effort were considerably lower than other populations as well (Peterson et al., 1988; Humphries and Pauley, 2005). Even populations considered “low density” reported considerably higher catches per unit effort (0.25-0.65 individuals/hour; Nickerson et al., 2002). Surveys in this study were typically conducted by four or five individuals which is comparable to other surveys, so it is unlikely that our lower CPUE was due to different levels of effort compared to other studies.

Hellbenders do not appear to make up a significant portion of biomass in the Blue River. In fact, the biomass estimates are much lower than previously reported, despite larger average body sizes in this study. This is most likely a result of the low densities found in the Blue River drainage. The sex ratio of the population was biased towards males, but within the range reported for other populations. Smith (1907) reported a skewed ratio of up to 3:1 males to females in the Allegheny River in Pennsylvania and Pfingsten (1990) found an even greater skew of 3.4:1. Conversely, both female biased and 1:1 ratios have been reported from other populations (Nickerson and Mays, 1973b; Peterson, 1987; Bothner and Gottlieb, 1991; Table 1). In Indiana, Kern (1984) reported a nearly even sex ratio of 1.03:1 males to females for the entire study area and 0.74:1 males to females at a known nesting site containing a large number of individuals. Under most situations, a male biased sex ratio would not be considered problematic; however, with a low density population, it could have drastic effects on the individual fitness of remaining individuals. Depending on the distribution of individuals, breeding opportunities could be hampered by the lack potential mates (Scribner et al., 1997; Gibbs, 1998; Jehle et al., 2001).

Reports of sexual size dimorphism for eastern hellbenders are inconsistent (Nickerson and Mays, 1973b; Taber et al., 1975; Mayasich et al., 2003; Humphries and Pauley, 2005). In the current study, females were significantly larger than males for all three standard measurements. The sexual dimorphisms we observed could be a by-product of life-history patterns (e.g., gravid females are older than males) or by inter-specific sex differences (e.g., females have longer SVL to store more ova). We cannot distinguish between these (or other) hypotheses with the existing data sets, although it is notable that our results are largely consistent with those reported in Kern (1984). Most hellbenders in the population appeared healthy and active and only two noticeably underweight individuals were located within two sites where other healthy individuals have been found. However, it is worth noting though that the two sites were within 2.4 km of each other and might be subject to similar environmental conditions. A mostly healthy population indicates suitable resources are present to support current population levels. Likewise, it provides some support for the idea that exogenous environmental stressors are not currently affecting the population's physical fitness. However, it does not preclude these factors as potential past causes of declines nor does it provide a biological endpoint for other potential consequences of these factors such as reproductive fitness.

Hellbender use of gravel substrates and large, flat rocks is widely documented (Hillis and Bellis, 1971; Blais, 1996; Humphries and Pauley, 2005; Burgmeier et al., unpubl data). Our findings are concordant with this pattern and indicate hellbenders use gravel disproportionately to other available types. This is possibly due to the increased abundance of macroinvertebrates at these same areas; however, more extensive research in this aspect of hellbender ecology would be needed to further elucidate habitat preferences. Distance to nearest shelter, distance to shore, and the number of suitable shelter rocks within a 6-m radius were all similar to numbers reported in a radio-telemetry study of the same population (Burgmeier et al, in press). These findings suggest that hellbenders choose areas with abundant cover and are rarely more than a body's length from an alternative shelter. Moreover, the abundance of available shelters would suggest that the lack of suitable shelters is not a likely cause for decline.

Hellbender numbers in Indiana have steadily declined over the past two decades. Our results reveal that the current population structure consists of a geriatric, low density population with little to no recruitment over the past two decades. While the low densities reported herein are likely a contributing factor to these declines, other causes are not readily apparent from this data set. Studies evaluating the current levels of genetic variation, habitat quality, and overall animal health are needed to fully assess the contributing factors to hellbender declines in the Blue River drainage of Indiana.

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CHAPTER 2

SPATIAL ECOLOGY OF THE EASTERN HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS ALLEGANIENSIS*) IN INDIANA

ABSTRACT

Few studies have examined the spatial ecology of the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*). We used radio telemetry to examine the seasonal home range, movement patterns, and habitat use of 21 individuals within the Blue River drainage of southern Indiana. Individuals were located up to three times weekly from July 2008 – October 2009. Mean 100% minimum convex polygon (MCP) home-range sizes were much larger than previously reported and largest during the summer. Male MCP's were significantly larger than females. Mean linear home range sizes were also significantly longer in the summer, but did not differ between the sexes. Hellbenders moved very little throughout the year (Mean = 14.1 movements/individual) and over relatively short distances (Mean = 27.5 m) to nearby shelter rocks. The majority of hellbenders were routinely located under large, flat shelter rocks; however, five individuals periodically used bedrock, downed trees, and submerged tree root masses along the riverbank. Habitat use of hellbenders was similar to that found in other studies with 79.5% of our locations found on a gravel substrate. Our results provide essential information about a declining, low-density population of hellbenders in need of management.

INTRODUCTION

A thorough understanding of spatial movement patterns and habitat use can have important implications for successful management activities. At present, much is known regarding the spatial ecology of mammals and birds, whereas fewer studies have examined amphibian spatial ecology. This is due, in part, to the secretive nature of amphibians (Pough, 2007), their limited availability during much of the year (Williams and DeWoody, 2009), complex life histories (Duellman and Trueb, 1986), and their small body size (Wells, 2007). Few techniques allow researchers to collect spatial data as efficiently and effectively as radio telemetry. While radio telemetry has been widely used with larger vertebrates, transmitter size has precluded its use on smaller amphibians until relatively recently. This technique has been particularly effective when working with cryptic species where traditional methods of observation are difficult or impossible (e.g., aquatic salamanders). To this end, we have now gained valuable insight into the habits of an aquatic amphibian which otherwise would likely have been overlooked (Peterman et al., 2008).

The eastern hellbender is a large, fully aquatic salamander found throughout the eastern United States (Petranka, 1998). They require cool, rocky, swift flowing streams and rivers with high levels of dissolved oxygen (Smith, 1907; Hillis and Bellis, 1971; Guimond and Hutchison, 1973). During the day, they spend most of their time under large, flat rocks, which they require for shelter and will defend from other conspecifics (Hillis and Bellis, 1971; Nickerson and Mays, 1973; Peterson and Wilkinson, 1996). Gravel and cobble substrates provide important areas for prey items and habitat for larvae. Hellbenders forage nocturnally and are thought to play an important role in influencing aquatic invertebrate populations, especially crayfish (Netting, 1929; Peterson et al., 1989; Humphries and Pauley, 2005). Hellbender populations have experienced dramatic population declines throughout their range (Mayasich et al., 2003). Despite this drastic reduction in range and population density, little research has been done regarding hellbender movements and home-range size in declining, low-density populations.

Previous attempts to document the spatial patterns of hellbenders have been limited in scope, occurred over a short duration, or suffered from inadequate sample sizes (Hillis and Bellis, 1971; Blais, 1996; Peterson and Wilkinson, 1996; Humphries and Pauley, 2005). Moreover, these studies focused on areas of high-population density (Hillis and Bellis, 1971; Peterson and Wilkinson, 1996; Ball, 2001; Humphries and Pauley, 2005). The factors influencing home-range size are largely unknown, but variance in population densities could greatly affect the movements and behaviors of individuals as resource availability changes (Hillis and Bellis, 1971). Future research will need to focus on geographic locations reflecting current hellbender population trends. As hellbender populations continue to decline and result in a concomitant decrease in population densities, empirical data focused on spatial patterns, movements, and habitat use in declining populations will become increasingly important.

The midwest region of the U.S. is likely experiencing the most dramatic declines in hellbender populations. In Indiana, historic hellbender distribution included most of the Ohio and Wabash River drainages (Petranka, 1998). However, during the past several decades, populations have been reduced to a single low-density population within the Blue River drainage. Given that Indiana populations occur at densities much lower than previously reported (Burgmeier et al., in prep), empirical data on the spatial ecology within this context is both unique and needed. The objectives of this study were to: 1) describe hellbender seasonal and spatial movement patterns, 2) estimate home range sizes between seasons and sexes, and 3) document habitat use in relation to river and benthic microhabitats in Indiana.

METHODS

Study Area and Site Selection

Eastern hellbenders were studied along a 112-km stretch within the Blue River drainage of southern Indiana. The Blue River intersects two of Indiana’s ecoregions originating in the Mitchell Karst Plain Section of the Highland Rim and crosses into the Escarpment Section of the Shawnee Hills region. The river winds through a complex of agriculture, forest, and rocky cliffs until ultimately converging with the Ohio River. The Blue River is comprised of long stretches of deep pools interspersed with riffles and runs. Average summer depth of our study area was 37.2 cm ± 20.5, while average wetted width was 19.3 m ± 5.0. The river includes long stretches containing a predominately bedrock bottom, while other areas consist of gravel and cobble. Most of the substrata, regardless of type, are covered by a thin layer of silt. Boulder-sized rocks (>256 mm diameter) suitable for hellbender refugia are spread throughout the river. A total of eight sites were selected based on habitat suitability, ease of access, and the known presence of hellbenders due to previous surveys by the Indiana Department of Natural Resources (IDNR). These sites have been surveyed annually by IDNR biologists for approximately 10-12 years and are known to be areas of recent hellbender activity.

Capture and Surgery

Hellbenders were collected from June through August 2008 and July 2009. Individuals were captured by hand or net within the river. Underwater goggles were worn to aid in visibility when needed. Individuals were placed ventral side up in a custom squeeze box, the “bender board”, to minimize struggling (Burgmeier et al., in press). We recorded total length (cm), snout-vent length (cm), weight (g), gender, and any noticeable physical abnormality on all captured individuals. All hellbenders were scanned with Biomark FS2001F-ISO and AVID Multiscan 125 PIT tag readers. If no tag was found, a Biomark 12.5 mm, 134.2 kHz tag was implanted in the dorsal side of the tail approximately 8 cm posterior to the hind leg. All animals were released at their point of capture immediately after processing.

Twenty-one individuals were selected from the eight sites to be implanted with radio-transmitters. All radio-telemetered individuals were adults ranging in snout-vent length from 26.6 cm – 38.5 cm and weighing between 370 g – 960 g. We generally followed the surgical procedures described in Stouffer et al. (1983), with notable exceptions to transmitter and suture type. A 13-g SI-2 transmitter (Holohil Systems Ltd. Carp, Ontario, Canada) was implanted and the incision was sutured with an absorbable Ethicon 3-0 PDS*II suture. The transmitters did not exceed 5% of total body weight. Following surgeries, animals were placed within a screened enclosure in slow-moving freshwater until recovery (all animals recovered from surgeries). Four additional transmitters were implanted to replace individuals that died, were lost, or experienced transmitter failure approximately 11 months into the study.

Radio telemetry and data collection

Individuals were tracked twice weekly (on average) during the spring, summer, and fall 2008 and 2009. Tracking was increased to three times weekly during the breeding season and decreased to once weekly during the winter. In September 2008, four individuals were tracked at one site during a 24 hour period to gather preliminary data on diel movement patterns and confirm our tracking efforts captured fine-scale diurnal movements. When individuals were located, surveyors recorded habitat measurements including GPS coordinates (Garmin GPSmap 76, Garmin LTD., Olathe, Kansas, U.S.A.; UTM, Accuracy s; 4 m), water temperature (^{o}C), water depth (cm), distance to shore (m), distance (cm) to nearest shelter rock >25.6 cm diameter, number of shelter rocks within 6-m radius, weather (sunny, partially cloudy, cloudy, raining), substrate type (bedrock, silt, sand, gravel, cobble), flow type (riffle, run, pool), and rock length (cm), width (cm), and depth (cm). Flow-rate data were acquired from the USGS National Water Information System (USGS, 2010). Wolman pebble counts were conducted at six of eight radio-telemetry sites to determine streambed substrate composition at the time of the study (Wolman, 1954). Counts were not conducted at the remaining two sites due to extensive flooding.

Data analysis

ArcGIS 9.2 (ESRI, Redlands, California) and the Hawth's Tools (Beyer, 2004) extension were used to develop intermovement distances, direction of movement, 100% minimum convex polygons (MCP) and linear home ranges (LHR) for all hellbenders with at least three or two movements, respectively (Mohr, 1947). Individuals that did not move during a given season were not included in standard analyses. Although there is some criticism of MCPs encompassing non-use areas, the sites used were mostly linear and confined by riverbanks making any such scenarios easily noticed and rectifiable (White and Garrott, 1990). In the few cases where MCPs did include areas of known non-use (i.e., land), they were conservatively modified before calculation to minimize home-range size overestimation. This modification results in a more realistic representation of hellbender home range as they are strictly aquatic and do not utilize terrestrial habitats. Linear home range (LHR) provides information regarding the length of stream used by individuals and is suitable for species living in fairly homogenous, linear environments such as small streams and rivers (Skains and Jackson, 1995).

Herein, we define LHR as the distance between the two most extreme locations of a single animal.

We estimated MCP and LHRs at multiple levels. First, we wanted to obtain an average home-range estimate using all individuals across all seasons. We also wanted to estimate seasonal home ranges. To obtain these seasonal estimates, we used the combined data sets for males and females for each of the four seasons based on changes in water temperature in a manner similar to Daugherty and Sutton (2005). Lastly, we wanted to compare the home-range sizes between males and females during the breeding and non-breeding seasons.

The number of locations found in both flow and substrate types were compared for all individuals to assess habitat use. We evaluated the influence of flow rates on the number of movements using linear regression. Wolman pebble counts were analyzed using the size-class pebble count analyzer developed by the United States Department of Agriculture (Potyondy and Bunte, 2002). A t-test was used to determine any significant differences between seasons or genders for home range and movements. A chi-square goodness of fit test was used to determine habitat availability versus habitat use. Where appropriate, results are presented as mean ± standard deviation. Data were log or square-root transformed in instances where normality assumptions were not met. Non-parametric Mann-Whitney U tests were performed when normality assumptions could not be attained via transformations. In cases of normality, but non-constant variance, a Satterthwaite's Approximate T-test was used. All statistical analyses were conducted in SAS 9.1.3 or JMP 8.0 and tests were considered significant at alpha<0.05.

RESULTS

We recorded 1193 total locations (mean = 57 ± 23.9/individual; median = 65) from 21 radio-tagged adult hellbenders (13 males: 4 females: 4 unsexed) from June 2008 - October 2009. Transmitter failure was low throughout the study with only one transmitter failing in 2009. However, we lost an additional three transmittered animals due to unknown causes during the spring of 2009. In each of these cases, the radio-tagged hellbender was never located, but the transmitters were found along the bank ridge several meters from the water. It is suspected that these animals were either illegally captured or killed by a predator.

Movement Patterns and Site Fidelity

A total of 297 movements were detected over the entire tracking period. For the 21 telemetered hellbenders, the mean number of movements per individual was 14.1 ± 9.1. Hellbenders moved a mean of 10.2 ± 3.4 times during the summer, significantly more than all other seasons ($t=5.437$, $p<0.00002$; Fig. 2.1). Very few movements were recorded outside of summer and no significant differences were detected between spring, fall, or winter ($F_{2, 52}=1.991$, $P=0.147$; Fig. 2.1). Nearly all spring movements took place within the final three weeks of the season leading into summer. Hellbenders moved significantly more (N=120) during the breeding season than during the non-breeding season (N=170) when season lengths were taken into account ($x^2=194.01$, $p<.05$). With the exception of one sample site, individuals typically moved within the same small areas throughout all seasons.

The mean intermovement location distance by 20 hellbenders was 27.5 ± 6.5 m. One individual of unknown gender moved downstream 347 m between successive tracking events shortly after its initial capture, but only moved once during the study and thus was excluded from the analysis. There were no significant differences between the number of upstream and downstream movements for the overall population ($x^2=0.016$, $p=0.9$). We found no relationship between discharge (cubic meters per second) and the initiation of movement ($r^2=0.032$, $p<0.05$). During one 24 hour tracking period conducted on four individuals, only a single movement was recorded by one individual shortly before 10:00 p.m.

Of 1193 total locations, only six were made on a hellbender that was actively moving outside of cover. Three of these locations (two summer and one fall) were from one individual of unknown gender near its usual shelter rock. The other three occasions were from three different hellbenders (two females and one male) at three separate sites. During the summer, one female was found moving in a leaf pile a few meters from her usual shelter rock. In late spring, we tracked one female to within a few meters from her usual large (2.7 m diameter) shelter rock and then she quickly moved

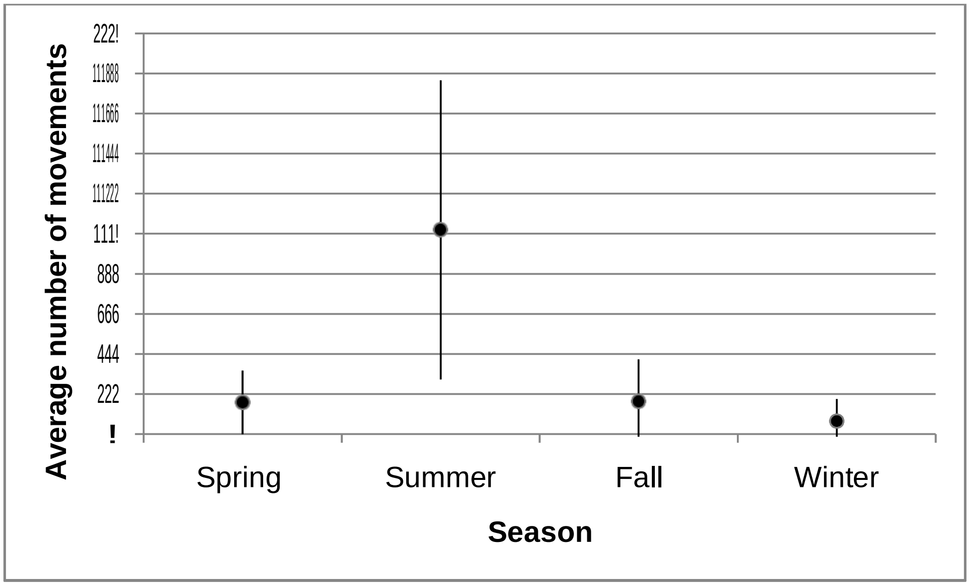

Fig 2.1. Average number of movements per season for 21 (summer/fall) and 17 (spring/winter) eastern hellbenders from southern Indiana. Circles and lines represent the mean and standard deviations for each season, respectively. The tracking period was from June 2008-October 2009.

approximately 20 m downstream under a small shelter. She returned to her normal rock within the week. The last individual was an untagged male being attacked by an American mink (*Neovison vison*) during mid-day. The hellbender was captured and checked for wounds. A single large laceration was found on its abdomen. This individual was not seen again during our study.

Home-Range Estimation and Overlap

Estimates of home-range size differed considerably between individuals, seasons, and sexes. The mean MCP for all individuals (i.e., data for the sexes were combined) with at least 35 locations spanning across the four seasons was 2211.9 ± 990.3 m^2 (N=16). Mean LHR for all individuals was 144.0 ± 57.7 m (N=21). There were significant differences in mean MCP home-range sizes between climatic seasons (x^2=9.30, p=.026; Fig 2.2a). In general, hellbenders maintained the largest MCP's during the summer, averaging 1544.8 ± 785.5 m^2 (N=18). During fall, the mean home-range size decreased to an average of 643.9 ± 442.2 m^2 (N=7). Home-range sizes were smallest throughout the winter and spring at 290.0 ± 402.8 m^2 (N=3) and 150.6 ± 121.0 m^2 (N=7), respectively. Spring MCP were significantly different than both summer (t=4.14, p=0.0004) and fall (t=2.63, p=0.0302), but no other seasonal differences were detected (Fig 2.2a). We detected a significant difference between summer and spring LHR (U=-1.96, p=0.0495), but no other seasonal differences were detected (Fig 2.2b).

When combining data from both sexes, the mean breeding MCP home range size was 1132.9 ± 635.7 m^2 (N=18), whereas the mean non-breeding MCP was 1395.3 ± 501.5 m^2 (N=16); these were not found to be significantly different (t_{32}=1.01, p=0.3184). Despite considerable differences between genders in both mean breeding and non-breeding MCP home-range sizes; none were detected at significant levels (Fig. 2.3a). No breeding season versus non-breeding season LHRs comparisons were significant (Fig. 2.3b). MCP sizes for males and females across all seasons were 2844.6 ± 1493.1 m^2 (N=9) and 675.8 ± 1648.4 m (N=3) respectively. Male MCP size was significantly larger than female (T=3.06, p<0.01).

Home-range overlap occurred at all sites and with multiple individuals of each gender, but only during the summer (which did include portions of both breeding and non-breeding seasons). The greatest overlap between two individuals (2685.4 m^2)

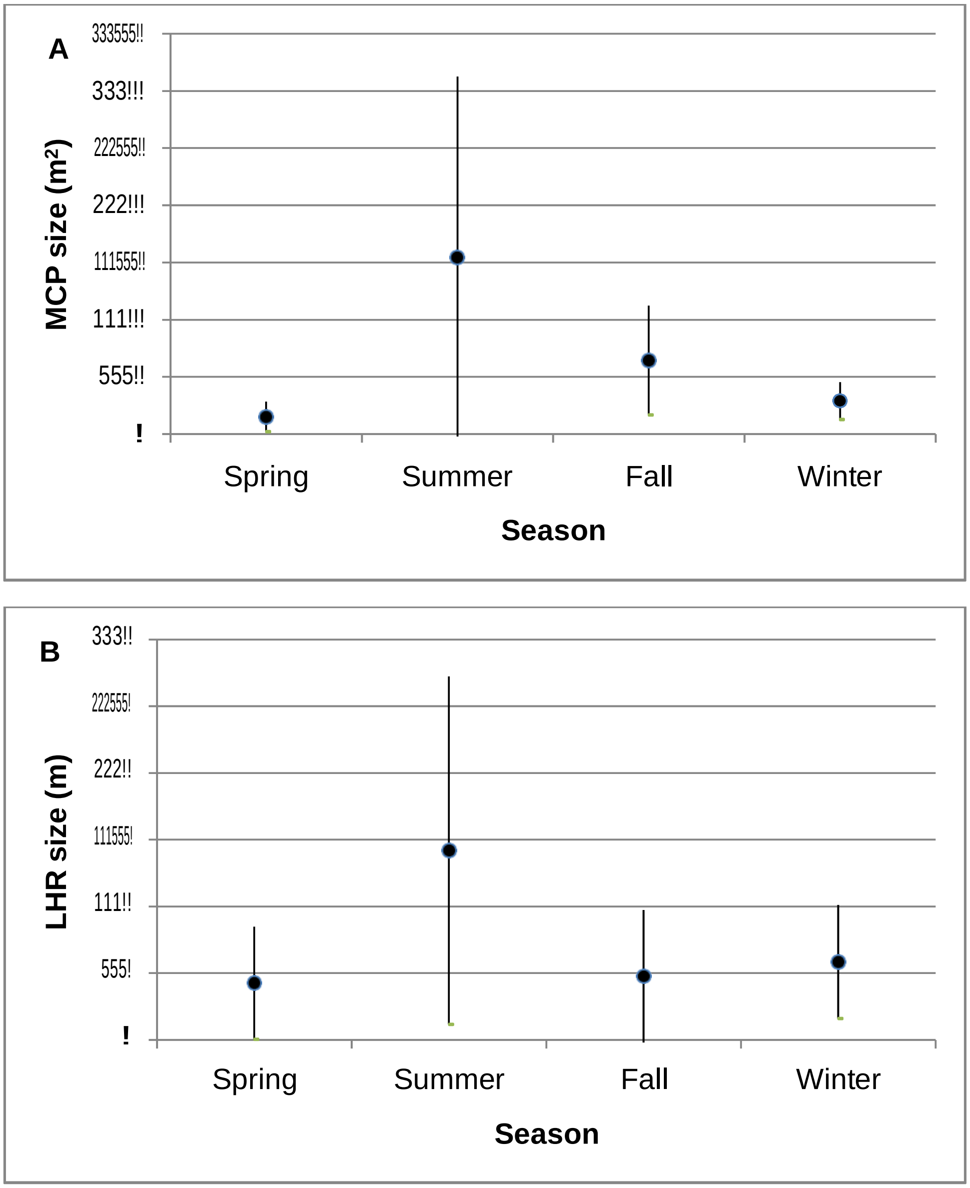

Fig. 2.2 a, b – (a) Average seasonal MCP size (m^2) for all individuals with at least three movements during a given season, (b) Average seasonal LHR size (m) for all individuals with at least two movements during a given season. Circles and lines represent the mean and standard deviations for each season, respectively. The tracking period was from June 2008-October 2009.

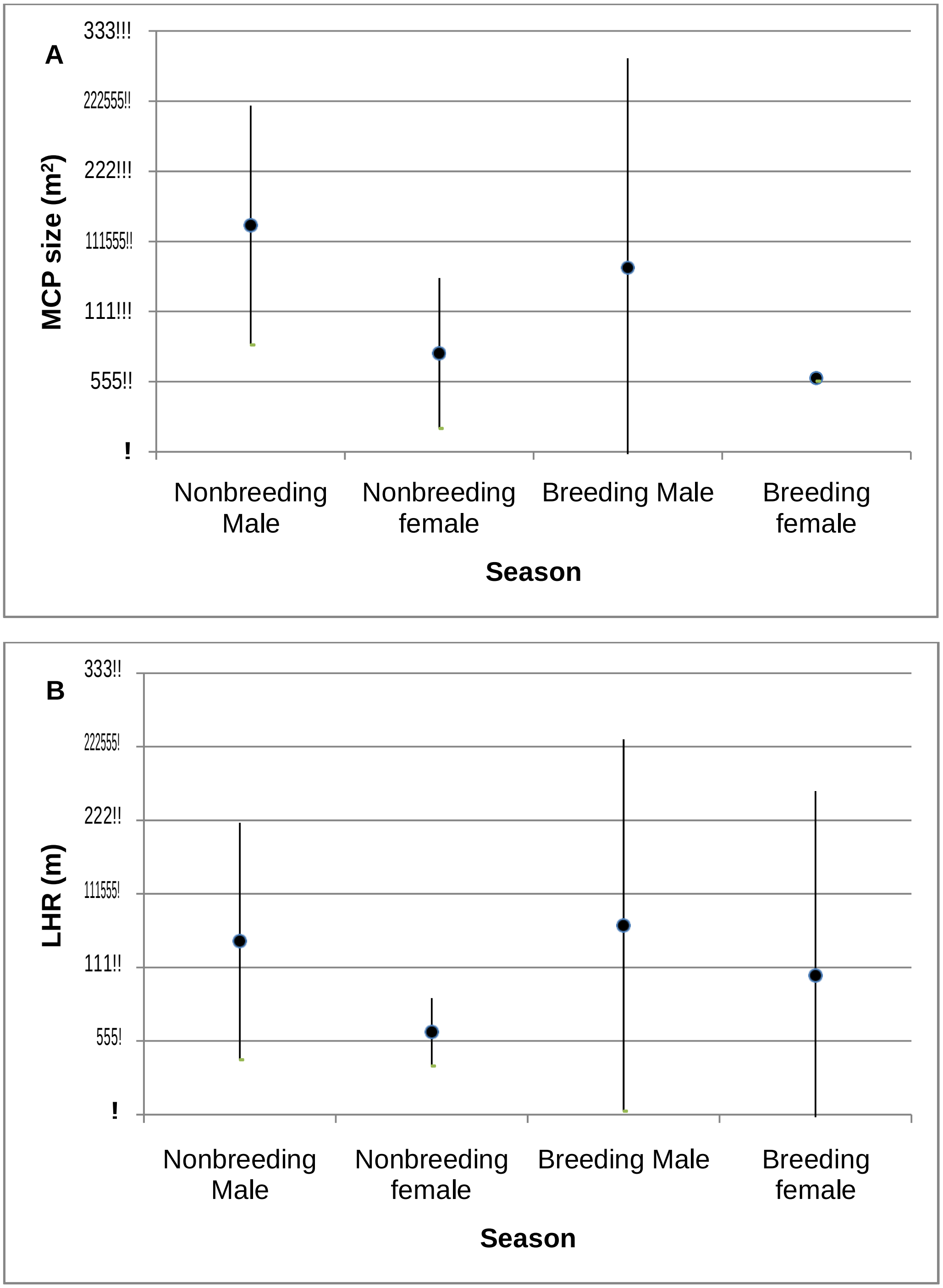

Fig. 2.3 a, b – (a) Comparison of average breeding versus non-breeding MCP size (m^2) by gender for all individuals with at least three movements during a given season (b) Comparison of average breeding versus non-breeding LHR size (m) by gender for all individuals with at least two movements during a given season. Circles and lines represent the mean and standard deviations for each season respectively.

occurred at one site with four radio-tagged animals (three males: one unknown). All individuals at this site overlapped with at least two other individuals. The smallest area of overlap was a 12.7 m movement by one individual into another individual's home range. Only once were two individuals (one male, one female) located under the same shelter. This occurred during the non-breeding season and the shelter was comprised of two large rocks stacked one on top of the other.

Habitat Use and Selection

Gravel was the most frequently used substrate throughout the study. Seventy-nine percent of all hellbender locations were associated with gravel, compared to 11.5% with silt and mud. This trend was consistent across seasons, but varied from substrate availability with gravel being overrepresented and all other categories underrepresented (x^2=931.53, $p<0.05$). Wolman pebble counts revealed that gravel makes up the majority of the substrate in most cases at 38.9% (Fig. 2.4.). Moreover, when the boulder size classes are excluded from our data set, the actual percentage of gravel as available substrate is approximately 42.1%.

Flow-type use was skewed heavily towards runs which contained 77% (N=925) of all locations. Pools were used 22% (N=267) of the time and riffles only 1% (N=5). These trends are inconsistent with availability with runs and pools being overrepresented and riffles underrepresented (x^2=407.53, $p<0.05$). Throughout the study, hellbenders were found at water depths ranging from 7.4 cm to greater than 177 cm. Mean summer depth at locations were 59.9 ± 2.1 cm, while overall mean depth was 66.6 ± 1.9 cm. At one site, all four individuals (3 males, 1 unknown) moved into deep pools during the breeding season. One individual returned to shallow water shortly thereafter, but all three males overwintered in these pools. All three moved back to their normal areas of activity in late spring or early summer.

The most frequently used shelter rocks detected via radio-telemetry were between 5000 and 15000 cm^2 (60.16%), but shelter sizes ranged from 1033.9 cm^2 to 159448.3 cm^2 (Fig 2.5). Shelter rocks used during the spring, fall, and winter were significantly larger than those used during the summer (x^2=39.69, $p<0.0001$). Four individuals were located under bedrock throughout the study, but only two made

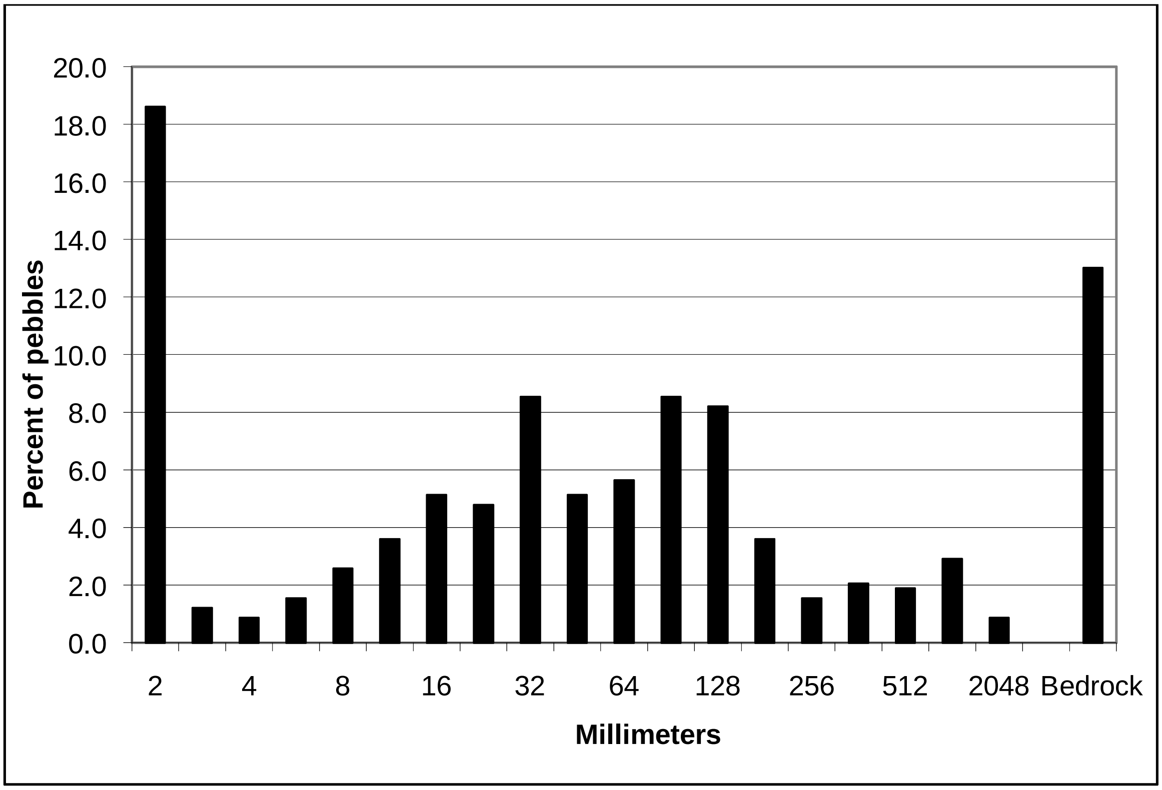

Fig. 2.4 – Study area particle size distribution for six sites in the Blue River, Indiana, during the 2008 study season determined via Wolman pebble counts. Vertical bars represent the percentage of the total particle substrate represented by each size class.

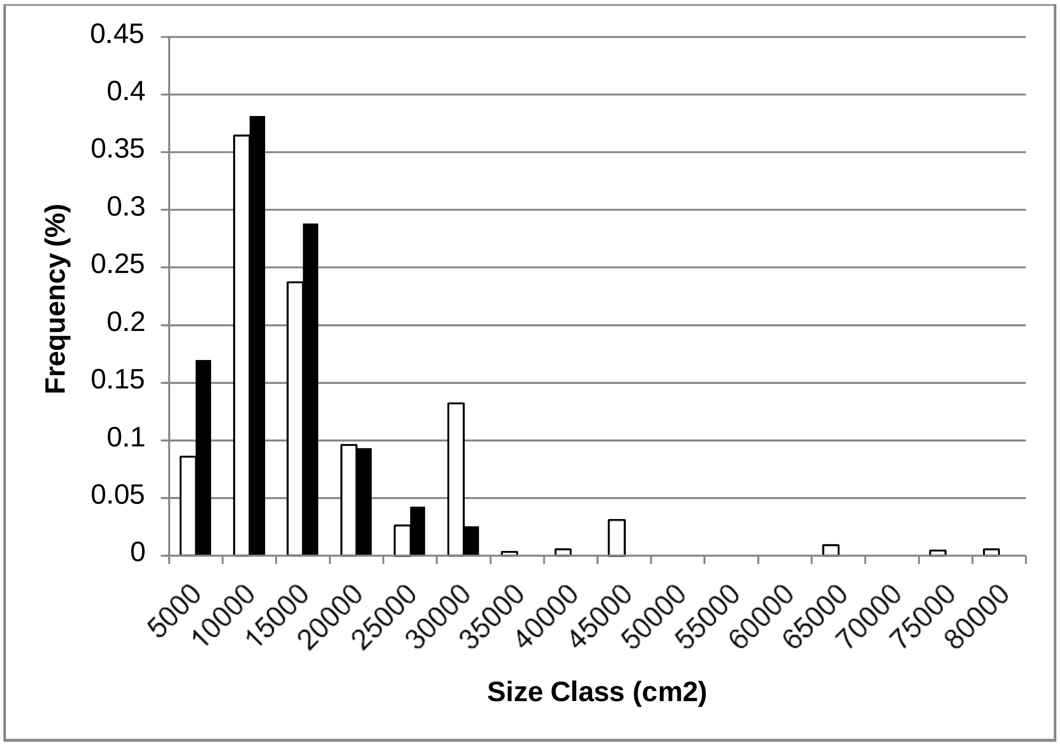

Fig. 2.5 – Frequency distribution of shelters used by hellbenders found during radio-telemetry study (gray bars) versus those found during a mark-recapture study (Burgmeier et al, in prep.; black bars).

extensive use of it. Both of these individuals were found in areas with large amounts of undercut bedrock, which provided ideal shelter. One female made frequent use of a large, downed tree located in the center of her home-range, while a male at a different site was found for a short time during the summer under a tree root mass along the riverbank before moving back under a frequently used large rock.

Shelter rocks detected during mark-recapture surveys were similar to those during radio telemetry with 83.90% being less than or equal to 15000 cm^2, but ranged from 1598.4 cm^2 to 26784 cm^2 (Fig. 2.5). Hellbenders disproportionately used large boulders (> 10485.76 cm^2) as shelters over smaller size classes (x^2=1803.39, $p<0.05$). Mean distance of shelter rocks to shore was 5.1 ± 0.2 m (N=584). The mean distance from located shelter rocks to the nearest adequate shelter rock was 17.2 ± 3.2 cm (N=540). The mean number of adequate shelter rocks within a 6-m radius of a located hellbender was 50.6 ± 4.7 rocks (N=405).

DISCUSSION

Movement Patterns and Site Fidelity

Hellbenders are thought to be relatively sedentary with only subtle differences in the number of movements during certain periods throughout the year. Ball (2001) reported that only 108 of 989 (11%) observations were movements in a North Carolina population of hellbenders. In this study, however, we found that roughly 25% (297 of 1,193) of our observations were movements. We detected significantly more movements during the summer months than all other seasons. Our results are concordant with Blais (1996), but are in stark contrast to Ball (2001) who reported increased activity in the spring, intermediate activity in the summer, and decreased activity in the fall and winter. Hellbenders are thought to spend a considerable amount of time searching for prey during late spring and early summer leading into the breeding season when they begin searching for nesting sites and mates (Smith, 1907; Nickerson and Mays, 1973; Blais, 1996). Indeed, those activities fall within our summer tracking season (May – Sept.) and represent the most likely explanation for the increased movements. To this end, when accounting for the difference in the length of the non-breeding and breeding seasons, hellbenders were found to move significantly more during the breeding season than during the non-breeding season. During the breeding season, it is expected that males would be searching for suitable nest rocks while females would be searching for males.

While increased movements are associated with feeding and breeding activities, other factors may potentially limit movement during other times of the year. For example, hellbenders may seek refuge under shelter rocks and limit their movements in direct response to extensive flooding events during late winter or early spring. Our analysis, however, suggests that hellbender movement was unaffected by either high or low flow events. Flow direction, particularly during high-flow periods, may also affect hellbender movements. Topping and Peterson (1985) reported significantly more upstream than downstream movements, but Peterson (1987) detected no significant difference. We detected no difference in the movement patterns with regard to stream direction, regardless of season or flow levels.

Hellbenders generally do not move large distances (Mayasich and Phillips, 2003), but there are several accounts of relatively long distance movements from 990 m (Nickerson and Mays 1973) up to 5 km (P. Petokas, pers. comm.). We documented a single long distance movement (347 m) by an individual of unknown sex. However, the majority of the movements in this study averaged 28 m, which is consistent with the mean linear movements reported in previous studies of 20.1 m (Humphries, 1999) and approximately 19 m (Hillis and Bellis, 1971). Most movements were between several preferred rocks within a small area. The lack of long distance movements can have profound impacts on a species with low population densities by limiting the amount of gene flow and reducing the overall effective population size (Wang, 2009).

Home Range Estimation and Overlap

Our results suggest that hellbenders in Indiana maintain much larger home ranges than those reported in other studies (Table 2.1). Several possible explanations exist for the increased home-range sizes. Several studies relied on mark-recapture methods for home-range estimation (Hillis and Bellis, 1971; Peterson and Wilkinson, 1996; Humphries and Pauley, 2005). This method possesses several drawbacks, most notably, that it is time consuming and results in relatively few locations per individual,

Table 2.1. Previous studies providing home-range estimates for eastern hellbenders.

Author	Date	# Ind.	# Loc. /Ind	Study Duration	Method	HR Estimator	HR Size (mean)	Location
Blais	1996	16	~30	3 – 36 weeks (varied per animal)	RT	LHR	23.2 – 110.3 m	NY
Coatney	1982	7	-	2 weeks	RT	Elliptical	90 m^2	MO
Gates	1983	5	-	4 months	RT	MAR	4 m	MD
Hillis & Bellis	1971	73		3 months (9 surveys)	MR	MAR	346.4 m^2	PA
Humphries & Pauley	2005	9		2 years (29 surveys)	MR	MCP	198 m^2	WV
Peterson & Wilkinson	1996	26		5 months (33 surveys)	MR	MCP	36m^2 (f), 95m^2 (m)	MO

Method: RT = radio-telemetry, MR = mark-recapture; HR (Home Range) Estimator: LHR = linear home range, MAR = mean activity radius, MCP = minimum convex polygon; HR Size: f = female, m = male.

especially in colder months. As suggested by Humphries and Pauly (2005), this creates a relatively conservative estimate and likely underestimates true home-range sizes.

Drawbacks to mark-recapture methods notwithstanding, another explanation for our increased home-range estimates is that we used radio-telemetry and collected a much larger number of locations over a much longer period. This decreases the likelihood of home-range underestimation with the use of MCP methods (Boulanger and White, 1990; Arthur and Schwartz, 1999; Humphries and Pauly, 2005). A third explanation is that home-range sizes may reflect population densities. Unfortunately, there are no experimental studies that have examined the relationship between density and home range for hellbenders. Kleeberger (1985) found that home-range size increased at high densities in *Desmognathus monticola*, a stream-dwelling salamander. As the population density increased, these salamanders searched a larger area for food and shelter. Humphries and Pauley (2005) calculated a mean MCP of 198 m^2 for a high density population (0.8-1.2 individuals/100 m^2) of hellbenders in West Virginia. Conversely, the home-range sizes found in this study are much larger, yet the population density is extremely low (0.038 individuals/100 m^2; Burgmeier et al., in prep.). Home-range sizes in this study were largest during the summer months and correspond with the breeding season. Given the low population densities, it is plausible that the increase in home-range size results from individuals moving greater distances in search of mates.

Finally, differences in home ranges between studies might be due to habitat differences among the study sites. Differing types and availabilities of refugia might affect the distance an individual needs to move to reach a new shelter. Kleeberger (1985) found no effect on the home-range size of *Desmognathus monticola* when cover objects were added to study plots. However, Spieler and Linsenmair (1998) reported that with increasing cover (i.e., bushes) the home-range size of the frog *Hoplobatrachus occipitalis* decreased considerably when compared with areas containing fewer bushes. Our study areas are replete with adequately sized shelter rocks. Males typically display territorial behavior over specific shelter rocks; however, this behavior might be reduced given the high density of available shelter and the lack of competition from other individuals, thus freeing individuals to move freely between rocks with little risk of injury. It is important to note that all tracking was conducted during the daylight hours when hellbenders are typically inactive. It is possible that movements could have occurred in between tracking periods, and therefore our estimates should be viewed as conservative

(Hayne, 1949). However, our results during a 24 hour summer tracking session showed very little movement and the IDNR has reported a lack of success during night time surveys in these same areas (Walker, Z, pers. comm.).

Few significant differences were found between seasons or genders for either MCP or LHR methods. This is likely due to the high amount of variance found within categories and the small female sample size. Hellbenders are sexually dimorphic for only a short period during the breeding season (Smith, 1907) which limited our ability to assess gender prior to surgeries. While every attempt was made to obtain equal sex ratios, surgeries were conducted almost exclusively outside the breeding season to minimize interference with reproduction. This precautionary effort made accurate gender differentiation impossible unless developing ova were encountered during the procedure.

Gender identification notwithstanding, we found that seasonal MCP size was significantly different between summer/fall and spring. This corresponds with known hellbender behavior as movements typically do not begin until late spring or early summer to forage and the breeding season does not end until early fall (Kern, 1986). Despite summer MCPs being twice as large as fall and winter MCPs, the high variance and low sample size due to inactivity in the cooler months resulted in a lack of statistical significance. Male 100% MCP size was found to be significantly larger than females. Peterson and Wilkinson (1996) found a similar relationship using MCPs, but Hillis and Bellis (1971) reported no difference between sexes while using mean activity radii. Blais (1996) found female LHR to be slightly larger than males. Given our small female sample size, any intersex gender comparisons should be viewed with caution. In light of this information, it is important to consider that the Blue River population is of low density and restricted to only adult individuals. Results could vary in populations with multiple age classes and higher population densities.

Home-range overlap has been reported in other studies, particularly during the breeding season (Blais, 1996; Humphries and Pauley, 2005). During this study, there was considerable overlap among individuals at each site. Overlap occurred at all sites, with multiple hellbenders, and increased with increasing numbers. At one site, all hellbenders overlapped with at least two other individuals. At most sites, there were numerous untagged individuals that would increase the amount of overlap. Territoriality was not quantified, but we did observe one instance that between successive locations a tagged individual was found under a rock previously used by a different individual for the past nine months. The apparently displaced individual had moved 5-m to a new location. The new occupant was male, but the sex of the displaced individual was unknown. We also observed numerous occurrences of tagged individuals using rocks that had at some point in the past been used by other individuals. Coatney (1982) reported that individuals typically avoided areas of overlap. In this study, two tagged individuals of opposite sex were found under the same set of rocks where the female had been located for the previous eight months. This occurred well over a month before the breeding season, and in this case, the shelter consisted of two very large, flat rocks stacked on top of each other. This frequent overlap is likely due to a combination of factors including high shelter density and decreased hellbender density. More shelter rocks are available per individual, thus reducing the need to defend specific areas and individuals are moving farther during the breeding season resulting in larger areas of overlap. However, the highest amount of overlap occurred at a site with four individuals, three of which moved upstream to the same deep pool during the breeding season. This shared movement accounted for a considerable portion of the overall overlap in this area.

Habitat Use

We found that eastern hellbenders in Indiana are typically found in cool, moderately flowing waters with a substantial amount of gravel or cobble substrate and large flat rocks as shelters. These results are consistent with studies conducted in other portions of their range (Ball, 2001; Nickerson et al., 2003; Humphries and Pauley, 2005; Keitzer, 2007). Humphries and Pauley (2005) reported that hellbenders were associated with gravel substrates and no individuals were found in heavily silted areas. While the majority of the hellbenders in this study were associated with gravel substrates, over 11.5% of the locations occurred in heavily silted areas. The Blue River is characterized by long stretches of gravel substrate surrounded by areas of moderate siltation. There is considerable siltation at some sites represented in patches within stretches of gravel substrate. These findings are not surprising given the abundance of gravel substrate at the sites and the increased presence of siltation relative to other populations.

Large, fast flowing riffles are often cited as critical hellbender habitat (Smith, 1907; Mayasich and Phillips, 2003; Humphries and Pauley, 2005), but the majority of locations in this study were associated with runs and not riffles. Riffle areas in the Blue River tend to be short, shallow, and comprise a small proportion of the overall available habitat relative to other flow types. However, we did detect a significant underrepresentation of riffle use when compared with availability. It is possible that the patchy distribution of riffles within our study area accounts for their relative lack of use when compared to the longer, more prevalent runs.

A subset of the radio-tagged individuals exhibited obvious shifts in habitat use during this study. Four individuals at one site moved to deep pools (>1.2 m) adjacent to the site in late summer or early fall and remained there until spring when they moved back to previous shelters in shallow water. The site was similar to others with regards to habitat structure; however, this site maintained a much higher density of hellbenders than most other sites. Given the timing of the movements, it is possible that these individuals were moving to deeper areas for nesting purposes to escape high flow areas. Nests have been reported at this site during previous surveys. It is unlikely that individuals use these deeper pools for relief from frigid water temperatures expected in the shallow areas of the site as a large spring feeds into the center of the site keeping water temperatures considerably warmer than surrounding areas. However, the deeper water might reduce the impact of increased winter flow rates on individuals trying to conserve energy. During surveys, shelter rocks within the site were disturbed several times throughout the season and inadequate accumulation of sediment might increase the amount of flow penetrating under rocks. Deep-water habitats have not been extensively surveyed in the Blue River, but further surveys targeted at these areas might garner important information for future conservation efforts.

Previous research has highlighted the importance of large, flat rocks as critical shelter for hellbenders (Hillis and Bellis, 1971; Humphries and Pauley, 2005; Keitzer, 2007). While our data are generally consistent in that regard, we have documented several striking discoveries. Overall, hellbenders seemed to utilize the medium and larger size classes of boulders (>2621.44 cm^2) at a much higher frequency than expected. This trend is even more apparent when comparing radio-telemetry versus traditional mark-recapture methods (Burgmeier et al., in prep.) to document hellbender use of shelter rocks. We discovered that hellbenders readily used shelter rocks far too large for researchers to lift during traditional rock-flipping surveys. At least six of the largest shelter rock size classes were underrepresented and could lead to a bias in the importance of smaller rock size classes. We also found that hellbenders used significantly larger rocks during non-summer months. It is possible that hellbenders are taking refuge under larger shelter rocks to reduce disturbance during colder months when energy conservation is important. In contrast, it may simply be that this population is composed of large, senescent adults and the smaller rock classes do not fully shelter these individuals or provide the necessary protection from the current or light. Lastly, we document that hellbenders will often utilize alternative structures for shelter. We located several individuals under large logs, bedrock, and under root masses along the riverbank. Similar behaviors were previously reported in Blais (1996). These habitat types are abundant in most lotic systems and could provide refuge for a number of individuals. This is an important consideration when conducting hellbender surveys. Failure to incorporate these structures in surveys could lead to underestimation of population sizes and spurious management recommendations.

Alternative shelters might be an important characteristic when choosing suitable shelters. Mean distance to the nearest shelter rock was 17.2 cm and the average number of suitable shelter rocks within a 6-m radius was 50.6 rocks. These numbers indicate hellbenders typically inhabit areas where alternative shelters are both accessible and abundant. Whether these findings are an artifact of sampling bias, habitat availability, or legitimate preference is worth further exploration.

As mentioned previously, hellbender densities in Indiana are low and the population is skewed towards large adults, and results may differ under different demographic scenarios. However, populations across the species range are beginning to experience shifts in demography similar to those found here. Data such as those reported herein provide valuable insight on the habits of eastern hellbenders that allow managers to develop conservation plans that target severely declining populations.

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CHAPTER 3

HEALTH AND HABITAT QUALITY ASSESSMENT FOR THE EASTERN HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS ALLEGANIENSIS*) IN INDIANA

ABSTRACT

The Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) has experienced precipitous population declines throughout its range, particularly in Indiana. The remaining population is limited to older, adult age classes characterized by a lack of recruitment. Numerous factors are speculated to be involved, but no empirical evidence substantiating these claims has been presented. We implemented a population-wide health assessment in Indiana examining both the physical well-being of individuals and the quality of their habitat. Physicochemical parameters were analyzed directly in the field and later in the laboratory, when appropriate. Samples were collected from June – October 2008 and June – September 2009 for reproductive analysis, blood screening, and disease prevalence. Of the 27 chemicals screened in water samples, three were found in the study site, including atrazine. Atrazine was found at levels reported to cause reproductive problems in other amphibians. Vitellogenin was detected only in females and proved a reliable indicator of gender. Sperm parameters were generally of high quality and similar to other populations. Most plasma parameters were similar between sexes, although there were significant differences in calcium and potassium concentrations. Abnormalities were common, occurring in 68.2% of individuals. No hemoparasites were found, but chytrid fungus (*Batrachochytrium dendrobatidis*) was detected on one individual. Our findings establish a baseline for hematology and water-quality parameters that can be used as a model for evaluating population health throughout the hellbender range.

INTRODUCTION

Amphibian populations throughout the world have been declining for several decades (Barinaga 1990; Stuart et al. 2004; Nyström et al. 2007). While habitat destruction and climate change remain primary areas of concern among biologists, it has become increasingly evident that some amphibians are declining as a result of deteriorating health, especially disease (Lips et al. 2006). Water quality can have a profound effect on individual health and potentially contribute to the continued decline of aquatic amphibian populations, many of which spend part or all of their lives in aquatic environments. Recent research suggests that stressors such as pesticides and other contaminants may result in male feminization, decreased survival, increased susceptibility to disease, and amphibian malformations (Hayes et al., 2002a; Storrs and Kiesecker, 2004; Relyea, 2005; Forson and Storfer, 2006; Hayes et al., 2006). Additionally, a species of chytrid fungus (*Batrachochytrium dendrobatidis*) has been found in many amphibian populations around the world and may be partially responsible for the decline and extinction of many infected populations. Herein, we examine the relationship between water quality and animal health using a long-lived, obligate paedomorphic salamander, the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis)*

Given its life history, the eastern hellbender is especially sensitive to issues of poor water quality. Hellbenders are restricted to cool, rocky, flowing rivers and streams with high levels of dissolved oxygen (Smith 1907; Hillis and Bellis 1971). Flowing water is necessary to facilitate optimal respiration via cutaneous oxygen diffusion (Guimond and Hutchison 1973, Ultsch and Duke 1990). The effects of chemical contamination on hellbenders are unknown, but continual exposure to endocrine disrupting chemicals could lead to feminization in males and potentially disrupt reproductive efforts (Hayes et al. 2002a). Vitellogenin assays are commonly used as a biomarker in fish to indicate exposure to estrogenic chemicals present in the ecosystem (Heppel et al., 1995; Palmer and Palmer, 1995; Sumpter and Jobling, 1995; Selcer et al., 2001). Vitellogenesis is the process whereby yolk is deposited in developing eggs and the associated vitellogenin protein is usually only present in females. However, vitellogenin production has been induced in laboratory studies in male red-eared sliders (*Trachemys scripta*), African-clawed frogs (*Xenopus laevis*), tiger salamanders (*Ambystoma tigrinum*), and eastern hellbenders when exposed to estradiol, diethylstilbestrol, and DDT (1-chloro-2-[2, 2, 2-trichloro-1-(4- chlorophenyl) ethyl] benzene (Palmer and Palmer 1995, Nespoli et al., unpublished). More recently, vitellogenin induction has been shown in the common frog (*Rana temporaria*) when exposed to ecologically relevant doses of ethinylestradiol (Brande-Lavridsen et al., 2008). This is especially important for permanently aquatic salamanders that could be exposed throughout their lifetime and continuous exposure during the pesticide application season.

Chemical contaminates not only act as estrogen mimics, but recent research has indicated that exposure to certain commonly used agricultural chemicals can result in significant effects in the semen quality of human males (Hauser et al., 2002; Swan et al., 2003). Given the hellbenders fully aquatic nature and mode of reproduction, pesticide exposure could greatly reduce the species reproductive output. Many existing hellbender populations are comprised primarily of adults in older age classes and are already exhibiting low reproductive output (Wheeler et al., 2003). Assessing the reproductive output of males is one method of addressing this lack of recruitment at the prezygotic stage.

Lastly, in order to evaluate amphibian declines in light of environmental factors, animal health assessments that incorporate hematological screens, parasites analysis, and estimates of disease prevalence at the population level are critical. Hematology has long been used by wildlife and veterinary professionals as a means to evaluate the health of animal populations and their individuals. It is capable of revealing a wide variety of information about an individual's overall condition and can be used to diagnose specific ailments (Ciesla, 2007). An additional measure of population health is estimates of physical abnormalities (Stocum, 2000; Blaustein and Johnson, 2003; Taylor et al., 2005). Abnormality rates have recently been reported that exceed the expected rates of 0 – 5% (Johnson et al., 2002; Levey, 2003; Miller and Miller, 2005; Williams and DeWoody, 2009). There are a wide range of purported causes including parasites, trauma, UV-B radiation, and chemical contaminants (Blaustein et al., 1997; Johnson et al. 2002; Blaustein and Johnson, 2003). While some hellbender abnormalities are thought to be the result of intraspecific confrontations, abnormalities are reported at rates much higher than expected (Hiler et al., 2005; Miller and Miller, 2005). Complete animal health screens also include disease prevalence. Chytrid fungus has a history of causing widespread population declines in other amphibians and has been implicated in the die-off of a large captive population of hellbenders (Unger, personal comm.). Moreover, it has recently been reported in areas of Georgia, Kentucky, Missouri, North Carolina, Ohio, Pennsylvania, and West Virginia. Chytridiomycosis is a highly infectious disease caused by chytrid fungus and is thought to disrupt cutaneous osmoregulation in amphibians resulting in lethal electrolyte imbalances (Voyles et al., 2009). The cool temperatures hellbenders prefer and the fungus' aquatic reproductive cycle puts the hellbender at an increased risk of infection if conditions are favorable.

Our study addresses these questions within the context of a declining hellbender population in Indiana. Hellbender populations in Indiana are now relegated to a single river within the Ohio River basin (Kern, 1984). The river passes through areas of intensive agricultural use, including livestock operations and row cropping, which has raised concerns about how water quality may influence the health and persistence of the population. The specific goals of the study were to: 1) monitor water-quality parameters including physical, nutrient, and chemical properties on a weekly basis, 2) examine blood plasma for the presence of vitellogenin and its use in gender determination, 3) establish baseline hematological characteristics of captured individuals, 4) evaluate sperm quality, and 5) document the occurrence and frequency of physical abnormalities, parasites, and chytrid fungus.

METHODS

Study Area and Surveys:

All surveys were conducted along a approximately 112 km stretch of the Blue River in southern Indiana. The Blue River runs through a complex of agriculture and forest until connecting with the Ohio River. For a detailed description of the study site, see Burgmeier et al. (in prep). Adult hellbenders were captured across 40 sites that were selected as part of a separate mark-recapture study (Burgmeier et al, in prep). Samples were collected from June 2008 – October 2008 and June 2009 – September 2009. An additional two days of sampling in September 2007 were conducted as part of the Indiana Department of Natural Resource's annual hellbender survey.

Water Quality and Analysis:

Seven IDNR survey sites were selected for continual water quality sampling. These sites were spread roughly equidistant throughout the study area and are representative of most land use types surrounding the river. Physical and nutrient properties were sampled at all seven sites while a subset of five sites was selected for a 27 chemical multiresidue pesticide screening of water samples (Table 1). Grab samples were taken once weekly using 500 mL nalgene bottles for nutrient sampling and 1 L amber glass bottles for pesticide samples. Samples were collected from November 2008 through August 2009. All water samples were taken as close to the thalweg of the river as safely possible at a depth of greater than 0.5 m. Water samples for pesticide analysis were shipped on ice within 24 hours of collection to A&L Great Lakes Laboratories, Inc. (Fort Wayne, Indiana, USA) and analyzed via EPA-525.2 Modified/NPD. A Quanta Hydrolab (Hach Company, Loveland, Colorado, USA) was used to determine the dissolved oxygen (mg/L, % saturation), specific conductivity (mS/cm), pH, temperature (^{o}C), and turbidity (NTU) at each site. A Hach DR 2400 and a Hach DR 2800 (Hach Company, Loveland, Colorado, USA) were used to analyze water samples for orthophosphate (mg/L PO_4^{3-}), ammonia (mg/L NH_3-N), and nitrate (mg/L NO_3^--N). Nutrient samples were kept on ice and analyzed within eight hours of sampling.

Vitellogenin:

Concentrations of total protein in plasma samples were determined according to Bradford (1976) using Coomassie Plus Bradford Assay Reagent (Thermo Fisher Scientific Inc., Rockford, Illinois, USA). Plasma proteins (2.5 - 4 µg) were separated using 4% stacking/8% resolving sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under discontinuous conditions (tris-glycine buffer, pH 8.3) with 200 V (Mini-Protean III, Bio-Rad Laboratories, Inc., Hercules, California, USA). Rainbow trout (*Oncorhynchus mykiss*) vitellogenin at 141.66 ng/µL (Cayman Chemical, Ann Arbor, Michigan, USA) was used as a positive control for the phosphoprotein staining assay. Following electrophoresis, gels were stained with Pro-Q Diamond Phosphoprotein gel stain (Molecular Probes, Eugene, Oregon, USA) at room temperature with gentle agitation according to the manufacturer's instructions. Gels were fixed overnight in 50% methanol and 10% acetic acid in ultrapure water (Millipore, Billerica, Massachusetts, USA). Gels were washed three times in fresh 100 mL baths of ultrapure water for 10 min each, incubated in 60 mL of Pro-Q Diamond phosphoprotein gel stain in the dark for 90 min, and de-stained two times in the dark with 100 mL 20% acetonitrile, 50 mM sodium acetate, and pH 4.0 for 30 min each. Bands were visualized under ultraviolet light at 365 nm and imaged using a Molecular Imager ChemiDoc XRS+ System (Bio-Rad Laboratories, Inc., Hercules, California, USA). Putative VTG protein bands were excised from the gels, trypsin digested (Sequencing Grade Modified Trypsin, Promega Corporation, Madison, Wisconsin, USA) and sequenced using matrix-assisted laser desorption ionization-time of flight/mass spectrometry (MALDI-TOF/MS, Applied Biosystems, Foster City, CA, USA). The resulting peptide sequences were searched against the MASCOT database (Matrix Science Ltd., Boston, Massachusetts, USA) to identify proteins.

Sperm Screening:

Sperm samples were collected from eight adult males (five from September 22-24, 2008 and three from September 16-19, 2009). To assess sperm quality, we examined motility, viability, and concentration. Sperm was collected from males actively producing milt and placed in 1.5 mL microcentrifuge tubes. All estimates of sperm quality were made on site in the field. Sperm motility was determined by placing 10 !L of milt on a slide and counting all motile (moving) sperm cells. A total of 200 sperm cells were counted and expressed as the percent of total motile cells. Sperm viability was quantified by staining with a 5% Eosin solution which preferentially stains dead cells (i.e., live cells are left unstained; Bearden & Fuquay 1999). A blow dryer was used to quickly heat fix the stain to prevent sperm cells from slowly dying and absorbing the stain. Sperm concentration was calculated using a hemocytometer by mixing 1 !L of undiluted sperm with 9 !L of water. Sperm cells were then counted using a phase contrast microscope over five diagonal squares on the hemocytometer. The total amount of sperm was then averaged and a formula (dilution factor X hemocytometer factor X number of sperm cells counted X conversion factor) was used to calculate the amount of sperm cells per ml (Unger, 2003).

Blood Collection and Analysis:

Blood was drawn immediately from all captured individuals, including previous captures. Approximately 1.3 mL of blood was drawn from the caudal vein approximately 2.5 cm posterior to the cloaca using a 25-gauge 1 in needle (Solis et al. 2007). No more than 1 mL of blood for every 100 grams of body weight was extracted (Wright and Whitaker 2001, Solis et al. 2007). Blood smears were made in the field and fixed in 100% methanol. Blood was placed in 3 mL heparinized vials and held on ice for later laboratory analysis. A heparinized capillary tube was used to extract blood from each vial. Capillary tubes were placed in a microhematocrit centrifuge and spun at 12000 G's for two minutes to assess packed red blood cell volume (hematocrit [%]). The remaining blood was centrifuged at 11200 G's for 30 seconds to isolate plasma. The separated plasma and red blood cells were placed in separate tubes and then placed in liquid nitrogen for short term storage. A Vetscan Classic (Abaxis, Union City, California, USA) was used to conduct plasma-chemistry analyses using the avian-reptile profile. The 12 parameters analyzed were albumin (ALB, g/dL), aspartate aminotransferase (AST, U/L), bile acids (BA, µmol/L), calcium (CA, mg/dL), creatine kinase (CK, U/L), globulin (GLOB, g/dL), glucose (GLU, mg/dL), potassium (K+, mmol/L), sodium (NA+, mmol/L), phosphorus (PHOS, mg/dL), total proteins (TP, g/dL), and uric acid (UA, mg/dL).

Abnormalities, Parasites, and Chytrid Fungus:

All individuals were examined for injuries, abnormalities, and ectoparasites in the field. In addition, a Giemsa stain with azure blue (EMD Chemicals, Gibbstown, New Jersey, USA) was used to stain blood smears. All slides were viewed under a 10x lens to scan for intercellular parasites and determine smear quality and then 1000 individual cells were examined under a 100x oil immersion lens to rule out parasite infection. Duplicate slides were examined for a subset of half of the slides to ensure quality control. Cotton swabs were used to acquire skin swabs for chytrid zoospores following the protocol by Briggs et al. (2007). All swab samples were submitted to Pisces Molecular LLC (Boulder, Colorado, USA) for endpoint PCR analysis (Annis et al., 2004).

Statistical Analysis:

A Student's t-test was used to test for differences between genders for each hematological parameter. Data were log or square root transformed in instances where normality assumptions were not met. If assumptions were not satisfied, a non-parametric Mann-Whitney U test or Satterthwaite t-test were used. Student's t-tests and Satterthwaite t-tests were conducted using SAS. Mann-Whitney U tests were conducted in JMP. Descriptive statistics (i.e., mean, variance, standard deviation, etc.) were developed using Microsoft Excel. Data are reported as mean ± 1 standard deviation. In the case of average pH, values were converted to their associated hydrogen ion concentrations, an average of these values was taken, and then converted back to pH (EIFAC, 1986).

RESULTS

Water Quality, Vitellogenin, and Sperm Screening

All levels of physical parameters were within suitable ranges for freshwater aquatic life (Table 1). Dissolved oxygen was high, with a mean level of 10.15 ± 2.14 mg/L and a mean percent oxygen saturation of 94.2 ± 11.51%. Orthophosphate was present throughout the year at a mean level of 0.21 ± 0.23 mg/L, while the highest levels were present during summer at a mean of 0.38 ± 0.24 mg/L. Nitrate was also found throughout the year at a mean level of 1.59 ± 0.76 mg/L. Ammonia was present in the system at low levels (Table 3.1). The 27 chemical multi-residue pesticide screening of water samples resulted in three pesticides at detectable levels. Atrazine was detected from April 1 – July 8, 2009, metolachlor from May 26 – June 30, 2009, and simazine from June 2 – June 12, 2009, with June being the month of highest concentration (Fig. 3.1). These chemicals, and all others tested, were below detectable limits (<0.5 µg/L) the remainder of the year.

Putative VTG protein was used as a biomarker on 81 samples from 73 individuals. Of the 73 individuals examined, 62 had previously been sexed in the field (43 males and 19 females). The VTG screening accurately matched 61 of 62 (i.e., > 98% accuracy) to the field identified gender. The discrepancy involved an individual identified in the field as female, but which tested negative for the putative VTG. Of the unknown individuals, seven males and four females were identified. The putative VTG was not present among the 43 known males that were screened.

Average sperm motility was 85.25 $\pm$ 21.3%. Sperm viability was high with 95 $\pm$ 5.4% of sperm categorized as live with intact acrosomes. Sperm concentration averaged 31.4 X $10^6 \pm 7.6 \times 10^5$ sperm/mL for both years. Collectively, sperm quality was found to be high across all sampled individuals.

Table 3.1: A listing of all tested water quality parameters and their estimated levels.

Parameter	**Detection Limit**	**Mean ± SD.**	**Median**	**Range**
DO (mg/L)	-	10.15	9.88	6.83, 14.98
DO % Saturation	-	94.2 ± 11.51	95%	56,124
pH	-	8.11 ± 0.21	8.16	7.53,8.66
SpC (mS/cm)	-	0.38 ± 0.07	0.37	0.15, 0.66
Temp (C)	-	13.51 ± 7.35	13.58	0.02, 25.94
Turbidity (NTU)	-	9.31 ± 15.48	4.40	0, 82.5
Ammonia (NH_3-N) (mg/L)	0.01-0.5 mg/L	0.02 ± 0.03	0.02	0.00, 0.33
Nitrate (NO_3^--N) (mg/L)	0.1-10.0 mg/L	1.59 ± 0.76	1.60	0.00, 3.50
Phosphate (PO_4^{3-}) (mg/L)	0.02-2.5 mg/L	0.21 ± 0.23	0.14	0.00, 1.70
Atrazine (µg/L)	0.5 µg/L	2.92 ± 2.85	2.00	0.50, 11.10
Metolachlor (µg/L)	0.5 µg/L	1.46 ± 0.65	1.40	0.60, 2.70
Simazine (µg/L)	0.5 µg/L	0.74 ± 0.22	0.70	0.50, 1.10
Alachlor	0.5 µg/L	ND		
Acetochlor	0.5 µg/L	ND		
Butylate	0.5 µg/L	ND		
Clomazone	0.5 µg/L	ND		
Cyanazine	0.5 µg/L	ND		
EPTC	0.5 µg/L	ND		
Ethalfluralin	0.5 µg/L	ND		
Fluchloralin	0.5 µg/L	ND		
Metribuzin	0.5 µg/L	ND		
Pebulate	0.5 µg/L	ND		
Pendimethalin	0.5 µg/L	ND		
Prometon	0.5 µg/L	ND		
Propachlor	0.5 µg/L	ND		
Propazine	0.5 µg/L	ND		

Table 3.1 continued

Trifluralin	0.5 µg/L	ND
Vernolate	0.5 µg/L	ND
Carbofuran	0.5 µg/L	ND
Ethyl Chlorpyrifos	0.1 µg/L	ND
Diazinon	0.1 µg/L	ND
Ethyl Parathion	0.1 µg/L	ND
Fonofos	0.1 µg/L	ND
Isofenphos	0.1 µg/L	ND
Malathion	0.1 µg/L	ND
Methyl Parathion	0.1 µg/L	ND

ND = Not detected at specified detection limit.

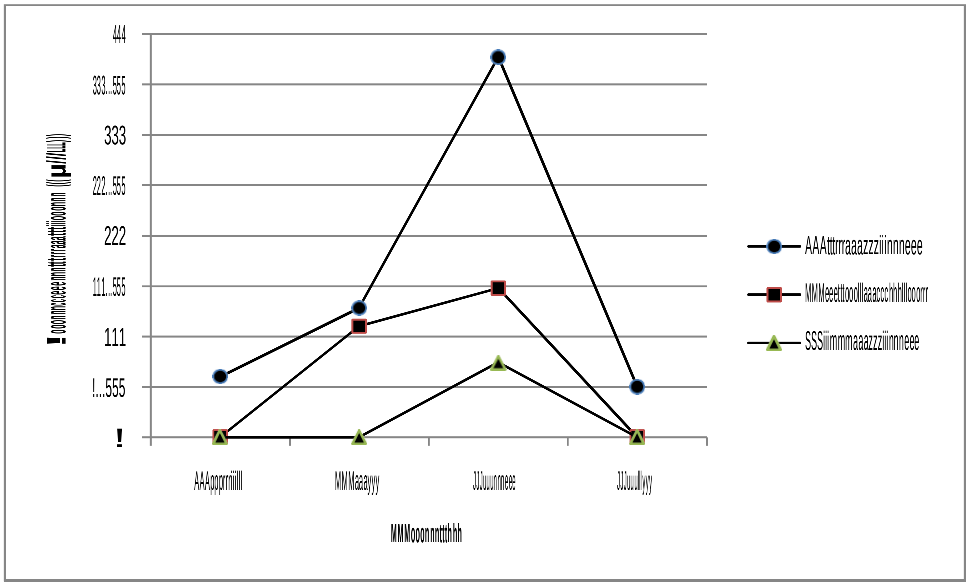

Fig. 3.1. Mean pesticide concentration per month for three chemicals detected during weekly samples in the Blue River, Indiana.

Hematology and Blood Chemistry

We collected 122 samples from 86 individuals over three seasons (53 male, 22 female, 11 unknown). Levels of most parameters were similar between genders (Table 3.2). Calcium ($t=5.26$, $df=33.9$, $p<.0001$) and potassium ($t=-2.3$, $df=80$, $p=0.0239$) levels differed significantly between males and females. No other comparisons were significant. Three apparently sick individuals (one bloated, two emaciated) exhibited notable differences between baseline population values and their individual values. Two individuals, one emaciated and one bloated, had differences in hematocrit (%) which is a measure of packed red blood cell volume. Differences existed also in AST (elevated, all three individuals), CK (elevated, one bloated, one emaciated), GLU (depressed, both emaciated), and PHOS (elevated one emaciated, depressed bloated). There was no apparent pattern with regards to the respective ailment.

Abnormalities, Parasites, and Chytrid

We examined 88 individuals for abnormalities and found that 68.2% (N=60) had at least one abnormality, 47.7% (N=42) had at least two, and 21.6% (N=19) had three or more. Abnormalities included missing digits and limbs, scars, open wounds, and abnormal or missing eyes. Missing or deformed digits were the most common abnormality occurring in 52.3% (N=46) of all individuals (Table 3.3). No significant differences in the number of abnormalities were detected between genders ($z=-0.997$, $p=0.32$). One individual worth noting appeared grossly distended nearly to the point of floating. This individual was observed in this condition in 2007 and 2008. We analyzed 168 blood smears from 80 individuals and found blood to be free of hemoparasites. Eighty samples from 77 individuals were analyzed for chytrid fungus with one sample from an adult male testing positive. Extracted blood was nearly clear and this individual exhibited severe anemia and hypoproteinemia with hematocrit values of 4% and undetectable protein levels. When compared with other individuals from the same population (Table 3.2), calcium (5.4 mg/dL) and creatine kinase (114 µg/L) levels were also greatly reduced.

Table 3.2: A comparison between genders for mean blood parameters.

Parameter	Males	Females	P-value
Hematocrit (%)	34.37 ± 7.59	31.65 ± 6.18	0.0821
AST (U/L)	74.52 ± 38.1	76.48 ± 47.12	0.8909
BA (µmol/L)	<35*	<35*	-
CK (U/L)	661.7 ± 788.7	488.52 ± 300.1	0.2732
UA (mg/dL)	<.3*	<.3*	-
GLU (mg/dL)	23.27 ± 6.41	21.61 ± 5.49	0.2297
CA^{++}(mg/dL)	7.89 ± 0.58	10.93 ± 2.1	<.0001
PHOS (mg/dL)	5.14 ± 1.33	5.11 ± 1.36	0.8232
TP (g/dL)	3.15 ± 0.62	3.19 ± 0.56	0.7428
ALB_BCG (g/dL)[a]	1.22 (<1) ± 0.08	1.19 (<1) ± 0.12	-
GLOB(g/dL)[b]	2.6 (>1.99) ± 0.36	2.34 (>2.03) ± 0.59	-
K^+(mmol/L)	5.07 ± 1.42	4.38 ± 1.18	0.0239
NA^+(mmol/L)[c]	110.83 (<110) ± 0.75	111 (<110) ± 1.20	-

a Number in parentheses is the detection limit for this parameter. Of 60 male samples and 29 female samples, 54 and 22 respectively were below this limit.

b Number in parentheses is the mean estimate based on the formula TP-ALB=GLOB which was used for individuals with ALB_BCG levels below detectable limits that also had readings for total protein (46 males, 22 females). This would represent the minimum estimate for these samples

c Number in parentheses is the detection limit. Of 54 male and 31 female samples, 48 and 23 respectively were below this limit.

Table 3.3: The type of abnormalities present in the population, the total number of each type, and the frequency of occurrence in individual animals.

Type	**Total per Type**	**Frequency[a]**
Missing/Deformed digits	64	0.52
Bite marks/scarring	32	0.28
Open wounds	21	0.18
Missing appendages	9	0.091
Other	7	0.08
Eye injury	5	0.057

a Frequency of individual hellbenders with specified type of abnormality (N=88)

DISCUSSION

Water Quality

Habitat destruction and degradation are regularly cited as the leading causes in the decline of many wildlife populations, including amphibians (Tilman et al., 1994; Dodd and Smith, 2003; Wheeler et al., 2003; Stuart et al., 2004). An important component of degradation is the decrease in the quality of aquatic systems due to chemical and nutrient inputs from various anthropogenic sources (Karr et al, 1985; Carpenter et al., 1998; Houlahan and Findlay, 2003). Considering the hellbender's aquatic life history, it is a prime candidate for decline due to poor water quality. All physical parameters measured in our study were well suited for aquatic life. Standard levels for orthophosphate in streams do not exist; however the USGS does recommended levels of total phosphates be below 0.1 mg/L (Mueller and Helsel, 1999). Orthophosphate levels in our study measured slightly higher than the recommended 0.1 mg/L total phosphate by the USGS. Moreover, it has been suggested that the reference level for total phosphate in streams be decreased and signs of eutrophication have been seen at levels far lower than we detected (Dodds and Welch, 2000). Levels of both orthophosphate and nitrate exceeded the EPA's recommended criteria for total phosphate and total nitrogen for ecoregion IX, subecoregion 71 of 30 µg/L and 0.8 mg/L, respectively (EPA, 2002). Despite these levels, there were no noticeable signs of eutrophication (e.g., high densities of submerged macrophytes) even during low water periods, suggesting that eutrophication is not a major concern in the Blue River.

Atrazine, metolachlor, and simazine were present in low levels continuously from late April through early July. At low concentrations, acute toxicity is of little concern, but chronic toxicity due to repeated, long-term exposures or bioaccumulation could produce adverse effects (Langerveld et al., 2009). Atrazine and simazine are not known to produce bioaccumulation risks, but exposure to ecologically relevant levels of atrazine has been shown to produce effects ranging from multiple gonads and demasculinization in male frogs to increased disease and parasite sensitivity in amphibians (Hayes et al., 2002a; Kiesecker, 2002; Forsen and Storfer, 2006). Williams and Semlitsch (2009) reported that low-dose exposures to atrazine and metolachlor did not result in a significant increase in larval mortality in three species of anurans, but notes that combinations of commonly used pesticides have elicited negative effects in some studies (Hayes et al., 2006; Relyea, 2009). The three pesticides detected in our study were present concomitantly for two weeks while atrazine and metolachlor were found together at detectable levels for up to one month. Our results indicated that atrazine was consistently present over the course of two months at levels that have resulted in reproductive abnormalities in frogs in previous studies (Hayes et al. 2002a; Hayes et al., 2003; Rohr et al., 2006). Whether this combination of chemicals has manifested noticeable effects in the population will be difficult to study given the species protected status, but our study has found no detectable effects concerning the endpoints of VTG production and sperm quality. Despite the hellbender's life history and the high level of agricultural production around our study area, our results indicate that the water quality of the Blue River is not likely the reason for the apparent lack of reproduction and the continued decline of the population. However, it will be important to continue monitoring to ensure eutrophication does not become a problem and that pesticide levels remain low.

Vitellogenin and Sperm Screening

The potential for endocrine disruption in amphibians has been a growing concern since the advent of modern technologies have allowed researchers to evaluate the effects of pesticides on various taxa. Atrazine has been reported in laboratory studies to induce effects including the castration and demasculinization of developing amphibian larvae (Carr et al., 2003; Hayes et al. 2002a, 2002b, 2003, 2006). However, Kloas et al. (2009) reported contradictory findings that atrazine had no significant effect on sexual differentiation. Unfortunately, the biomarkers used in these laboratory studies require sacrificing the individual for testing. For less invasive field studies requiring the survival of the animal, vitellogenin has been proposed as a viable alternative and its production has been induced in male herpetofauna at ecologically relevant doses of some pesticides (Palmer and Palmer, 1995; Brande-Lavridsen et al., 2008). We found no evidence of VTG production in known males. VTG production has been shown to end in males once exposure to a particular estrogenic substance has ceased (Tata, 1979; Hayes, 2005). The lack of VTG detection among males does not entirely eliminate endocrine disruption as a potential problem in the population, but does provide evidence to suggest that it is likely not the cause behind the continued decline and apparent lack of reproduction.

Hellbenders in Indiana typically come into breeding condition in late August – early October. Outside of this 8 - 9 week period, gender determination is impossible using less invasive field methods. In the absence of poor water quality, however, vitellogenin assays may serve as valuable tools that provide a means for gender determination due to its female specificity (especially for species that are sexually dimorphic for only a short period of time). Our findings clearly illustrate the utility of VTG as a means of gender determination up to two months prior to the breeding season in the hellbender. We had only one discrepancy between field identification and putative VTG identification. The non-matching individual was recorded as a female in the field, but tested negative for the putative VTG protein. The most likely explanation is a gender misidentification in the field as the date of capture was near the beginning of the breeding season when males and females would typically be morphologically distinct, but their remains a possibility they might not. Another possible explanation is the lack of VTG production due to a lack of egg production. Little is known regarding the production of VTG in hellbenders, although it is thought the vitellogenin phase in amphibians lasts approximately nine months (Redshaw, 1972; Ingersol, 1982). If eggs were not being produced, VTG might remain undetectable. Goodbred et al. (1996) reported undetectable levels of VTG in some female common carp (*Cyprinus carpio*) while others within the same population produced detectable levels. VTG has been detected throughout the year in female crested newts (*Triturus carnifex*) (Zerani et al., 1991); however, the VTG cycle of female hellbenders is currently unknown outside of the sampling period of this study and requires further investigation.

Sperm quality has been reported to decrease with senescence in humans (Kidd et al, 2001; Eskenazi et al., 2003). This is especially concerning as the hellbender population of interest in this study consists of only adults approximately 15 years or older and no signs of successful reproduction (i.e., lower age classes) have been reported for 25 years (Kern, 1984; Burgmeier et al., unpubl data). However, estimates of sperm health indicated reproductively active and “healthy” males. Overall motility and viability estimates were high, indicating the majority of hellbender milt sampled contained live motile sperm cells. Sperm concentration estimates were on average lower, but similar to previous estimates for eastern hellbenders from Missouri and North Carolina (Unger, 2003). Variation in local seasonal male breeding conditions could explain these numbers. However, it is likely that these estimates of sperm concentration, along with relatively high rates of motility and viability, are enough to successfully fertilize a clutch of eggs under available nest rocks in Indiana.

Hematology and Blood Chemistry

Our results indicate that few differences exist in blood chemistry between genders in the Blue River hellbender population. Calcium differed between genders with levels in females significantly higher than in males. Calcium levels have been reported to be associated with folliculogenesis in females and likely explains the discrepancy (Campbell, 1996). Males however, had significantly higher potassium, which although not explicitly stated, was a pattern noticeable in Solis et al. (2007). In general, our numbers were similar to those reported from populations of eastern hellbenders by Solis et al. (2007) with a few noticeable exceptions. In our study, AST, CK, and UA values were considerably lower than the previous study. AST is the liver, heart, and skeletal muscle and can indicate liver disease. CK is found in muscles and can increase during stress. UA is a waste product found in amphibians, reptiles, and birds and increased levels can indicate dehydration. Solis et al. (2007) anesthetized their individuals and performed routine processing before blood withdraw which could explain increased levels of CK and UA. Creatine kinase and UA have been shown to be affected by handling in racing pigeons (*Columba livia domestica*) with substantial increases in CK and decreases in UA (Scope et al., 2002). However, Halliday et al. (1977) showed increases levels of UA in domestic chickens (*Gallus domesticus*). Baseline differences between populations might exist that explain all or part of the differences seen, but further research would need to be conducted to rule out handling stress. Our numbers, when used in conjunction with those of Solis et al. (2007), provide an important baseline for assessing the individual and population health of this species for future monitoring and management programs.

Abnormalities, Parasites, and Chytrid

Abnormalities are common within amphibian taxa (Stocum, 2000; Blaustein and Johnson, 2003; Taylor et al., 2005). Any given population can be expected to exhibit a background abnormality rate of 0 - 5% (Meyer-Rochow and Asahima, 1988). However, hellbenders in Indiana exhibit a rate of 68.2%, which greatly exceeds that which would be expected. Although high for most amphibian populations, it is similar to previous studies of hellbenders which report rates of 41% and as high as 90% (Hiler et al., 2005;

Miller and Miller, 2005). Moreover, most injuries in the Indiana population seem to be the result of physical trauma rather than ontogenetic malformations. None of the observed malformations approached the severity of the squamous cell carcinoma found in an Ozark hellbender in Missouri (Harshbarger and Trauth, 2002). Hellbenders are long-lived, territorial, and regularly come into conflict for suitable shelter rock habitats. This, combined with contact with predators and human recreationalists, likely explains most abnormalities. It will be important to monitor the frequency and type of abnormalities in the future as the more extreme types may indicate a serious underlying problem.

Parasite infections are routinely reported among many amphibian species (Nickerson and Mays, 1973; Wright and Whitaker, 2001; Solis et al., 2007). While it has been reported that Ozark hellbenders have shown some signs of parasitemia, eastern hellbenders have shown little in terms of parasite infections (Johnson and Klemm, 1977; Solis et al., 2007). No ectoparasites or hemoparasites were found for hellbenders examined in the current study. It does not appear that hemoparasites are a potential cause for the steady decline of hellbenders in Indiana. It is important to note that none of the parasites screened in the current study are those typically thought responsible for producing abnormalities in other amphibians (Johnson et al., 2002; Johnson and Lunde, 2005).

Reports of chytrid in naturally occurring wild populations is problematic given its ability to spread quickly and eradicate entire populations (Lips et al. 2003; Lips et al. 2006). Chytrid fungus has been reported from hellbender populations in several states, but no cases of symptomatic infections have been confirmed. Chytrid was found on one individual within our population. The infected individual showed no clinical signs of infection, however, blood results revealed that the individual was severely anemic and hypoproteinemic. Voyles et al. (2009) reported a significant negative correlation between chytrid infection intensity and plasma potassium, sodium, and calcium levels in green tree frogs (*Litoria caerulea*). The single infected individual from the current study followed the same general pattern of reduced calcium and creatine kinase levels, but potassium levels could not be determined due to hemolysis and sodium was below detectable limits (<110 mmol/L) . Our findings suggest possible renal or hepatic issues, but an official diagnosis cannot be made and these symptoms cannot be linked to chytrid infection without further examination. Despite chytrid being present at low levels, hellbenders have been regularly studied in Indiana for the past several decades with no reported symptomatic cases. It is unlikely that chytrid fungus is the major cause of the decline in Indiana, but it will be important to monitor its presence in order to prevent or dampen the effects of a future outbreak.

Management Implications

Health issues are of growing concern to researchers as hellbender populations continue to decline. Our study provides an extensive summary of several important factors often associated with amphibian health and effectively precludes them as the cause of continued hellbender decline in Indiana. Our results revealed the Blue River maintains good water quality throughout the year. Additionally, individuals within the population seemed healthy with no signs of endocrine disruption, low levels of parasitemia, and high sperm quality comparable to known breeding populations from other watersheds. Comprehensive health assessments could provide a means of determining which factors potentially contribute to either the decline or suppression of other hellbender populations and might serve to detect problems in otherwise healthy populations so management actions can be implemented prior to decline. It is important to rule out all complicating variables when designing management plans in order to increase the chance of successfully fulfilling goals. On a fine scale, our study provides important information for state agencies and management organizations in Indiana to evaluate the next step in hellbender conservation in the state. More broadly, our study provides a framework for others looking to assess overall population health and eliminate potential causes of decline so future managers can increase their focus and target the necessary components for successful conservation activities.

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VITA

VITA

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INTERESTS:
Ecology and conservation of reptiles and amphibians, Invasive species ecology, Habitat conservation and restoration

EDUCATION:
2007-2010 M.S., Wildlife Science, Purdue University (expected May)
2002-2006 B.S., Wildlife Science, Purdue University

AWARDS AND HONORS:
2009 FNR Graduate Research Symposium Award, 2nd place
2005 Gruel Memorial Scholarship

PROFESSIONAL AFFILIATIONS:
2008-Present American Society of Ichthyologists and Herpetologists
2002-2003 The Wildlife Society

EXPERIENCE:
2007-Present **Graduate Research Assistant, Wildlife -** Purdue University, full-time 40+ hrs/wk

- Hellbender populations in Indiana have experienced precipitous declines throughout the past several decades. In order to make the most informed management decisions for the future of the population, we set out to gather information about the status, habits, and health of the population. Mark-recapture surveys were used to estimate various population parameters while radio-telemetry was used determine home-range size, movements, and habitat use. A series of biological samples was taken to assess such health parameters as blood chemistry, endocrine disruption, and the presence of chytrid fungus. Available habitat was mapped and water quality samples were examined for physicochemical and nutrient content. Throughout this position, I was in charge of the training and management of up to six technicians and/or volunteers at any given time.

2006-2007 **Field Technician** - Indiana DNR, Division of Fish and Wildlife, part-time 37.5 hrs/wk

- Capture, radio-telemetry, tissue and data collection of radio-tagged eastern box turtles and timber rattlesnakes
- Deployment, monitoring, maintenance, and data collection of common snapping turtle hoop net sets and aquatic salamander leaf litter bag sets
- Conducted transect and aural surveys for multiple herpetofauna species including green treefrogs and the Indiana state endangered copperbelly water snake, Kirtland snake, and green salamander
- Conducted vegetation surveys to document habitat use for eastern box turtles and timber rattlesnakes
- Completed reports for publication in annual Indiana DNR extension publication
- Management of large databases using Microsoft Excel and Access

2007 **Research Technician** - Purdue University, full-time 40+ hrs/wk

- Conducted raccoon latrine surveys for eventual raccoon roundworm analysis
- Distributed raccoon roundworm vaccines in a systematic manner for raccoons
- Radio-telemetry and data collection of Allegheny woodrats
- Trapped Allegheny woodrats for genetic sample acquisition and mark-recapture

2004-2006 **Herpetology Laboratory Technician** - Purdue University, part-time 15 hrs/wk

- Collected, prepared, and cataloged over 100 reptile and amphibian museum specimens
- Assisted instructor and other technicians herpetofaunal surveys throughout Indiana and Kentucky

2005 **Independent Contract Biologist** - Indiana DNR, Division of Fish and Wildlife, full-time 40 hrs/wk

- Capture of the exotic, invasive common wall lizard at the Falls of the Ohio State Park using bait and lizard nooses
- Performed visual encounters surveys of areas surrounding park to document distribution of the common wall lizard
- Frequent interaction with public to convey the project’s purpose and promote scientific education

2005 **Teaching Assistant** - Purdue University, part-time 20 hrs/wk

- Conducted herpetology lectures and associated field activities
- Led students in field exercises focused on vertebrate survey and trapping methods and habitat and vegetation surveys
- Acted as a liaison between faculty, staff, and students
- Assisted instructors and students in the completion of assigned exercises, examinations, and field exercises
- Inventory and maintenance of field tools and equipment

2004 **Ecologist Aide -** Indiana DNR, Division of Nature Preserves, full-time 40 hrs/wk

- Conducted visual encounter surveys for three rare snake species

- Worked in small groups and alone using chainsaws and various herbicide application techniques to selectively remove unwanted and invasive plants in close proximity to rare and endangered plant species

2003 **Biological Intern -** US Fish and Wildlife Service, Muscatatuck NWR, full-time 40 hrs/wk

- Assisted with the monitoring of Wetland Reserve Program and Conservation Reserve Program lands
- Monitoring and maintenance of cover boards, drift fences, and pitfall traps for amphibians and reptiles including the Indiana state endangered four-toed salamander
- Conducted North American Amphibian Monitoring Program anuran call surveys
- Performed nest monitoring, banding, and brood counts for various bird species
- Captured anuran species for deformity, malformity, and parasite evaluation
- Performed water quality and invertebrate sampling of refuge tributaries
- Conducted visual encounter surveys for butterflies
- Assisted in refuge programs for public education and recreation

RELEVANT SKILLS

- Conducted and instructed on monitoring and survey methods for herpetofauna, mammals, and birds
- Proficient in the identification, handling, trapping, and marking of reptiles, amphibians, and aquatic invertebrates
- Responsible for the hiring, training, and management of 10 project personnel
- Collected tissue for genetic analysis from eastern hellbenders, eastern box turtles, timber rattlesnakes, and Allegheny woodrats
- Experience identifying Anurans and some songbirds by call
- Experience conducting vegetation surveys and identifying trees and herbaceous species
- Performed basic transmitter implantation surgeries on hellbenders, mudpuppies, and tiger salamanders
- Conducted and trained field technicians on both field and laboratory water quality testing using handheld meters and portable spectrophotometers
- Performed basic wetland delineation and habitat characterization in riparian habitats
- GPS and orienteering skills
- Experience with Program MARK, Arc GIS, SAS, JMP, BehavePlus, and the management of large databases and production of documents using Microsoft Office Suite
- Extensive experience interacting with landowners and the general public
- Experience in chainsaw use and herbicide application
- Experience driving four-wheel drive vehicles on and off road

PROFESSIONAL PUBLICATIONS AND PRESENTATIONS

Publications:

- Burgmeier, N., Unger, S., Williams, R., and T. Sutton. The Bender Board: A New Design for the Restraint and Measurement of Hellbenders. Herpetolical Review (In Press).

- Water Quality and hellbender health assessment paper (in preparation)

- Hellbender Spatial Ecology paper (in preparation)

- Hellbender Population Estimation paper (in preparation)

Talks:

- Burgmeier, N. and R. Williams. Dec. 2009. Health Assessment of Indiana Hellbenders. 70th Midwest Fish & Wildlife Conference, Hilton Hotel, Springfield, IL.

- Burgmeier, N. and R. Williams. June 2009. Movements Patterns, Habitat Use, and Home Range Size in Eastern Hellbenders. 4th Hellbender Symposium, Cumberland Falls State Resort Park, Corbin, KY.

- Burgmeier, N. and R. Williams. Dec. 2008. Movements Patterns, Habitat Use, and Home Range Size in Eastern Hellbenders. 69th Midwest Fish and Wildlife Conference, Hyatt Regency, Columbus, OH.

Posters:

- Burgmeier, N. and R. Williams. July 2009. Spatial Ecology, Population Size, and Health Status of Eastern Hellbenders. 2009 Joint Meeting of Ichthyologists and Herpetologists, Hilton Portland and Executive Towers, Portland, OR.

- Burgmeier, N. and R. Williams. April 2009. Spatial Ecology, Population Size, and Health Status of Eastern Hellbenders. 2009 Purdue University Forestry and Natural Resources Poster Symposium, Purdue University, West Lafayette, IN.

EXTENSION ACTIVITIES

Publications:

- Burgmeier, N., Unger, S., Sutton, T., and R. Williams. 2010. Hellbenders: Indiana's Living Relic. (In press).

- Burgmeier, N. Unger, S. and R. Williams. Hellbender Ecology and Genetics. Indiana Department of Natural Resources Wildlife Diversity Report. (In press).

- Burgmeier, N. and R. Williams. Spatial Ecology, Population Status, and Health Assessment of the Eastern Hellbender in Indiana. Purdue Forestry and Natural Resources Compass. (In press).

Presentations:

- Burgmeier, N. April. 2010 (scheduled). Snot Otter, Grampus, Devil Dog: The Hellbender! Miss Skelton's 4th Grade, Wilson Elementary School, 2915 Charlestown Pike, Jeffersonville, IN.

- Burgmeier, N. Dec. 2008. Snot Otter, Grampus, Devil Dog: The Hellbender! Miss Skelton's 3rd Grade, Pleasant Ridge Elementary School, 1250 Monroe St., Charlestown, IN.

WORKSHOPS
2009 Conservation Leaders for Tomorrow

VOLUNTEER ACTIVITIES

- **Wildlife Habitat Evaluation Program** (WHEP) 2007, Invited judge
- **Friends of the Muscatatuck,** River clean-ups