

Long-term growth and monitoring of the Eastern Hellbender (*Cryptobranchus a. alleganiensis*)
in Eastern West Virginia.

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ABSTRACT

Long-term growth and monitoring of the Eastern Hellbender (*Cryptobranchus a. alleganiensis*) in Eastern West Virginia.

Amphibian declines have been well documented, specifically in the last few decades. The Hellbender, *Cryptobranchus alleganiensis*, one of North America's largest salamander species, has suffered dramatic declines throughout much of its range, with estimated declines of up to 77 percent recently documented in some populations. With diurnal and nocturnal searches and mark-recapture techniques, I collected data on the status of Eastern Hellbender populations in Eastern West Virginia. We re-sampled a study site on the West Fork of the Greenbrier River that was first examined in 1998 by Jeff Humphries. Long-term growth and survivorship data were collected and compared to 1998 data. Of the 29 hellbenders tagged within the West Fork site in 1998, 11 were recaptured in 2009. Eleven year mean growth of recaptured hellbenders is 3.38 cm (range 0.6cm - 4.5 cm, n=11). A notable demography shift in both sex and size occurred from 1998 to 2009. In 1998 the population exhibited a 1.1:1 sex ratio, while the 2009 population exhibited a 2.1:1 ratio. A marked shift in size class was noted, with the 2009 population exhibiting shifts to larger size classes. However, evidence of reproduction was found in 2009 where it was lacking in 1998, potentially suggesting a viable population. The recapture data will allow for a better understanding of the growth and age of large size classes of hellbenders.

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OVERVIEW OF FINDINGS

I processed thirty-eight hellbenders, encompassing 112 person-hours, on the West Fork of the Greenbrier in Pocahontas County, West Virginia between April 25, 2009 and October 11, 2009. Eleven of the original 29 hellbenders tagged within the study site in 1998 by Jeff Humphries were re-captured in the 2009 season. Table 1 is a list of all hellbenders captured, including their tag number, location, sex, total length, snout-vent length, mass, body condition, reproductive condition, and visible injuries. I used both diurnal rock-lifting and night surveys. Thirty of the hellbenders were captured within the original 1998 study stream reach, including all eleven 1998 recaptures. I found 19 “new” hellbenders within the site. Of all hellbenders captured on the West Fork, 25 were male, 12 were female, and one was of undetermined sex. The sex ratio was 2.1:1. Of these, 29 were captured once, 8 were captured twice, and one was captured four times. Eight males and one female comprised the eleven re-capture events. I captured twelve hellbenders (8 males, 4 females) during night surveys, and 26 (8 females, 16 male, and unknown sex) during diurnal rock-lifting and snorkeling surveys. There was a marked size class shift in the 2009 population towards larger individuals compared to the 1998 population. Also, there was a dramatic shift in sex ratio toward males in 2009, compared to a ratio of 1.06:1 eleven years prior. Of the 29 hellbenders tagged within the West Fork site in 1998, 11 were recaptured in 2009. This is the first documentation of long-term philopatry for Eastern Hellbenders. Eleven year mean growth of recaptured hellbenders is 3.43 cm (range 0.6cm - 4.5 cm, n=11). Contrary to the 1998 study, I found evidence of reproduction. I found two nests sites, and an adult hellbender possibly guarding approximately 75 six-month old larvae. This may be a rare example of extended parental care. Upon examining a loose egg near a nest rock, I noted the embryo just beginning to cleave into four sections. Therefore, I am able to date with relative certainty that the date of oviposition is September 3rd or Sept 4th. While I did notice several demographic shifts, I did not document a decline. Also, the presence of 19 new hellbenders within the study site suggests stability within the stream system. Overall, the West Fork of the Greenbrier likely represents a relatively stable and viable population of hellbenders.

INTRODUCTION

Species Description

The Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*) is a large, fully aquatic salamander that prefers streams that are swift-flowing, highly oxygenated, and exhibit limited disturbance (Green 1933; Bishop 1941; Nickerson and Mays 1973; Pfingsten and Downs 1989; Conant and Collins 1998). Habitat specialists, hellbenders need large, flat rocks for cover and nesting (Hillis and Bellis, 1971; Nickerson and Mays, 1973; Nickerson et al., 2002; Humphries and Pauley, 2005).

The Eastern Hellbender ranges from southern New York to northern Georgia, west to Alabama, and Mississippi, and a population in central Missouri (Nickerson and Mays, 1973; Petranka 1998). The Ozark subspecies (*Cryptobranchus alleganiensis bishopi*) has populations in southern Missouri and Northern Arkansas (Nickerson and Mays, 1973). The Ozark subspecies is characterized by smaller spiracles, dorsal blotches instead of spots, increased mottling on the chin, a smooth pectoral lateral line system, and a smaller overall body size (Grobman, 1943; Nickerson and Mays, 1973; Petranka, 1998).

One of North America's largest salamanders, Eastern Hellbenders are characterized by a heavily wrinkled, dorso-ventrally flattened body, keeled tail, and a large flat head (Green and Pauley, 1987). Hellbenders average 50 cm in length, with a record length of 74 cm (Nickerson and Mays, 1973; Green and Pauley, 1987; Petranka, 1998). While their coloration varies, hellbenders normally exhibit a dark, spotted or blotched dorsum, and a plain, light venter. Utilizing cutaneous respiration, hellbenders have loose, highly vascularized skin. Folds along the dorsal and ventral aspects of the body increase surface area and allows for maximum gas exchange (Bishop, 1941). This method of respiration limits hellbenders to cool, swiftly flowing streams where gas exchange is maximized (Ultsch and Duke, 1990).

Hellbenders exhibit slow growth, delayed maturity and low fecundity (Wheeler et al. 2003). This life history strategy results in slow recovery from perturbations, leading to difficult and unique

conservation challenges (Congdon et al. 1993, 1994). Due to these specific adaptations and strict habitat requirements, hellbenders are very sensitive to disturbance. Changes to stream systems, specifically anthropomorphic alterations, often result in declines or extirpation of hellbenders. Several studies have noted recent declines in hellbender populations across their range (Nickerson and Mays, 1973; Trauth et al., 1992; Wheeler et al., 2003; Briggler et al., 2007; Foster et al., 2009). Trauth et al. (1992) noted a decline from 370 hellbenders to 20 in the Spring River in Arkansas in only a 10 year span. Wheeler et al. (2003) observed a 77% decline in both hellbender subspecies over a 22-year period in Missouri.

Declines

Hellbender declines can be attributed to several major causes. Perhaps the most significant factor in these declines is habitat degradation (Nickerson and Mays, 1973; Williams et al., 1981; Trauth et al., 1992; Nickerson et al. 2002; Wheeler et al., 2003; Humphries and Pauley, 2005). Logging, urban development, and agricultural practices reduce canopy cover, and have the potential to increase water temperatures. Such alterations decrease the amount of dissolved oxygen and can lower the respiratory ability of hellbenders (Utlisch and Duke, 1990). Such practices also increase siltation, another major potential cause of hellbender declines. Welsh and Ollivier (1998) suggest an increase in sedimentation was a major cause of decline in the Pacific Giant Salamander (*Dicamptodon tenebrosus*). Interstitial spaces of the stream substrate become filled with sediments and reduce macroinvertebrate prey, cover, and habitat for larvae. While there is a paucity of information on larval hellbender habitat requirements, sedimentation likely similarly affects hellbender larvae through alteration of this microhabitat and prey availability (Nickerson et al., 2003). Dundee (1971) suggested that siltation, as well as general and thermal pollution, was likely responsible for eliminating hellbenders from the Ohio River drainage.

Two other causes of hellbender decline for this region are acid mine drainage and impoundment (Nickerson and Mays, 1973; Bury et al., 1980). Mine wastes cause anoxia and acidification to streams,

while impoundment and channelization alters the streams' natural flow. Once swift, highly oxygenated streams become slower and warmer, decreasing dissolved oxygen levels. Humphries (1999) suggests that the lack of hellbenders in healthy streams today can possibly be explained by past acid mine drainage issues that have since undergone remediation. Killing by fishermen who may wrongfully view hellbenders as poisonous is also another potential source of decline (Nickerson and Mays, 1973).

No one cause is completely responsible for declines in hellbender populations. It is likely a combination of factors, which will vary for different hellbender populations across their range. Management and conservation decisions should be based on the specific stressors affecting a certain population.

Hellbenders in West Virginia

Hellbenders historically are known from the Ohio River and were considered common in sections near Ohio and West Virginia (Green, 1934). However, it is highly unlikely that hellbender populations still exist in large rivers like the Ohio due to channelization, impoundment and siltation (Humphries, 1999). Eastern Hellbenders are considered endangered or a species of concern in most states where they occur. In West Virginia, hellbenders are considered a species of concern (S2) by the West Virginia Division of Natural Resources. The current major threats listed for hellbender populations in West Virginia are habitat degradation, over-collection, and acid mine drainage. Over-collection, specifically in West Virginia, may pose a significant threat to hellbenders. West Virginia provides no protection for the hellbender, and general collection of all aquatic life requires only a Class B fishing license. As there is currently a high demand for hellbenders in the pet, medicinal, and food trade in Asia, with some salamanders selling for up to \$3,500 per animal (Trauth et al., 1992; Briggler et al., 2007), over-collection is likely a major cause of decline. However, to collect for scientific purposes a permit is required, yet

collection is unlimited for anyone with a West Virginia fishing license (Grandmaison et al., 2003). No other state allows such unregulated collection practices.

Study Objectives

This study seeks to further our knowledge and understanding of the ecology of the Eastern Hellbender in West Virginia. Specifically, it is my goal to document current demographic and population status and trends of a hellbender population on the West Fork of the Greenbrier, Pocahontas County, West Virginia. Historical data for this population was provided by Jeff Humphries in his 1998-1999 Marshall University masters degree thesis research. I compared data I collected in 2009 to data collected in 1998. Specifically, I am interested in long-term (eleven-year) hellbender growth, philopatry, population dynamics, reproduction, and overall ecological trends. It is also my goal to lay further groundwork for future studies and add to our overall knowledge of this fascinating and unique salamander species.

METHODS

Study Site Description

This study took place on the West Fork of the Greenbrier River, 1.7 miles north of Durbin, in northern Pocahontas County, West Virginia (Fig.1). The Greenbrier River is a tributary of the New River, which is in the Mississippi River drainage. The West Fork begins on the eastern slopes of Shaver's Mountain, flows southwest, and forms a watershed inside the Monongahela National Forest. The Monongahela National Forest provides protection from development for this section of stream and its headwaters. The West Fork watershed exhibits limited disturbance, mainly in the form of horse trails, campsites and recreational fishing. It should be noted that just upstream of the study area is a popular fishing hole that is frequented regularly by trout fishermen.

The immediate surrounding watershed and riparian zone is characterized by a steep slope with a densely vegetated mixture of hemlock, rhododendron, and northern hardwood species on the south side of the site. The north side of the site is characterized by a narrow floodplain, sparsely vegetated by rhododendron, various shrubs and a few large hardwoods. Also on the north side of the study site is a forest service road that doubles as a horse and hiking trail. Non-forest service authorized vehicles are not permitted on this road and vehicular travel is therefore limited.

The stream, which encompasses the study site, is characterized by shale or limestone bedrock, which underlies the entire stream. The stream substrate is comprised mainly of gravel and cobble, with sections of sand and silt in the slower sections. Throughout the stream are many large, flat rocks, varying in dimension from the size of a car tire to the size of a large dining room table. Normally consisting of limestone or igneous rock, it is these rocks that provide shelter and habitat for hellbenders. However, other aquatic organisms, including crayfishes, mudpuppies (*Necturus maculosus*) and various stream fishes can also be found under these large rocks. Hydrodynamically, the stream consists of a series of fast flowing rapids, fast flowing runs, and a few deep pools. During normal flow, the majority of the stream is less than 80 cm deep. The deepest pools are generally less than 2.5 m deep.

My study site encompassed Jeff Humphries's 1998 site, which was a stream section 216 meters long and approximately 20 meters wide. However, I extended the site both upstream and downstream approximately 50 meters to increase the likelihood of capturing 1998 tagged hellbenders. Three relatively deep, slower flow runs and two areas of higher flowing rapids characterized the original 1998 site. Flooding and increased flow occurred during and after major precipitation events, and the stream was flooded and inaccessible almost the entire month of May. Stream temperature ranged from 3°C in winter to 24°C in August; however the stream normally remained below 21°C most of the summer. It should be noted, that anecdotally the stream appeared to have more silt to Jeff Humphries on the several occasions he aided in field work in 2009 compared to 1998. Mean stream discharge for 2009 varied from a high of 487.1 cfs (cubic feet per second) in May 2009 to a low of 28.9 cfs in September 2009 (Table 2).

Humphries used a 9m x 9m (3 quadrants wide by 24 quadrants long) grid system to map locations of hellbenders. I re-established this grid system lengthwise for the 2009 season using reflective flagging tape along the shoreline every ten meters. Flags were numbered from 0 to 11, with .5 increments representing 10 meters. The top (upstream) of the study site started with flag 0 and flag numbers increased and stopped at 11 at the bottom of the study site (Fig.2). This allowed me to precisely document the locations of 1998 hellbender recaptures for later comparison with their original capture locations eleven years prior.

Study Site Surveys

I implemented both nocturnal and diurnal surveys on my study site; however the majority of time and effort was focused on daytime searches. Daytime searches typically started from 10:00 -11:00 AM until 4:00 -6:00 PM, or until searchers were either extremely cold and/or exhausted. A minimum of two people were required for effective daytime surveys. I was in the stream with a mask and snorkel looking under large rocks while the other searcher/searchers slowly lifted the rock (Fig. 3). Almost without exception the use of a log peavey was required to aid in lifting the large and heavy rocks. If a hellbender was under a rock, I captured it by hand and quickly transferred it into a mesh bag. I then took the animal to shore and collected the following data: date and time of capture, exact global positioning satellite (GPS) position within the grid, the presence of a previously implanted Passive Integrated Transponder (P.I.T.) tag, sex, total length, snout-vent length, mass, injuries/scars, and in males the degree of swelling of the cloaca (not swollen, slightly swollen, swollen, very swollen). Various other descriptive data was noted if apparent, i.e. the presence of milt, presence of eggs, general overall health. I measured total length and snout-vent length with a 10 cm diameter PVC pipe cut in half with measuring tape glued inside (Fig. 4). This aided in keeping the hellbender straight during measurement. I used a 300g, 600g or 1000g Pesola scale attached to a heavy-duty plastic bag (tared) to measure mass to the nearest gram. If the

hellbender was a new capture, I injected it with a P.I.T. tag. I also took DNA samples for use in a Purdue University genetic study by cutting a small section of skin from the top of the tail. The sample was then placed in a vial with 70% alcohol, and date, sex, and location were noted. Once I recorded all data, I promptly returned the hellbender to the site of capture. Great care was taken to return the rock to its original position and orientation, and I released the hellbender directly beside the entrance to underneath the rock. It often took two to three days to exhaustively search the study site with this diurnal survey method.

I also conducted nocturnal visual surveys for active hellbenders. Typically, I began searching for hellbenders just after dark, with up to as many as five people searching. Starting downstream of the site, I would work upstream using headlamps and flashlights to search the bottom for active hellbenders. Once a hellbender was found, all searching halted and I recorded the aforementioned data. I then released the hellbender at the original site of capture and then would resume the search. We continued searching until we had completely explored the whole study site. A typical survey lasted 45 minutes to 2.5 hours, depending on the number of hellbenders captured and people surveying. It should be noted that this method is often difficult to feasibly and reliably implement during above normal stream flow. I often was able to effectively survey the study site during the day, however, at night the higher flow made it impossible to clearly see the stream bottom. I was often only able to search certain areas of the site during anything other than optimal conditions.

Crayfish Luring Capture Technique

During night surveys, I would often see a hellbender with its head just barely sticking out from underneath an extremely large rock (Fig. 5). This rock was too massive to lift in a daytime survey, and this hellbender never seemed to be active and leave this rock at night. On two separate occasions I was able to lure this hellbender out using a crushed crayfish placed approximately 10-20 cm outside of the

entrance to the rock. I then waited for 10 to 15 minutes for this hellbender to slowly leave the rock enough to effectively grab and capture by hand. After discovering the success of this technique, I used it on other occasions to lure out hellbenders from underneath cover rocks at night. Surprisingly, direct and extended exposure to search lights did not seem to deter the hellbenders from venturing out to eat the crayfish. Also interesting to note, I was only able to perform this technique a maximum of two times for the same hellbender in a single season. After one to two captures using this method, the hellbender would retreat under the rock if searchers came within approximately a meter of its rock. This retreat behavior was still present up to two months from last capture using the crayfish luring method. This may suggest some form of learned behavior and an ability to remember negative stimuli for at least a period of 3 to 4 months. While this technique was not necessarily part of the sampling protocol, it was an effective capture technique in certain cases.

Marking Technique

After a hellbender's initial capture, I tagged each hellbender with a P.I.T. tag. All tags were 12 mm, 125 kHz Biomark TX1411SSL. Each tag was sterilized in 100% rubbing alcohol prior to being implanted, and I injected the tag into the fatty dorsal region of the base of the tail (Fig. 6). I then recorded the tag number with a HomeAgain brand microchip reader and returned the hellbender to its original site of capture. If the hellbender was captured under a rock, an effort was made to return it to that specific rock. The processing time took approximately 10 to 15 minutes per new hellbender. If I recaptured a tagged hellbender, I simply scanned the tag with the reader and collected the same measurements. Wounds from the injection of P.I.T. tags were minimal and healed with no sign of complications. There was no evidence that any tags shifted or were expelled, and this method of tagging appeared to have little to no adverse effects on the animal. P.I.T. tags from hellbenders tagged in 1998 also showed no sign of shifting.

RESULTS

Surveys and Search Effort

Twenty-eight survey events (19 diurnal, 9 nocturnal) and 112 adjusted person hours resulted in the capture of 38 hellbenders on the West Fork of the Greenbrier from April 24th to October 10, 2009. I captured twelve hellbenders (8 males, 4 females) during nocturnal surveys, and 26 (8 females, 17 males, and unknown sex) during diurnal rock-lifting and snorkeling surveys. Person hours were adjusted during diurnal surveys to include only those actively searching. This was normally the equivalent of two people, one person to lift the rock, and one to snorkel underneath. Even if there were more than two people, others were not actively searching and were considered observers. I quantified survey efforts this way in order to avoid biased search effort data. Often times I would have up to seven people in the stream, however, only one rock was lifted at a time. Actual person hours were 186 for the 2009 season. Surveys ranged in their effectiveness for finding hellbenders, ranging from zero hellbenders per hour to 1.25 hellbenders per hour.

Morphometrics

I captured and measured 38 hellbenders in 2009. Mean total length was 45.86 cm. Mean total length for males was 43.70 cm and ranged from 36 cm to 49 cm. Mean total length for females was 49.67 cm and ranged from 43 cm to 57 cm. I found a hellbender of unknown sex that was 23 cm in total length and was the smallest hellbender captured during my study (Fig 10).

Mean mass of all hellbenders was 537.5 grams, ranging from 79.0 g to >1000 g. (One very large female was over 1000 g, and I did not have a >1000 g scale at the time.) It should be noted that the spring

did go past the 1000 g mark, but did not bottom out the scale. Therefore it is likely that an approximation of 1000 g for this animal will not adversely affect the data. Mean mass for males was 504.45 and ranged from 260 g to 720 g. Mean mass for females was 697.31 and ranged from 420 g to 1020 g.

1998-2009 Demographic Shift

I found a shift in size classes of hellbenders in the 2009 study towards larger salamanders (Fig. 12). However, I did find 1st year gilled larvae in this study. This represents the smallest size class (4-6 cm total length), but they were not included as I could not capture them without jeopardizing them to potential predators and general undue stress. Therefore I did not include them in my size class comparison figure. I found one individual that was 23 cm in total length; however, sex could not be confidently determined and was not used in analysis.

I found a notable sex shift toward male hellbenders in the 2009 study. In 1998, Jeff Humphries captured 19 males, 18 females, and 4 of unknown sex (Fig. 11). The sex ratio for hellbenders in the study stream was 1.06:1. In 2009, I captured 25 were male, 12 were female, and one was of undetermined sex (Fig. 12). The sex ratio was 2.1:1.

Eleven-Year Hellbender Growth

I found 11 hellbenders (8 males and 3 females) that had been previously tagged in 1998 within the study site (Table 1). Mean growth over 11 years was 3.38 cm, with growth ranging from 0.6 cm to 5.2 cm (Fig. 13). All recaptured 1998 hellbenders were over 40 cm in total length in 2009. Average annual growth would therefore be estimated at 0.31 cm. Mean gain in mass was 103.4 g, with a range of -225 g to 210 g (Fig. 9). One particular salamander, (P.I.T. # 008-866-349), lost 225 g in mass, and was in very poor condition when captured (Fig. 16).

Case Study of a Male Hellbender

I captured a male hellbender (#0A00081134) four times during the course of my study. He was first captured May 24, and was recaptured July 7th, August 10th, and September 7th of 2009. This was the only hellbender recaptured more than once, and is the only hellbender that I could follow and examine throughout the 2009 season. As the season progressed this hellbender showed a trend toward losing weight as mating season approached (Fig. 17). Also, I was able to note the occurrence and healing of wounds over time. On his initial capture, this hellbender showed no significant wounds or scarring. However, on the next capture in July, he exhibited fresh bite scars on his head, and three cuts below his right eye. I captured him again in August and he had a fresh scar behind his left front leg, and scarring by his right eye. The bite scar on his head was barely visible. On the final recapture in early September, he showed no signs of scarring. His cloaca was extremely swollen and he was milting upon being handled (Fig. 18). It is clear that while he showed a 15% percent drop in mass (620g to 525g) throughout the season, it did not seem to affect his ability to reproduce. The stresses of preparing for breeding season are likely the cause of this hellbenders change in body mass. This is likely not uncommon for male hellbenders approaching breeding season.

Crayfish Survey

I conducted a crayfish survey on August 14th to help determine the abundance of crayfish, the staple food source of hellbenders, within the study site. I documented the presence/absence of crayfish under 100 rocks greater than 30 cm wide chosen at random (Fig. 21). I also noted the presence of fishes under rocks. Rocks with hellbenders underneath were not counted in the survey. The vast majority of crayfish encountered in the West Fork were the New River Crayfish, *Cambarus (Hiaticambarus) chasmodactylus* (Fig. 22).

Evidence of Reproduction and Parental Care

In contrast to the 1998 study, I documented evidence of reproduction both in and out of the study site. On May 24, approximately 50 to 75 larval hellbenders were found under a large rock along with a large adult hellbender in the downstream section of the study site (Fig.7). The larvae were all approximately 4 to 6 centimeters in total length. The sex of the adult hellbender was not determined because it evaded capture. It is presumed that it was a male guarding fall 2008 hatchlings (J. Humphries, personal communication). This is potentially a rare example of extended parental care of larvae by hellbenders. I also found a larval hellbender near a large rock that had just been lifted. I did not attempt to capture it for fear of injury. However, I was able to view it long enough to confirm its identity as a first year larval hellbender. It also was approximately 4 to 6 centimeters in total length and was found at a depth of 0.5 meters. It is not clear if this particular larva was initially under the rock or in the interstitial spaces of the gravel and cobble that surrounded the large rock.

I also documented two separate nesting sites, one within the original study site, and one several 100 meters upstream of the study site. The first nest site was found on September 5th at the upstream end of the site (Flag 1), and was discovered by the presence of a few loose eggs protruding from the side of the rock (Fig.7). This was fortunate as I was able to identify a nest rock without lifting it and potentially compromising the nest site. This also allowed me the unique opportunity to examine a loose and unguarded egg. The egg had just very recently been fertilized as the embryo had just started to cleave into 4 sections (Fig. 8). This leads me to approximate the date of oviposition between September 3rd and September 4th. Another nest site was discovered several 100 meters upstream of the study site on September 6th. A male (#985120030063764) was guarding approximately 200-300 eggs beneath a large rock.

Mudpuppies (*Necturus maculosus*)

Throughout the course of my study, I found 3 mudpuppies. All sightings occurred in mid-August between the 11th and 15th. All were found underneath large rocks that would also be suitable hellbender habitat. The largest one was 9.2 cm TL (Fig. 14).

Wounds, Scars, Polydactylism and Mortality

Just over half (20) of the hellbenders captured exhibited wounds or scars. Injuries included missing limbs (stubs), missing toes, bite wounds and scarring from bite wounds, eye injuries, a broken shoulder, notches in tail, and open gashes. One male was missing his front left foot, rear left foot, and two toes on his rear right foot (Fig. 15). I recaptured this individual once, and both times he appeared to be in good general health. The most common injuries or past injuries were bite marks and scars to the head, with 11 hellbenders exhibiting evidence of being bitten in the head by another hellbender. Another hellbender had a broken left shoulder and a large gash under his front left leg (Fig. 16). This hellbender was a recapture from 1998 and had lost over 35% of his body mass (-215 g). There did not appear to be a correlation with sex to this type of injury as 3 females had bite scars on their heads. These findings were consistent with findings made by Jeff Humphries in 1998, as 49% of captured hellbenders had either missing limbs or toes, and 29% had major wounds or scars from major wounds present (Humphries, 1999).

I also found polydactylism present in my study. One female hellbender had nine toes on her rear right foot (Fig. 19). This has been seen in North Carolina hellbender populations and is most likely a complication of limb regeneration (Jeff Humphries, personal communication).

I documented a case of hellbender mortality. On September 5th, 2009 I found a dead male hellbender (42.5 cm total length, 32 cm snout/vent length) upstream from the study site. This male had a

swollen cloaca, and was assumed to be in decent reproductive status. I found him partially protruding from under the upstream end of a large rock, and he was partially disemboweled and just starting to decompose (Fig. 20). It appeared that his stomach had been cut with a knife, and there was no evidence that anything had fed on his remains. While his cause of death cannot be known for certain, it is likely he was killed by a fisherman.

Philopatry

I documented site fidelity among hellbenders in my study. Over a third (11 of 29) of the hellbenders tagged within the study site in 1998 were still found within the site 11 years later. One hellbender, a large female, was found within a meter of the same rock where she was found in 1998 (Humphries, personal communication).

DISCUSSION

Several studies have noted the decline of hellbender populations in recent years (Wheeler et al., 2003; Briggler et al., 2007; Foster et al., 2009). Anecdotal accounts of historical records and abundance compared with recent data have indicated a strong decline and loss of several historical populations of hellbenders across their range. In this study, I sought to find out if the hellbender population of the West Fork of the Greenbrier was declining.

In my study, demographic data from 1998 were compared to data collected in 2009 to document any demographic changes within the hellbender population in the West Fork of the Greenbrier. I found a dramatic shift in sex ratio, with males dominating the population more than two to one (Fig.12). A 2009 New York study also documented a shift toward males in several populations (Foster et al., 2009). In that

study, demographic data from the 1980's was compared to 2004 and 2005 data. They noted an overall sex shift from 0.8:1 historic sex ratio to a 1.8:1 ratio. Female hellbenders declined in all of their study sites. This shift towards a male dominated population was also coupled with a general decline in hellbender abundance (Foster et al., 2009). A recent Pennsylvania study also found a hellbender population with a 2:1 sex ratio (Llewellyn and Petokas, unpublished data).

Methodology Comparison of West Fork Greenbrier Studies

I employed diurnal rock-lifting surveys for the majority of sampling in my study. This is in contrast to the Humphries 1998 study where night surveys comprised the majority of sampling. I encountered several problems during night searches. Unless weather and stream conditions were optimal, limited visibility affected my ability to effectively search the entire study site. For almost half of my field season, the stream level was too high and the flow too fast to allow me sufficient visibility to search effectively. Many nights I was only able to search the very shallow sections of the stream. Also, if it had recently rained, the stream would rise quickly and hinder visibility. In addition, night surveys are biased and target only active hellbenders. Smaller size classes are likely either not as active at night, and/or are more difficult to see on the stream bottom (Personal observation). The increased use of nocturnal surveys in 1998 likely limited the detection of smaller size classes of hellbenders (Humphries and Pauley, 2005). This also likely limited the detection of nests and gilled larvae.

Diurnal rock-lifting was the most efficient and successful method in a recent study comparing three hellbender capture methods (Foster et al., 2008). While this study did not test nocturnal visual searches, it was clear that rock-lifting was the best method. This technique coupled with snorkeling is the method thought to produce multiple age groups of non-gilled and adult hellbenders, and all sizes of gilled larvae within short sampling periods (Nickerson and Krysko, 2003). However, during breeding season, this method may disrupt nest sites and disturb or kill eggs and larvae (Williams et al. 1983). I was aware

of this drawback, and during the breeding season where eggs were being deposited and guarded, I modified my technique slightly. Beginning late August, I would have the rock-lifter very slowly lift a potential nest rock to approximately 10 centimeters off the stream bottom. I then implemented the use of a waterproof flashlight to peer underneath rocks after the silt had settled. I feel this slight modification limited the disturbance to a potential nest sight by not completely uncovering and disrupting a nest (Personal observation). Twice during my study, I located nest sites. Upon discovery, I would estimate the number of eggs, and slowly return the rock back to its original position. On one occasion I was able to easily grab the guarding male, collected data, and returned him to his nest.

Long-term growth and Future Monitoring

Hellbenders are long-lived salamanders, living up to 55 years in captivity (Nigrelli, 1954). They are also one of the largest salamanders in the world, reaching lengths of up to 74 cm (Bishop, 1941; Nickerson and Mays, 1973). Displaying indeterminate growth, hellbenders grow their entire lives. However, very little is known about their longevity under “non-captive conditions” (Nickerson and Mays, 1973). A length-age relationship was derived from Ozark hellbenders, *Cryptobranchus alleganiensis bishopi*, in the North Fork of the White River, Missouri (Peterson et al., 1983). Age estimates are possible for hellbenders up to around 40 cm using the following equation:

$$\text{Age}_{(\text{months})} = (1/-0.0127)\ln(5.2193 + (-0.0127)TL_{(\text{mm})}) + 120 \text{ (Peterson et al., 1983)}$$

This estimate, however, is based upon the Ozark subspecies, which generally is slightly smaller on average than the Eastern subspecies (Nickerson and Mays, 1973). This would likely skew estimates for the Eastern Hellbenders found in this study. A later review of two studies (Taber et al., 1975; Peterson et al., 1983) found that Eastern Hellbenders growth is greater and more variable than the Ozark subspecies (Peterson et al., 1985). Long-term growth data of large salamanders is severely lacking. Age estimates of hellbenders >43 cm is difficult due to the variation of individual growth, and the decreasing growth in

these larger size classes (Westrheim and Ricker, 1978). Therefore, growth of large classes of salamanders is largely unknown. This raises several study questions. How fast do hellbenders on the West Fork of the Greenbrier grow? Do salamanders vary in growth rates among individuals in a population? Is this Eastern West Virginia population representative of other hellbender populations?

Of the 29 hellbenders tagged within the site in 1998, I found 11 in 2009. All were greater than 40 cm. Mean 11-year growth for all 11 1998 hellbenders was 3.66 cm. Growth rates varied among individuals, with one hellbender growing only 0.6 cm, and another 5.2 cm (Table 1). Four individuals were very similar in size in 1998 (40, 40.5, 40.5, 40.8 cm). However, growth was 3.2, 4, 5, and 1.7 cm, respectively. The two largest salamanders (55 and 56 cm, both female) also showed great variation in growth. The 55 cm hellbender measured in my study grew 4 cm in 11 years, while the 56 cm animal grew only 0.6 cm. The 55 cm hellbender grew 6.6 times more than her counterpart. Also, it should be noted that 0.6 cm is small, and could easily be within the margin of error during the measuring process. Effectively it is possible, though not likely, that this female did not grow at all.

In order to determine if food availability may be a factor, I conducted a crayfish survey. Crayfish make up a vast majority of the hellbender's diet and were found to make up approximately 87.5% of stomach content in 40 Ozark hellbenders (Nickerson and Selby, 1969 unpublished data). Another study found crayfish to be present in 81% of the stomachs of 108 hellbenders (Peterson et al., 1989). As hellbenders were found to be the primary predator of crayfish in streams they occupy (Dierenfeld et al., 2009), crayfish density data is an important aspect of hellbender ecology. Crayfish populations are considered low if 0-30% of rocks revealed crayfish, moderate if 31-60% revealed crayfish, and high if $\geq 60\%$ of rocks revealed crayfish (Nickerson et al., 2003). I found crayfish were in moderate abundance at my site, with 43 percent of the rocks sampled having at least 1 crayfish underneath. The very large *Cambarus (Hiaticambarus) chasmodactylus* was the crayfish species I most often encountered in the West Fork of the Greenbrier. There was a moderate abundance of crayfish throughout the study period, and I doubt prey availability is a problem for hellbenders at my study site.

Even if I take the greatest amount of growth documented in 11 years, 5.2 cm, and liberally estimate annual growth, it still is only 0.47 cm a year. This is a very slow growth rate for a salamander that can reach lengths in excess of 70 cm. Current published estimates of age for non-captive hellbenders are approximately 30 years (Taber et al., 1975; Petranka 1998). However, based on findings in this study, I believe many salamanders I found to be over 50 years in age.

Throughout my study, 12 hellbenders were able to evade capture. It is certainly possible, and probably likely, that some of those were hellbenders tagged in 1998. My work laid the foundation for future demographic studies on this stream by tagging 27 “new” hellbenders. Philopatry, reproduction, and movement can also be examined and compared to data from this study in the future. Several questions require future investigation, including but not limited to: Is the sex ratio shift towards males cyclical, or does it represent a long-term trend for the population? Based on reproduction findings, is there sufficient juvenile recruitment in this stream? Is this adult dominated population representative, or are juveniles not detected? Is this population truly stable, and if so, will this population continue to remain stable?

The sex ratio shift I noted remains enigmatic. One hypothesis, which remains untested, is the potential of an unknown pollutant which may have a masculinizing effect on hellbenders in the stream (M. Nickerson, personal communication). I believe that this is unlikely, as the study stream is within the Monongahela National Forest has limited disturbance from pollutants. It is likely that this shift in sex ratio is an accurate representation of the demographic composition in this stream as the survey method I employed is the most comprehensive of all hellbender surveying methods (Nickerson and Krysko, 2003; Foster et al., 2008).

Perhaps the most likely explanation for the sex ratio shift I found is the result of female emigration and movement. A recent West Virginia study found that female hellbenders moved more frequently, and moved greater distances than males (Greathouse, 2006). This increased female movement may explain my low number of female recaptures from 1998. Female hellbenders tagged in 1998 may

simply have moved out of the site. This however does not explain the overall sex ratio (both “new” and 1998 hellbenders) I documented in my study. Future studies could help determine if this is a true long-term trend for this population.

Potential Impact of Surveys

As the survey progressed, I noticed that it became increasingly more difficult to find hellbenders within the study site. I also noticed that many of the rocks that previously sheltered hellbenders often no longer were used by hellbenders. On many occasions, several of the rocks that produced hellbenders were now being used by rock bass and other fish. I took great efforts to return the rock to as close to its original positioning as physically possible, but often that was very difficult as many of these rocks were sealed by sediment. Also, upon lifting large shelter rocks, several smaller rocks would shift and trying to recreate the exact original position and orientation of that rock was impossible. The disturbance of this “seal” likely creates a subpar micro-habitat for use as a hellbender shelter rock. The amount of time until that rock becomes suitable for shelter again warrants future investigation. The 1998 study primarily implemented nocturnal surveys and likely had less of an impact on hellbender habitat. I focused more on diurnal surveys and lifted more rocks than the previous study. The lower impact of the 1998 study likely did not displace many hellbenders from the study site, thus allowing for them to be found 11 years later. The potential return of a particular hellbender to a particular rock after disturbance could also be a future study question. When biologists speculate about the potential causes of decline in hellbender populations, particularly ones that have been actively surveyed for several decades, we must not under-estimate the impacts of scientific surveys.

Conclusions and Management Recommendations

Overall, I did not document a decline in abundance in this population of hellbenders on the West Fork of the Greenbrier. Furthermore, I documented two nest sites, evidence of potential extended parental care, and gilled larvae. Also, 19 new hellbenders were found in the study site. These findings strongly suggest that hellbender populations on the West Fork of the Greenbrier are currently viable and relatively stable. While the evidence of reproduction I observed is in contrast to the previous study 11 years prior, it is likely due to a difference in search methods. I believe that this stream could potentially serve as a reference stream, and may represent a system in which hellbender populations can remain stable under continued protection and monitoring. This is not to say that this stream is pristine and represents historical conditions. The West Fork of the Greenbrier likely represents some of the best hellbender habitat in West Virginia and should be managed and monitored to ensure hellbender populations remain stable. Sedimentation from an adjacent forest service road, incidental killing by fisherman, and collection are still likely causes of potential future decline for hellbenders in the West Fork of the Greenbrier if not regulated.

LITERATURE CITED

- Bishop, S. 1941. The Salamanders of New York. New York State Mus. Bull. 324-365.
- Briggler, J., J. Utrup, C. Davidson, J. Humphries, J. Groves, T. Johnson, J. Ettling, M. Wanner, K. Traylor-Holzer, D. Reed, V. Lindgren, and O. Byers (Eds.) 2007. Hellbender Population and Habitat Viability Assessment: Final Report.
- Bury, R.B., C.K. Dodd, Jr., and G.M. Fellers. 1980. Conservation of the Amphibia of the United States: a review. Resource Publication 134, U.S. Fish and Wildlife Service, Washington, D.C., 34p.
- Conant, R.A. and J.T. Collins. 1998. A field guide to reptiles and amphibians of eastern and central North America, Third edition.
- Congdon, J.D., A.E. Dunham, and R.C. Van Loben Sels. 1993. Delayed sexual maturity and demographics of Blanding's Turtles (*Emydoidea blandingii*): implications for conservation and management of long-lived organisms. *Conservation Biology* 7:826-833.
- Congdon, J.D., A.E. Dunham, and R.C. Van Loben Sels. 1994. Demographics of Common Snapping Turtles (*Chelydra serpentina*): Implications for conservation and management of long-lived organisms. *American Zoologist* 34:397-408.
- Dierenfeld, E., McGraw, J., Fritsche, K., Briggler, J., and J. Ettling. 2009. Nutrient composition of whole crayfish (*Orconectes* and *Procambarus* species) consumed by hellbender (*Cryptobranchus alleganiensis*). *Herpetological Review* 40(3):324-330.
- Dundee, H. A. 1971. *Cryptobranchus* and *Cryptobranchus alleganiensis*. Catalogue of American Amphibians and Reptiles. SSAR 101.1-101.4.
- Foster, R.L., A.M. McMillan, A.R. Breisch and K.J. Roblee. 2008. Analysis and comparison of three capture methods for the Eastern Hellbender (*Cryptobranchus alleganiensis alleganiensis*). *Herpetological Review* 39(2):181-186.
- Foster, R.L., A.M. McMillan, and K.J. Roblee. 2009. Population status of hellbender salamanders (*Cryptobranchus alleganiensis*) in the Allegheny River drainage of New York State.
- Grandmaison, D., Mayasich, J., and C. Phillips. 2003. Eastern hellbender status assessment report. Natural Resources Research Institute. Duluth, MN.
- Greathouse, J. 2006. Monitoring of the Eastern hellbender, *Cryptobranchus alleganiensis alleganiensis*, in five Ohio River tributary streams in the Northern Panhandle of West Virginia. (Technical report submitted to West Virginia Division of Wildlife). 93 pp.
- Green, N.B. 1934. *Cryptobranchus alleganiensis* in West Virginia. *Proc. W.Va. Academy of Science* 7:28-30.

- Green, N.B. and T. K. Pauley. 1987. Amphibians and Reptiles in West Virginia. University of Pittsburgh Press. Pittsburgh, PA.
- Grobman A.B. 1943. Notes on salamanders with the description of a new species of *Cryptobranchus*. Occasional Papers of the Museum of Zoology 470:1-13.
- Hillis, R.E. and E.D. Bellis. 1971. Some aspects of the ecology of the hellbender *Cryptobranchus alleganiensis alleganiensis*, in a Pennsylvania stream. Journal of Herpetology 5:121-126.
- Humphries, W.J. 1999. Ecology and population demography of the hellbender, *Cryptobranchus alleganiensis*, in West Virginia. M.S. Thesis, Marshall University, Huntington, WV.
- Humphries, W. J. and T. K. Pauley. 2005. Life history of the Hellbender, *Cryptobranchus alleganiensis*, in a West Virginia stream. American Midland Naturalist 154:135-142.
- Nickerson, M.A., K.L. Krysko, and R.D. Owen. 2002. Ecological status of the hellbender (*Cryptobranchus alleganiensis*) and the mudpuppy (*Necturus maculosus*) salamanders in the Great Smoky Mountains National Park. Journal of North Carolina Academy of Science 118:27-34.
- Nickerson, M. A. and K. L. Krysko. 2003. Surveying for hellbender salamanders, *Cryptobranchus alleganiensis* (Daudin): A review and critique. Applied Herpetology 1:37-44.
- Nickerson, M. A. and C. E. Mays. 1973. The Hellbenders: North American “giant salamanders”. Milwaukee Public Museum. Milwaukee, WI.
- Nickerson, M.A. and M. Selby. 1969. Unpublished data. Taken from Nickerson, M. A. and C. E. Mays. 1973. The Hellbenders: North American “giant salamanders”. Milwaukee Public Museum. Milwaukee, WI.
- Nigrelli, R. 1954. Some longevity records of vertebrates. Transactions of the New York Academy of Sciences. 1954(16):296-299.
- Peterson, C. L., R. F. Wilkinson Jr., M. S. Topping, and D. E. Metter. 1983. Age and growth of the Ozark Hellbender (*Cryptobranchus alleganiensis bishopi*). Copeia 1983(1):225-231.
- Peterson, C. L., Topping, M. S., Wilkinson, R. F., Taber, C. A. 1985. Examination of long term growth of *Cryptobranchus alleganiensis* predicted by linear regression models. Copeia 1985(2): 492-496.
- Peterson, C. L., Topping, M. S., Wilkinson. 1989. Seasonal food habits of *Cryptobranchus alleganiensis* (Caudata: Cryptobranchidae). The Southwestern Naturalist 34(3):348-441.
- Petranka, J. W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, DC.
- Pfingsten, R.A. and F.L. Downs, eds. 1989. Salamanders of Ohio. Ohio Biological Survey. New Series Vol. 7 No. 2.

- Taber, C. A., R. F. Wilkinson Jr., and M.S. Topping. 1975. Age and Growth of Hellbenders in the Niangua River, Missouri. *Copeia* 1975(4):633-639.
- Trauth, S.E., J.D. Wilhide and P. Daniel. 1992. Status of the Ozark hellbender, *Cryptobranchus bishopi* (Urodela: Cryptobranchidae), in the Spring River, Fulton County, Arkansas. *Proceedings of the Arkansas Academy of Science* 46:83-86.
- Ultsch, G.R. and J.T. Duke. 1990. Gas exchange and habitat selection in the aquatic salamanders *Necturus maculosus* and *Cryptobranchus alleganiensis*. *Oecologia* 83(2):250-258.
- Welsh, H.H. and L.M. Ollivier. 1998. Stream amphibians as indicators of ecosystem stress: a case study from California's redwoods. *Ecological Applications* 8: 1118-1132.
- Westrheim, S.J. and W.E. Ricker. 1978. Bias in using an age-length key to estimate age-frequency distributions. *Journal of the Fisheries Research Board of Canada* 35:184-189.
- Wheeler, B. A., E. A. Prosen, A. Mathis, and R. F. Wilkinson. 2003. Population declines of a long-lived salamander: A 20 + - year study of hellbenders, *Cryptobranchus alleganiensis*. *Biological Conservation* 109:151-156.
- Williams, R. D., J. E. Gates, and C. H. Hocutt. 1981. An evaluation of known and potential sampling techniques for hellbender, *Cryptobranchus alleganiensis*. *Journal of Herpetology* 15:23-27.

APPENDIX

P.I.T. tag #	Sex	TL 1998	TL 2009	Growth(cm)	Mass (g) 1998	Mass (g) 2009	Δ Mass (g)
006-798-526	M	38.3	42.5	4.20	330	401	71
0A00085974	M	39	43.5	4.50	335	545	210
0A00085612	M	40	43.25	3.25	280	425	145
0A00054800	M	40.5	44.5	4.00	500	465	-35
0A00081134	M	40.5	45.5	5.00	350	564	214
0A00083847	M	40.8	42.5	1.70	381	541	160
0A00083243	M	42.8	46.5	3.70	430	630	200
008-127-071	F	45.3	50.5	5.20	405	615	210
008-866-349	M	47.5	48.5	1.00	640	415	-225
007-829-630	F	51	55	4.00	655	760	105
0A00083714	F	55.4	56	0.60	893	975	82

Table 1. Eleven-year growth data of the 11 1998 hellbenders recaptured in 2009. Mean growth (total length) over 11 years was 3.38 cm, with growth ranging from 0.6 cm to 5.2 cm. Average gain in mass was 103.4 g, with a range of -225 g to 210 g.

00060, Discharge, cubic feet per second,												
YEAR	Monthly mean in cfs (Calculation Period: 2008-09-01 -> 2009-09-30)											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2008									8.48	7.03	57.3	529
2009	410	290	273	429	487	121	51.9	105	28.9			
Mean of monthly Discharge	410	289	273	429	487	121	52	105	19	7	57	529
** Incomplete data have been used for statistical calculation												

Table 2. Discharge data for the West Fork of the Greenbrier in cubic feet per second. Taken from USGS gauging station 03180500, Greenbrier River at Durbin, WV.

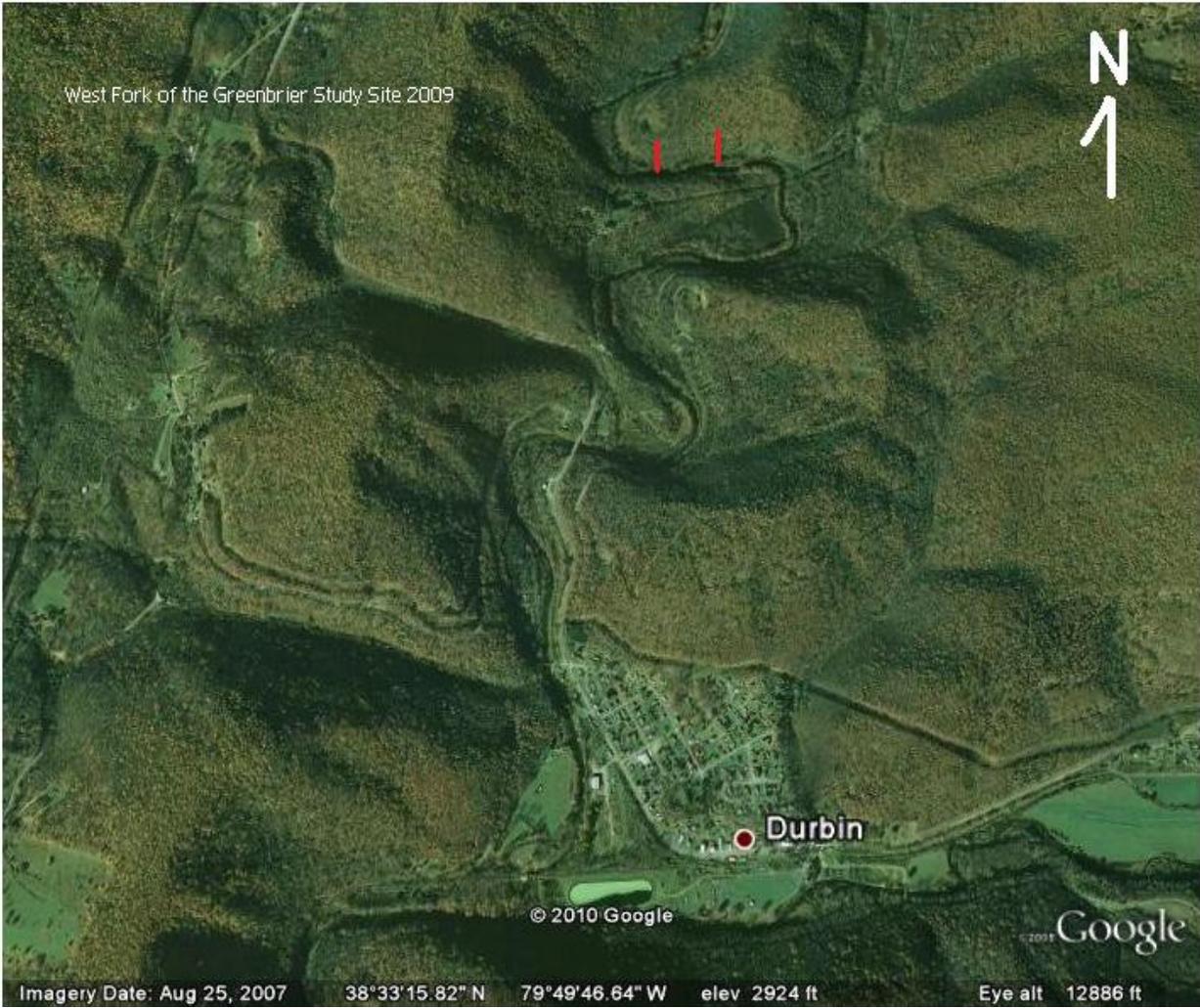


Figure 1. Location of study site along the West Fork of the Greenbrier River, Pocahontas County, West Virginia. Study reach is located between the two red lines.

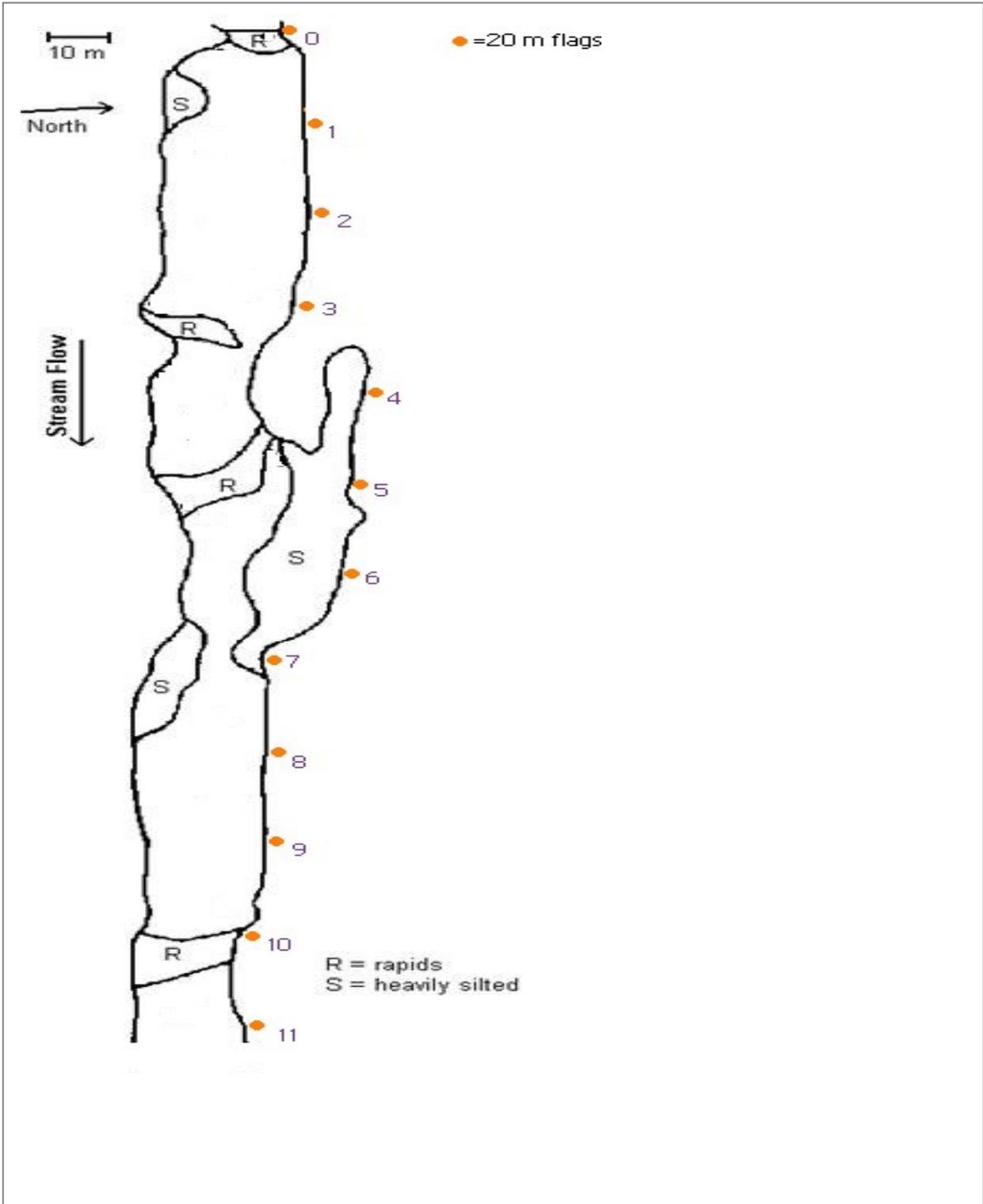


Figure 2. Diagram of study reach. Flags were placed every 20 m to aid in documenting hellbender capture locations.



Figure 3. Photo of diurnal rock-lifting survey technique. Photo by George Grall.



Figure 4. Measuring apparatus and a recently captured hellbender. Photo by David Fields.



Figure 5. Hellbender at night with only head exposed. Note the scarring near the mouth. Photo by Doug Horchler.



Figure 6. Marking Technique. Insertion of a P.I.T. tag into the fatty dorsal region of the tail. Photo by Casey Bartkus.

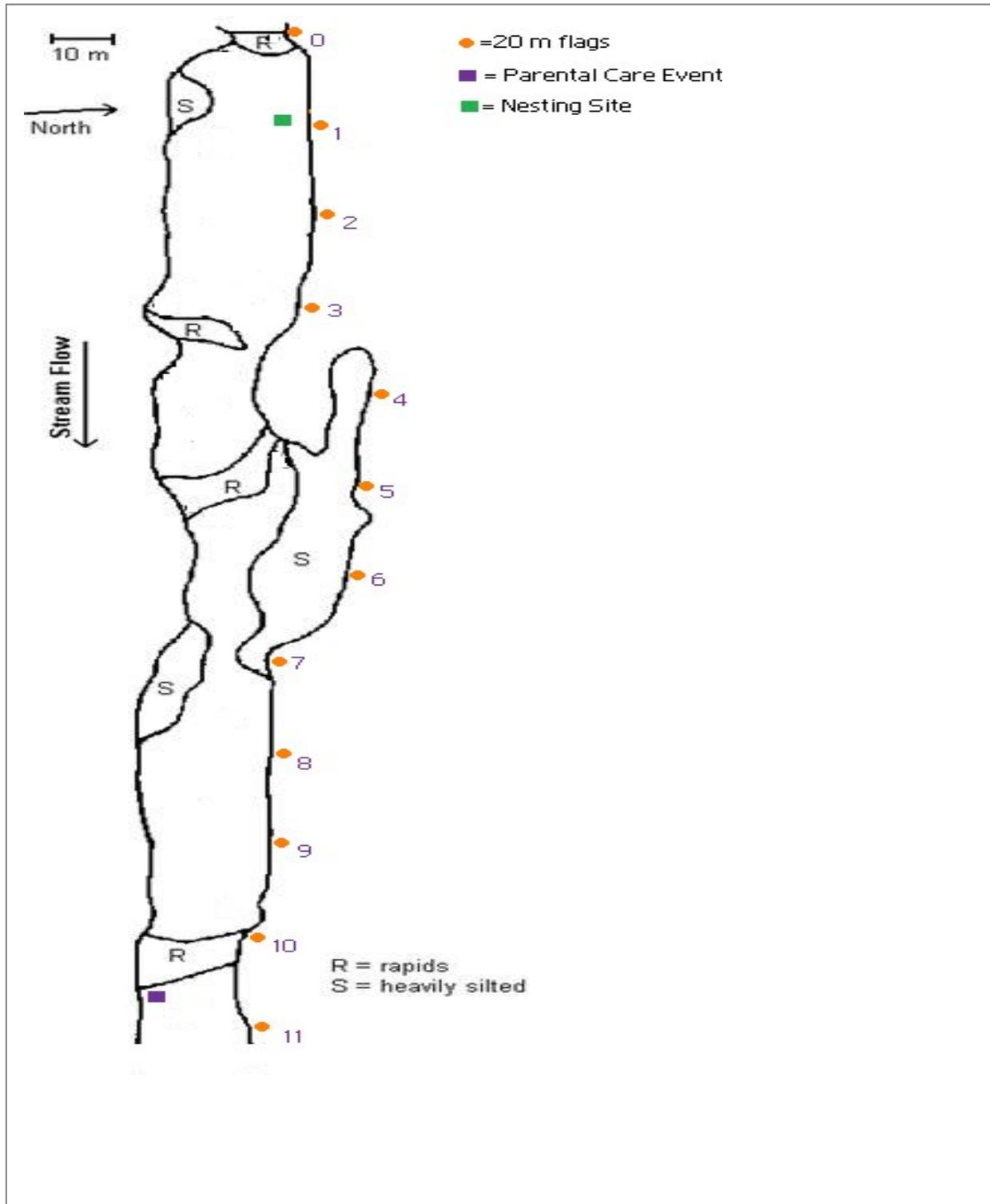


Figure 7. Documented evidence of reproduction within study reach in 2009. Note both a nest site and a potential parental care event.



Figure 8. A loose hellbender egg found protruding from a nest in the West Fork of the Greenbrier on September 5th, 2009. Note how the embryo is beginning to cleave. Photos by Doug Horchler.

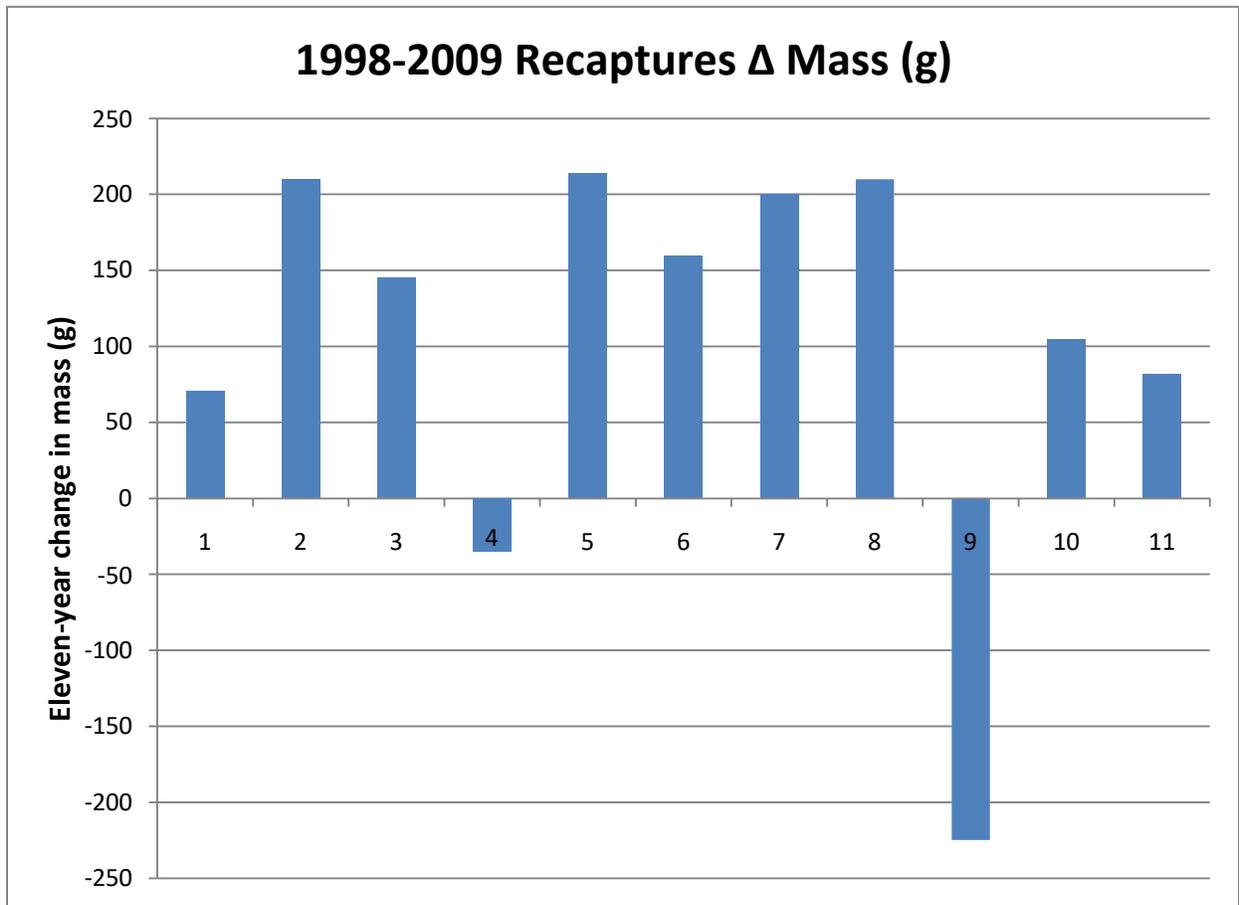


Figure 9. Change in mass (g) of the eleven 1998 recaptured hellbenders from the West Fork Greenbrier.



Figure 10. Hellbender # 985120027944372 was the smallest hellbender (23 cm T.L., 79 g) captured during the study. Photo by Jeff Humphries.

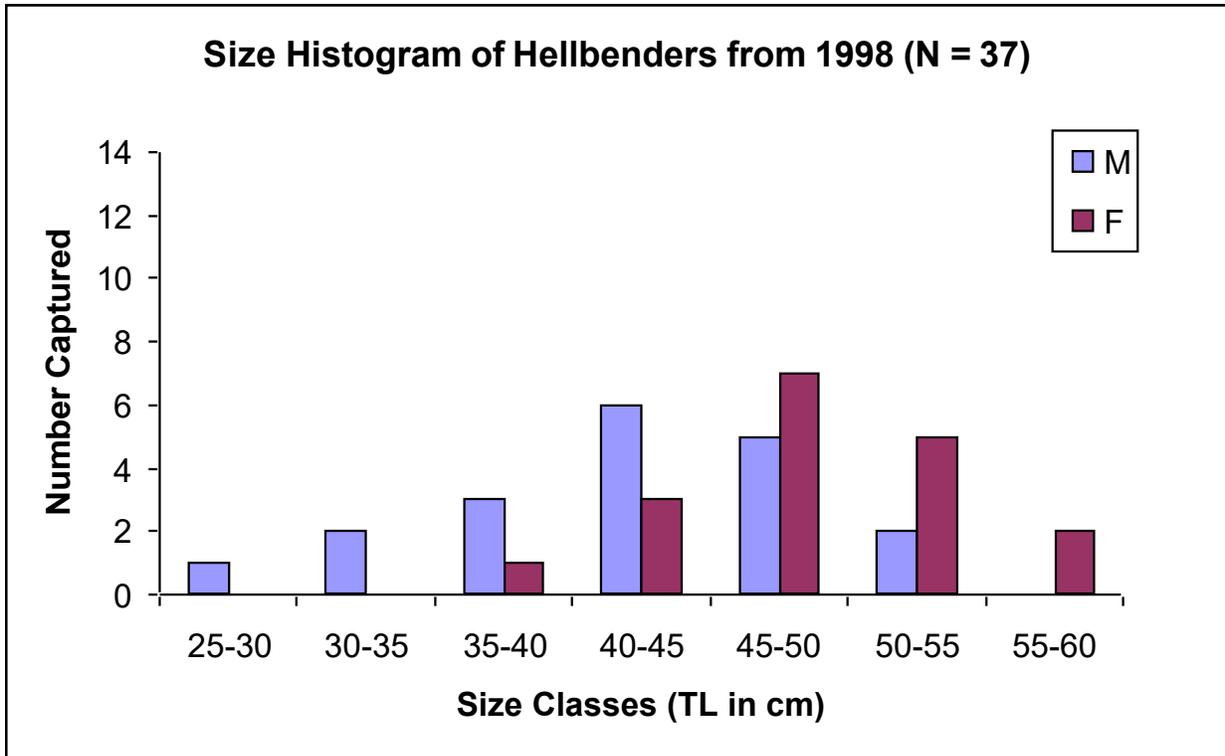


Figure 11. Demographics for the 1998 West Fork of the Greenbrier hellbender population. Note the almost even sex ratio (1.06:1), and a normal size class distribution for each sex.

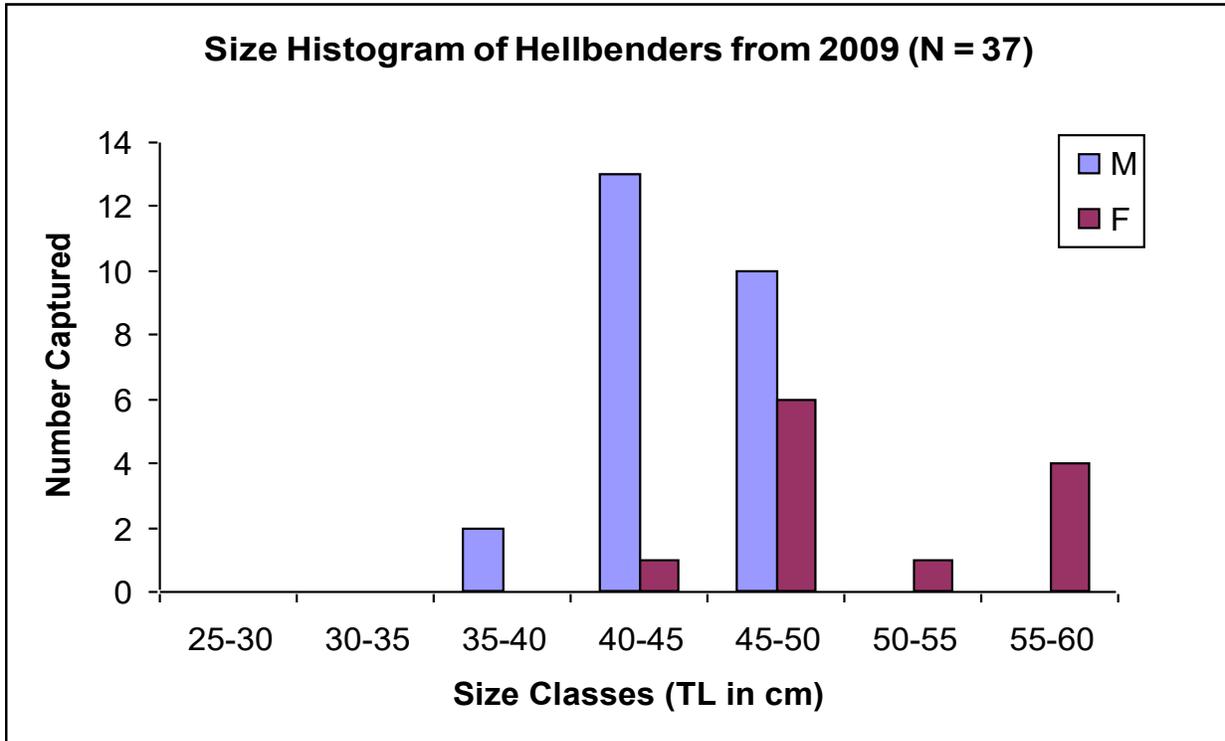


Figure 12. Demographics for the 2009 West Fork of the Greenbrier hellbender population. Note the sex shift to a male dominated population (2.1:1), and shift toward larger size classes of hellbenders.

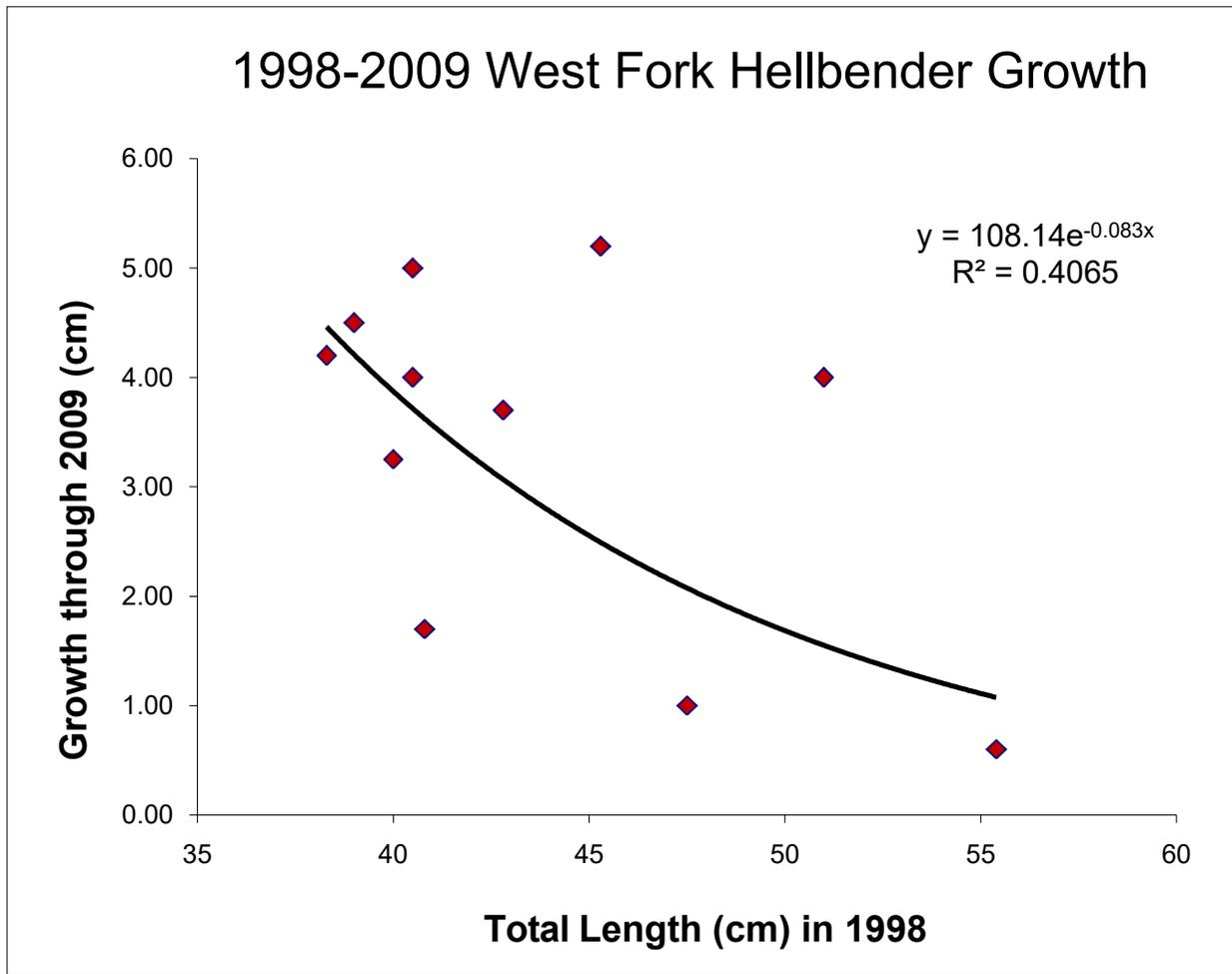


Figure 13. Eleven-year growth curve for 1998 hellbenders recaptured in 2009 (n=11) on the West Fork of the Greenbrier. Average 11 year growth was 3.38 cm (range= 0.6-5.2 cm).



Figure 14 . Mudpuppy (*Necturus maculosus*) found August 11th, 2009 underneath large rock. Total length was 9.2 cm. Photo by Geoge Grall.



Figure 15. Hellbender #0A0008347. Captured on the West Fork of the Greenbrier in May 2009. Note the missing feet on both front left and rear left legs. Photo by George Grall.



Figure 16. Hellbender #008-866-349. This hellbender had a broken left shoulder and a large gash under his left front leg, significant scarring on his head and body, and in very poor and weakened condition when recaptured in 2009. He had lost over 35% of his body mass from 1998 (-225 g). Lower left photo by Scott Jones, other three photos by Kevin Messenger.

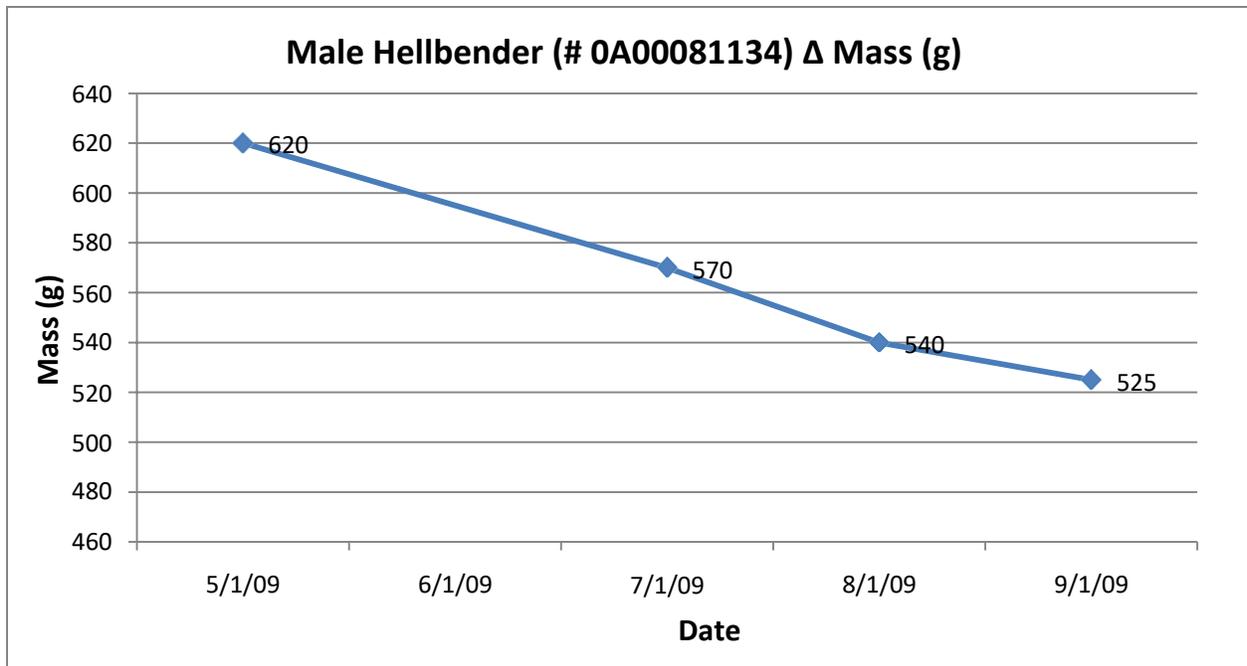


Figure 17. Hellbender (#0A00081134), a male, showed a steady decline in mass from his initial capture on May 24th, 2009. This loss of approximately 15% of his body mass likely represents stress in preparation for the breeding season.



Figure 18. Male hellbender (#0A00081134) captured on September 7th, 2009. Note the very swollen cloaca and the emission of milt upon being handled. Photo by Doug Horchler.



Figure 19. An example of polydactyly. This female hellbender had nine toes on her rear right foot. Photo by Doug Horchler.



Figure 20. Dead hellbender discovered September 5th, 2009 upstream from the study site. Note that this hellbender is a male and has a very swollen cloaca. Cause of death is unknown. Photos by Doug Horchler.

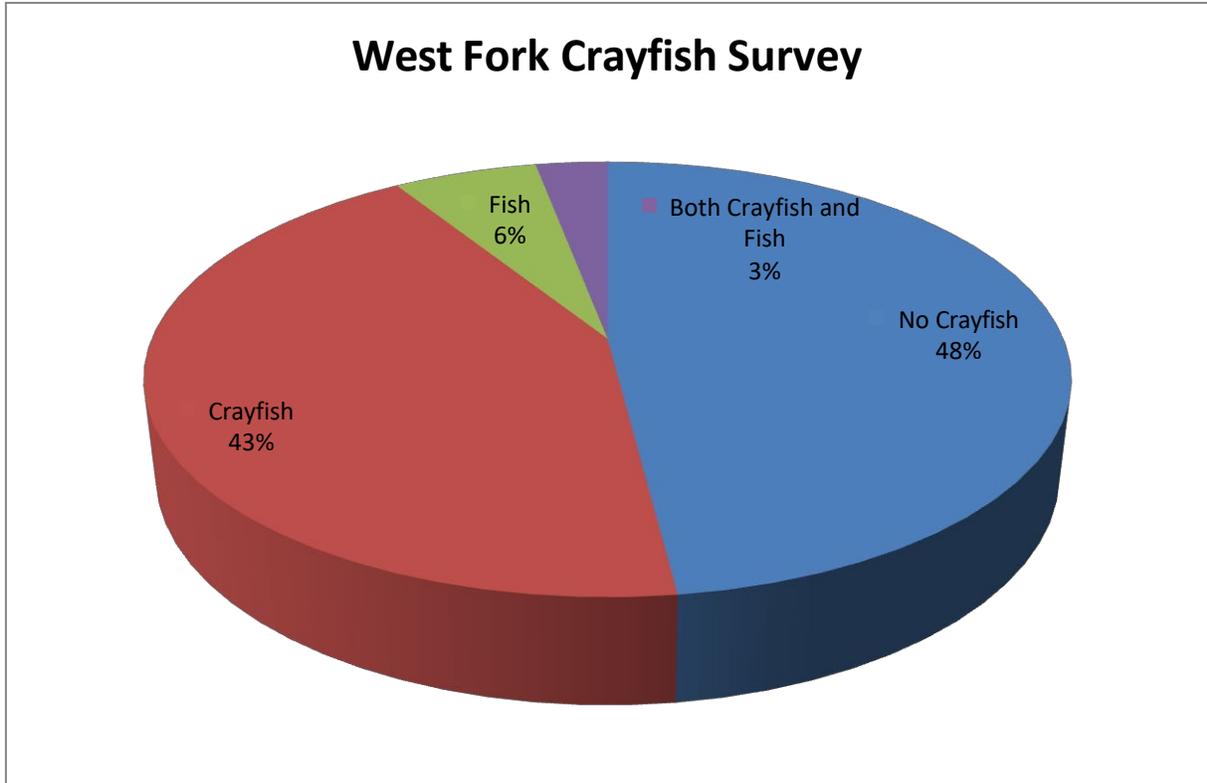


Figure 21. Results of August 2009 crayfish survey. I noted the presence/absence of crayfish under 100 rocks greater than 30 cm wide chosen at random. More than half of the rocks surveyed had either crayfish and/or fish present underneath them.



Figure 22. The New River Crayfish, *Cambarus (Hiaticambarus) chasmodactylus*. This species of crayfish is the likely the staple prey item for hellbenders in the West Fork of the Greenbrier. Photo by Kevin Messenger.