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Habitat Preferences of the Eastern Hellbender in West Virginia

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Habitat Preferences of the Eastern Hellbender in West Virginia

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By

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ABSTRACT

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By S. Conor Keitzer

The Eastern Hellbender, *Cryptobranchus alleganiensis alleganiensis*, is a species of concern in West Virginia and is in need of management. An important component of conservation efforts will involve identifying suitable habitat for protection. The goal of this research was to locate populations and examine hellbender habitat preferences to help managers identify habitat for protection. Populations were located using rock turning surveys from May through November, 2006. Hellbenders may be sensitive to water chemistry, so the dissolved oxygen, pH, turbidity, specific conductivity, and water temperature were measured. Substrate composition may influence populations, so substrate was characterized with Wolman pebble counts. Crayfish relative abundance was measured because they are an important prey item. Mean habitat characteristics of sites where hellbenders were present and absent were compared with t-tests. Habitat variables were ordinated in principal component analysis and examined in 2-dimensional ordination space to determine if sites where hellbenders were present grouped. Populations were found at 12% of sites, indicating that populations have declined in many streams. Hellbenders preferred sites with a large amount of gravel and cobble, cool water temperatures, low specific conductivity, and lower pH values. Gravel and cobble substrates may provide habitat for larval hellbenders and invertebrate prey items. Cool streams allow for more efficient cutaneous gas exchange. Low specific conductivity may indicate undisturbed conditions, suggesting hellbender populations were concentrated in less disturbed streams. Acidic conditions can alter prey communities and affect amphibian survival, so it was surprising to find populations in more acidic streams, although levels were above those known to harm stream ecosystems. Streams with similar habitat characteristics should be protected to conserve this unique salamander.

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Chapter 1
Overview

The long-term survival of a species depends on populations successfully recruiting new individuals through reproduction, which is intimately linked to environmental conditions. Habitat specialists, such as the hellbender, are species which have narrow environmental tolerance ranges and therefore live in relatively specific habitats. Understanding habitat preferences of these species has important conservation implications. Protection of required habitat will increase the likelihood of successful reproduction and contribute to the species' long-term survival.

Large scale declines in hellbender populations have been observed throughout their range, indicating the need for management of this species (Gates et al., 1985; Nickerson et al., 2002; Pfingsten, 1990; Wheeler et al., 2003; Williams et al., 1981). An important component of this management will involve identifying and protecting suitable habitat. The majority of the information regarding hellbender habitat requirements comes from a study conducted by Nickerson and Mays (1973) on healthy populations in Missouri. Most hellbender studies have focused on demography and behavior, with few studies examining habitat requirements (but see Hillis and Bellis, 1971; Humphries and Pauley, 2005; Nickerson et al., 2003).

The objective of this study was to quantitatively assess environmental characteristics of sites where hellbenders were present to determine habitat preferences. This information should help management agencies identify hellbender habitat for protection and contribute to the long-term survival of this unique species.

Chapter 2
Introduction

The Eastern Hellbender

The Eastern Hellbender, *Cryptobranchus alleganiensis alleganiensis*, is a large salamander found in swiftly flowing streams in the central and eastern United States (Figure 1). They possess several adaptations to lotic environments which are helpful in identification (Figure 2). Hellbenders have dorsoventrally flattened bodies and heads, which offer a minimum of resistance to flowing water. Their paddle-like tail can be used for swimming at surprising speeds for short distances to avoid predators (Nickerson and Mays, 1973). Although hellbenders posses large lungs, the majority of gas exchange occurs through the skin. Highly vascularized folds of skin are present and increase the surface respiratory area (Guimond and Hutchinson, 1973). They also exhibit a rocking behavior, particularly when stressed, which has been shown to further enhance gas exchange (Harlan and Wilkinson, 1981). This method of respiration is believed to limit hellbenders to cool, swiftly flowing streams where gas exchange is maximized (Ultsch and Duke, 1990).

The northern extant of the Eastern Hellbenders' range is southern New York, extends southward to northern Georgia and Alabama, and westward into Missouri (Nickerson and Mays, 1973) (Figure 3). Hellbenders have been found in the Savannah, Susquehanna, Ohio, Tennessee, Missouri, and Meramec River systems (Phillips and Humphries, 2005). Populations can be found statewide in West Virginia except for the Potomac and James River systems, which are east of the Allegheny front (Green and Pauley, 1987).

Although nocturnal (Noeske and Nickerson, 1979) and rarely encountered, hellbenders can be abundant where they occur (Hillis and Bellis, 1971; Humphries and Pauley, 2005; Nickerson and Mays, 1973, Peterson et al., 1988; Taber et al., 1975). The population estimate for a 4.6 km section of a Missouri stream was 428 individuals/km and a biomass estimate of 156 kg/km. A 4,600 m^2 riffle of that same stream had an estimated population of 1 individual/8-10 m^2 and a biomass estimate of 98.2 kg (Nickerson and Mays, 1973). Peterson et al. (1988) found population densities to range from 0.9-6.1 hellbenders/100 m^2 from four rivers in Missouri. Hillis and Bellis (1971) captured 152 individuals in a 220 m x 70 m study area from a Pennsylvanian stream.

High population density and biomass estimates suggest that hellbenders are an important component of stream ecosystems (Humphries and Pauley, 2005). Hellbenders feed predominantly on crayfish, but will eat a variety of invertebrates such as snails, insect larvae, adult insects, worms, and mollusks (Alexander, 1927; Green, 1933; Netting, 1929; Nickerson and Mays, 1973; Reese, 1903). Vertebrate prey items include minnows, suckers, anurans, aquatic reptiles, small mammals, lamprey, and other hellbenders (Alexander, 1927; Netting, 1929; Nickerson et al., 1983; Nickerson and Mays, 1973; Reese, 1903). Hellbenders, particularly eggs and larvae, are potential prey for a variety of species. Known predators include Northern Pike, catfish, turtles, water snakes, and humans (Nickerson and Mays, 1973). The specific role of hellbenders in ecosystems has not been examined, but they appear to occupy a high trophic level. Species that occupy high trophic levels can influence production in ecosystems through trophic cascades, which have been observed in a variety of habitats, including freshwater streams (Carpenter et al., 1985; Carpenter et al., 1987; Huryn, 1998; Pace et al., 1999). The diet of hellbenders suggests they may play an important role in shaping invertebrate communities, principally by influencing crayfish populations (Humphries and Pauley, 2005). Hellbenders may exhibit top-down control of stream food webs and influence productivity through consumption of primary and secondary consumers (Figure 4).

Despite declines in densities, populations were abundant in many river systems as recently as the 1970s (Nickerson and Mays, 1973). However, it appears that since this time, populations have declined substantially throughout the hellbenders' range (Gates et al., 1985; Nickerson et al., 2002; Nickerson and Mays, 1973; Pfingsten, 1990; Trauth et al., 1992; Wheeler et al., 2003; Williams et al., 1981). Today, the only state where hellbenders are not considered a species of concern or endangered is South Carolina, where the status is unknown (Table 1). In West Virginia they are an S2 species, which means they are very rare or imperiled. It may also mean there are factors present which make them vulnerable to extirpation.

Reasons for Declines

The specialized adaptations of hellbenders to relatively specific habitat conditions (Guimond and Hutchison, 1973; Nickerson and Mays, 1973; Taketa and Nickerson, 1973; Ultsch and Duke, 1990; Williams et al., 1981) make them susceptible to rapid environmental changes. In addition, their low genetic diversity (Merkle et al., 1977; Routman, 1993; Routman et al., 1994) indicates hellbenders may be unable to adapt to long-term environmental changes (Williams et al., 1981). Habitat degradation is therefore believed to be a major reason for population declines (Humphries and Pauley, 2005; Nickerson and Mays, 1973; Nickerson et al. 2002; Trauth et al., 1992; Wheeler et al., 2003; Williams et al., 1981). Despite this belief, there have been few studies that address habitat requirements (but see Hillis and Bellis, 1971; Humphries and Pauley, 2005; Nickerson et al., 2003; Nickerson and Mays, 1973).

In general, hellbenders require cool, fast-flowing streams with a heterogeneous substrate (Nickerson et al., 2003; Nickerson and Mays, 1973). Nickerson and Mays (1973) found the probable optimal conditions were temperatures between 9.8-22.5 C, pH from 7.6-9.0, and dissolved oxygen from 8.4-13.6 ppm. It appears that adult and larval hellbenders utilize different stream microhabitats (Nickerson et al., 2003). Adults require access to large flat rocks for cover and nesting (Hillis and Bellis, 1971; Humphries and Pauley, 2005; Nickerson and Mays, 1973; Nickerson et al., 2003). Hellbenders will actively defend these rocks and they may be a limiting resource (Hillis and Bellis, 1971; Nickerson and Mays, 1973; Peterson and Wilkinson, 1996). Larval hellbenders require smaller rocks for cover and will also utilize interstitial spaces in gravel and cobble (Nickerson and Mays, 1973; Nickerson et al., 2003). These areas also provide habitat for a variety of aquatic invertebrates (Bourassa and Morin, 1995; Williams, 1978), which make up the bulk of the hellbender diet (Alexander, 1927; Green, 1933; Netting, 1929; Nickerson and Mays, 1973; Reese, 1903).

There are a number of potential reasons for hellbender declines, but habitat degradation is probably the most important (Humphries and Pauley, 2005; Nickerson and Mays, 1973; Nickerson et al. 2002; Trauth et al., 1992; Wheeler et al., 2003; Williams et al., 1981). Land use practices such as logging, urban development, and agriculture have the potential to increase water temperatures and lower the respiratory ability of hellbenders (Utlsch and Duke, 1990). Temperatures above the thermal maximum range of 32.7-36.6 C can be fatal (Hutchinson et al., 1973).

Acid precipitation and acid mine drainage can increase stream acidity, which has been shown to decrease trout and benthic invertebrate abundance, alter vertebrate and invertebrate communities, and impact aquatic food webs (Baker et al., 1996; Cagen et al., 1993; Hall et al., 1980). Acidic conditions have been shown to affect the development and hatching success of amphibian eggs and larvae (Freda and Dunson, 1985; Gosner and Black, 1957; Ling et al., 1986). It is possible that acidic conditions will affect hellbender eggs and larvae in a similar manner. Acidification can therefore potentially impact hellbenders by altering aquatic food webs and by directly impacting an individual's survival.

Modification of stream flow for transportation, agriculture, and hydroelectric power can alter stream ecosystems (Benke, 1990; Robinson et al., 2004). These changes can negatively affect larval and juvenile fish survival and change fish communities (Bain et al., 1988; Scheidegger and Bain, 1995; Schlosser, 1985; Travnichek et al., 1995). Changes to stream flow can also alter invertebrate drift and species diversity (Minshall and Winger, 1968; Robinson et al., 2004). Stream flow alteration could therefore impact hellbenders by changing the abundance and diversity of vertebrate and invertebrate prey. Reduction in flow may also affect the respiratory ability of hellbenders because gas exchange is increased by flowing water (Nickerson and Mays, 1973; Ultsch and Duke, 1990; Williams et al., 1981).

Sedimentation is one of the most common non-point sources of pollution and is believed to be a major contributor to hellbender declines (Nickerson and Mays, 1973; Williams et al., 1981). A variety of activities such as agriculture, urban development, logging, and wildfires may increase the sediment load of streams (Kerby and Kats, 1998). Increased sediment loads have been shown to alter fish, salamander, and invertebrate communities and can affect stream ecosystem processes (Angradi, 1999; Berkman and Rabeni, 1987; Kerby and Kats, 1998; Kreutzweiser et al., 2005; Lemly, 1982; Rabeni and Minshall, 1977; Sponseller and Benfield, 2001). It may directly impact survivorship of hellbender larvae by increasing the embeddedness of gravel and cobble, forcing larvae to utilize less secure areas (Nickerson et al., 2003). Sedimentation also has the potential to bury larvae and limit their ability to breathe. Sedimentation probably impacts hellbenders by altering prey communities, limiting larval access to interstitial spaces, and decreasing the availability of large rocks (Nickerson et al., 2003; Williams et al., 1981). These environmental factors do not act in isolation of each other and synergistic interactions probably occur which magnify their impact.

It is unlikely that habitat degradation alone is responsible for all population declines. Over-collection by the pet trade and scientific researchers has also been implicated (Humphries and Pauley, 2005; Nickerson and Mays, 1973; Phillips and Humphries, 2005; Trauth et al., 1992; Wheeler et al., 2003). For example, in the mid 1980’s over 100 individuals were removed by commercial collectors in two days from a Missouri stream. Surveys from this same site in 1991 resulted in no hellbender captures (Trauth et al., 1992). There are several examples of large collections for scientific research as well, including the collection of over 650 individuals from a stream in Pennsylvania, although I am unaware of the current status of this population (Swanson, 1948). Hellbenders are often killed by fisherman due to the mistaken belief that they are poisonous or harmful to the fishery (Nickerson and Mays, 1973). Nickerson et al. (2002) noted that hellbenders can be negatively affected by fishery management practices, such as the use of chemicals to reduce non-game fish populations. They also suggest that a reduction in crayfish populations as a result of a large otter population may be contributing to declines in a Tennessee stream.

It is possible that physical abnormalities as a result of intraspecific competition, failed predation, accidental injury, and birth defects may reduce individual reproductive effort (Miller and Miller, 2005). A study by Unger (2003) found that males from a declining population had lower sperm concentrations (sp/ml) than males in a stable population. The apparently low survival rate of eggs and larvae (Taber et al., 1975) suggests that anything that further affects reproductive success could significantly impact populations.

There is no single reason for hellbender declines; instead it is probably due to the interaction of multiple factors. Additionally, the relative importance of each factor will vary spatially and temporally, which can make effective conservation management difficult. While there are a variety of potential causes, habitat degradation is probably the major threat to hellbender populations

The Eastern Hellbender in West Virginia

Green (1934) believed hellbenders were more abundant in West Virginia than any other part of the Ohio River drainage during the 1930s. Although populations were declining throughout their range by the 1970s, Nickerson and Mays (1973) believed large populations were still present in some West Virginian streams. However, prior to surveys by Humphries and Pauley (2005) in the late 1990s, the only information I am aware of regarding population distribution comes from scattered reports by the West Virginia Division of Natural Resources (WVDNR), West Virginia Biological Survey (WVBS), and anecdotal evidence (Figure 5). According to these reports, hellbenders have been found in 12 of the 32 major watersheds (Table 2). Although there are no records, populations were probably found in the other major watersheds as well, except for river systems east of the Allegheny front (Green and Pauley, 1987).

Humphries and Pauley (2005) provided the first population density estimate for West Virginia. They estimated a population of 31 individuals in a 3,883 m^2 section or 0.8 individuals/100 m^2 and an estimated biomass of 39.2 kg/ha. This density is lower than reported by Nickerson and Mays (1973), but probably represents a healthy population. This study confirmed that large and apparently stable populations still exist in some West Virginian streams.

WVDNR reports from the last 10 years show that populations may still be present in Buffalo Creek, North Fork of the Cherry River, Desert Fork, Back Fork of the Elk River, Fish Creek, West Fork of the Greenbrier River, Horseshoe Run, North Fork of the Hughes River, Little Laurel Creek, and Shavers Fork. However, recent surveys by Makowsky (2004) only found hellbenders at 3 of 32 sites. He found adult hellbenders in the Cranberry and Elk Rivers and eggs were found in the Holly River. Unfortunately, the lack of information regarding past population densities and distribution makes it impossible to know whether declines have occurred. Declining populations in other parts of the hellbender's range (Gates et al., 1985; Nickerson et al., 2002; Nickerson and Mays, 1973; Trauth et al., 1992; Wheeler et al., 2003; Williams et al., 1981) and results of recent surveys (Makowsky, 2004), indicate that populations may have declined, or are at risk of declining, in at least some West Virginian river systems.

Study Objectives

Hellbenders are considered a species of concern in West Virginia and are in need of management. Research is needed to gain a better understanding of habitat requirements and population distribution for effective conservation management. The objectives of this research were to (1) provide the WVDNR with information about populations in central and southern West Virginia and (2) quantitatively assess habitat preferences of the Eastern Hellbender. This information will aid the WVDNR in management decisions regarding hellbender conservation.

Chapter 3
Methods

Study Area

The area surveyed consisted of counties south of Randolph, Upshur, Lewis, Braxton, Calhoun, Roane, Jackson, Logan, and Cabell counties (Figure 6). This area encompasses approximately 15,120 km^2 and includes portions of the Appalachian Plateau, Allegheny Front, and Allegheny Mountain physiographic provinces. The large area surveyed allowed for a gradient of environmental variables to be sampled. The major watersheds in this area are the Greenbrier, Upper and Lower New, Gauley, Tug Fork, Upper and Lower Guyandotte, Coal, Upper and Lower Kanawha, Elk, and Big Sandy.

Hellbenders have been found in the Cranberry River, Williams River, Gauley River, Greenbrier River, East and West Forks of the Greenbrier River, Elk River, Back Fork of the Elk River, Mud River, North Fork of the Cherry River, Guyandotte River, Second Creek, Glade Creek, and Twelvepole Creek in this area. There is also evidence that they were in the New River, Bluestone River, and Tug Fork River (L. Rogers, 2006, personal communication). This area includes relatively undisturbed sites, such as the Cranberry River in the Monongahela National Forest (MNF) and disturbed sites, such as the Guyandotte River in the town of Pineville. Although sites within the MNF receive some protection from disturbances, even these sites have been exposed to the effects of logging. The majority of the forests in West Virginia were logged at some point as a result of the forestry boom following the Civil War, including the MNF (Miller and Maxwell, 1913).

Surveys

Surveys were conducted from May through November, 2006. Sites were searched by 1 or 2 surveyors wearing snorkeling gear (mask, snorkel, and wetsuit if needed). Rocks were slowly turned with the aid of a log peavey and specimens were captured by hand. Nickerson and Krysko (2003) found that snorkeling was the only method that captured hellbenders of all age classes in a review of survey techniques. The number of surveyors was multiplied by time searched to determine search effort. Relative abundance was determined by dividing the number of specimens encountered by search effort and has been found to correlate well with mark-recapture density estimates (Peterson et al., 1988). Sites were searched until hellbenders were encountered or for at least 3 hours if no specimens were found.

Total length (mm), weight to the nearest gram, markings/deformities, sex (if possible), depth (m) at site of capture, length (cm) and width (cm) of rock under which specimen was captured, and rock opening orientation (upstream or downstream) were recorded. Specimens were tagged with a Passive Integrated Transponder (P.I.T.) in a fatty portion of tissue at the base of the tail posterior to the hind legs. Tags were injected with a syringe and needle sterilized in ethyl alcohol and specimens were released at site of capture (Figure 7).

The life history of hellbenders suggests they may be affected by stream substrate, so Wolman pebble counts were used to characterize stream substratum (Wolman, 1954). Water chemistry of a stream may affect hellbenders as well, so water temperature (C), pH, dissolved oxygen (mg/L), percent dissolved oxygen, specific conductivity (ms/cm), and turbidity were measured with a Hydrolab Quanta (Hydrolab Corp.). Relative abundance of crayfish was also recorded for each site because crayfish make up a significant portion of the hellbender diet.

Night surveys were conducted from August through September, 2006. These surveys consisted of 1 to 3 surveyors using headlamps while walking in the stream to locate specimens. Box traps and hoop nets were baited with catfish bait and set at 2 sites. However, this method was time consuming and I had little confidence that areas were being effectively surveyed, so no further trapping attempts were made.

Sites were chosen in streams where populations were known from reports by the WVDNR and WVBS to determine if they were still present. If possible, the exact site where populations had been found was searched. New sites were subjectively chosen if they looked like good hellbender habitat.

Data Analysis

The length and width of the capture rock were multiplied for a rough estimate of the rock's area. Hellbenders were placed into size classes based on total length. Males reach sexual maturity at approximately 300 mm and females at a slightly larger size of 380 mm (Taber et al., 1975). Larvae partially metamorphose (lose gills) when they reach total lengths of 100-130 mm (Petranka, 1998). Wolman pebble counts were first converted into particle size categories (Table 3). The number of particles in size categories was then used to determine the particle size percentile classes for statistical analysis (Bunte and Abt, 2001). The size classes represent the size at which 5% (D_5), 16% (D_{16}), 25% (D_{25}), 50% (D_{50}), 75% (D_{75}), 84% (D_{84}), or 95% (D_{95}) of particles were below that size, so they varied depending on the stream substrate. For example, a site with a large number of particles falling in the fine sediment category will have a lower D_5 score than a site that has very few fine sediment particles and lots of large cobble.

Site specific habitat characteristics were ordinated in principle component analysis (PCA) with a variance-covariance centered matrix (McCune and Mefford, 1999). The only particle size class used in the analysis was the D_{50} because particle size scores are highly correlated. Percent dissolved oxygen was also not included because it is highly correlated with dissolved oxygen (mg/L) and water temperature. The PCA was graphed in 2-dimensional ordination space to examine whether sites where hellbenders were present (HP) separated from sites where they were absent (HA). Habitat characteristics were examined with t-tests to determine if there were differences in HP and HA sites (Statistix 7.0. Analytical Software. 1985, 2000). A Euclidean distance measures cluster analysis with Ward's group linkage method was used to determine how similar sites were based on habitat characteristics (McCune and Mefford, 1999). Only the characters found to be significant with t-tests were used in the cluster analysis.

Chapter 4
Results

Hellbenders were found at 8 of 67 sites, the majority of which were in the Monongahela National Forest (Figure 8). If sites are limited to streams where they have been found in past, then hellbenders were found at 8 of 41 sites (Table 4). Two additional locations were found by other researchers over the course of the study in the Kanawha River and a Williams River site. It appears that populations are patchily distributed in streams (Figure 9). My surveys confirm that hellbenders are still present in the Back Fork of the Elk River, East and West Forks of the Greenbrier River, Cranberry River, Gauley River, and Williams River. Hellbenders were only found in streams in which they were already known to occur from past reports by the WVDNR and BSMU (Table 5). Rock turning surveys during the day were the only method used to successfully locate specimens.

Thirteen hellbenders were observed, 4 of which escaped so the size class could not be determined, although they appeared to be adults (Table 6). A larval hellbender was found in the Williams River, a juvenile was found in the Gauley River, and the rest were adults. Eggs were not found at any sites. The highest relative abundances were in the West Fork of the Greenbrier River (0.800 hellbenders/hour) and the Back Fork of the Elk River (0.703 hellbenders/hour).

Only 1 hellbender was able to be sexed, a male from the Cranberry River. Most specimens were found at depths a little over 0.5 m (n = 13, mean = 62.2 cm) and under large rocks (n = 13, mean = 1. 028 m^2). The majority of rock openings were oriented downstream (n = 10). Five hellbenders had what appeared to be scarring from bite marks and 2 were missing the second digit of the right hind foot. One adult had a large, fresh circular shaped wound on its head (Figure 10). Another adult regurgitated what looked like a salamander, but it was too digested to identify.

The PCA ordination suggests that there were differences in habitat characteristics of HA and HP sites (Figure 11). HP sites grouped, although there was considerable overlap with HA sites. The majority of the grouping appears to be a result of the first principle component (PRIN 1), which explained 96.4% of the total variation (Appendix B). Although there is a large spread over which HP sites occurred along this axis, it appears there is a lower limit, below which no HP sites are located. The D_{50} score was most relevant along this axis and HP sites were positively correlated with D_{50} score (Table 7). Principle component 2 (PRIN 2) explained 2.8% of the overall variation and there was little grouping of HP sites on this axis. Turbidity was the most important environmental variable on this axis. The t-tests showed that HP sites had significantly higher D_{16}, $D_{25,}$ and D_{50} scores and significantly lower pH, water temperature, and specific conductivity (D_{16}: $p = 0.0190$, D_{25}: $p < 0.0001$, D_{50}: $p = 0.0035$, pH: $p = 0.0016$, water temp.: $p = 0.0066$, specific conductivity: $p < 0.0001$) (Table 8, Figure 12, & Figure 13). A conservative interpretation of the cluster analysis identified 6 groupings that included HP sites (Figure 14). Based on the cluster analysis, it appears that Pond Fork of the Little Coal River, Mash Fork of the Big Coal River, and the mainstem of the Greenbrier River may also be capable of supporting populations (Figure 15).

Chapter 5
Discussion

Large scale declines in Eastern Hellbender populations have been observed throughout their range for the last 30 years (Gates et al., 1985; Nickerson et al., 2002; Nickerson and Mays, 1973; Pfingsten, 1990; Trauth et al., 1992; Wheeler et al., 2003; Williams et al. 1981). Although it is impossible to say for certain, because we simply lack information regarding past population densities and distributions (Humphries and Pauley, 2005), it appears that populations are also declining in many streams in central and southern West Virginia. Hellbenders were only found at 12% of all sites surveyed and 19.5% of sites from streams in which hellbenders are known to occur from past reports. This low rate of encounters seems to indicate population declines if hellbenders were as common as Green (1934) believed they were in the 1930s.

However, these results should be interpreted with caution. There is the possibility that hellbenders were present at a site and not observed. This may have occurred if hellbenders were under large rocks that could not be turned or if they escaped when sediment released from turning rocks obscured vision. I think the use of a snorkeling mask allowed for most hellbenders to be observed, even if vision was briefly obscured, but it is possible that some escaped without being seen. Hellbenders are known to utilize cracks in the bedrock (Nickerson and Mays, 1973) which were not searched. I did not see many bedrock cracks while sampling though, so this was probably not a major problem in these surveys. In most cases, I believe that hellbenders were observed if they were present.

The absence of hellbenders at a site should not be extrapolated to the whole stream. Hellbenders appear to be patchily distributed in streams and it seems probable that populations were present in streams that I did not find them; they were just not at the sites sampled (Figure 9). For example, Makowsky (2004) found eggs in the Holly River, but I was unable to find any hellbenders there in my surveys. I believe that if Holly River had been surveyed more extensively, hellbenders would have been found. This example is probably true for a number of streams and more extensive surveys are needed to determine if populations exist in these streams (Table 4 & Table 5).

Although populations have probably declined, there are still apparently healthy populations in West Virginia. The majority of sites where hellbenders were found were in the Monongahela National Forest, which is encouraging because they receive some protection from many land use practices (Figure 8). It was also encouraging to find a larval hellbender in the Williams River and a juvenile in the Gauley River, because it means successfully reproducing populations exist. Hellbenders live up to 25 years in the wild, so adults may persist in environments which are not capable of supporting successful reproduction (Nickerson and Mays, 1973; Williams et al., 1981).Therefore, the presence of adults does not necessarily represent a healthy population, although I believe the high relative abundances of hellbenders in the West Fork of the Greenbrier River and the Back Fork of the Elk River represent healthy populations even though no larvae or juveniles were found.

The most likely threat to these populations is a change in stream quality as a result of logging activity. Most populations were located in the Monongahela National Forest, so they are protected from many land use activities that can degrade habitat, but logging still occurs. Logging has the potential to increase sediment loads and water temperatures. Sedimentation can alter stream ecosystems by changing invertebrate and vertebrate communities (Angradi, 1999; Berkman and Rabeni; 1987; Kreutzweiser et al., 2005; Lemly, 1982) and is believed to be a major reason for hellbender declines (Nickerson and Mays, 1973; Williams et al., 1981). Sedimentation could potentially affect hellbenders by decreasing the quality of larval and nesting habitat and by altering prey availability. Higher water temperatures could affect the respiratory ability of hellbenders and decrease their individual fitness (Ultsch and Duke, 1990). However, forestry best management practices are designed to limit these effects and logging may only have a minimal impact on populations. These streams should continue to be monitored however, so that if declines are observed action can be taken to ensure they are not extirpated.

The specific habitat requirements of hellbenders suggest their distribution will be influenced by the availability of preferred habitat. The patchy distribution observed in this study may therefore relate to site specific habitat characteristics. In general, hellbenders were found at sites with higher particle size scores and lower water temperatures, pH values, and specific conductivities (Table 8, Figure 11, Figure 12, & Figure 13).

It was not surprising that hellbenders preferred sites with higher particle size scores, which are usually associated with the more heterogeneous stream substrate required by hellbenders (Nickerson et al., 2003; Nickerson and Mays, 1973). In this study, hellbenders were generally found under large flat rocks oriented downstream and in relatively shallow water of rapid flow, similar to previous studies on habitat use (Hillis and Bellis, 1971; Humphries and Pauley, 2005; Nickerson et al., 2003; Nickerson and Mays, 1973). These areas probably allow for a maximum amount of gas exchange, protect individuals from stream flow disturbance, and provide nesting and cover for adults. However, it appears that the amount of large rocks was not a limiting factor in the streams I sampled Table 8, Figure 12, and Figure 13. Higher particle size classes were generally made up of boulders typically used by adults in my surveys. There were no real differences between sites where hellbenders were present or absent at these higher size classes, indicating that the amount of large rocks was not a limiting factor. Instead, it appears that hellbenders prefer sites with higher scores at the smaller size classes, specifically at the D_{16}, D_{25}, and D_{50} classes. This may relate to the availability of larval and prey habitat.

Nickerson et al. (2003) found that larval and adult hellbenders utilized different microhabitats. Larvae were usually found under small rocks, often in mixed substrates of cobble and gravel. They were predominantly found in deep pools, but were also found near stream margins and areas of sub-surface percolation which may provide protection from predators. These areas are also utilized by larval fishes and macroinvertebrates (Allan, 1997), illustrating the importance of this microhabitat in maintaining stream ecosystems. Hellbenders preferred sites with higher D_{16}, D_{25}, and D_{50} size class scores, which contained gravel and cobble in my surveys. This suggests that having a large amount of larval habitat is important for maintaining hellbender populations. Interestingly, the larval hellbender captured in this survey was found under a fairly large rock, more often utilized by adults (Table 5). Nickerson et al. (2003) also found larvae under larger rocks and believed this may have been the result of the limited availability of preferred sites under small rocks, which forced larvae to use less secure cover. Larvae may also have been under smaller rocks which were disturbed when the larger rock was turned.

In addition to providing habitat for larval hellbenders, gravel and cobble substrates are an important habitat for macroinvertebrates (Allan, 1997; Rabeni and Minshall, 1977; Reice, 1980; Williams, 1978). Macroinvertebrates are a key component of stream ecosystems and transfer energy to higher trophic levels occupied by vertebrate predators, including hellbenders (Allan, 1997). Macroinvertebrate diversity and abundance is higher in mixed substrates of gravel and cobble (Allan, 1997; Rabeni and Minshall, 1977; Reice, 1980; Williams, 1978). A significant portion of the hellbender diet is made up of macroinvertebrates and their distribution may be related to prey availability (Alexander, 1927; Green, 1933; Netting, 1929; Nickerson and Mays, 1973; Reese, 1903). Gravel and cobble substrates are therefore important in maintaining populations because they provide habitat for larval hellbenders and prey items.

The most likely threat to the habitat required by larvae and invertebrate prey is sedimentation. Fine sediments can fill interstitial spaces used by larval hellbenders and invertebrates, degrading the quality of this habitat. This could force larval hellbenders into less secure sites, making them more vulnerable to predation (Nickerson et al., 2003). Sedimentation could also lead to the direct mortality of individuals if they become buried and are unable to breathe. It may also alter invertebrate prey communities, which could negatively affect the quality and quantity of available food to support populations (Angradi, 1999; Berkman and Rabeni; 1987; Kreutzweiser et al., 2005; Lemly, 1982). Large amounts of fine sediments associated with sedimentation would drag down particle size scores, perhaps explaining why populations were not found at sites with lower particle size scores.

Although stream substrate was an important habitat characteristic, it appears that water characteristics were also important factors (Table 8, Figure 12, & Figure 13). The respiratory ability of hellbenders is greater in cooler waters, which may explain why hellbenders preferred sites with lower water temperatures (Ultsch and Duke, 1990). Summer temperatures at some HA sites reached up to 29.4 C, which is well above the proposed optimal temperature range of 9.8-22.5 C and approaches the critical thermal maximum range of 32.7-36.6 C (Hutchinson et al., 1973; Nickerson and Mays, 1973). The highest temperature reported for HP sites was 19.3 C, which includes summer months and supports the idea that hellbenders require cool streams. Hellbenders are slow to acclimate to temperatures changes and seasonal increases found in some streams may be too stressful on individuals to support populations (Hutchinson et al., 1973). This may also explain why hellbenders were found in a very narrow range of temperatures. Several of the highest temperatures were recorded from streams where hellbenders are known to occur from past reports, but were not found in my surveys. For example, a site where hellbenders have been found in the Greenbrier River reached a summer temperature of 28 C. This leads me to believe that temperatures in this stream have increased since hellbenders were last observed. This may also be true for several other streams and may explain why hellbenders were not found at many sites where they used to be present.

Specific conductivity measures the ability of water to pass an electric current and is related to the geology of an area, but may also be influenced by land use practices (Dow and Zampella, 2000; Lenat and Crawford, 1994; Sponseller and Benfield, 2001). Urbanization, agricultural practices, and logging can increase the amount of NO_3^-, NH_4^+, P, CA^{2+}, and Mg^{2+} present in streams, which results in a higher conductivity. Generally, less disturbed sites (i.e. forested streams) have a lower specific conductivity than disturbed sites. Dow and

Zampella (2000) found that specific conductivities in the range of 0.07-0.14 *u*S/cm were associated with disturbed streams. HA sites had a high mean specific conductance (0.2214 *u*S/cm), indicating that many of these areas were highly disturbed (Table 8, Figure 12, & Figure 13). Conversely, HP sites had a much lower mean specific conductivity (.0356 *u*S/cm), indicating that these sites are relatively undisturbed. Differences may have been caused by geology rather than disturbance; although HP sites were usually in forested areas. Habitat degradation as a result of land use could potentially impact hellbenders in a number of ways and this study suggests that disturbed areas do not support populations. However, research designed to specifically address the effects of land use on hellbender populations is needed to determine if this is true.

Acidic conditions in streams can alter invertebrate communities and decrease the survival rate of fish and amphibian eggs and larvae (Baker et al., 1996; Cagen et al., 1993; Freda and Dunson, 1985; Gosner and Black, 1957; Hall et al., 1980; Ling et al., 1986). It was therefore surprising to find hellbenders in more acidic conditions than HA sites, some of which were lower than has previously been reported (Table 8, Figure 12, & Figure 13). However, these conditions were still above the acidity levels (pH $<$ 4-5) shown to negatively affect amphibian and fish survival rates. Some of these sites were approaching conditions known to harm stream ecosystems. It appears that hellbenders can survive in stream conditions that are more acidic than has previously been reported. However, pH levels should be monitored because conditions in some streams are approaching levels that may alter stream communities and could affect the long-term survival of populations.

The results of the cluster analysis indicate that the mainstem of the Greenbrier River, Pond Fork of the Little Coal River, and Marsh Fork of the Big Coal River contained areas with similar habitat characteristics to those where hellbenders were found (Figure 14 & Figure 15). This suggests that these streams may contain habitat suitable for supporting populations. Marsh Fork of the Big Coal River and Pond Fork of the Little Coal River were not surveyed extensively, so it is possible that populations were present in areas not surveyed.

The mainstem of the Greenbrier River was surveyed fairly extensively, but hellbenders have been found there in the past and populations may still exist in areas of suitable habitat. The interpretation of the cluster analysis was conservative, so there are a number of other streams that probably contain suitable habitat as well. The cluster analysis also suggests that hellbenders were present at some sites and missed or that other factors may be influencing habitat suitability.

Hellbenders preferred slightly acidic sites with a heterogeneous substrate, cool water temperatures, and low specific conductivity. A combination of these characteristics is required to support populations. For example, a stream with heterogeneous substrate but warm water temperatures is unlikely to support a healthy population. While these characteristics are important components of hellbender habitat, they are certainly not the only relevant habitat characteristics. The overlap of HA and HP sites in the PCA ordination and their relationships in the cluster analysis suggest that other factors not measured were also affecting populations (Figure 11 & Figure 14). The absence of populations at apparently suitable sites indicates that further research is needed to determine what factors were responsible for the absence of hellbenders at these sites.

Additionally, caution should be used when extrapolating information from the small sample size of this study. In most cases, the narrow ranges of habitat variables probably do not represent the entire range of tolerances that hellbenders are able to survive in. For example, hellbenders have been found in temperatures slightly higher than the range found in this study (Nickerson and Mays, 1973). However, the patchy distribution suggests populations are concentrated around available resources and the habitat characteristics identified as being important in this study probably represent good hellbender habitat. Therefore, streams with similar habitat characteristics will likely be capable of supporting populations and should be protected.

Chapter 6
Conclusions

Eastern Hellbender populations appear to be declining in central and southern West Virginia. Declines may be a result of the relatively specific habitat requirements of hellbenders, which makes them susceptible to changes in their environment (Guimond and Hutchison, 1973; Nickerson and Mays, 1973; Taketa and Nickerson, 1973; Ultsch and Duke, 1990; Williams et al., 1981). Hellbenders appear to require stream substrates with large areas of gravel and cobble, probably because they provide habitat for larval hellbenders and invertebrate prey (Allan, 1997; Nickerson et al., 2003; Nickerson and Mays, 1973; Rabeni and Minshall, 1977; Reice, 1980). Hellbenders preferred cooler streams and were found in a narrow temperature range between 17.36 C and 19.34 C. This may be related to the respiratory ability of hellbenders which is maximized in cool water and their inability to acclimate well to temperature changes (Hutchinson et al., 1973; Ultsch and Duke, 1990). Hellbenders also preferred specific conductivities around 0.0365 mS/cm. This may relate to stream disturbance, with the lower values where hellbenders were found indicating less disturbed streams (Dow and Zampella, 2000). Hellbenders were found in more acidic streams than they have previously been reported from, with some streams approaching conditions known to harm stream ecosystems (Baker et al., 1996; Cagen et al., 1993; Hall et al., 1980). Although discussed individually, these habitat characteristics do not occur in isolation of each other. Instead, they interact and it appears that a combination of suitable habitat characteristics is needed to support populations.

Alterations to these habitat characteristics, whether from human or natural causes, could negatively impact populations in a variety of ways. Habitat alterations probably do not affect one characteristic at a time, but instead alter several characteristics which can act synergistically to impact populations. For example, logging can increase sediment loads and water temperatures. Increased sediment loads could degrade the quality of larval habitat and alter prey communities. At the same time, increased stream temperatures could decrease individual fitness and lower reproductive success. The combined impact of these effects could render a habitat unsuitable to support a healthy population.

Although populations seem to be declining, there are apparently healthy populations in several streams. These populations were patchily distributed, probably concentrating in areas of high resource availability. The majority of these sites were in less disturbed areas, indicating the need to protect forested streams. The habitat characteristics found to be important in this study may represent good hellbender habitat and streams with similar habitat characteristics should be protected to help conserve this unique salamander.

Chapter 7
Management Recommendations

The results of this study support the listing of the Eastern Hellbender as an S2 species, due to declining populations and specific habitat requirements which make it vulnerable to extirpation. However, there are apparently healthy but disjunct populations existing in central and southern West Virginia, largely within the Monongahela National Forest. Surveys should continue to monitor these populations as well as attempt to locate new populations for protection. Diurnal rock turning surveys were the most effective method for locating specimens, although other researchers have had success with trapping and nocturnal surveys (Humphries and Pauley, 2005; Nickerson and Krysko, 2003). In addition to streams where hellbenders were found in this study, the cluster analysis suggests that the mainstem of the Greenbrier River, Pond Fork of the Little Coal River, and Marsh Fork of the Big Coal River should be surveyed more extensively because they may be capable of supporting populations (Figure 14 & Figure 15). Forested habitat should be protected because populations were rarely observed in more disturbed areas. Land use practices that increase sediment loads should be limited because they have the potential to degrade larval and invertebrate prey habitat needed to maintain populations. For example, the juvenile hellbender found in the Gauley River was less than a mile downstream from a new bridge being built. Sediment levels were much higher downstream of this bridge (Pers. Obs.) and could potentially impact the long-term survival of this population. Activities that could increase stream temperatures should also be limited because hellbenders need fairly cool and stable water temperatures. Acidic stream conditions are a potential threat, but did not appear to be a problem in these surveys.

Tables

Table 1. State listings of the Eastern Hellbender. These listings indicate that the Eastern Hellbenders are declining throughout its' range.

State	Status
Alabama	Rare/Possibly Endangered
Arkansas	Endangered
Georgia	Threatened
Illinois	Endangered
Indiana	Endangered
Kentucky	Special Concern
Maryland	Endangered
Mississippi	Rare/Possibly Endangered
Missouri	Critically Imperiled
New York	Special Concern
North Carolina	Special Concern
Ohio	Endangered
Pennsylvania	Immediate Concern
South Carolina	Unknown
Tennessee	In Need of Management
Virginia	Special Concern
West Virginia	Very Rare or Imperiled

Table 2. Watersheds that hellbenders have been found on based on reports by the WVDNR and WVBS. Numbers in () represent multiple records for that year. * represent watersheds within the study area.

Watershed	Hellbenders	Year(s)
Big Sandy River*	Unknown	
Cacapon River	No	
Cheat River	Yes	1910, 1932, 1936, 1997(2), 1998, 2001
Coal River*	Unknown	
Dunkard Creek	Unknown	
Elk River*	Yes	1963, 1998, 2005
Gauley River*	Yes	1937(2), 1938, 1959, 1986, 1995, 1996, 1997, 1998, 2001
Greenbrier River*	Yes	1935, 1955, 1967(2), 1993, 1994(2), 1996, 1997(2), 1998, 2005
James River*	Unknown	
Little Kanawha River	Yes	1968, 1974, 1983, 1969, 1998
Lower Guyandotte River*	Yes	1955, 1959
Lower Kanawha River*	Unknown	
Lower New River*	Yes	1995
Lower Ohio River*	Unknown	
Middle Ohio North River	Unknown	
Middle Ohio South River	Unknown	
Monongahela River	Unknown	
North Branch of the Potomac River	No	
Potomac River Drains	No	
Shenandoah Hardy River	No	
Shenandoah Jefferson River	No	
South Branch of the Potomac River	No	
Tug Fork River*	Unknown	
Twelvepole Creek*	Yes	1955, 1957, 1970
Tygart Valley River	Yes	1935, 1938
Upper Guyandotte River*	Yes	1937
Upper Kanawha River*	Yes	1951
Upper New River*	Unknown	
Upper Ohio North River	Unknown	
Upper Ohio South River	Yes	1955, 1998, 2000
West Fork River	Unknown	
Youghiogheny River	Unknown	

Table 3. Particle size classes used for Wolman pebble counts.

Material	Size Range (mm)
Silt/ Clay	0 - 0.062
Very Fine Sand	0.062 - 0.13
Fine Sand	0.13 - 0.25
Medium Sand	0.25 - 0.5
Coarse Sand	0.5 - 1.0
Very Course Sand	1.0 - 2.0
Very Fine Gravel	2.0 - 4.0
Fine Gravel	4.0 - 6.0
	6.0 - 8.0
Medium Gravel	8.0 - 11.0
	11.0 - 16.0
Coarse Gravel	16.0 - 22.0
	22.0 - 32.0
Very Coarse Gravel	32.0 - 45.0
	45.0 - 64.0
Small Cobble	64.0 - 90.0
Medium Cobble	90.0 - 128.0
Large Cobble	128.0 - 180.0
Very Large Cobble	180.0 - 256.0
Small Boulder	256.0 - 362.0
	362.0 - 512.0
Medium Boulder	512.0 - 1024.0
Large Boulder	1024.0 - 2048.0
Very Large Boulder	2048.0 - 4096.0
Bedrock	

Table 4. Streams surveyed that are known to contain hellbender populations and the results of my surveys. The number () is how many of sites hellbenders were found. * are for average relative abundances if specimens were captured at more than one site in the same stream or if a site was visited multiple times.

River	Most Recent Date Found	My Surveys	# of Sites Searched	Relative Abundance (# per hour)
Back Fork of the Elk River	2005	Yes	2(1)	0.703
Cranberry River	2003	Yes	4(1)	0.682
Dry Fork	1910	No	1	
East Fork of the Greenbrier River	1994	Yes	1(1)	0.229
Elk River	1963	No	2	
Gauley River	1938	Yes	5(2)	0.437*
Glade Creek	1995	No	1	
Greenbrier River	1996	No	9	
Guyandotte River	1949	No	4	
Holly River	2003	No	2	
North Fork of the Cherry River	2001	No	2	
Second Creek	1955	No	1	
Twelvepole Creek	1970	No	2	
West Fork of the Greenbrier River	2005	Yes	2(1)	0.800*
Williams River	1996	Yes	4(2)	0.515*

Table 5. Streams searched where no hellbenders were found. Dates are for last known record.

Site Name	Most Recent Year Found	# of Sites Searched
Twelve Pole Creek	1970	2
Anthony Creek - Blue Bend	Unknown	1
Birch River	Unknown	1
Bluestone River	Unknown	3
Camp Creek	Unknown	1
Cherry River - Coal Siding	Unknown	2
Clear Fork	Unknown	1
Dry Fork	1910	1
Dry Fork of Tug Fork River	Unknown	1
East River	Unknown	1
Elk River	1963	2
Elkhorn Creek	Unknown	1
Glade Creek	1995	1
Glade Creek of New River	Unknown	1
Greenbrier River	1996	9
Guyandotte River	1949	4
Left Fork of Holly River	2003	2
Indian Creek	Unknown	2
Marsh Fork of Big Coal River	Unknown	1
Meadow River	Unknown	1
Mountain Creek	Unknown	1
New River	Unknown	2
North Fork of Cherry River	2005	2
Paint Creek	Unknown	2
Panther Creek	Unknown	1
Pond Fork of Little Coal River	Unknown	1
Second Creek	1955	1
South Fork of Cherry River	Unknown	1

Table 6. Information for hellbenders captured during surveys. The mean total length and mass are for adults only. A = Adult, L = Larvae, J = Juvenile.

Age Class	Rock Depth (m)	Rock Area (m^2)	Rock Opening	Total Length (mm)	Weight (g)
A	0.65	0.3474	Both	349	394
A	0.57	4.9216	Up	673	1424
A	0.68	0.6388	Down	532	854
N/A	0.37	0.8955	Down	Unknown	Unknown
N/A	0.50	0.6044	Down	Unknown	Unknown
L	0.56	0.6192	Down	57	1
A	1.14	0.5657	Down	502	980
A	0.34	1.0305	Down	509	980
N/A	0.29	0.7134	Down	Unknown	Unknown
A	0.89	0.7148	Up	424	378
N/A	0.48	0.9312	Down	Unknown	Unknown
A	0.50	0.8432	Down	360	280
J	1.11	0.4706	Down	216	62
Mean	**0.62**	**1.0228**		**478**	**756**

Table 7. Eigenvectors of the PCA ordination. D50 was the most relevant on PRIN 1 and turbidity was most relevant on PRIN 2.

Habitat Characteristic	PRIN 1	PRIN 2	PRIN 3	PRIN 4	PRIN 5	PRIN 6
Crayfish	0.0001	0.2123	0.9751	-0.0404	-0.0483	-0.0116
Water Temp C	0.0053	-0.1706	0.069	0.9686	-0.1504	-0.0718
SpC	-0.0005	-0.0021	0.0019	0.0049	0.0011	0.2144
DO mg/L	0.0006	0.037	-0.0625	-0.141	-0.9872	0.018
pH	-0.0008	-0.0196	0.0194	0.0706	0.0062	0.9738
Turbidity	-0.0409	-0.9605	0.2001	-0.1874	-0.0222	-0.0095
D_{50}	0.9992	-0.0384	0.0078	-0.0126	0.0005	0.0008

Table 8. Mean values of habitat characteristics ± 1 S. E. Habitat characteristics were anaylyzed with t-tests. Bold variables were significantly different at the 0.05 level.

Habitat Variable	Absent	Present	p-value
Crayfish/hr	6.3278 ± 0.9607	7.0479 ± 2.5590	0.7961
DO mg/L	8.2031 ± 0.3534	8.4262 ± 0.2864	0.6268
pH	**7.2544 ± 0.0910**	**6.4163 ± 0.1447**	**0.0016**
Turbidity	27.595 ± 2.0967	17.004 ± 3.2183	0.0744
Water Temperature	**19.943 ± 0.6954**	**17.355 ± 0.5634**	**0.0066**
% DO	90.345 ± 3.8663	91.560 ± 4.1558	0.8324
SpC	**0.2214 ± 0.0257**	**0.0365 ± 0.00272**	**<0.0001**
D_5	8.6229 ± 1.1190	6.9538 ± 2.0720	0.5973
D_{16}	**22.771 ± 2.4214**	**55.375 ± 10.759**	**0.0190**
D_{25}	**37.169 ± 3.1756**	**84.500 ± 12.408**	**<0.0001**
D_{50}	**159.78 ± 10.993**	**259.50 ± 38.444**	**0.0035**
D_{75}	404.47 ± 26.001	519.75 ± 76.668	0.1340
D_{84}	654.37 ± 44.575	832.00 ± 93.686	0.1640
D_{95}	1512.6 ± 169.89	1152 ± 128.00	0.0981

Figures

Figure 1. Hellbenders from the East Fork of the Greenbrier River (top) and the Williams River (bottom).

Figure 2. Morphological adaptations of hellbenders to a benthic lifestyle in lotic environments. The flattened body and head are adaptations to stream flow and help keep hellbenders on the bottom. The flattened tail aids in swimming. The lateral folds are highly vascularized and increase respiratory ability.

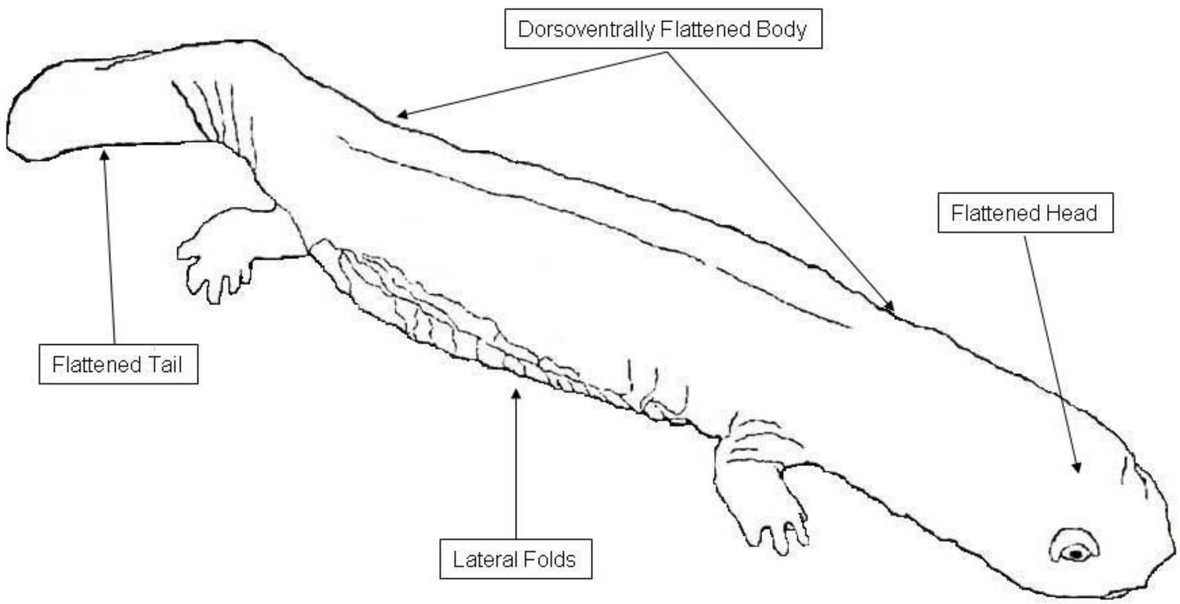


Figure 3. Range of the Eastern Hellbender in the U.S. (adapted from Nickerson and Mays, 1973) and probable range in West Virginia (adapted from Green and Pauley, 1987).

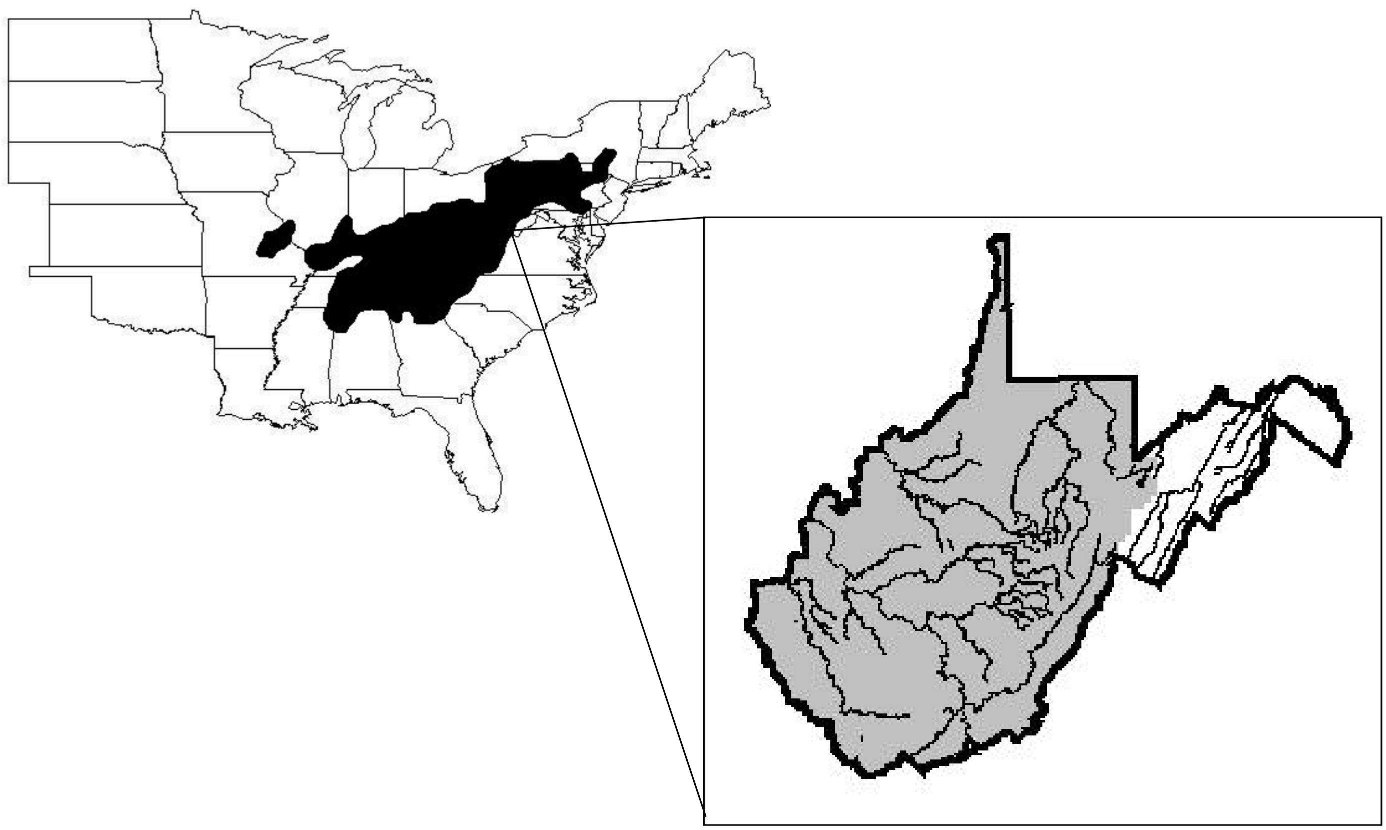


Figure 4. Hypothesized and simplified food web involving hellbenders based on dietary studies (See text for details). Figure adapted from Burton and Likens, 1975.

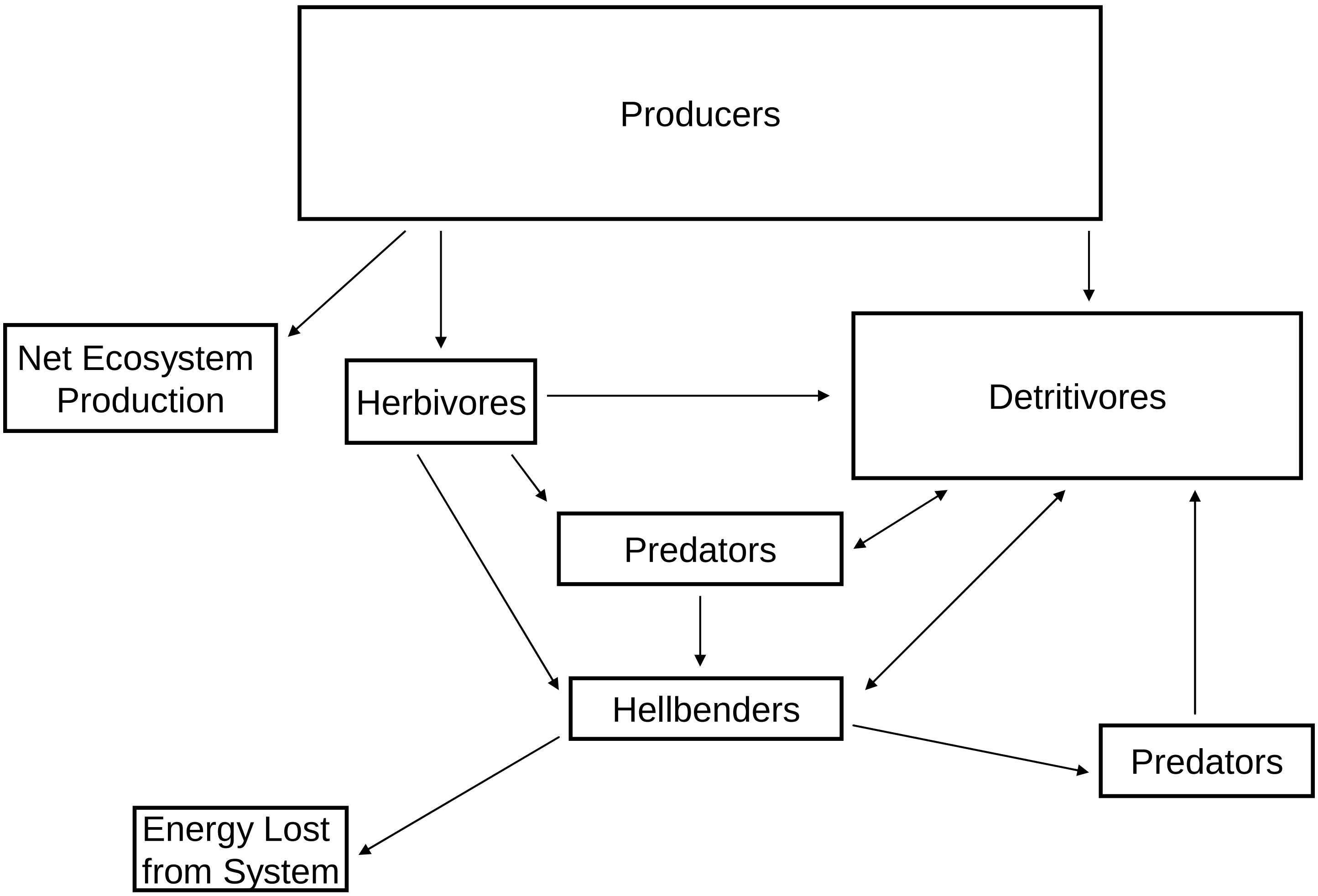


Figure 5. Rivers and streams hellbenders have been found based on reports by the WVDNR and WVBS.

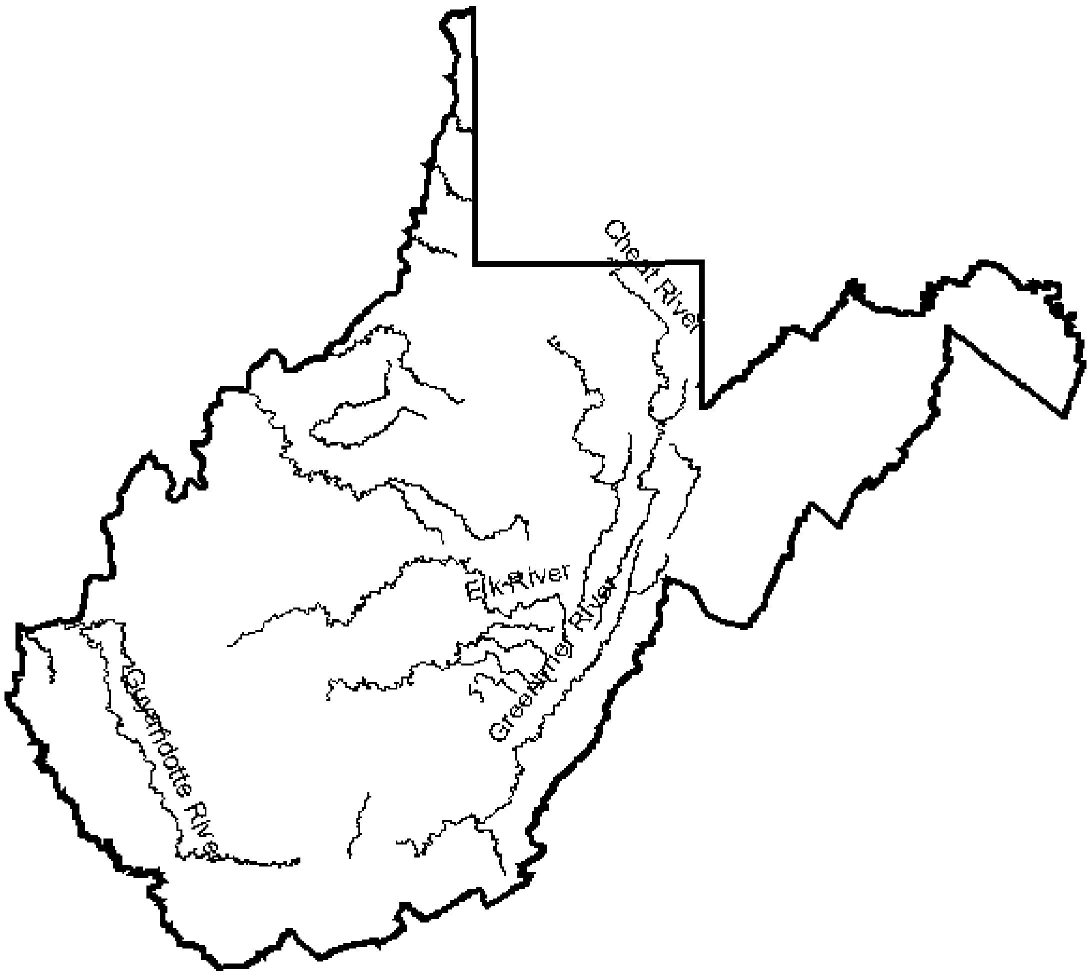


Figure 6. Area surveyed and streams in which hellbenders have been found. The area surveyed was approximately 15, 120 km^2 and contains many of the river systems in which hellbenders have been found.

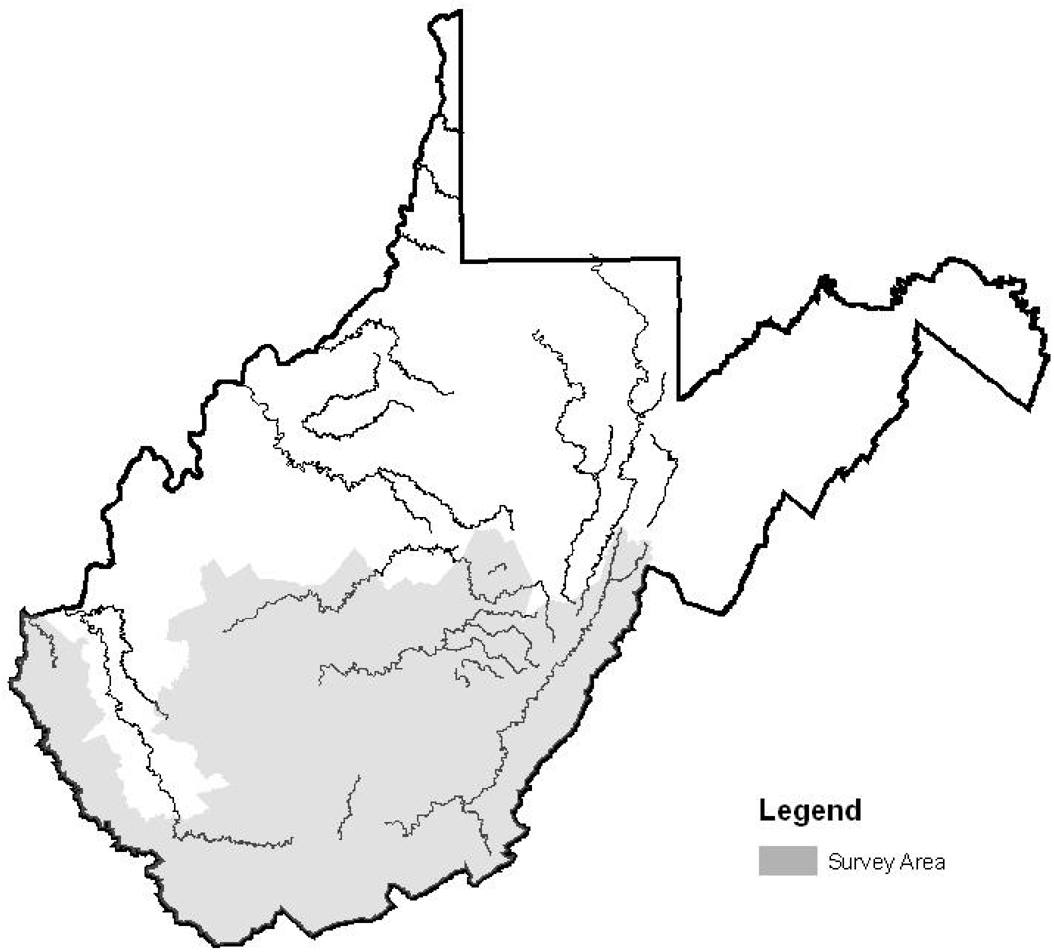


Figure 7. Method for P.I.T. tagging individuals. Tags were inserted in a fatty portion of tissue at the base of the tail posterior to the hind legs. Photo by Tim Baldwin.

Figure 8. Locations and results of population surveys. The majority of sites where hellbenders were present were in central West Virginia, in and around the Monongahela National Forest (MNF). Symbols are larger than actual search areas to protect exact locations.

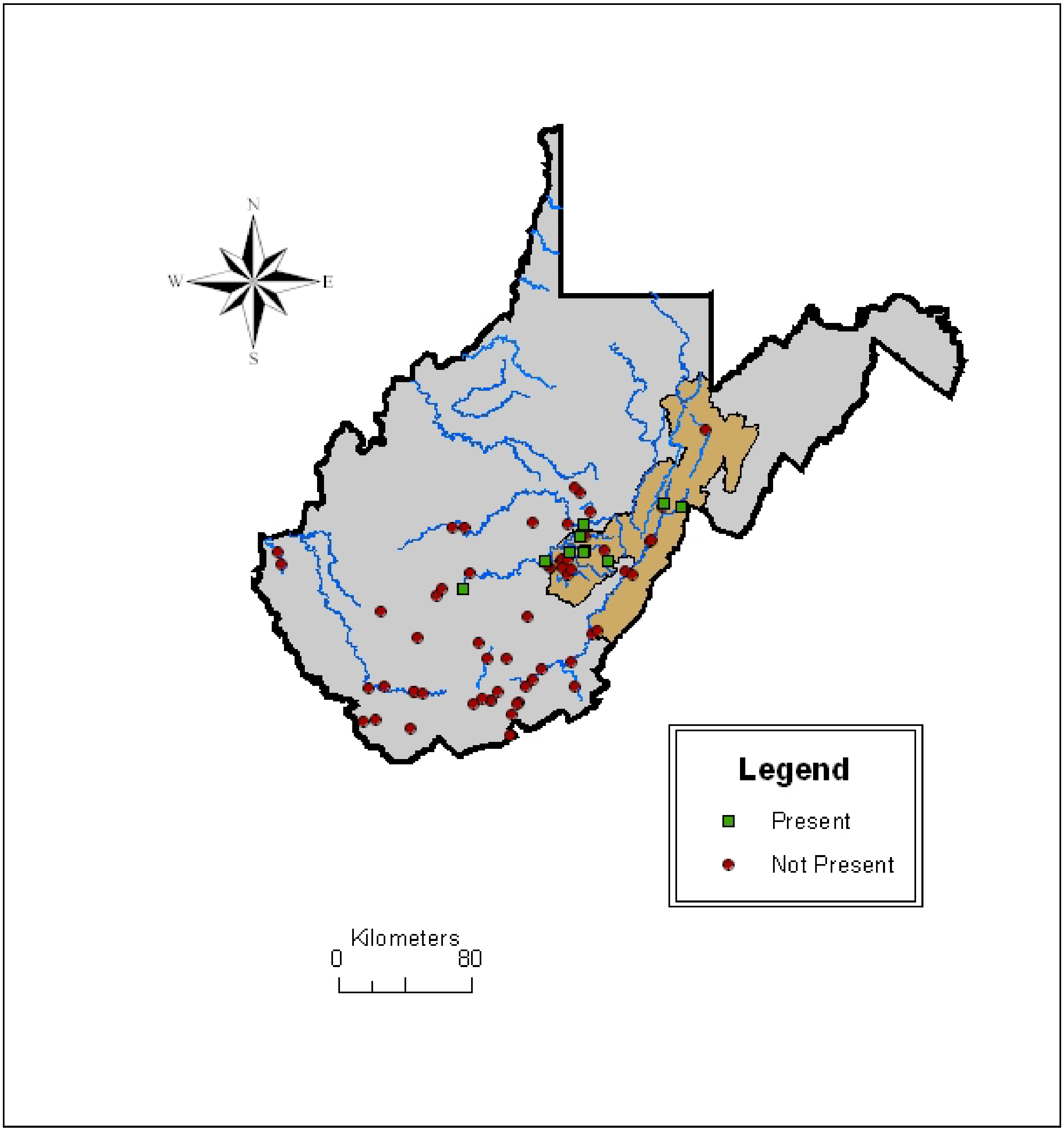


Figure 9. Example of the patchy distribution of populations, which may relate to resource availability.

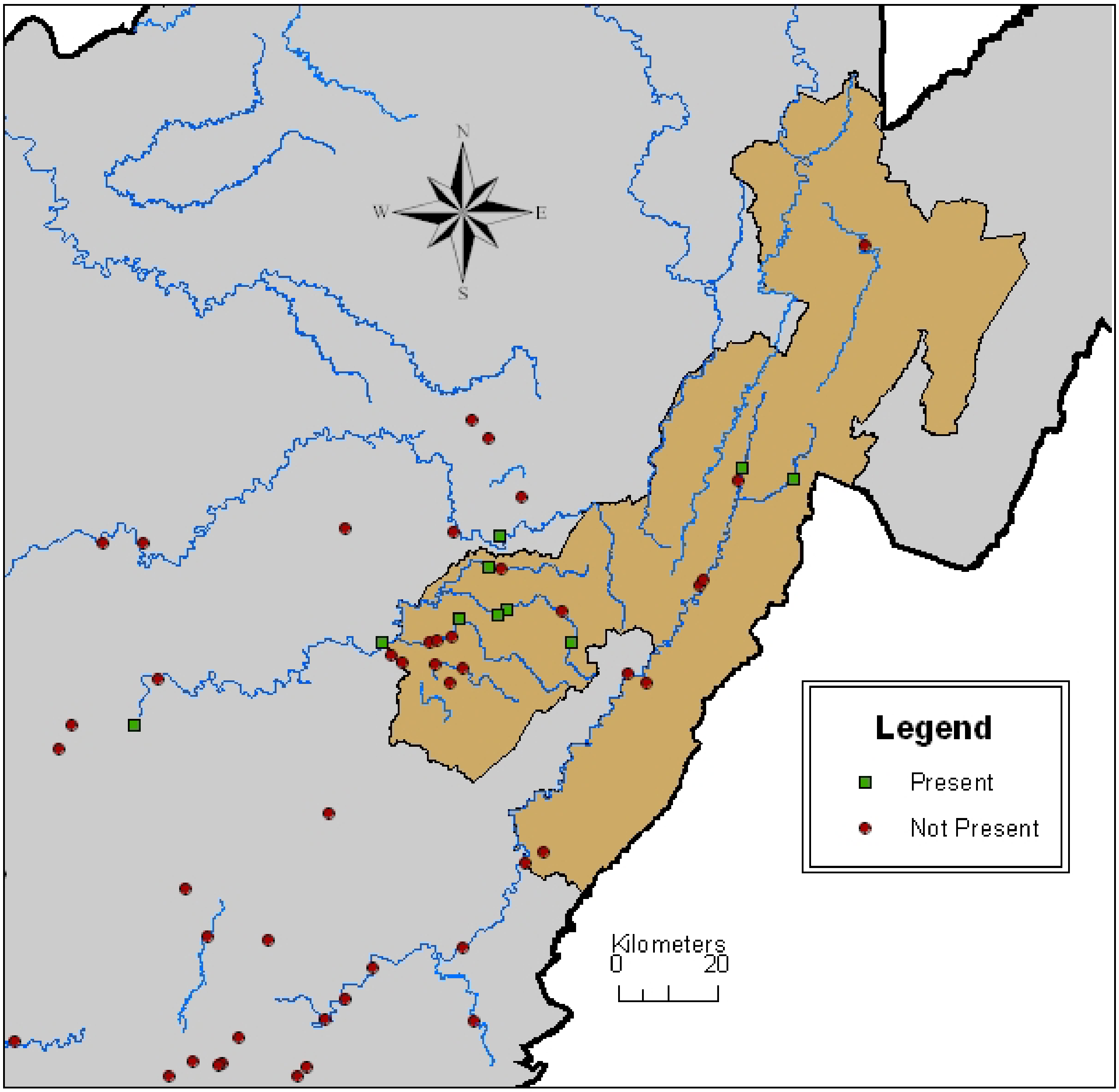


Figure 10. Example of scarring often observed on individuals. Rock turning may have resulted in the large circular shaped wound on this hellbender. Scars from other injuries can also be seen.
Photo by Tim Baldwin.

Figure 11. PCA ordination of all sites surveyed. PRIN 1 explained 96.4% of the total variation and PRIN 2 explained 2.8 %. Sites where hellbenders group along the PRIN 1, but there is considerable overlap with sites where they were absent.

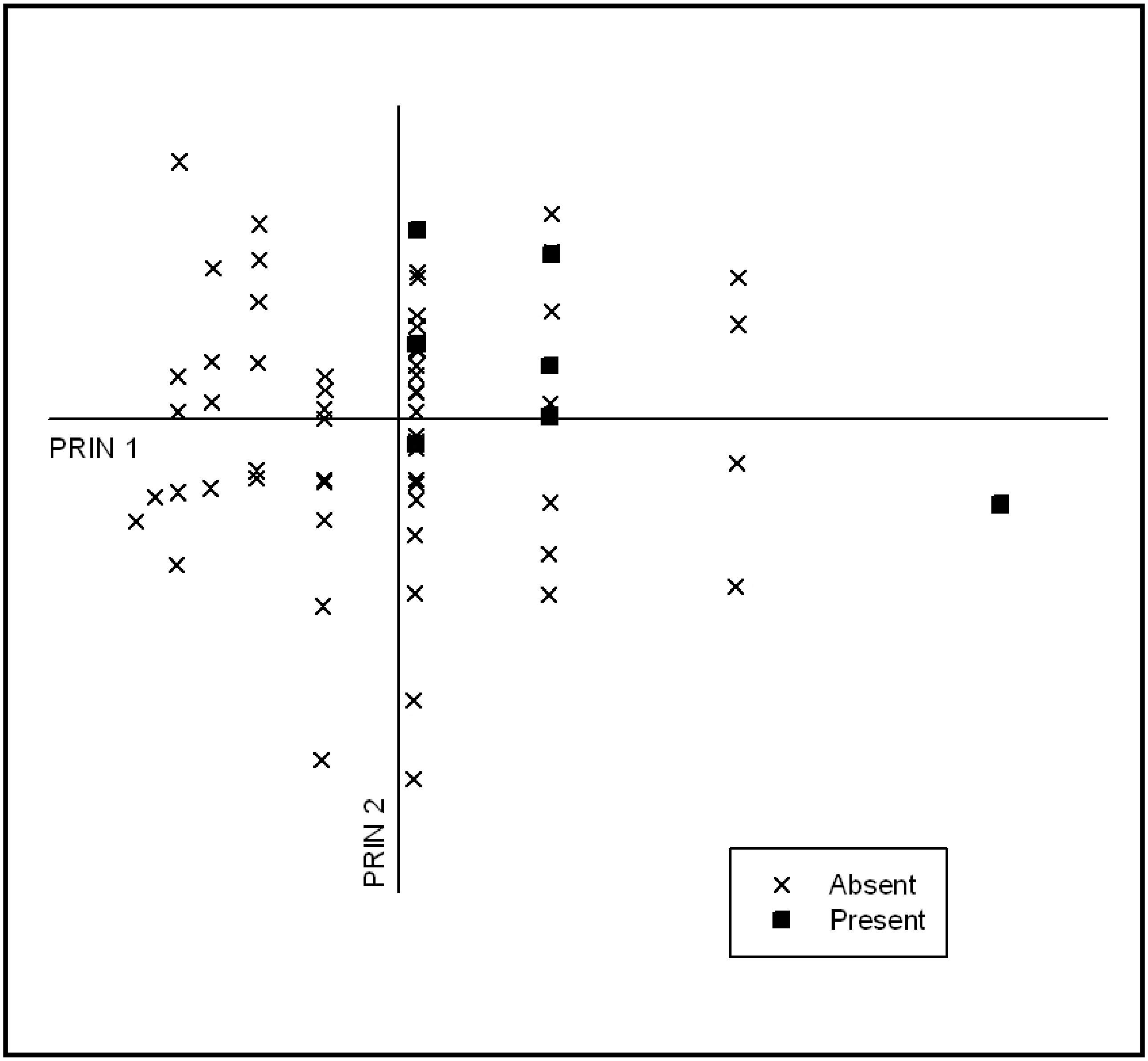


Figure 12. Mean values of habitat characteristics ± 1 S.E. for sites where hellbenders were present and absent. Values are Ln+1 transformed to show them all on one graph. * are significantly different at the p = 0.05

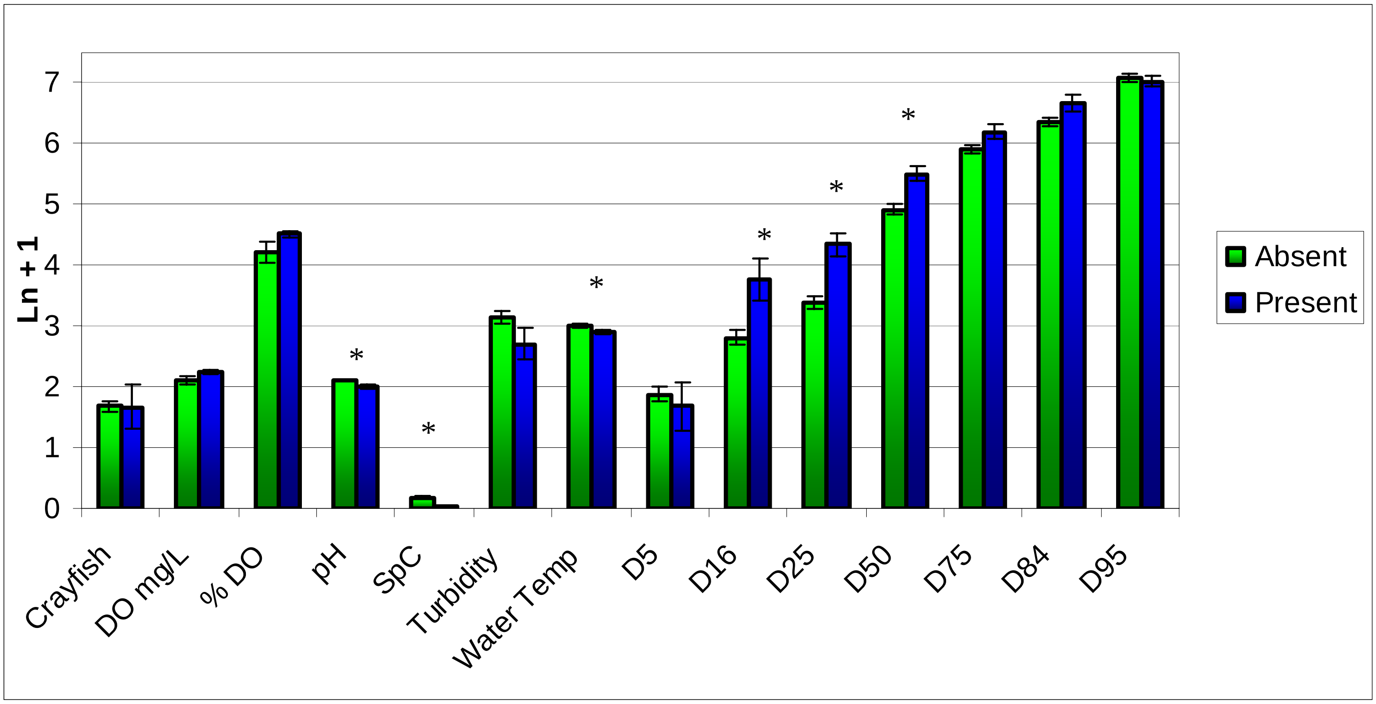


Figure 13. Boxplots of significant habitat variables. The particle size scores were generally higher, but overlap occurred. Water characteristics were lower and had small ranges. Dark circles are outliers and x's are extreme outliers.

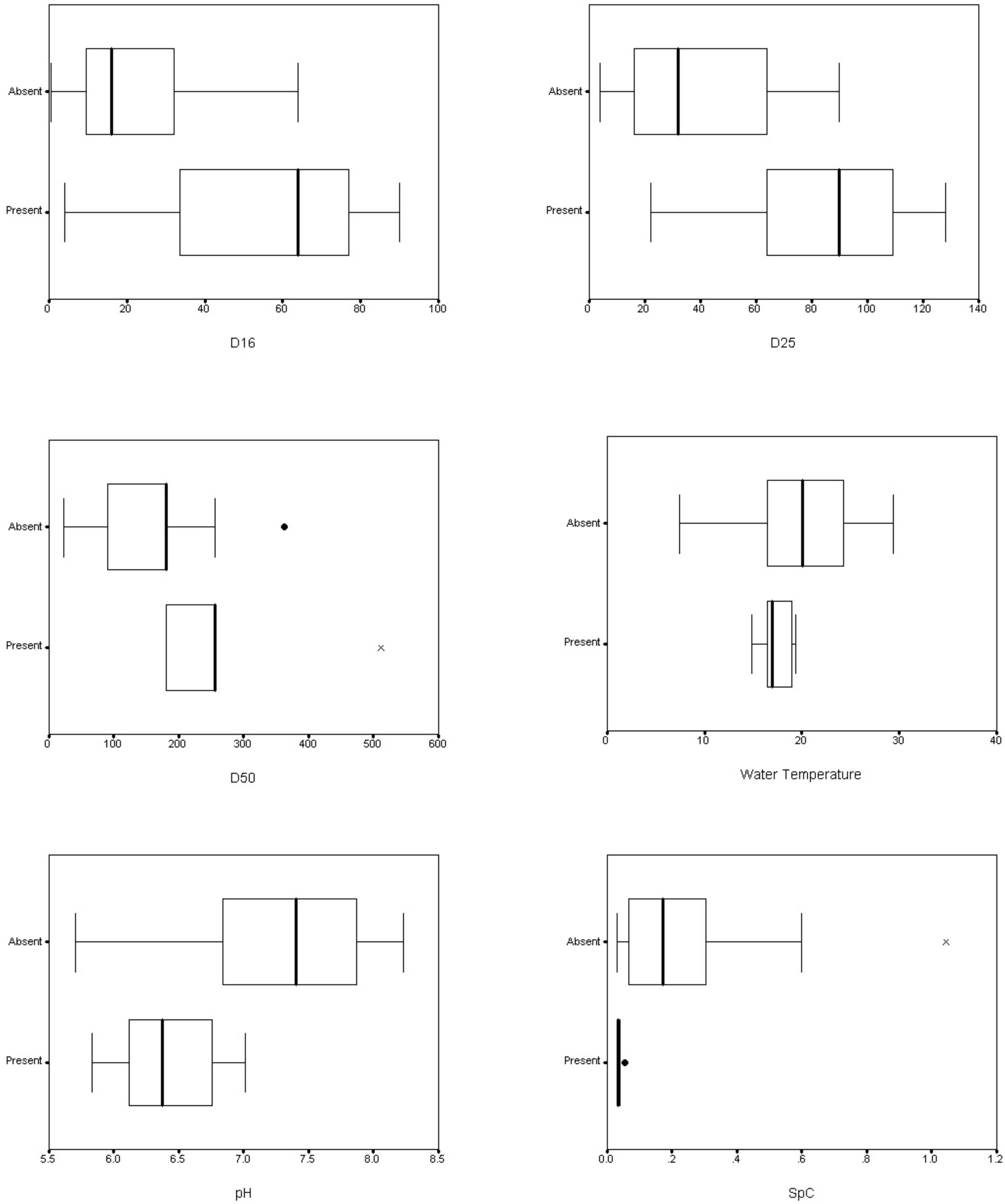


Figure 14. Results of cluster analysis showed that sites where hellbenders were present from 6 groups. In addition to those, areas in the mainstem of the Greenbrier River, Pond Fork of the Little Coal River, and Marsh Fork of the Big Coal River had similar habitat characteristics and may support populations.

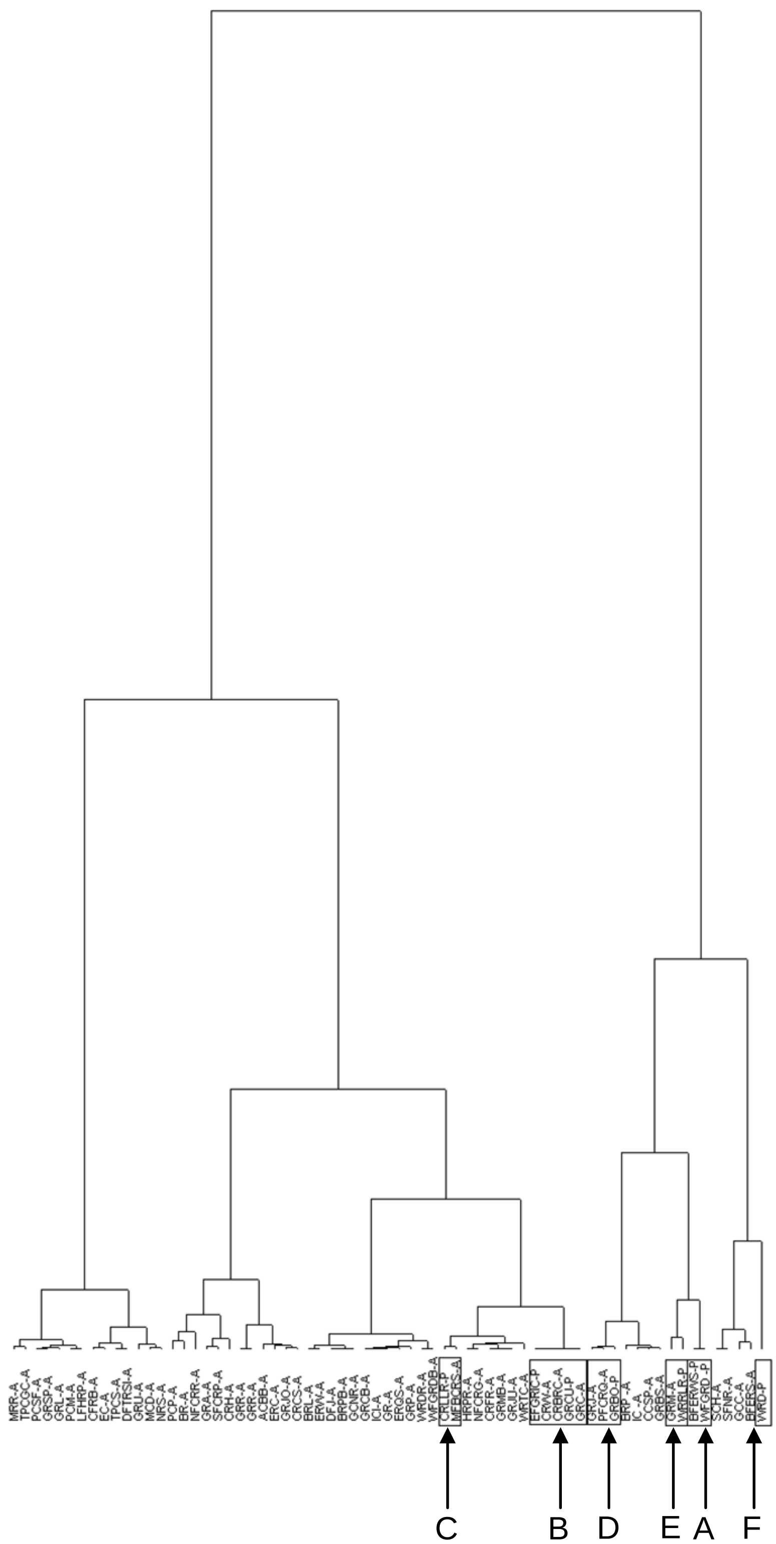
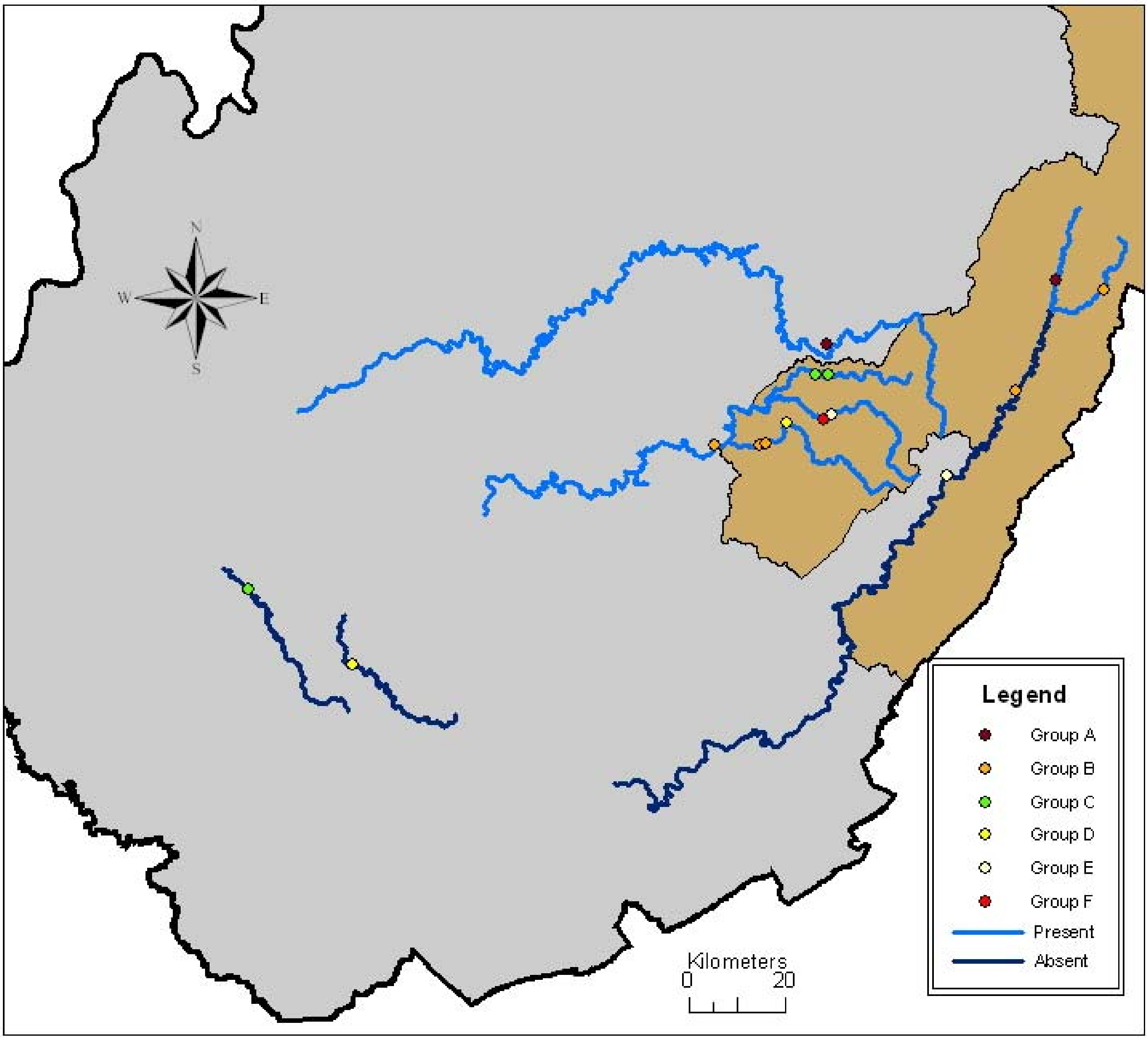


Figure 15. Locations of groupings identified in the cluster analysis. Streams where hellbenders have been found should be protected, while those where hellbenders were not found should be surveyed more extensively because they may potentially contain populations.



Appendix A

Hellbender information, including P.I.T tag numbers. Exact locations are not given.

ID	Date	TL(mm)	WT(g)	Site	Stream
Larval	7/12/2006	56.5	1.3	22	Williams River
Subadult	9/22/2006	216.0	62.0	61	Gauley River
4464043A	7/25/2006	360.0	280.0	27	Cranberry River
542E7A3830	5/29/2006	349.0	394.0	10	East Fork of Greenbrier River
4533597465	6/4/2006	673.0	1424.0	14	Back Fork of Elk River
452F3D0152	6/4/2006	532.0	854.0	14	Back Fork of Elk River
Escape	6/4/2006	N/A	N/A	14	Back Fork of Elk River
Escape	7/11/2006	N/A	N/A	21	Gauley River
44640C1D	7/16/2006	509.0	980.0	24	West Fork of Greenbrier River
Escape	7/16/2006	N/A	N/A	24	West Fork of Greenbrier River
452E7E7C5F	7/16/2006	424.0	378.0	24	West Fork of Greenbrier River
Escape	9/15/2006	N/A	N/A	24	West Fork of Greenbrier River
45343A3929	7/12/2006	502.0	980.0	23	Williams River

Appendix B

Principle Component Results and Coordinates for survey sites, exact locations are not given.
A = Absent, P = Present.

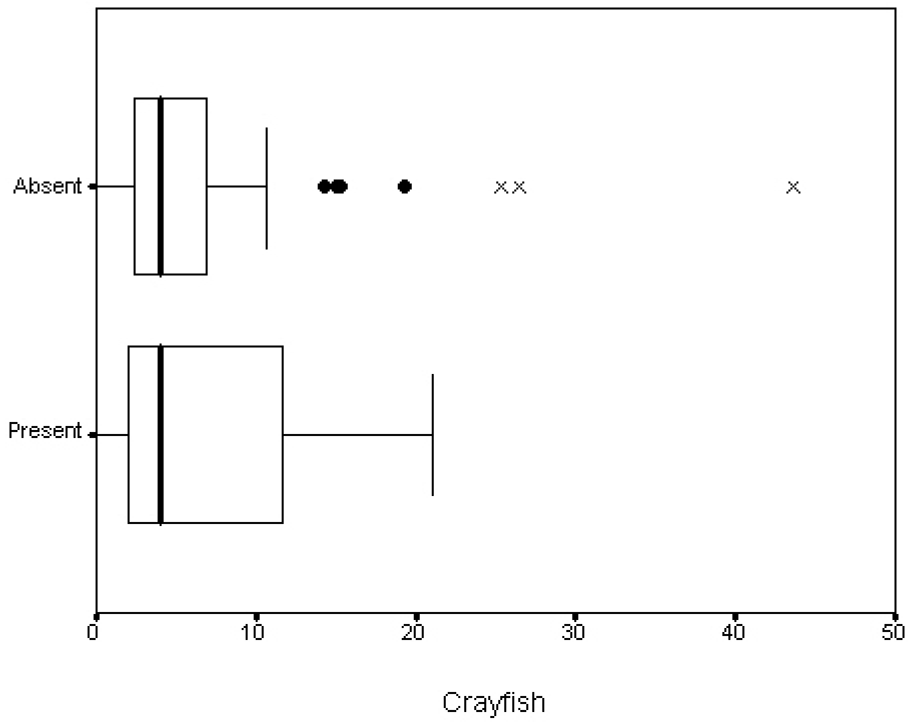
Axis	Eigenvalue	% of variance	Cum. % of Variance
1	567317.5	96.4	96.4
2	16587.209	2.8	99.2
3	2922.076	0.5	99.7
4	1277.543	0.2	99.9
5	382.124	0.07	99.99
6	21.35	0.004	100
7	1.342	0	100

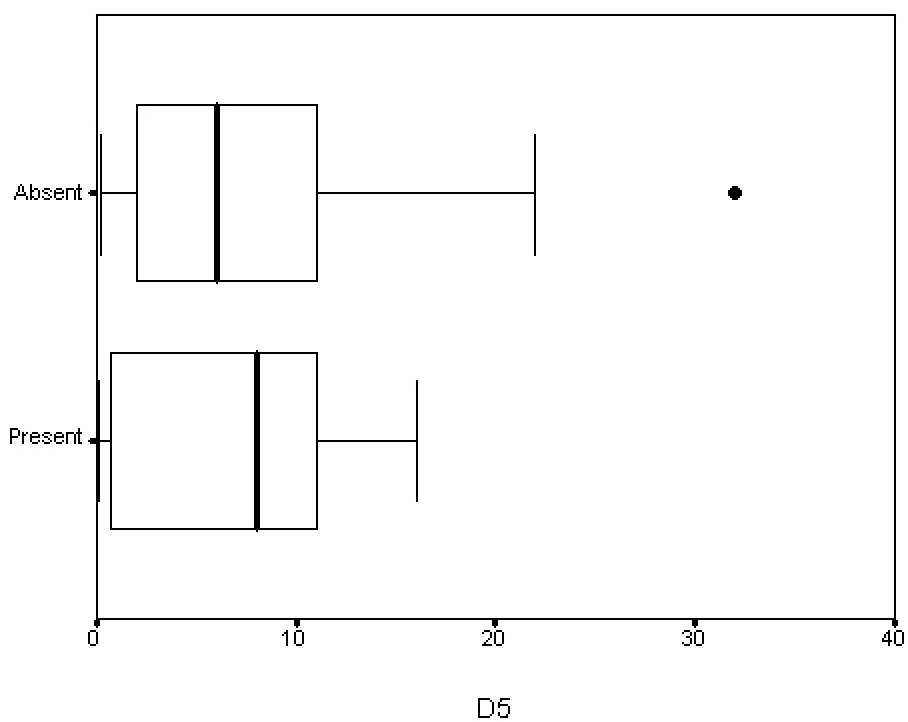
Site	Hellbenders	PRIN 1	PRIN 2	PRIN 3	PRIN 4	PRIN 5	PRIN 6
Twelve Pole Creek A	A	-140.2	-9.8068	-5.1008	-4.9591	-9.0771	-1.0691
Anthony Creek	A	-43.666	1.4923	3.7926	2.3563	-0.0679	0.038
Back Fork of Elk River A	A	191.03	18.132	16.544	-1.3387	0.5346	-1.0793
Back Fork of the Elk River B	P	85.18	21.094	2.602	0.2587	1.5381	-0.8162
Birch River	A	-80.834	24.982	-6.8509	-7.294	-0.4581	1.2383
Bluestone River A	A	9.0891	18.232	-6.7296	1.2333	1.3557	0.6082
Bluestone River B	A	83.563	-22.141	2.0401	3.5679	-2.2179	0.4375
Bluestone River C	A	6.9095	-35.667	2.7358	-1.432	-2.0703	0.1763
Camp Creek	A	85.274	26.344	13.62	-1.4876	-0.2625	0.1479
Cherry River A	A	-44.015	-7.8083	3.7595	3.0232	-0.6504	-0.422
Cherry River B	A	-43.454	5.5696	-1.1859	3.597	-0.7425	-0.5304
Clear Fork	A	-107.61	2.2553	-1.0715	5.922	-1.4266	0.2977
Cranberry River A	A	8.7292	9.0446	-6.8413	0.5186	-0.3673	-0.5766
Cranberry River B	A	8.2247	-3.5309	-2.6596	1.5132	0.3069	-0.3952
Cranberry River C	P	8.7415	9.7503	-8.0807	-1.4142	-0.3177	-0.4226
Cranberry River D	A	8.624	7.0046	-4.021	0.1416	-0.3425	-0.7596
Dry Fork	A	8.7619	8.8969	-4.6559	4.962	-0.1463	0.1472
Dry Fork of Tug Fork	A	-107.54	7.4263	2.2302	-2.4814	-1.8621	1.2391
East Fork Greenbrier River	P	9.1585	24.191	9.7612	-1.317	1.3156	-0.9595
East River	A	8.6925	13.282	10.139	-4.7018	-0.7319	1.0519
Elk River A	A	-45.606	-43.291	2.8215	-15.447	-1.4493	0.012
Elk River B	A	8.1834	-2.0908	-4.0153	-6.9509	-0.0484	0.4267
Elkhorn Creek	A	-108.12	-8.6537	0.1567	0.0727	7.9302	0.3267
Gauley River A	P	84.632	6.8856	2.0235	-0.2144	0.1145	0.004
Gauley River B	P	8.1681	-3.1148	-1.4546	-3.4652	0.6736	-0.1951
Gauley River C	A	85.257	21.404	-7.0616	-0.5406	2.2459	-0.8077
Gauley River D	A	-43.928	-7.5456	-1.2694	5.985	-0.0474	-0.1356
Gauley River E	A	-80.877	20.449	-8.3325	2.1362	1.6211	-0.9627
Glade Creek	A	190.26	-5.5072	-4.1327	-2.9698	0.3017	0.003
Glade Creek of New River	A	8.4976	3.5891	0.2406	3.2301	-1.2571	0.6674
Greenbrier River A	A	9.1345	18.879	-4.5642	4.198	0.6017	1.0453
Greenbrier B	A	-43.493	3.8598	-2.917	4.7148	-1.3445	0.4708
Greenbrier River C	A	-44.143	-12.646	-1.6178	4.2804	-0.4411	-0.2326
Greenbrier River D	A	83.8	-16.965	-0.9679	4.8015	0.2419	0.1785
Greenbrier River E	A	8.2807	-2.7582	-3.8514	2.799	-0.6715	-0.6354
Greenbrier River F	A	7.969	-10.059	-0.5231	2.4692	0.1665	-0.4791
Greenbrier River G	A	-127.42	-18.47	-0.607	6.2467	-0.4792	-0.2877
Greenbrier River H	A	84.054	-10.459	-1.4452	4.7064	-1.3812	-0.015

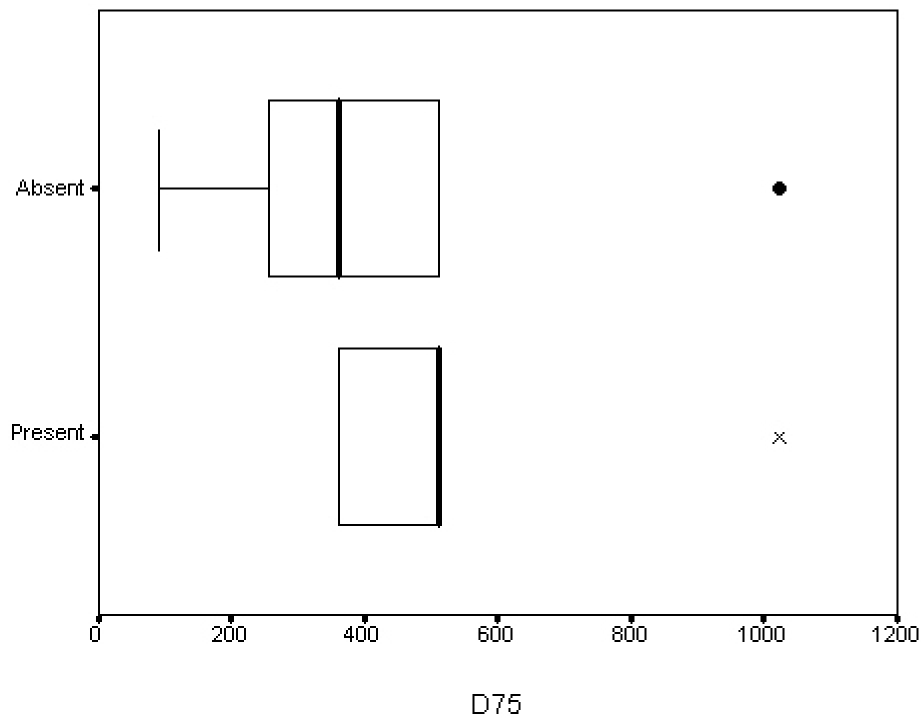
Greenbrier River I	A	8.0157	-8.0085	6.2197	4.0729	-1.0854	0.0327
Guyandotte River A	A	6.462	-45.706	8.0248	-2.6844	6.4982	-0.0927
Guyandotte River B	A	7.52	-22.066	0.716	4.2423	-1.589	0.0867
Guyandotte River C	A	-44.554	-23.66	-0.6009	5.8849	-2.1097	0.038
Guyandotte River D	A	-127.14	-9.1809	2.0483	2.2432	7.4678	0.2409
Holly River	A	8.3363	3.6069	0.019	-9.8021	-1.4281	-0.2772
Indian Creek A	A	84.973	13.941	-3.6147	1.8761	0.9649	0.389
Indian Creek B	A	7.7738	-14.633	-0.3421	1.0423	-1.4734	0.31
Left Fork of Holly River	A	-126.67	5.6508	-3.0596	-9.0258	-0.2036	-0.3656
Marsh Fork of Big Coal River	A	8.0084	-7.5532	9.8335	4.3111	-2.2649	0.1468
Meadow River	A	-150.46	-12.839	11.706	-5.1282	-0.5448	-0.6054
Mountain Creek	A	-82.07	-6.4053	3.6589	-2.7254	8.199	-0.1902
New River	A	-81.928	-7.3217	-2.9276	6.9406	1.0334	0.1886
North Fork of Cherry River A	A	8.7465	11.996	-3.569	-5.0095	0.2954	0.1683
North Fork of Cherry River B	A	-81.125	15.036	-5.0596	0.9526	-0.6727	-0.5593
Paint Creek A	A	-126.78	1.1129	-6.0505	-5.8378	-0.6466	0.7444
Paint Creek B	A	-81.484	7.3137	-6.6529	-4.8009	-0.1868	0.8717
Panther Creek	A	-125.73	32.915	31.068	3.3174	-2.4211	0.2732
Pond Fork of Little Coal River	A	84.542	2.1262	-2.4886	4.4193	-1.6284	1.014
Sandstone Alls	A	189.65	-21.214	2.0355	0.4783	-0.0051	0.6943
Second Creek	A	190.94	12.219	-1.0998	-0.6361	0.5168	1.1239
South Fork of Cherry River	A	-43.662	0.2421	-2.7411	2.4401	0.5744	-0.3206
Twelve Pole Creek B	A	-106.94	19.362	-10.713	1.0612	0.6552	-0.3749
West Fork of Greenbrier River A.	P	84.399	0.5914	-3.0796	-2.667	-0.1274	-0.107
West Fork of Greenbrier River B	A	8.4643	3.7492	-4.0553	-2.5254	0.4906	0.0588
Williams River A	A	8.4203	5.8115	7.3011	-5.3006	-0.4034	0.0688
Williams River B	P	340.18	-10.73	-4.4182	-3.7362	0.1294	-0.3749
Williams River C	P	84.415	0.3504	-3.3219	-0.8356	-0.4037	-0.6031
Williams River D	A	8.3809	1.0449	-1.3457	0.7126	-0.7179	-0.3145

Appendix C

Box plots of habitat characteristics that were not statistically significant. Dark circles are for outliers and x's are for extreme outliers.



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1200
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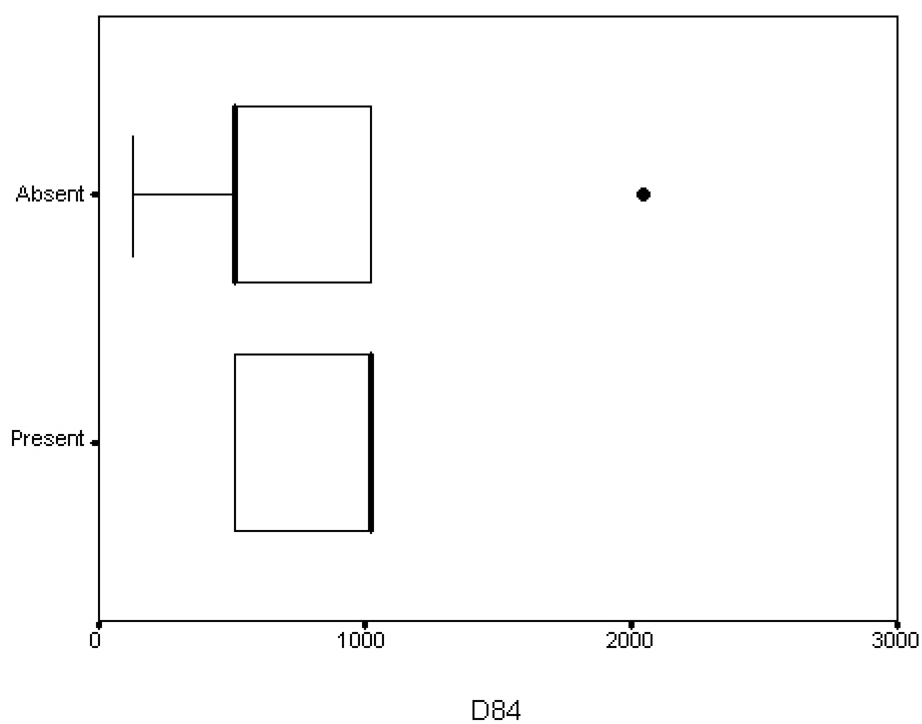
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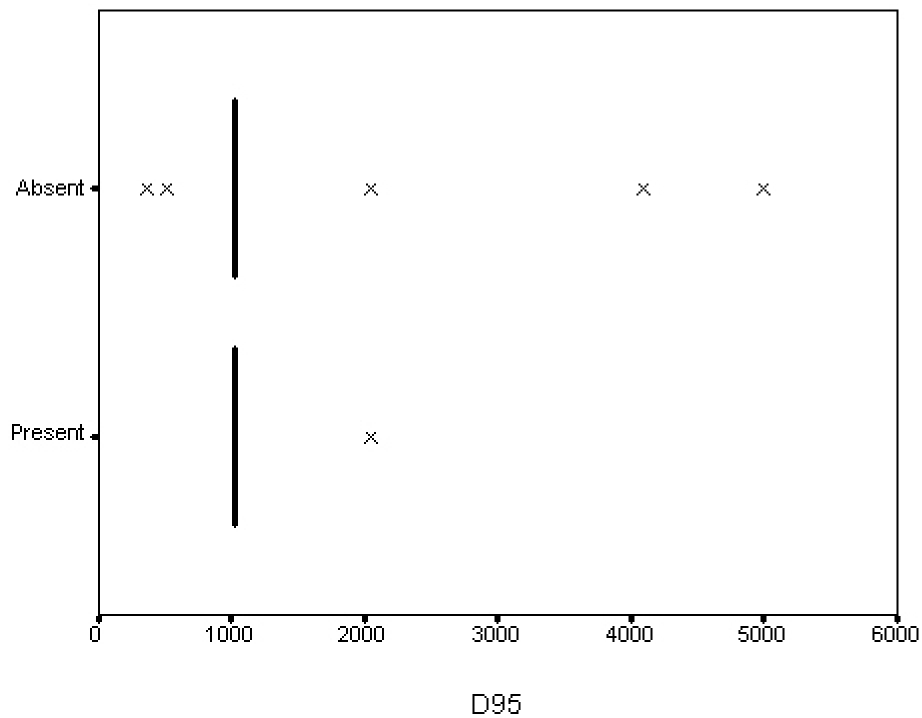
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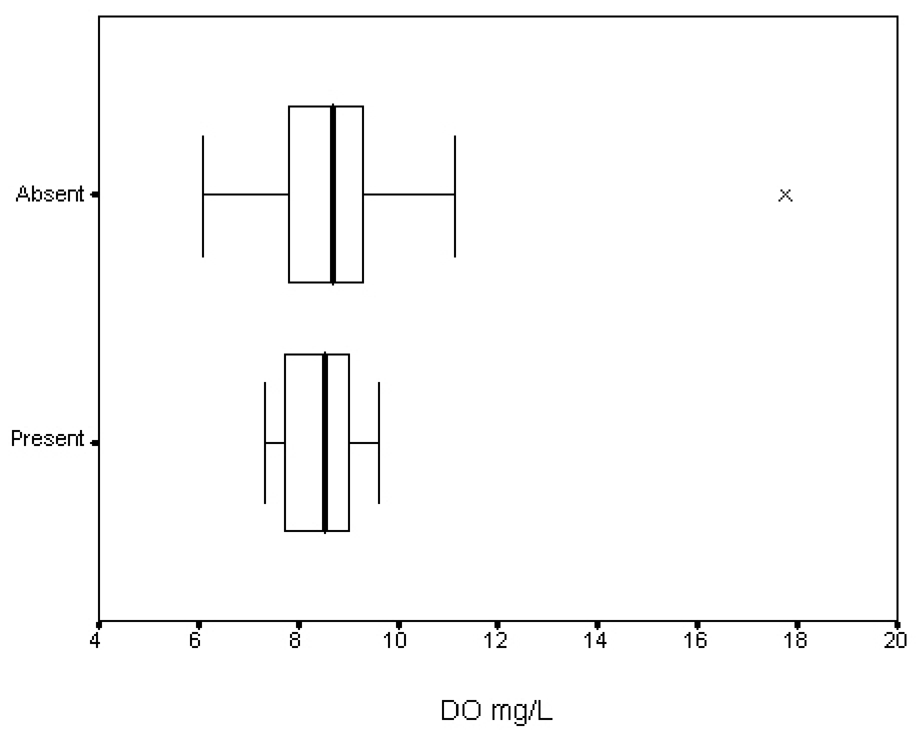
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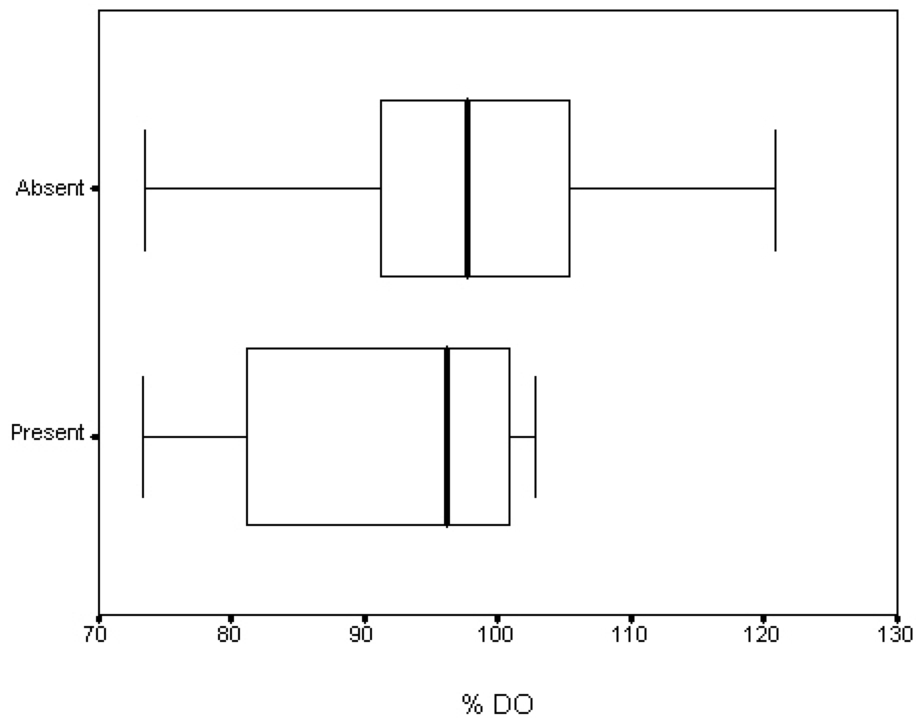
6
8
10
12
14
16
18
20
DO mg/L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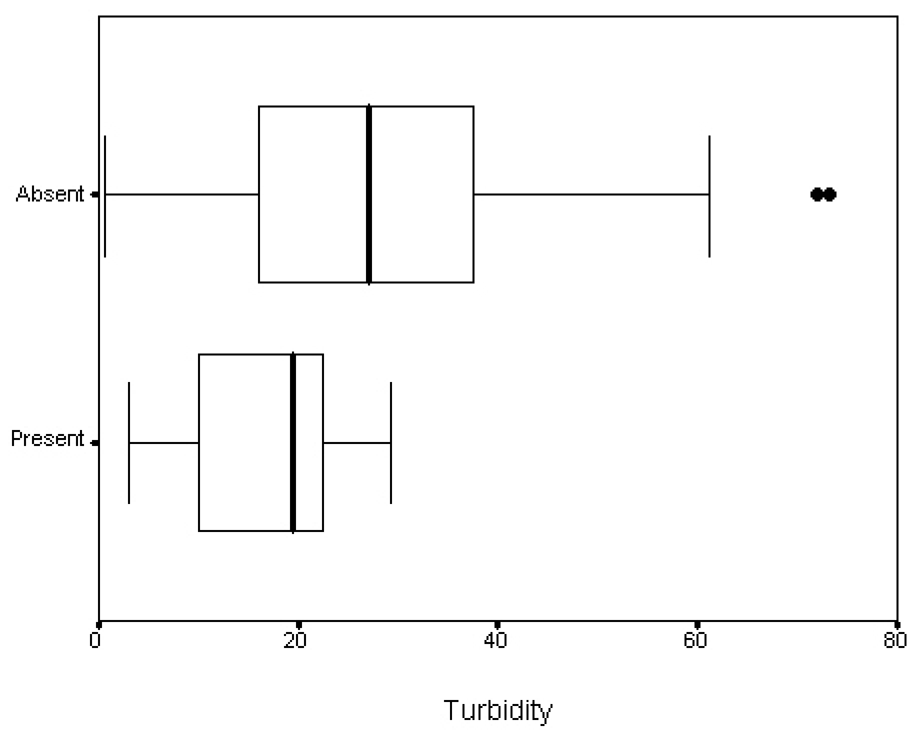
Present
70
80
90
100
110
120
130
% DO


Absent
Present
0
20
40
60
80
Turbidity

Appendix D:

Curriculum Vitae

S. Conor Keitzer

Contact Information
Biology Department
One John Marshall Drive
Huntington, WV 25755
keitzer@marshall.edu
(352) 262-4494

Education

Biology M.S. Student
Expected graduation is May 2007
Current GPA: 3.94
Marshall University, Huntington WV

B.S. in Wildlife Ecology and Conservation with a Minor in Zoology
May 2004
GPA: 3.75
University of Florida, Gainesville FL

GRE Scores
Verbal: 630
Quantitative: 690

Research Experience

Thesis Research, Marshall University
May 2006 – Present
Surveying for Eastern Hellbenders (*Cryptobranchus a. alleganiensis*) in West Virginian streams to determine current distribution and examining habitat preferences.
Supervisor: Dr. Thomas K. Pauley

Morphological Investigation of the West Virginia Small Woodland Salamanders (Plethodontidae), Marshall University
Spring Semester 2006
Compared morphological characters of the small woodland salamanders of West Virginia using principal component analysis.
Supervisors: Dr. Thomas K. Pauley and Dr. D. Evans

Research Assistant, Marshall University
August 2005 – Present
Long-term research project that is investigating populations of Cheat Mountain Salamanders (*Plethodon nettingi*) on ski slopes.
Supervisor: Dr. Thomas K. Pauley

Research Assistant, Marshall University
August 2005 – November 2005
Research investigating stream salamander species and their potential as indicators of stream health.
Supervisor: Dr. Thomas K. Pauley

Fishery Biotechnician, USGS
January 2005 – June 2005
Participated in research examining fish community structure on sand banks and deep-water coral reefs of the Gulf of Mexico.
Supervisor: Dr. Kenneth J. Sulak

Research Assistant, USGS
May 2003 – December 2004
Field assistant for research involving Gulf Sturgeon (*Acipenser oxyrinchus desotoi*) in the western panhandle of Florida.
Supervisor: Dr. Kenneth J. Sulak

Research Assistant, University of Florida
June 2002 – May 2003
Field assistant for research investigating impacts of roads on wildlife in central Florida.
Supervisor: Dr. Daniel Smith

Teaching/Other Experience
North American Amphibian Monitoring Program
August 2006 – Present
Organized volunteers and reviewed frog call survey data

Marshall Herpetology Journal Club
2006 – Present
Co-founded a graduate student journal club which meets weekly to discuss journal articles.

4-H Camp Counselor
June 2006
Taught ecology classes for campers 9-12 years of age.

Graduate Teaching Assistant, Marshall University
August 2005 – December 2006
Introduction to Biology (BSC 120 and 121) laboratory sections.

Treasurer of the University of Florida Student Chapter of The Wildlife Society
April 2002 – May 2003
Supervisor: Dr. Tanner

Presentations/Posters

Association of Southeastern Biologist Conference. 2007. Columbia, SC. Presentation.

West Virginia Academy of Science Conference. 2007. Marshall University, Huntington, WV. Presentation.

Midwest Ecology and Evolution Conference. 2007. Kent State University, Kent, OH. Presentation.

Association of Southeastern Biologist Conference. 2006. Gatlinburg, TN. Poster.

Grants/Awards
West Virginia Division of Natural Resources Natural Heritage Grant - $7,000
Marshall University Summer Research Grant - $500

Professional Societies
Association of Southeastern Biologists
Ecological Society of America
Society for the Study of Amphibians and Reptiles

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