1-1-2004

Natural History and Sexual Dimorphism of the Eastern Hellbender, Cryptobranchus A. Alleganiensis

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NATURAL HISTORY AND SEXUAL DIMORPHISM OF THE EASTERN HELLBENDER, *CRYPTOBRANCHUS A. ALLEGANIENSIS*

Thesis submitted to
The Graduate College of
Marshall University

In partial fulfillment of the
Requirements for the degree of
Master of Science
Biology

by

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May 2004

ABSTRACT

“NATURAL HISTORY AND SEXUAL DIMORPHISM OF THE EASTERN HELLBENDER, *CRYPTOBRANCHUS A. ALLEGANIENSIS*”

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The Eastern Hellbender’s natural history and morphology was examined. Field studies were done to examine the efficacy of different techniques (focusing on capturing larva) and diet analysis. Lab studies were done to determine if any sexual dimorphism exists in hellbenders using simple measurements (and ratios) and if this dimorphism could be used to reliably determine gender. Field studies were inconclusive due to the inability to find a reliable sample because of record rainfall. Lab studies revealed that a dimorphism does exist for one ratio (TG/TL, P=.048) and for several measurements. These measurements alone were not applicable for determining gender. Other ratios recorded definite trends signaling weak dimorphisms, but combinations of these ratios using principal component analysis were unable to conclusively separate genders. Therefore, while measurements and one ratio suggest a sexual dimorphism, this dimorphism is not distinctive enough to separate genders reliably.

DEDICATION

The author wishes to dedicate this thesis to Donald J. Shure whose kind words, support, and benevolence have revealed to me many things, but most of all that what I am doing was possible. I can only hope to become such an accomplished scientist, friendly individual and respected teacher.

ACKNOWLEDGMENTS

Over the course of my thesis, many people donated their time to help me in the field. These include Nancy Dickson, Keith Johnson, Bill Sutton, Zac Loughman, Carri Williamson, Elizabeth Fet, Cynthia Lucas, Linh Phu, Casey Swecker, Paul Lord, and Brian Sang. Mark Watson was very helpful with information and my committee was supporting and understanding. My former advisor Larry Wilson got me started in the right direction and has always been there to give me needed advise. I would like to extend a special thanks to the WVDNR for a generous grant to study hellbenders as well as information pertaining to them. During the course of my studies, several professors helped immensely with statistics, procedures, and general guidance. For this, I would like to thank Dan Evans, Chuck Sommerville, and Suzanne Strait. Finally, I would like to thank the Biology office, especially Vicki and Mary Jo, who were always helpful and there when I needed them.

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CHAPTER I

Review of Literature

Introduction

The Eastern Hellbender, *Cryptobranchus alleganiensis alleganiensis*, is a large, aquatic salamander found in large, cool streams (Guimond & Hutchison, 1973; Hillis & Bellis, 1971; Hutchison and Hill, 1976.) It is one of 3 extant species belonging to the Family Cryptobranchidae. The other 2 species, *Andrias davidianus* and *A. japonicus*, are found in China and Japan respectively. Another subspecies of hellbenders, *C. a. bishopi*, is restricted to Missouri and Arkansas and can be differentiated from the Eastern Hellbender by hematology, proteins, and mottling on the chin (Jerrett and Mays, 1973; Worthman and Nickerson, 1971.) The Eastern Hellbender is found throughout Ohio River Drainage in the Appalachian Mountains and Cumberland Plateau. It is known to occur in Mississippi, Alabama, Georgia, Tennessee, North Carolina, South Carolina, Missouri, West Virginia, Virginia, Kentucky, Ohio, Illinois, Indiana, New York, Maryland, Pennsylvania, and possibly Kansas (Brandon & Ballard, 1994; Dundee & Dundee, 1965; Fitch, 1947; Gates *et al*., 1985; Nickerson & Mays, 1972b.) While they have a large range, they are considered to be genetically uniform (Merkle *et al*., 1977; Routman, 1993.)

The hellbender is a long-lived animal, with individuals thought to achieve more than 30 years (Nigrelli, 1974: Peterson *et al*., 1988; Peterson *et al*., 1985; Taber *et al*, 1975.) They have a flattened head, small eyes, wrinkly skin, and a large longitudinally compressed tail (Green & Pauley, 1987.) Hellbenders are diploid, with spermatogonium containing 62 chromosomes (Makino, 1935.) They require high levels of dissolved oxygen to maintain proper blood oxygen tensions and meet metabolic demands. If blood oxygen tension levels drop, hellbenders are known to "rock" in the water, thereby increasing water flow across their skin (Harlan and Wilkinson, 1981). They are denizens of clean water and their presence indicates good stream quality.

Normally, adults (larvae are all a shade of black) are dull brown to black with large, dark spots, but orange and even red specimens have been reported (Fauth *et al*., 1996.) While some have speculated that these aberrant colors are a sign of poor health or increased stress, most feel that select populations simply have varying coloration, possibly due to diet (Green, 1933.) In West Virginia, the Hellbender is usually dark brown to black and is considered a species of special concern (ranked S2) by the West Virginia Department of Natural Resources. Recently, researchers have noticed declines in population size as well as extirpation of populations throughout its range (Mayasich *et a*l, 2003.) Concurrently, reports of abnormalities are becoming more common. These include epidermal papillomas (Trauth *et al*, 2002), missing limbs or toes, bifurcated limbs, and blindness (Wheeler *et al*., 2002.) There are probably multiple causes for these declines and abnormalities, but most can be attributed to habitat destruction and degradation (Petranka, 1998). Nevertheless, hellbenders are a very resilient creature. Nickerson (1980) found that specimens that were starved for months could be released back to their site of capture and re-establish themselves in the population. However, without appropriate habitat, even their resilience will not save them.

Considerable research has been done on aspects of the hellbenders natural history, but no intense research has been done examining for the presence of any sexually dimorphic characteristics. Normally, researchers examining specimens determine gender based on the swollenness of the cloaca. Sexually mature males for variable months of the year (usually around 3 in the fall) will be noticeably more swollen than females (see Fig. 2.) While this is a fairly reliable method for sexing mature specimens, it provides no information on smaller individuals. Furthermore, it is not known if all males breed every year or if they stop breeding (and swelling) after a certain age. King (1939) reported that males have more extensive folding on certain parts of the body than females, but this was only examined in one population and the measurement seemed difficult to repeat. Bishop (1941) reported that males are sometimes broader and heavier than females of the same length in a New York population. Other methods, like mass/length ratios have been reported, but these data often conflict and are based on uncertain gender determining methods that I will discuss later. Also, studies reporting dimorphism have only used specimens from one population. Essentially nothing is known about sexing smaller specimens. Presently, the only way to sex small specimens is to determine the type of gonads present. However, specimens may not be mature enough to have developed sex organs.

Part of this study will focus on determining if sexual dimorphism exists. Examples may be observed with morphological measurements, such as total length, primary or secondary reproductive structures (e.g. swollen cloacas in males during the breeding season), or size ratios, such as mass/total length. Sexual dimorphisms need not be influenced solely by reproduction. Shine (1989) reported how some dimorphisms, specifically head-width, could develop due to dietary divergence. Alternatively, it could be that male competitive advantage led to the dimorphism. For example, instead of different diets leading to dimorphism, males with wider head could have been sexually selected due to their ability to win more male-to-male combats. Whatever the reason, such dimorphisms do commonly exist in many sexually reproducing species. And for hellbenders, measuring specimens and determining gender would not be difficult, due to their large size.

Deciding whether to use preserved or live specimens is a dilemma. Preserved specimens are replete, available, and ready for dissection. The problem is that they vary in how long they have been preserved, how long they were kept alive before they were killed, their preservation method, and their storage method. These differences can have varying affects on a specimen's morphology (Nickerson, 2003.) These specimens could tell whether a dimorphism exists, though, and support whether further research into live specimens would be worthwhile. If live specimens were utilized, then simply defining a protocol would standardize all the problems encountered working with preserved specimens. Killing specimens of a species already in sharp decline, particularly the number needed for a study like this seems to be a terrible idea, especially considering that preserved specimens would at least reveal whether dimorphism exists. Therefore, it seems advisable to start with preserved specimens, determine if dimorphism can be found, then determine the best way to acquire the data needed to create an equation applicable to live specimens.

Male-to-male aggression is commonly associated with sexual dimorphism. For example, if the males combat one another for rights to breed then it would be advantageous to attain a larger overall size. If they do not combat, then the females usually attain a larger size since a larger female can produce more offspring. In caudata and other salamanders, this is the general trend (Shine, 1979; Bruce, 1993.) Past research has shown that hellbenders may not follow this trend. Aggressive male-to-male combat and scars that resemble conspecific bite marks has been observed and reported, but females are thought to attain a larger overall size than males. Not only is this unusual, but hellbenders are one of the only known species of amphibian where males are singly responsible for guarding the eggs. The behavior is similar to that of some fish as is the process of egg deposition and fertilization. First, a female enters the nest and lays her eggs. This happens during the fall, but apparently male Ozark Hellbenders are capable of fertilizing eggs throughout the winter (Peterson, 1989.) If she is reluctant, then the male attempts to contain her until she becomes receptive. The male subsequently fertilizes the eggs (externally) and chases the female away from the nest. As an adaptation to external fertilization, the sperm actually does not become active until introduced into water (Baker, 1963). Other females may then deposit their eggs, adding more eggs to the male's chamber (up to 700 per female (Topping & Ingerson, 1981), thousands per nest.) This has been observed in nature (Smith, 1912a.) Smith (1912a) also reported that the eggs a female lays are distinct in both color shade and size from other females. However, it has not been substantiated that the "attending" male actually fertilized the eggs. The evolutionary reason for this behavior is not known. It has been suggested (Nickerson and Mays, 1973b, Dundee and Dundee, 1965) that the male guards the eggs from predation, especially from conspecifics based on reports of males chasing off other males as well as spent females (Petranka, 1998.) Another possible explanation is that the males are not truly guarding the eggs, but instead the breeding spot. Since multiple females may visit a male each season there is good reason for a male to protect his claim and wait for other females. As for why a male would chase away a spent female, it seems logical that before another female can enter the nest the male must vacate it first. Also it seems that hellbenders are solitary animals. In fact, there are no reports (in the literature) of two hellbenders being captured under the same rock outside of the breeding season. So, it could be that males are instead guarding the nesting spot and at the same time maintaining their solitary lifestyle. A third theory why males might guard their offspring is that the male exudes anti-microbial compounds to help repel infections. Adult hellbenders, like many amphibians, are known to be very resistant to cutaneous microbial infection. Smith (1912a) reported that during artificial rearing of eggs fungal infections were a serious problem. These were reared in artificial creeks that housed other adult hellbenders. Fish eggs, which are also raised under water, also commonly experience mold and fungus infections (Moyle and Cech, 2004.) Many fish also attempt to protect their eggs from suffocation by removing debris that collects on the eggs. These two problems are the largest causes for fish egg mortality. And since hellbenders and some fish live breed in similar habitats, the same problems might affect hellbenders. By secreting antimicrobial compounds and tending the eggs, males may be significantly lowering the occurrence of offspring loss by either of these events.

While hellbender males may actually be good parents, they commonly engage in a practice detrimental to egg survival (and possible their own offspring's survival). Specimens (both male and female, but mainly female) captured during the fall commonly have eggs in their digestive system (Smith, 1912a). It may be that the eggs are unfertilized or that the male simply needs nutrition to sustain himself during the incubation period. Or it may be that hellbenders are actually eating their unhatched eggs.

Smith (1912a) also reported that egg strings contain empty capsules at the beginning and end of the strings. These empty capsules contain everything a normal capsule contains (like yolk) except the actual genetic material. Therefore, they are still highly nutritious. Some are the same size and shape of regular eggs, although they may be much smaller. So it may be that males and/or females attempt to consume these unfertilizable capsules (as well as other eggs that do not get fertilized) due to possibly a chemical cue. There have been no reports, though, as to whether the eggs found in the stomachs of adults are fertilized or unfertilized.

After hatching, hellbenders quickly become an opportune meal to many predators. Cannibalism is common, and the low number of juveniles observed in the wild (Bothner and Gottlieb, 1991) leads many to believe that survival rates are extremely low for the first couple of years. Smith (1912b) reported that larvae are 2.3 to 2.5 cm at hatching and grow to 5-7 cm in one year under laboratory conditions. Their diet, like adults, is probably anything that can be taken into their stomach. Diet analyses commonly report stones and other organic debris (Green, 1935; Netting, 1929; Makowsky, 2001.) Smith (1912b) even reported a 12 cm larva that regurgitated a 5 cm larva.

To possibly counter these high predation rates, juvenile hellbenders grow rapidly. They reach around 20 cm by 3 years of age in nature and some are sexually mature in an estimated 5 years. After reaching maturity, growth seems to decline quickly. At 25 years of age, Peterson *et al*. (1983) estimated growth rates at about 1 mm per year. It has not been reported exactly when the hellbenders skin starts to produce a toxic secretion, but adults are known to generate such chemicals. This may happen at any point following fertilization. Brodie (1971) found mucus collected from the dorsum of hellbenders was very bitter and dehydrating to the tongue and that injected secretions proved lethal in mice. He felt that the taste would probably be a deterrent against potential predators. Nickerson and Mays (1973b) reported that they could not find any antibiotic properties in these secretions, but some form of anti-microbial property is still believed possible. Other possible advantages the mucus provides are lubrication for the skin if the hellbender leaves the water and a slippery medium to aid in sliding under rocks. Most researchers who have worked with them would probably agree that the mucus probably aids in escape from any animal attempting capture. This mucus, combined with their wedge-shaped head and flattened body enables them to squeeze into small cavities under rocks.

Past research on the diet of the hellbender has found that crayfish comprised most of the hellbenders gut contents (Green, 1935; Netting, 1929; Nickerson & Mays, 1973b; Makowsky, 2001.) Other items that have been reported are fish, insects, and hellbender eggs (Nickerson *et al.*, 1983; Peterson *et al*., 1989b.) It could be, though, that hellbenders only eat crayfish in the wild because they are a convenient food source. Being that both animals take refuge under the same structures and that crayfish usually thrive in "hellbender" rivers, it is likely that the two frequently come in contact with one another. And since the hellbenders other prey items (excluding eggs) are probably more difficult to capture, it might be that crayfish are not actually the preferred food item of the hellbender but instead the most convenient. Hellbenders simply ingest them often due to their high availability compared with other prey sources.

Another explanation for the abundance in crayfish is that they simply take longer to digest than other items. Researchers found that in salamanders (Jaeger, 1990) and fish

(Moyle and Cech, 2004) different prey items have different digestion rates. These differential digestion rates are based on several factors, but the main one in this case would be the slowly digested exoskeleton of the crayfish. The food data collected on hellbenders up to this point only looks at stomach (and sometimes intestine) contents, not actual ingestion rates. It could be that several fish are eaten for every crayfish during a given time period, but diet analysis by means of stomach content analysis would not reveal this. Instead, due to the low digestibility of crayfish, it would appear that crayfish compose a larger proportion of ingested prey than they actually do. Controlled experiments examining digestion rates could help to provide evidence supporting this explanation.

To locate and capture hellbenders, researchers employ several methods. These are electroshocking (AC or DC,) turning rocks, visual searches by wading, trapping, and diving/snorkeling. Williams *et al*. (1981) reported that electroshocking was by far the most effective means of capturing hellbenders. The hellbenders captured by this method experienced no observable ill effects, but the effects on larvae and eggs were unknown. Bothner and Gottleib (1991) and Pfingsten (1990) conversely experienced poor results with electroshocking and found that wading and turning rocks was much more productive. Nickerson and Krysko (2003) reported that skin diving (a method Williams did not attempt) is far more effective than electroshocking. Nevertheless, they felt that the environment should dictate the method including substrate type. Turning rocks would only work if there were rocks small enough to turn. Nickerson and Krysko also discouraged electroshocking for hellbenders. They felt that the risks to juveniles and unlaid eggs were far too high. This was based on studies by Cho *et al*. (2002) working with Chinook Salmon. These researchers found that electroshocking for Chinook Salmon increased egg mortality and led to higher instances of spinal aberrations. See Pauley *et al.* (2003) for a more thorough discussion of techniques and advantages/disadvantages of search methods.

While searching for hellbenders, it should be noted that a hellbender's daily routine changes with the season and time of day. Noeske and Nickerson (1979) found that hellbenders are much more active at night than during the day. Humphries and Pauley (2000) found that the nocturnal activity of hellbenders is dictated by season in West Virginia. They noticed that during the spring and fall, hellbenders are much more abundant than during the summer and winter. Others have also noted this aspect throughout the hellbender's range. It should be noted that only adults were used in these studies. Very little is known about the activity cycles of larvae. They may avoid adults by adopting a more diurnal lifestyle. Alternatively, they may reduce their activity levels during the spring and fall. This lack of knowledge about larvae could be part of the reason why researchers find so few of them. Trying new search techniques could provide insight into the natural history of larvae hellbenders.

Humphries and Pauley noted that hellbenders appear to have a very small range. Several other studies have supported this conclusion. Humphries and Pauley reported a home range of <100 m on average while Peterson (1987) recorded no net movement (upstream or downstream) at all. Nickerson and Mays (1973a) reported a range of modally c. 900 m. Peterson and Wilkinson (1996) found home ranges to vary for both individuals and sexes. Males recorded both greater overall and greater mean home ranges (up to 211 m^2.) Both sexes showed a similar distribution of home ranges, as small as 0 m^2. These individuals were caught under the same rock every time the researchers turned it during the study.

This, coupled with the findings of other researchers that hellbenders have low genetic variation makes it seem that hellbenders recently experienced a genetic bottleneck. Merkle *et al.* (1977) examined 24 genetic loci and found almost all to be monomorphic, which illustrates low genetic diversity. Routman's (1994) mtDNA research, though, found high (.865) between-population variation and below average within-population variation. These findings do not contradict each other, but instead suggest only that the molecular sites studied by Routman evolve at a faster rate than the sites studied by Merkle. Shaffer and Breden (1989,) though, reported that this low variation is expected considering that non-transforming salamanders usually have significantly lower genetic variation than transforming ones. They concluded that this could be a reason for increased extinction rates and lower species abundance of non-transforming urodels.

CHAPTER II

Materials and Methods

1. Study Sites

Thirty-two sites were visited during this study (Table 1, Fig. 6.) The search method(s) used at each site was determined by stream morphology. The sites were chosen based on likelihood of finding hellbenders. Most streams contained clean water that was suitable habitat for hellbenders. Many had been (or were currently) severely impacted by acid mine drainage (AMD), sewage and chemical dumping, and higher than normal silt loads. Another common occurrence was the cutting of trees along the banks. While this has mostly indirect affects on hellbenders, the increased light warms the water, decreases bank stability, and leads to erosion and increased sediment loads.

2. Field Specimen Collection

Three different search methods were employed during the collection portion of this study. These were done both during the day and at night. Search times per site varied, but usually 4 search-hours elapsed at each site. Once specimens were captured, measurements and data collection techniques were utilized. All hellbenders captured were immediately released after examination in what appeared to be excellent condition to the spot of capture. No injuries or mortalities occurred to any specimens.

Wading- Dawning waders, searches were done by wading through the shallower sections of stream (less than 2 feet deep.) During the day, a net and log peavey were carried and rocks were flipped either by hand or if necessary using the log peavey. If a rock was flipped, a net was first placed downstream. The rock was then rotated up perpendicular to the current. Specimens seen were manipulated into the net and brought to shore for analysis. Rocks were returned to their original position. At night, only a flashlight and dip net were used since rocks were usually not flipped. Instead, interest was focused on sighting specimens foraging out from under cover objects. When sighted, hellbenders were also manipulated into the net and brought to shore for analysis.

Snorkeling/SCUBA- In deeper pools, SCUBA or snorkeling equipment was employed. SCUBA equipment was avoided unless the pools were over 8 feet deep. In shallower pools during the day, the equipment consisted of a lobster bag, a Princeton Tec dive light, an H_20 Odyssey mask, Ocean Master dry snorkel, and U.S. Diver fins, Orca Purge booties, and an Akona 6.5 mm Farmer John wetsuit. In deeper pools, the same equipment listed above was combined with a Sherwood Avid buoyancy compensator, a Dacor Viper Gold regulator and octopus, and a Sherwood aluminum 80 cubic foot tank. A dive flag was always placed within 50 feet of the divers. All sites were dove during the day first to more safely reconnoiter the stream for strainers, undercut rocks, and other dangers. During snorkeling/SCUBA searches, researchers attempted to located active foraging specimens out from under cover objects. Flipping rocks was initially also attempted, but this method was discarded due to its difficulty. If a hellbender was spotted, the specimen was manipulated into the lobster bag. The most successful method for this was to get close enough to cause the hellbender to flee. Usually, when this happens, the hellbenders would swim in the opposite direction of the perceived threat for a few meters then glide to the bottom. During descent to the bottom was the best time to catch them by placing the lobster bag underneath them. This method was mainly utilized at night, but day searches were also attempted.

Electroshocking- Electroschocking was attempted at several rivers as part of a Fish Biology class. While fish were the target species, participants also watched for hellbenders. This method employed several people and was at times risky due to fast currents and unstable substrates. Shocking was done with a backpack DC shocker. One to 2 people helped provide stability for the shocker depending on water currents. A seine was set up downstream of the shocker. The shocker then proceeded to shock towards the seine. Two to 3 people with nets were simultaneously scooping any aquatic vertebrates that were stunned by the current.

Data collection- Once a specimen was captured, it was placed into a shoebox-sized plastic contained filled with stream water (the water was changed every few minutes during data collection.) The specimen was then checked for stomach contents by gently easing a glycerine-coated rubber tube down its throat into its stomach (larvae were not checked for stomach contents.) A 60-mL syringe then slowly injected river water into the stomach. Excess water flowed back up the throat exiting from the mouth. Normally, two full syringes were injected. Specimens usually elicited a vomiting reflex before the process was complete. All particulate matter was then collected from the water by a strainer and placed into a vile containing 70 % ethanol. Measurements were then taken. Mass was determined by placing the specimen in a tarred plastic zip loc bag and connecting it to either a 10g, 100g, or 600g Pesola scale. Total length and snout-vent length were measured by placing the specimen in a 3” PVC pipe that had been cut in half and had a measuring tape affixed to the center. The specimen was slid on the bottom until their snout was at the end for total length, or till the anterior part of the cloaca was at the end for snout-vent length. Head width was measured with a pair of Pro-Max digital calipers. This measurement was taken at the widest portion of the head without displacing tissue. TG was measured by wrapping a string around the abdomen 25 % of the way posterior from the front legs to the rear legs. The string length was then measured with a tape measure. Any unusual abnormalities, coloration, or injuries were noted. Once complete, the specimen was returned to the site of capture by either releasing them where it was foraging or persuading them back under the rock from which it was captured. Notes on stream declivity, weather, and air/water temperature were then taken. Coordinated were determined using a Magellan Meridian handheld GPS and recorded in NAD83 format.

3. Lab Specimen Analysis

For the dimorphism part of the study, only preserved specimens were used. These came from both the West Virginia Biological Survey Museum and Carnegie Museum of Natural History collection. Specimens were collected from Pennsylvania (n=63), Virginia (n=28), and West Virginia (n=18). These ranged from less than 5-years old to over 100-years old. Preservation methods for specimens were not known but were considered varied. All specimens were in 70% ethyl alcohol at the time of measurement. Initially, 15 measurements were taken on the 16 WV specimens. These measurements included common field measurements (mass, head width, total length, snout-vent length, and thoracic girth) as well as other morphological measurements commonly taken during salamander dimorphism studies. These included head height, eye-to-eye distance, eye-to-nostril distance, eye-to-cross-nostril distance, nostril to nostril distance, tail height, first toe length, third toe length, and axilla to groin length. For the remainder of the specimens, only common field measurements were taken.

Mass was determined by placing the specimen onto an Ohaus digital 1-kg scale. For specimens too large, a 5-kg triple beam balance was utilized. Head width was measured with a pair of Pro-Max digital calipers. This measurement was taken at the widest portion of the head without displacing tissue. Thoracic girth was measured by wrapping a string around the abdomen 25 % of the way posterior from the front legs to the rear legs. The string length was then measured with a tape measure. Total length and snout-vent length were also measured with a string. By placing the string along the middle of the specimen, accurate measurements for both total length and snout-vent length could be obtained despite specimen contortion. Head height was attained by measuring the greatest distance from top of head to bottom of jaw with calipers. For eye-to-eye, eye-to-nostril, eye-to-cross-nostril, and nostril-to-nostril measurements the calipers measured the least distance between each body part. For eye-to-cross-nostril, the distance from the left eye to the right nostril was measured. Tail height was measured by pressing the tail against a ruler and unfolding the keel so that maximum tail height was attained. The first toe and third toe measurements were done from the end of the toe to the approximate knuckle. These measurements were done on the rear foot on whichever toes were the longest. Axilla to groin was measured with a ruler from the back of the front leg to the front of the rear leg. If the specimen was curled due to preservation, this measurement was taken on the outer side after straightening of the specimen was attempted. Each measurement was taken twice and the mean of the measurements was taken. One-hundred and five specimens were measured for this study. Four specimens were not used due to inability to confirm gender. Specimens included were at least 50 grams since smaller specimens were not sexually developed enough to guarantee gender identification. Nine specimens captured in Virginia from June 6-8, 1988 included live measurements that were taken before preservation. These measurements were compared with current measurements to determine what affects, if any, preservation had on morphology. These specimens were all captured and preserved synchronously in an unknown manner, so no comparison between other techniques or time elapsed since preservation could be made.

4. Data Analysis

Before data was analyzed for significant differences between males and females, the cube root of mass was substituted for mass. This was done since mass is considered a 3-dimensional measurement while the other measurements are 1-dimensional. Since not all ratios or measurements passed normality tests, the data was transformed using both log and arcsin transformations (Sokal and Rohlf, 1969.) All ratios, standard measurements, and LN measurements between males and females were then checked for significance using t-tests or appropriate non-parametric tests in Sigma Stat. Regression analyses were also run to determine if either gender was more variable morphologically. Two multivariate techniques were applied, principal component analysis (PCA) and discriminate analysis. Discriminate analysis is a more powerful test which weighs characters based on their maximum ability to separate groups. If this does not produce a difference, there is a strong likelihood there are no morphological dimorphisms. This would support accepting the null hypothesis that no dimorphism exists. On the other hand, PCA is more conservative in that it separates the data without assignment into a particular group (gender in this case.) If there is an obvious clustering of males and females into disparate groups, than this is a powerful indicator of dimorphism which is not biased by group assignment. This would support rejecting the null hypothesis. Different combinations of 3 ratios (mass/total length, head width / snout-vent length, & thoracic girth / total length) were then entered into SAS and PCA and discriminate analysis were run. These ratios entered into SAS for PCA were decided upon using step-wise comparisons in discriminate function analysis.

CHAPTER III

Results

1. Field Studies

The results from stream searches are shown in Table 1. Hellbenders were found in only three streams: the Cranberry (Fig. 1), the Holly, and the Elk Rivers. This is probably due to the high stream flow rates West Virginia streams recorded in 2003 (Table 2.) For example, where the Greenbrier was searched flow rates were 1.72 times the normal recorded mean. Only one adult was found, this being in the Cranberry River. Two larvae were found, one in the Cranberry and the other in the Elk River. Both larvae were found in the middle of the river under rocks large enough for sub-adults to hide under. An egg string was found in 2002 on the Holly River and consisted of 12 eggs in a single string. These were found entangled in some rocks in the river during a search. They were not part of a nest but instead a stray strand that had probably floated away from a nest. Since no male was present to protect them, they were brought back to the lab to attempt captive hatching. The eggs developed mold within 5 days and were then preserved and deposited in the Marshall University Herpetology Museum.

Searches in shallow shoals and lower-order tributaries yielded no hellbenders, although two-lined salamanders (both *Eurycea bislineata* and *E. cirrigera*) and species in the *Desmognathus* genus, i.e. *D. fuscus* and *D. monticola*, were encountered frequently. These searches occurred where historical records of hellbenders or good habitat for hellbenders exist. One researcher would spend about 10 minutes per lower-order tributary flipping any rocks or debris that a larva could fit under. These searches were only done during the day.

The only successful methods to locate hellbenders were SCUBA diving and turning rocks while wading (Table 1.) SBUBA diving was successful at night, but not during the day. The individual captured was found at about 11:00 p.m. in the open but not moving. It was at the deepest section of river in a hole about 3 m deep. It remained this way for several minutes until it was disturbed.

On average, each dive took about 2 hours to complete, including set-up. No other amphibians were seen during these searches, but several crayfish and fish (most commonly Rock Bass, *Ambloplites rupestris*, Brown Trout, *Salmo trutta*, and Brook Trout, *Salvelinus fontinalis*) were encountered. Rock flipping was done usually for 2 man hours per site during the day. Numerous small insects, crayfish, fish, and salamanders (*E. cirrigera*, *D. monticola*, *D. fuscus*) were frequently capturd. The other methods, including electro-shocking and wading searches were unsuccessful. Electro-shocking trips were labor intensive, requiring 7-8 people working together. The only amphibians captured were an American Bullfrog (*Rana catesbeiana*) and a Southern Two-lined Salamander (*E. cirrigera*.) Crayfish and fish were also caught frequently. Sites were usually shocked for 30-60 minutes. Wading searches were conducted for about 3 hours per site and produced no findings.

2. Lab Studies

Initially, 15 measurements were taken on WV specimens. These consisted of 6 common field measurements and 9 measurements commonly taken during dimorphism studies of salamanders. Since no significant differences were found between genders for the 9 measurements in West Virginia specimens (Table 4,) these were ignored and not taken for the remainder of the specimens. The other 5 measurements taken for all specimens as displayed in Table 3. Specimens dissected showed distinct differences between sexes based on raw measurements (Head width, Thoracic girth, total length, snout-vent length, and mass) but little separation based on ratios (with either snout-vent length or total length in the denominator, Table 5.) For each measurement, i.e. thoracic girth, males were commonly smaller than females (Figs. 7-10.) The only ratio (Table 6) that showed a significant difference between males and females was torso girth/total length (P=0.048, 0.046 for log transformed data.) Figures 11 through 26 display how males and females compared for each ratio, both standard and log transformed. Consistently, males showed lower mean ratio values than females (Table 5.) Two of these ratios, snout-vent length /total length and tail length/ total length failed normality and were excluded from the rests of the tests. Arcsin and log transformations were unable to normalize the data. Canonical scores for males and females are displayed in figures 27 and 28.

Principal components analysis yielded no separation between males and females for any of the components (Figs. 29 and 30.) Eigenvector values for each component are displayed in table 6.

When the pre-preservation measurements of the 9 specimens were compared to my measurements, results demonstrate that specimens consistently shrank in snout-vent length, total length, and mass (Table 8.) Other measurements, such as head width and thoracic girth, were not compared since they were not recorded before preservation.

CHAPTER IV

Discussion

1. Field Studies

Due to record rains during 2003 (Table 2,) field searches were very unyielding. This is discussed more thoroughly in the third section of the discussion. Therefore, hellbender populations probably occur in many of the rivers where none was found. Of the three specimens that were captured, only one of them was large enough to stomach flush. This animal had the vertebral remains of a fish inside its stomach. Larvae were not flushed due to their size and fragility.

It is noteworthy that hellbender larvae were found. When Humphries (2000) searched in 1998, he captured 75 specimens, none of which was a larva. The minor success this study exhibited for finding larvae was probably due more to method than anything else. Humphries mainly used nocturnal searches while wading. This method would obviously make it difficult for anyone to see a larval hellbender. Also, a possible selective strategy for larvae would be to hide when adults are active. This would make it even less likely for a researcher to find larvae utilizing this method.

Larvae were captured during this study by utilizing 2 different methods. The first larva was found accidentally during mussel searches utilizing SCUBA. The researchers participating in the study were thoroughly examining the substrate and beneath cover objects for mussels, when the larval hellbender was found under a rock. The second larva was found by turning rocks sideways in relatively strong current with a net placed downstream. In this situation, the hellbender was not seen until the net was withdrawn from the water and searched. While adults can usually fight stream currents, larvae often succumb until they reach calmer water. Therefore, it seems that based on larva morphology the best methods for finding larvae are based on water velocity. If water flow is slow, SCUBA searches are likely to be productive. In quicker flowing water, flipping rocks and letting the current sweep hellbenders into the net is probably the most productive option. Based on the limited number of larvae found so far (see also Makowsky, 2001,) it appears that larvae utilize the same habitat as adults. Both larvae captured during this study were under rocks large enough to house an adult.

Most search hours were spent turning rocks during the day. This method was attempted from May through October. While this method yielded 1 larva, none adult was seen. While this task is often labor-intensive and difficult, many researchers have reported good success using this method. Once again, high water levels made these searches very difficult and were probably the main reason why no adults were seen.

Even though no adults were observed, eggs were found during a day search. Twelve eggs were found on the Holly River at 11:00 a.m. on 23 September, 2002. Apparently, they had either separated from a larger string or were accidentally released. Whether or not they were fertilized could not be ascertained, but they were not found under a rock. Instead, they were found floating at the surface.

Searches focusing on lower-order tributaries yielded no hellbenders. This is surprising since habitat was often available and water levels would have made them more favorable. Tributaries were only searched during the day, though, so it could be that larvae utilize these tributaries at night to avoid active adults and/or locate prey.

The electro-shocker was used by multiple researchers for ~ 15 hours in good hellbender habitat as part of a fish biology class in the fall of 2003. Fish and many other aquatic vertebrates were stunned and captured in the seines downstream such as crayfish, insects, and sometimes Southern Two-lined Salamanders (*Eurycea cirrigera*.) While other authors have reported great success for capturing hellbenders using an electro-shocker, this method did not yield specimens. Salamanders captured included several Southern Two-lined Salamanders, *Eurycea cirrigera*. And like the other non-fish captures, these were set free with no observable ill-effects. So, even though hellbenders were not actually being searched for, habitats were searched that could have supported a population. Whether populations were not present, not affected by the electro-current (the water usually had few dissolved ions and was a poor conductor), or stunned specimens simply did not flow into the seine is undeterminable. It does seem, though, that shocking is not a very productive method in West Virginia, at least during high discharge. Why this is different from other results is probably to the differences in environment. The study done by Williams et al. (1981) was in Pennsylvania 23 years previous to this study during the spring. So, it could be that the differences between streams and possibly seasonal activity accounted for the disparate results.

SCUBA searches were done in several rivers (Table 1) during both day and night from June through September. These were often more labor intensive then the other search methods and took several hours of preparation and clean-up. During day searches, very few vertebrates were seen and no amphibians were seen at all. Night searches yielded far more life, including fish, crayfish, and other aquatic insects. No other salamanders were seen during these searches. Several searches had to be stopped due unsafe search conditions caused by high stream discharge. The one adult that was found was observed sitting on the bottom out in the open of a deep pool (3m) in the Cranberry

River at 10:00 p.m. It sat still for several minutes and did not move until it was disturbed. Once disturbed, the hellbender swam over the bottom about 1 m and coasted down to the bottom. This happened several times before it was captured in a net. The specimen was very alert, much more alert than hellbenders are during the day (based on past searches, Makowsky 2001) and fled before it could be restrained each time. This technique would probably be much more affective in "normal" conditions when flow rates are down and there is greater visibility. It is also probably the best way to observe specimens without disturbing them in the wild.

Another method, though not attempted, is the use of traps. Several researchers have mentioned using them, but nothing has been published reporting their efficiency compared with other methods. Benefits are that they can be used in any water condition (besides high flow) and require little effort. There are two main drawbacks to using traps. First, they require either an expensive investment or time and materials to build. Second, they may allow predators, such as snapping turtles, to enter and attack any other animals captured. Concurrently, the traps must be checked regularly to ensure that trapped turtles do not suffocate. For bait, many researchers have reported using native trout that are cut up into quarters.

Based on the inability to find hellbenders during this study, it seems that the methods used are not effective when stream discharge is high. In the future, search attempts should take this as well as two other aspects into: what life stage is being sought and in what type of stream is one working. The best options for larvae have already been described, so adults will be the focus here. While Nickerson reports that diving can be very productive, it is only the case in moderate to slow currents. Fast currents, very shallow water levels, and cold temperatures all make diving impractical. But, it is the only method to sample deep holes (> 4m deep.) It can be done during day or night, but turning large rocks while diving can be very awkward and dangerous. Wading and turning rocks has the advantage that no special skills (like SCUBA certification) are needed and that it can be done year around with the proper equipment. Even during the coldest months, rivers can be sampled in a pair of chest waders and arm-length gloves. Based on past searches, it seems that wading should be coupled with rock turning during the day but not at night. Therefore, nocturnal searches can not only be productive (Humphries & Pauley, 2000), but do not require the effort or disturbance to habitat that rock turning does. During high discharge, though, these methods are not very productive. Trapping, while not attempted here, could be useful in such conditions. Overall, there is no one good way to always search for hellbenders (Table 8.)

2. Lab Studies

To determine if dimorphism exists, comparison of 15 measurements was initially proposed. This included 6 measurements commonly taken in the field (mass, total length, tail length, snout-vent length, thoracic girth, and head width) and 9 other measurements commonly taken during dimorphism studies. Since most of the other measurements seemed difficult to obtain on live specimens without anesthetizing them, their usefulness in determining gender was tested using only the WV specimens. None of the measurements showed significance using t-tests (Table 4,) so they were not taken for the remainder of the specimens since this study was designed to be field applicable.

Log and arcsin transformations yielded the same results, but only log transformed data is presented. Log transformed ratios were included to show these results but they will not be distinguished from standard ratios for the remainder of the paper since they support the same conclusions.

The 105 specimens used in this study showed significant dimorphism between sexes. In the sample analyzed, mean male size (head width, total length, snout-vent length, thoracic girth) was smaller than female (Table 5). These data, while significant for 3 (Table 6) of the measurements, were not suitable for any definite separation of sexes. There was far too much overlap plus this separation was most likely due to the lack of small females in the sample. Obviously, this is not because small females do not exist, but that they were under-represented in the sample. For instance, Fig. 8 compares the snout-vent length's of males and females. The graph accurately shows that no females in the sample recorded lengths of under 28 cm. One possible explanation for this is that females utilize habitats that are not commonly searched by researchers until they reach around 28 cm. Also, while the mean of males in the sample were consistently smaller than of females, the overlap of size was by far too great for snout-vent length to be useful to determine gender (Figs. 7-10).

Analysis of total length's for all specimens did not support the conclusion that either gender reaches an overall larger size. Instead, it appears that males and females reach a similar overall size (Fig. 7.) Although, while no dimorphism can be observed concerning overall size attained with the specimens studied, that is not to say that in the wild this does not occur. In this study, the largest specimen was a male with a total length of 58 cm, 20 cm shortest than the largest confirmed record. The second largest specimen was a female 56 cm in total length. Therefore, dimorphism may exist but only at larger sizes. For these reasons, ratios were examined to determine if any dimorphism was present in specimens of the same length.

When ratios for males and females were compared, with the exception of head width/snout-vent length, males recorded a lower average value than females. The only ratio that was found to differ significantly between genders (Table 6) was thoracic girth/total length. Even in this case, overlap between sexes (Fig. 19) was too great to accurately determine gender. Therefore, principal component analysis (PCA) and discriminate function analysis was run in SAS to determine whether distinct morphologies for females and males could be found based on several ratios. Both tests were used here (see methods for reasoning) to determine to what extent separation could be attained. Figures 29 and 30 display the results of principal component analysis using these ratios.

Obviously, no separation of the sexes is observable using these procedures. Far too much overlap of the sexes reveals that intra-gender variation is just as large as inter-gender variation. Based on this and the ANOVA's discussed above, it seems that there is no way to positively determine the gender of specimens based solely on the measurements used in this study due to this variation. Even discriminate function analysis, which is more powerful than PCA, was unable to overcome the variation within genders and separate males from females (Figs. 27 & 28.) Also, there is no reason to infer that live specimens would exhibit a dimorphism using the same ratios even though proportions may change during preservation since there is no reason why both genders would not change in the same way (Table 8.) Therefore, although the thoracic girth/ total length ratio was found to differ significantly between males and females (95 % confidence,) the overlap between genders (and intragender variation) was too great to conclusively suggest that a discernable dimorphism exists. Maybe with a larger sample and more accurate measurement techniques separation will become more obvious.

Morphological change associated with preservation was found in all specimens that were examined (n=9.) Not only did the measurements differ, but they differed to different degrees (Table 8.) For example, while length was reduced by 5.9 %, mass was reduced by 12.6%. Plus, the only specimens that had pre-preservation measurements were all preserved at the same time 20 years ago. Males and females did shrink different amounts, but these differences were not significant. So while it is conclusive that specimens shrank, the rate at which they shrank is not determinable since no other specimens preserved for a different time period were available. It is not known either what procedure was used to preserve the specimens (i.e., fixed in formalin, ethanol, propanol, etc.) which can have an affect on morphological changes as well.

Regression analyses were also run on all measurements for both genders (Fig.31) together and separately to determine exactly intra- and inter-gender variation (Figs. 32-35.) These analyses correlated mass, head width, thoracic girth, and snout-vent length with total length. Snout-vent length and total length showed the strongest correlation with an R^2 value of .92. The other measurements did not correlate as strongly, but they still exhibited supportive R^2 values. When males and females were compared, males always showed a higher correlation coefficient than females. Morphologically, this means that females were more variable than males. This is especially interesting considering that males were more variable in total length than females. This might be explained for thoracic girth values since eggs might have an affect on this ratio. The affect is minimal though since the measurement was taken where eggs are not present in the thoracic cavity. For the other measurements the only explanation is that females are more variable than males. This variation is the reason why males and females can not be sexed based on measurements or ratios.

Another finding during this study concerns how specimens are sexed in the field. Most accounts for hellbenders (Nickerson and Mays, 1973; Petranka, 1998) report that males and females can be differentiated in the field by the presence or absence of a swollen cloaca (Fig’s. 2 & 3.) This is restricted to the breeding season, though, and the months just before and after. Finding during this study reveal that male hellbenders can vary greatly in the extent to which males are swollen at any one time. The 4 males pictured were all collected on 3 June, 1988 from a steam in Virginia. The top two, based on cloaca size, appears to be a female based on cloaca size. All specimens, though, turned out to be males. Therefore, it seems that males can be positively identified by the presence of a swollen cloaca, but specimens without a swollen cloaca could be either a male or female.

At this point, there seems to be no reliable way to determine gender for most specimens. Future studies might want to determine if a hormone that is preset in one gender can be easily detected in the blood. This could make field sex determination a more reliable method.

3. Problems Encountered During the Study

Two problems were encountered during these studies. These were present in both the field studies and the lab studies. The only problem with the field studies encountered unfortunately was impossible to overcome. Record high rains (Table 2) and consequently flooded streams during the 2003 field season made searching not only extremely difficult but at times dangerous. For the year, stream flow was on average 144 % higher than it normally is in the rivers data was available. Flooding resulted in high silt loads that impaired any visual searches (night searches, diving, even wading) and elevated water levels that made access to the appropriate rocks while wading very difficult. This was encountered because most of the rocks that were shallow enough to turn were usually not under the water during normal flow, but on dry terrestrial shoreline. Only on a couple of occasions were wading searches able to be conducted in the parts of rivers when hellbenders must occupy during normal stream levels.

During flooding, these otherwise terrestrial rocks apparently do not make good hiding places for hellbenders. There are 3 likely reasons that could explain this observation. It could be due to the tenet that hellbenders move little and therefore do not utilize the rocks that are normally not available to them. Or, it could be that the newly available rocks are untenable for some reason, possibly because the substrate under them that has too much silt. Finally, the hellbenders could be avoiding the strong currents that occur during raised water levels by remaining under their "normal" home rock. Whatever the reason(s), it seems that hellbenders do not commonly occur under the rocks that only high waters make available to them.

The other problem encountered occurred in the lab portion of the study and concerned the inability to take accurate measurements on preserved specimens. This was most notable when measuring head width. When trying to measure specimens, the common procedure is to tighten the measuring device enough to remove gaps but not so much as to displace any tissue. And normally, on hellbenders, the tissues present on both sides of the head (skin, muscle, etc.) do not always preserve in the same shape or does it accurately portray live tissue. Commonly, muscles contract into odd positions which make it difficult to get exact measurements. Since these irregularities of tissue were found in both males and females, they were ignored and measurements were taken as if there was no problem. This could have either made no difference in the findings or it possibly could have disguised a present dimorphism. It could be that males have on average thicker muscles than females on the sides of their heads yet this is undetectable due to changes associated with preservation.

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Appendix-I

Figures

Figure 1. Shallow rapids ideal for hellbenders on the Cranberry River.

Figure 2. Male (left) compared with female (right) reproductive structures.

Figure 3. A picture of four specimens lined up together. All four specimens were captured on the same day. While it appears that the two bottom specimens are males and the top two are females, all four specimens are male. Also notice the variation within males.

Figure 4. Picture of a female (left) and male (right) hellbender.

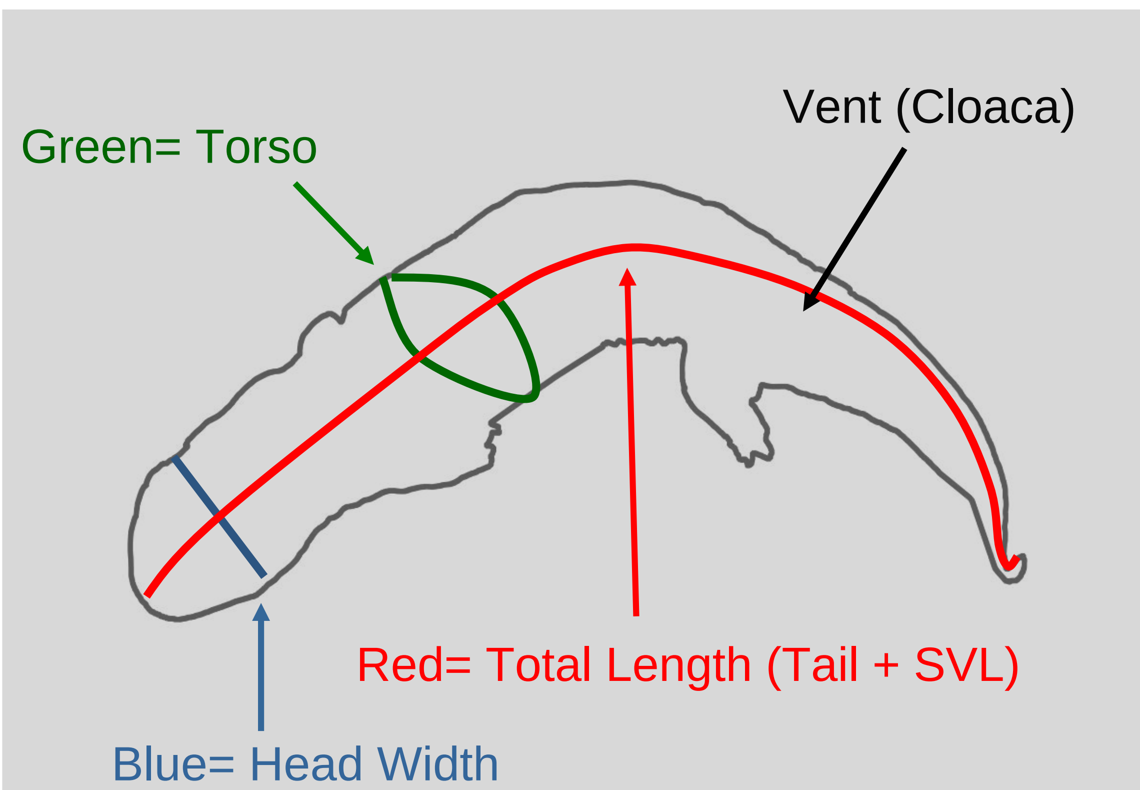

Figure 5. Measurements taken for both field analysis and lab specimen analysis.

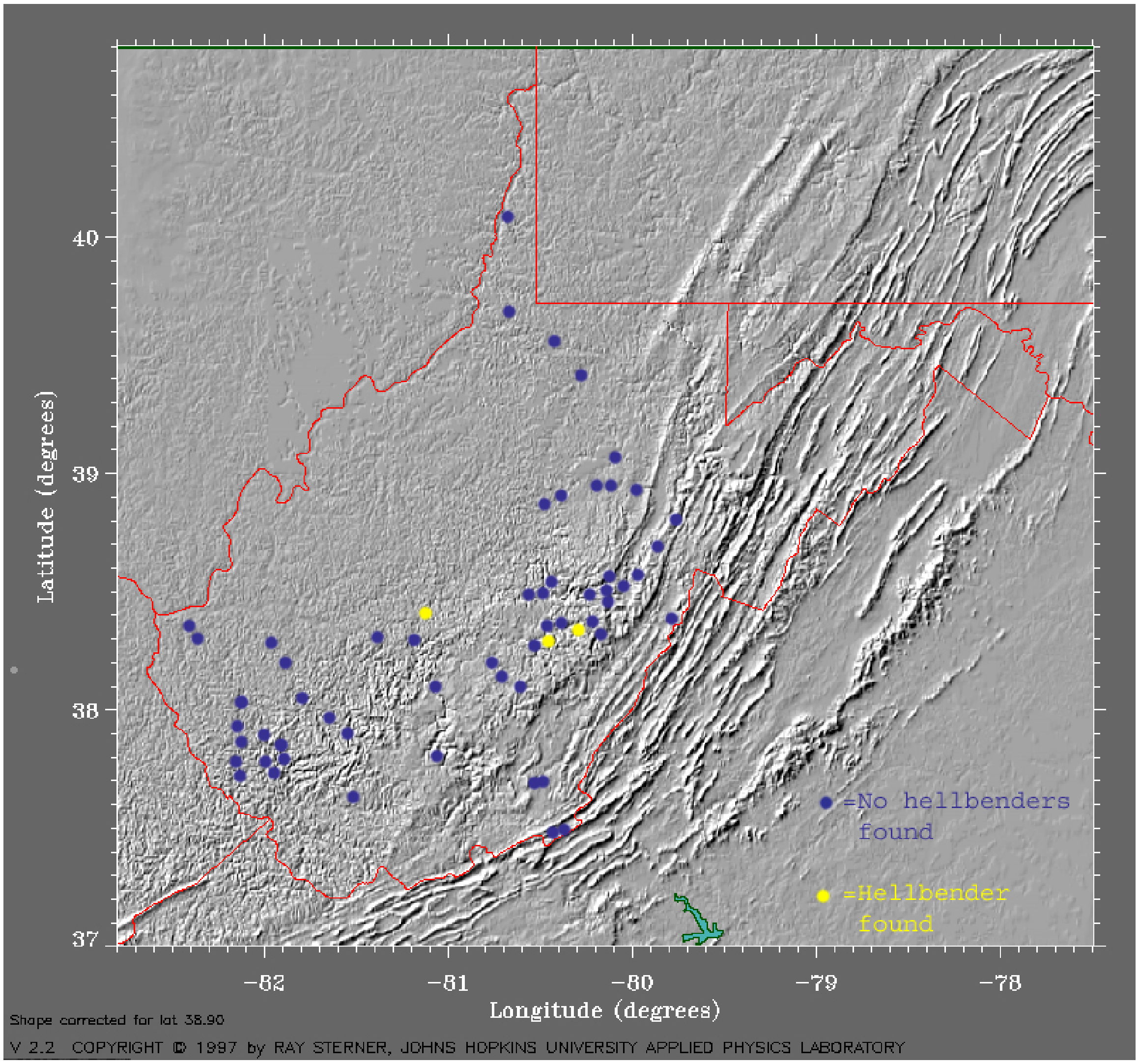

Figure 6. Map of sites visited and searched for hellbenders. Sites close to one another on the same river were represented with one dot. Some sites were visited multiple times and some had multiple search methods were employed.

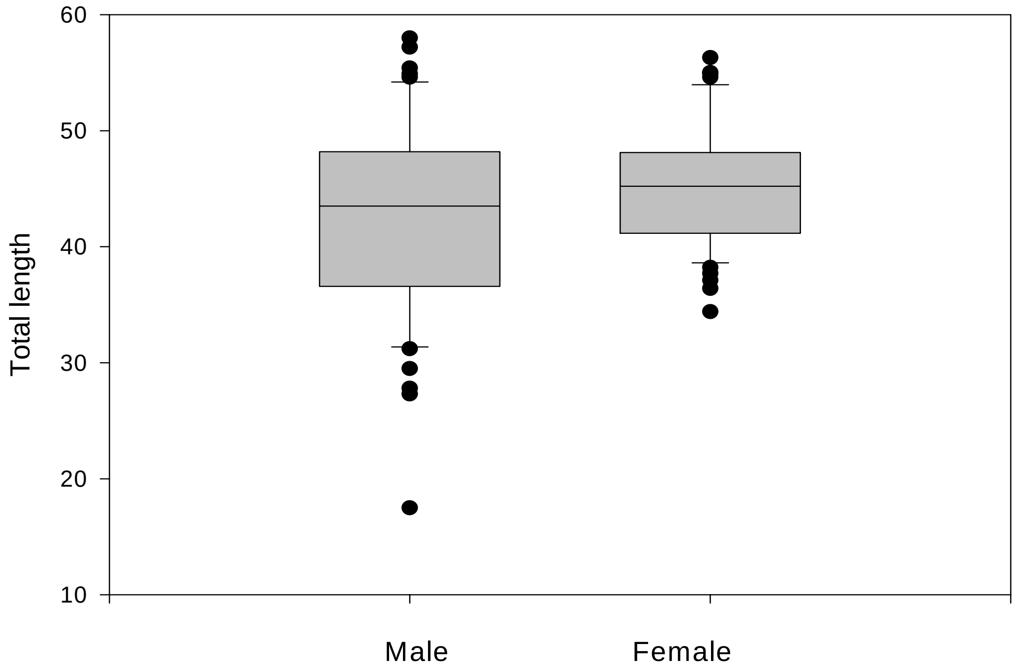

Figure 7. Comparison of total lengths of males and females.

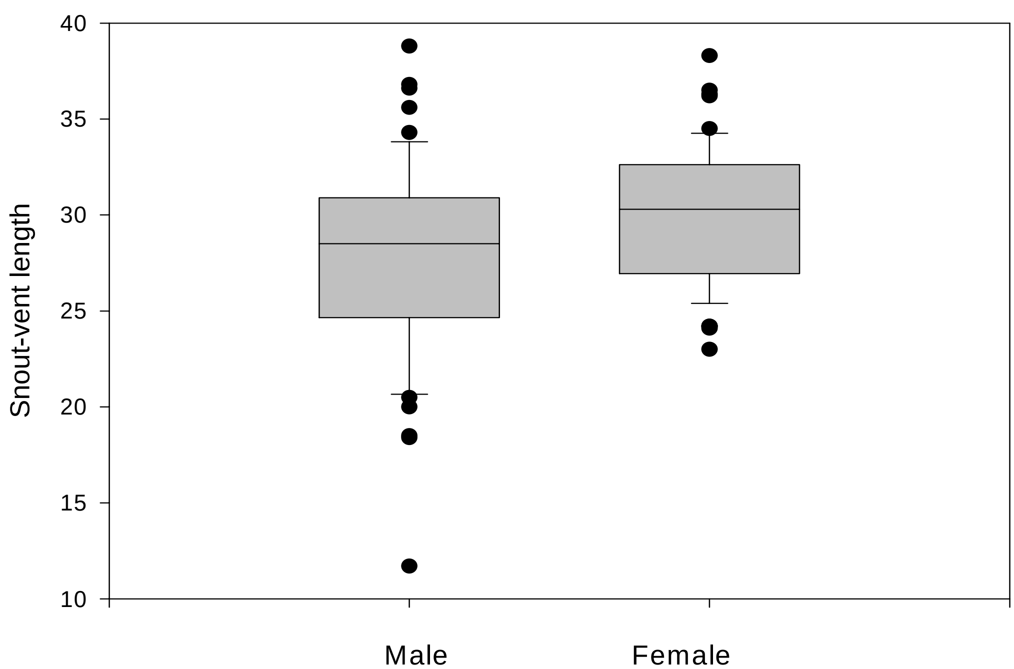

Figure 8. Comparison of snout-vent lengths of males and females.

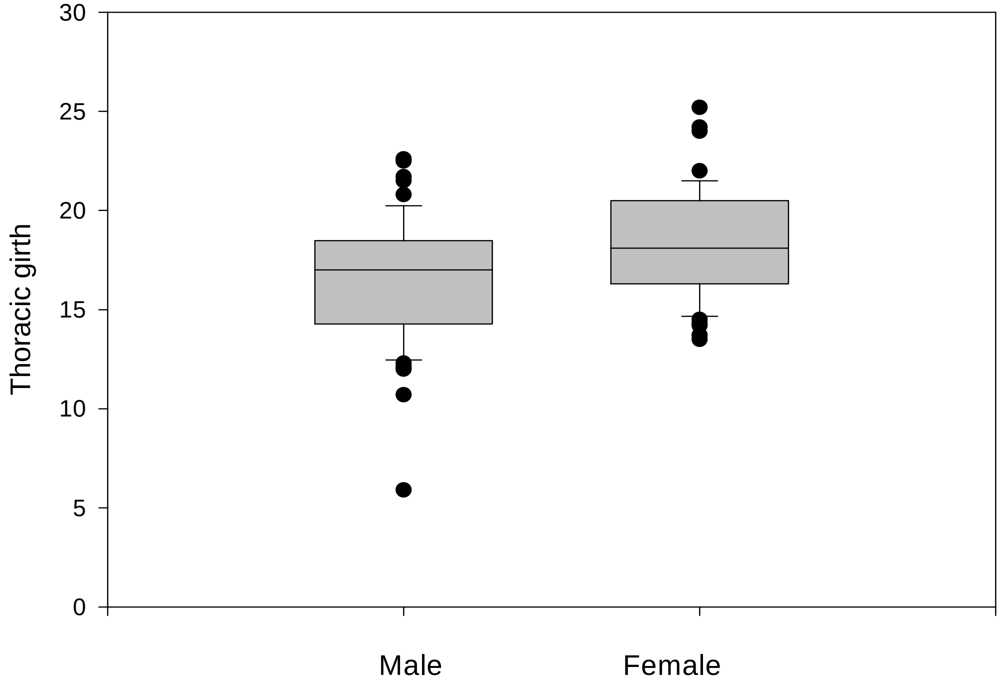

Figure 9. Comparison of thoracic girths of males and females.

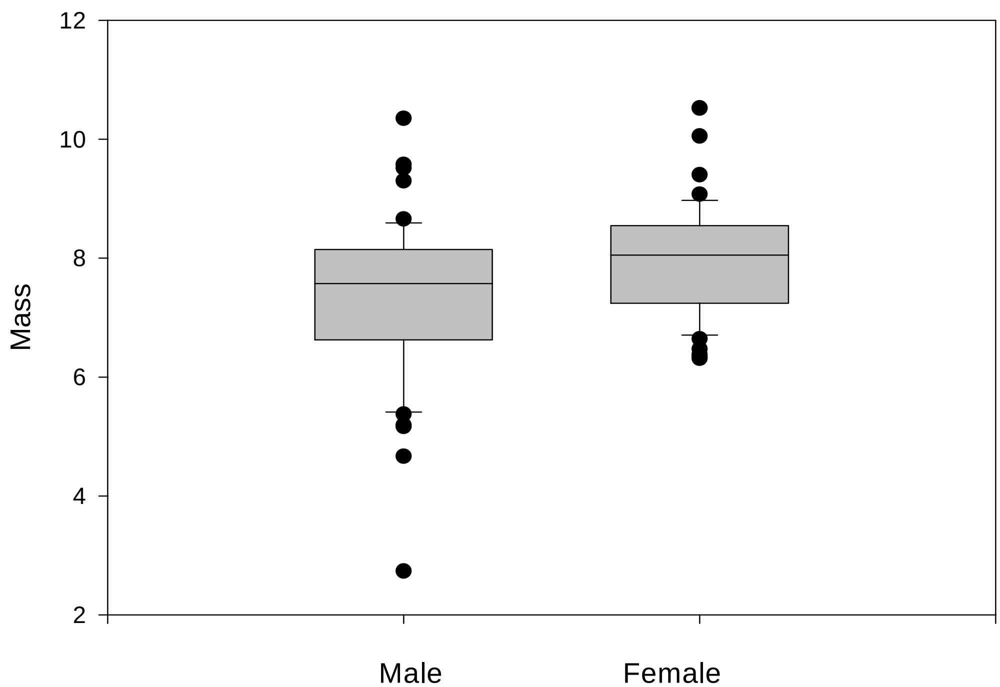

Figure 10. Comparison of masses of males and females.

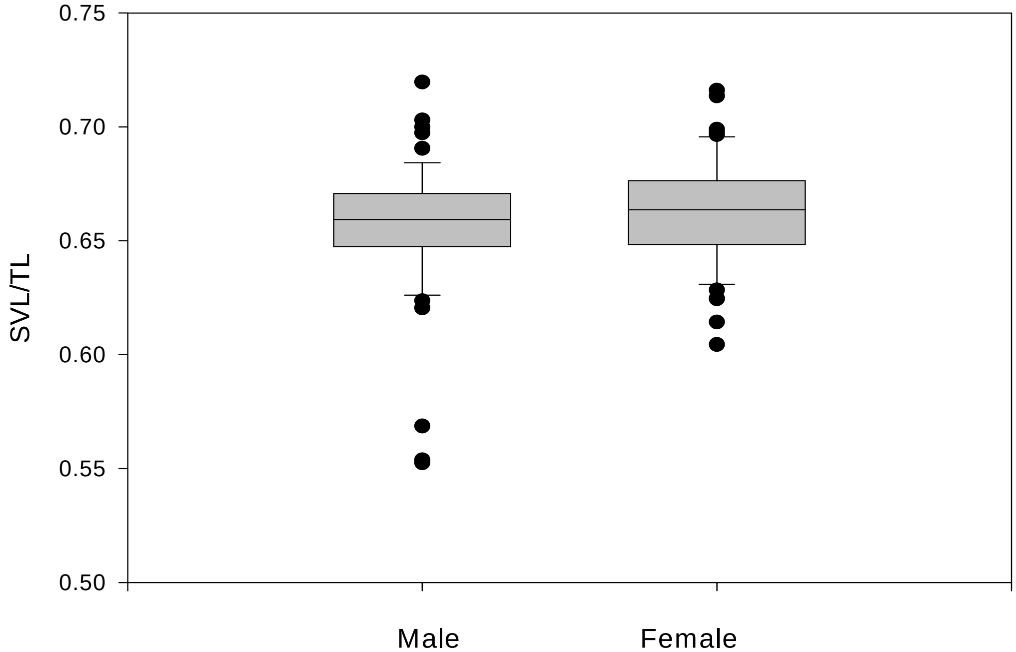

Figure 11. Snout-vent length/ total length ratios for males and females.

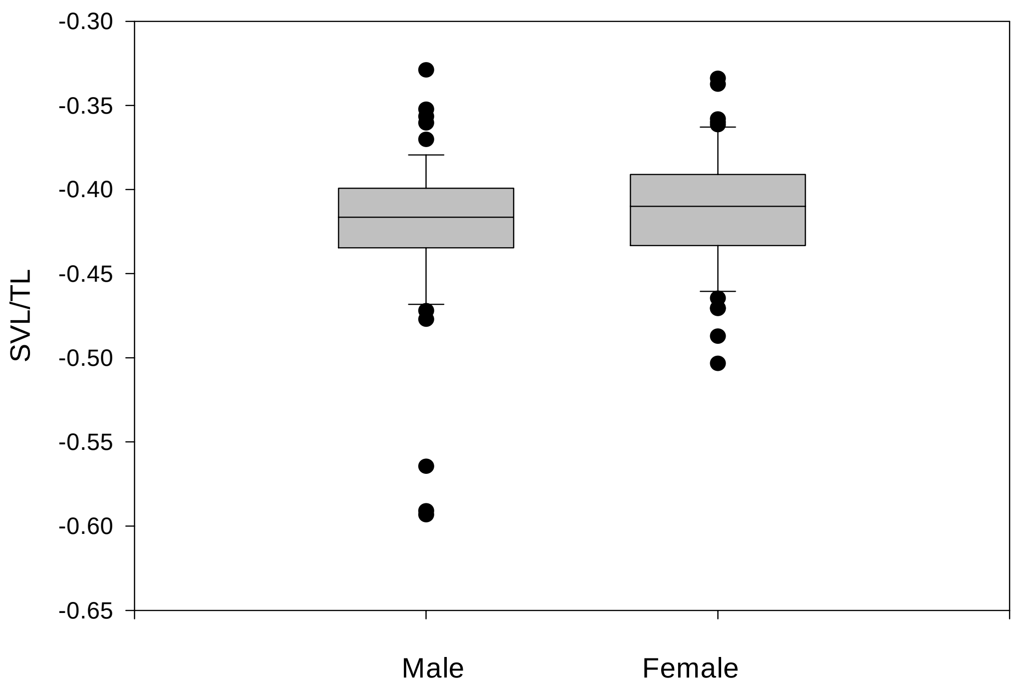

Figure 12. Snout-vent length/ total length ratios for males and females that have been log transformed.

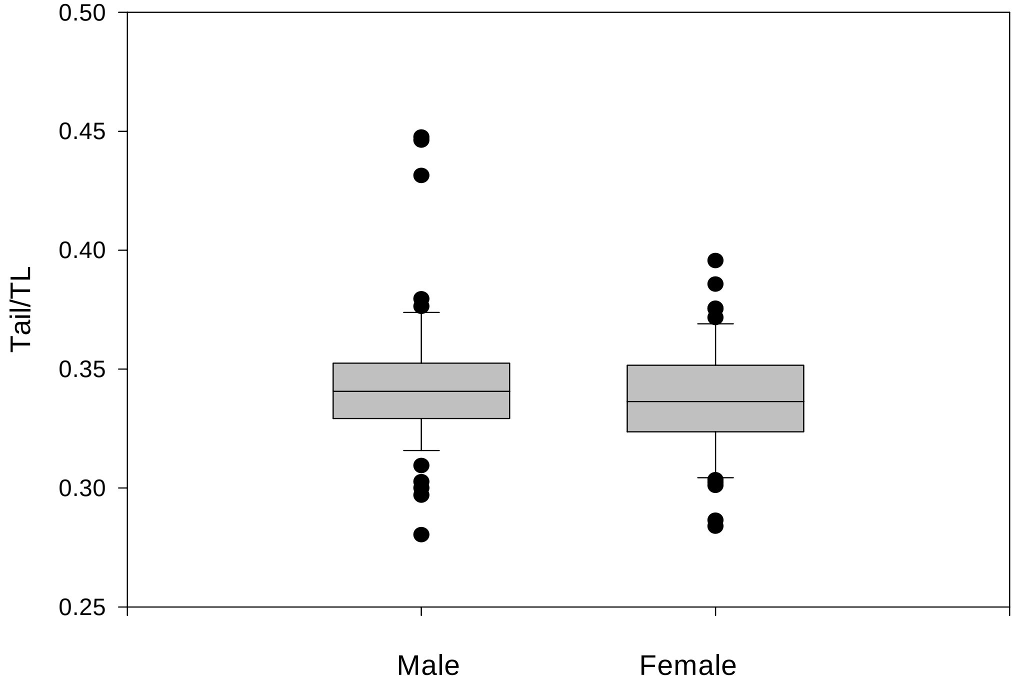

Figure 13. Tail length/ total length ratios for males and females.

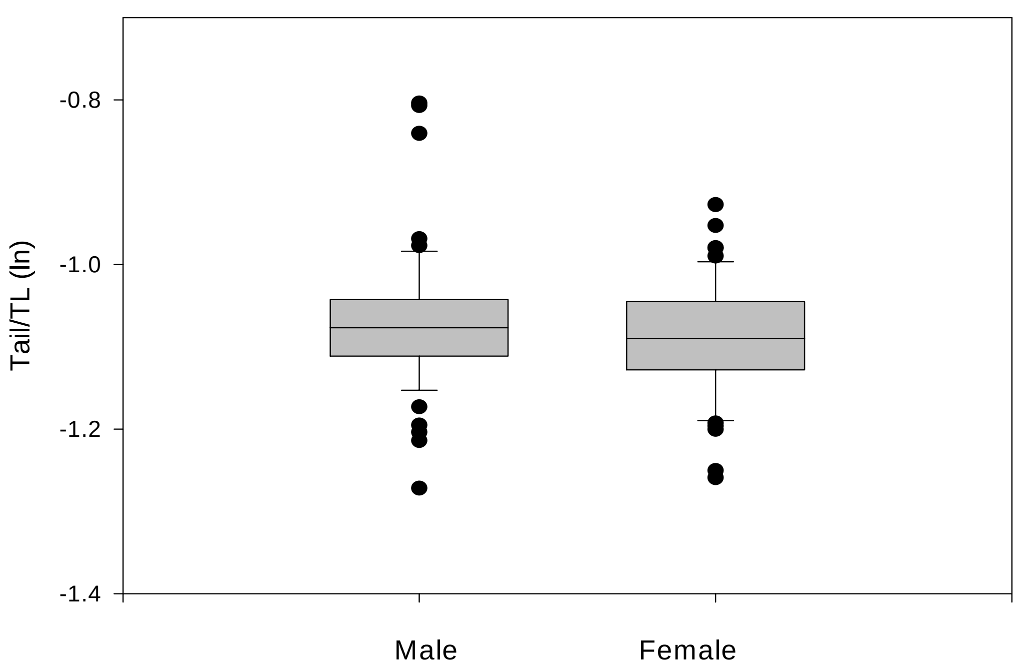

Figure 14. Tail length/ total length ratios for males and females that have been log transformed.

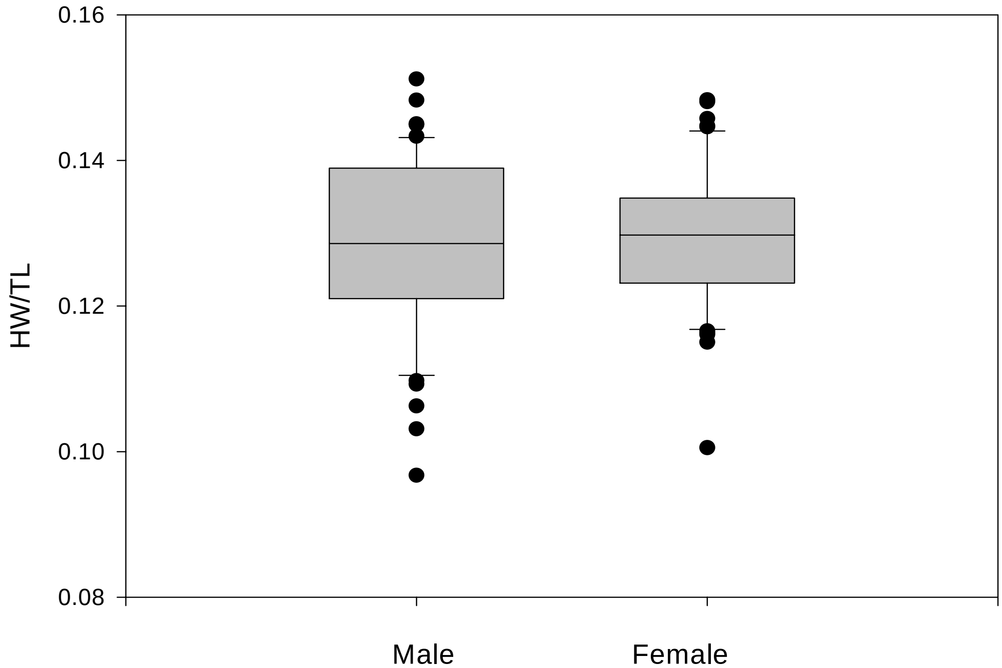

Figure 15. Head width/ total length ratios for males and females.

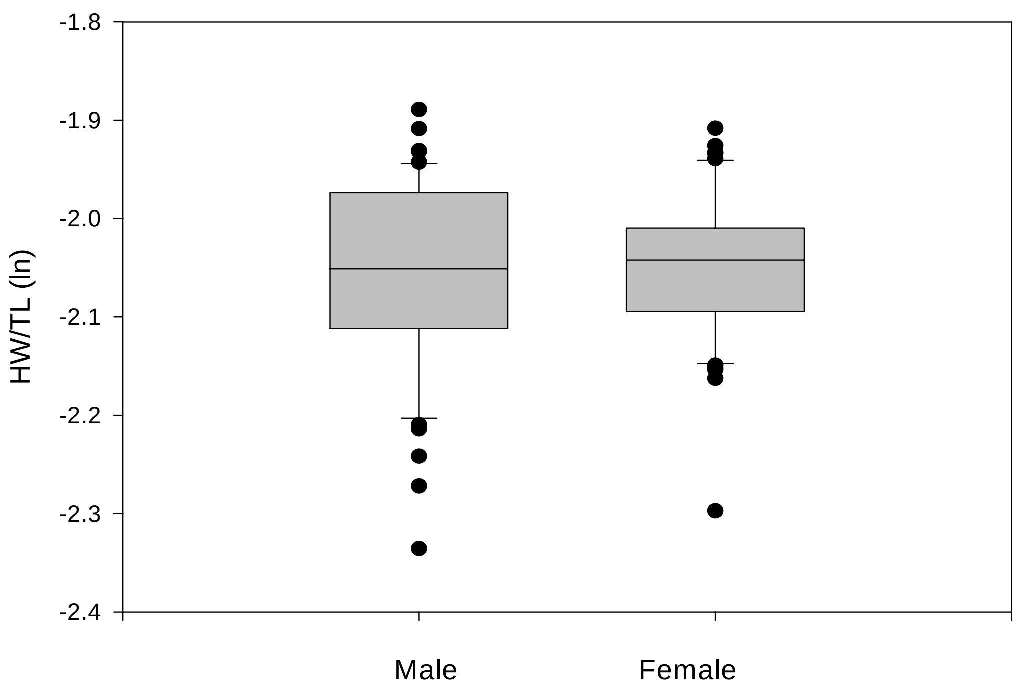

Figure 16. Head width/ total length ratios for males and females that have been log transformed.

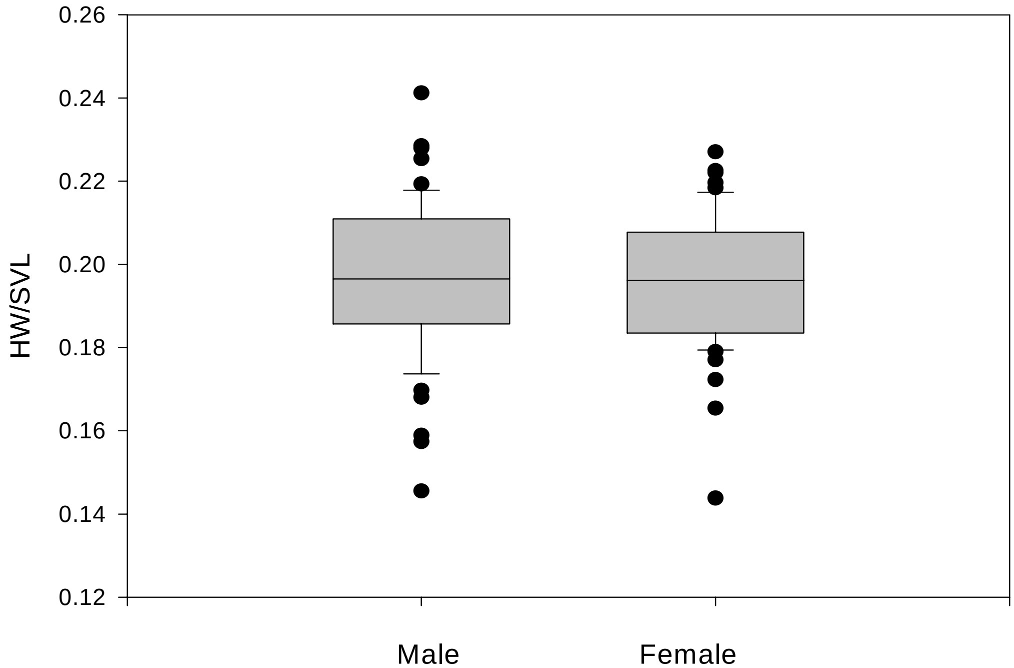

Figure 17. Head width/ snout-vent length ratios for males and females.

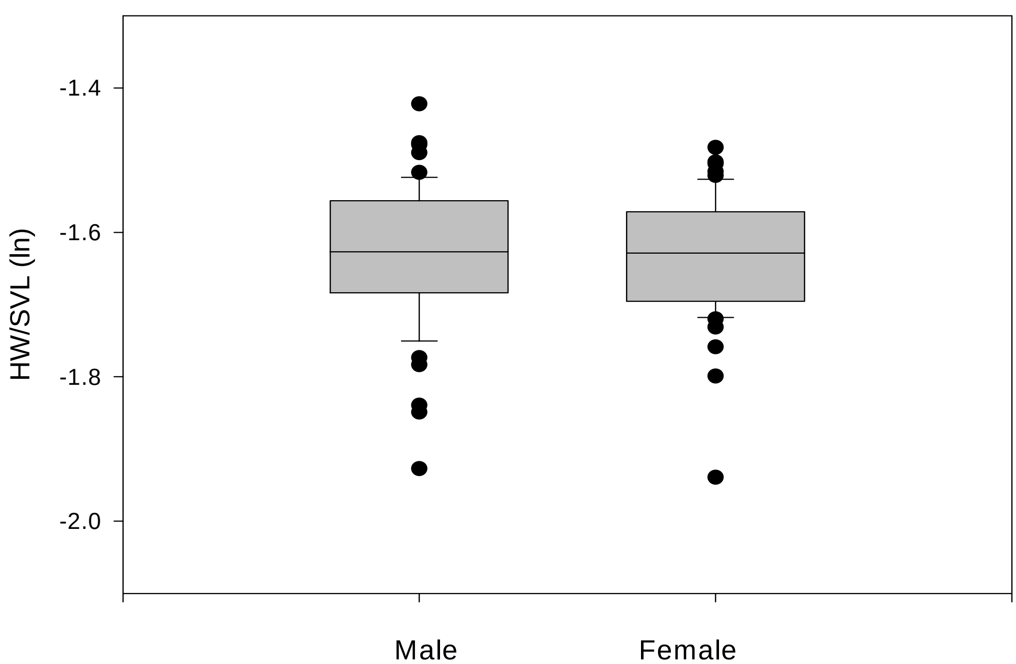

Figure 18. Head width/ snout-vent length ratios for males and females that have been log transformed.

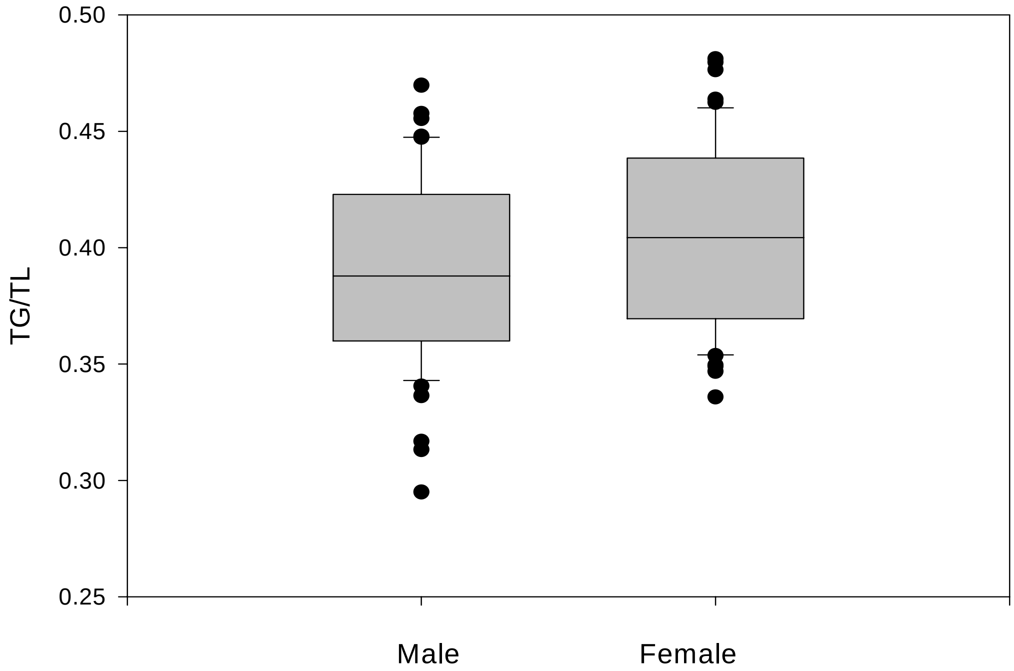

Figure 19. Thoracic girth/ total length ratios for males and females.

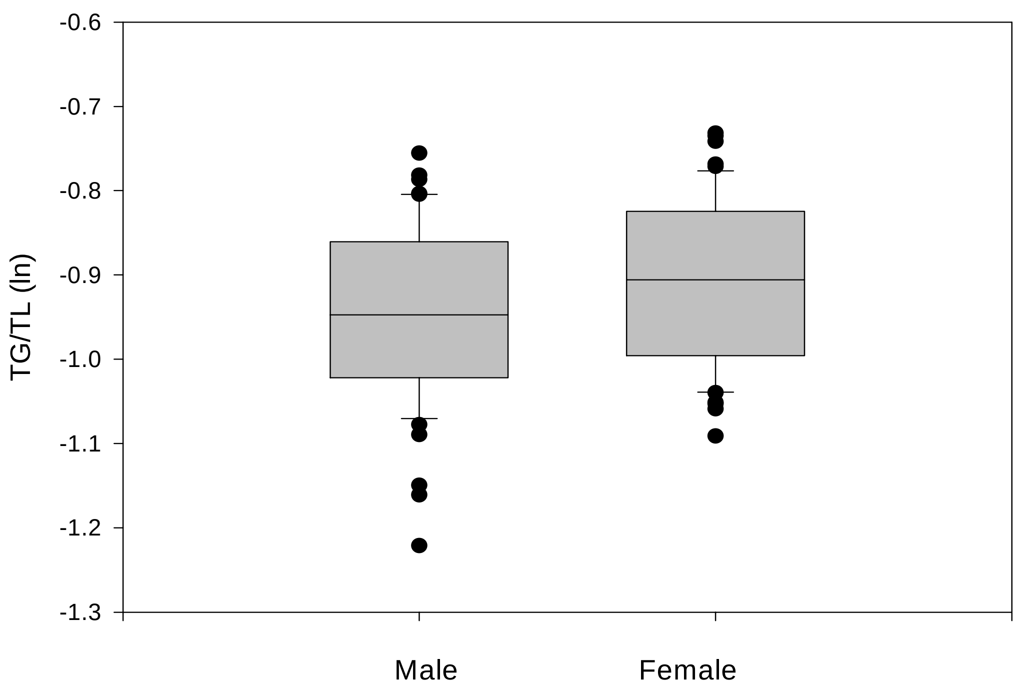

Figure 20. Thoracic girth/ total length ratios for males and females that have been log transformed.

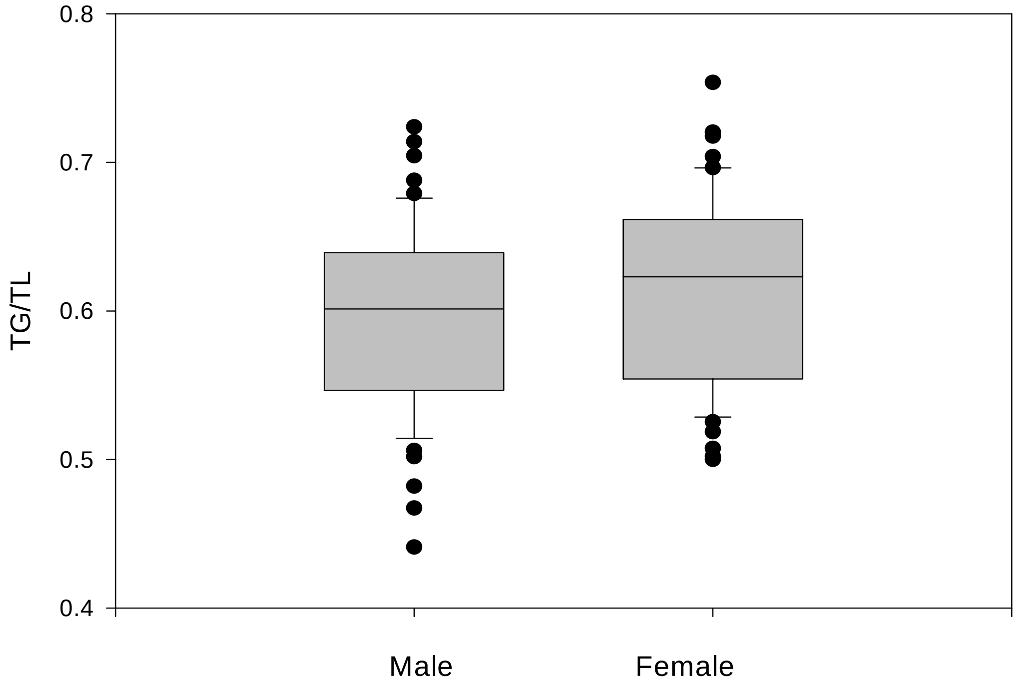

Figure 21. Thoracic girth/ snout-vent length ratios for males and females.

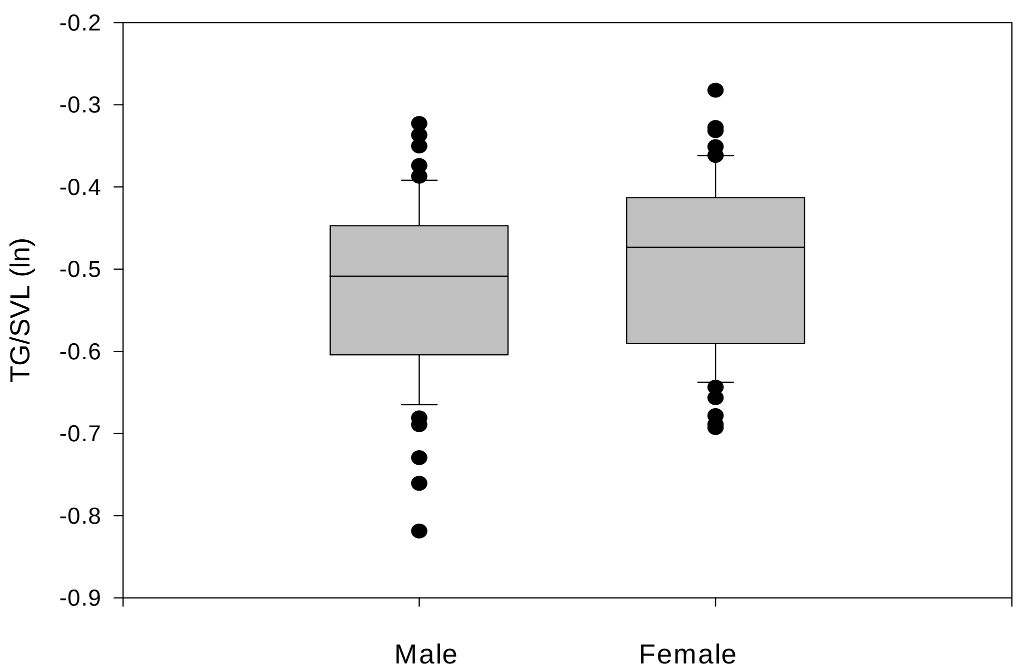

Figure 22. Thoracic girth/ snout-vent length ratios for males and females that have been log transformed.

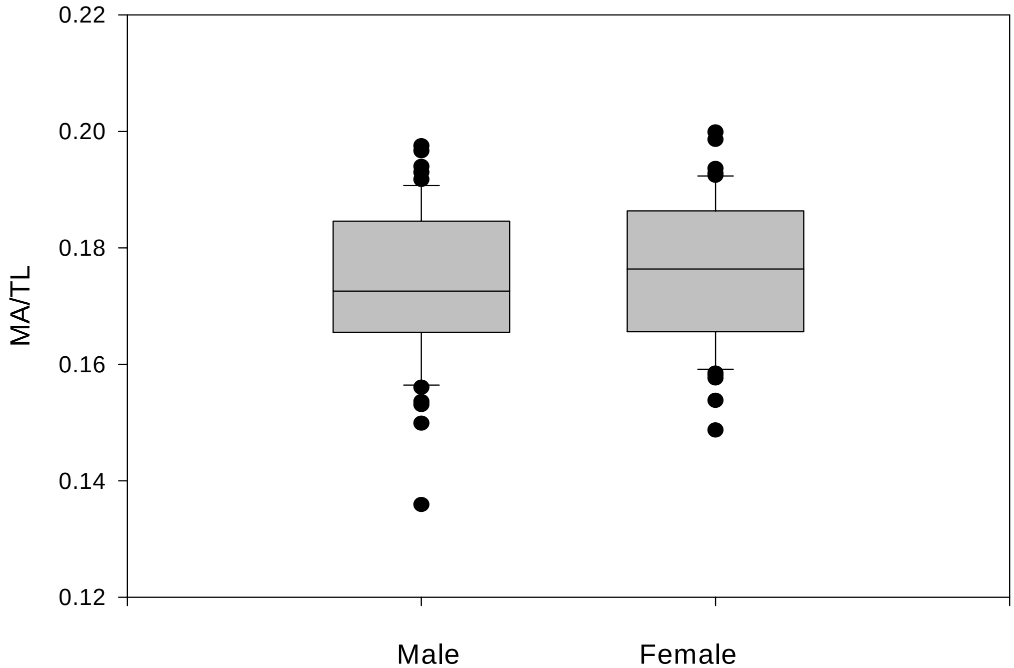

Figure 23. Cube root of mass/ total length ratios for males and females.

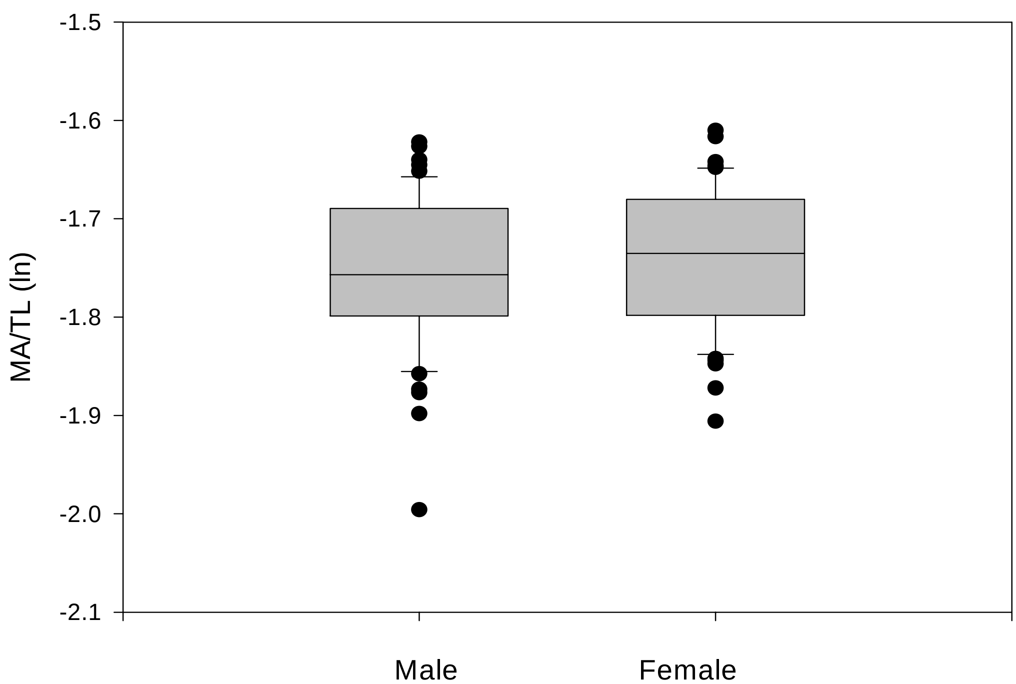

Figure 24. Cube root of mass/ total length ratios for males and females that have been log transformed.

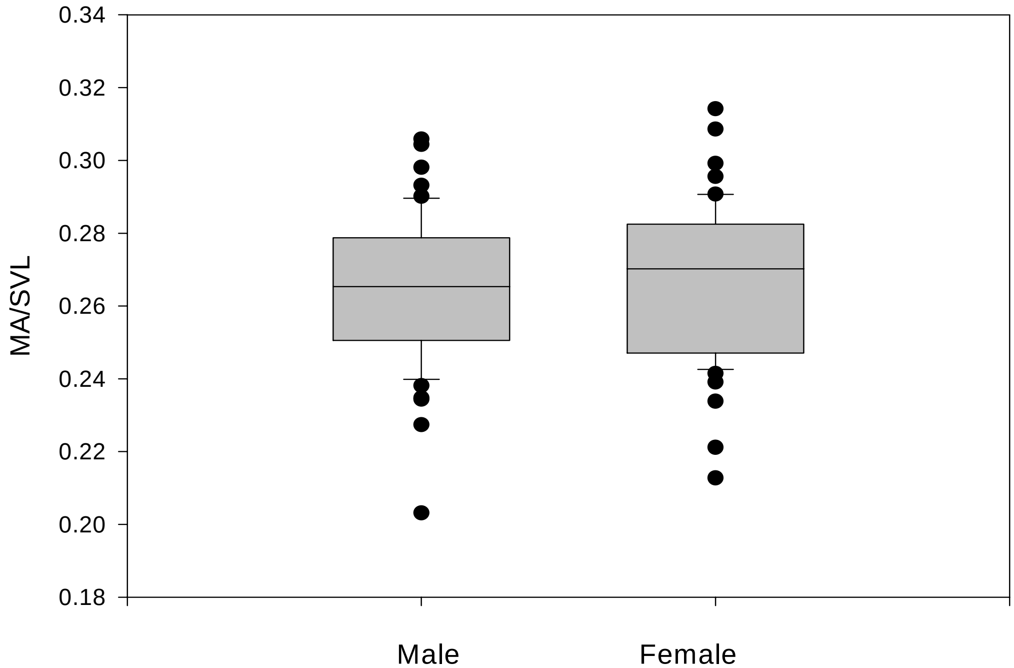

Figure 25. Cube root of mass/ snout-vent length ratios for males and females.

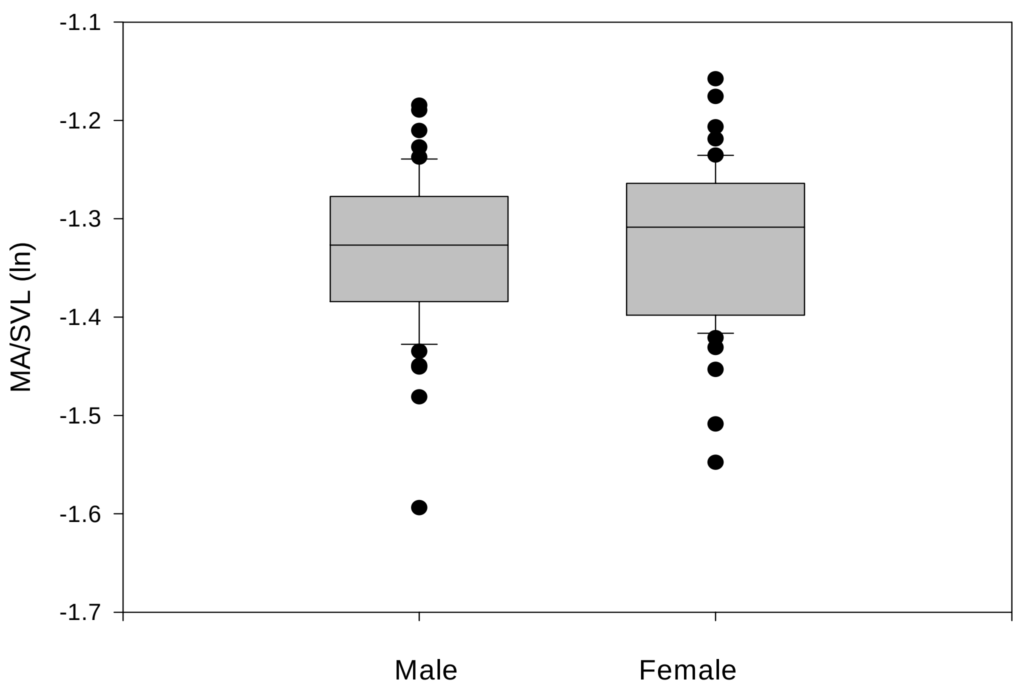

Figure 26. Cube root of mass/ snout-vent length ratios for males and females that have been log transformed.

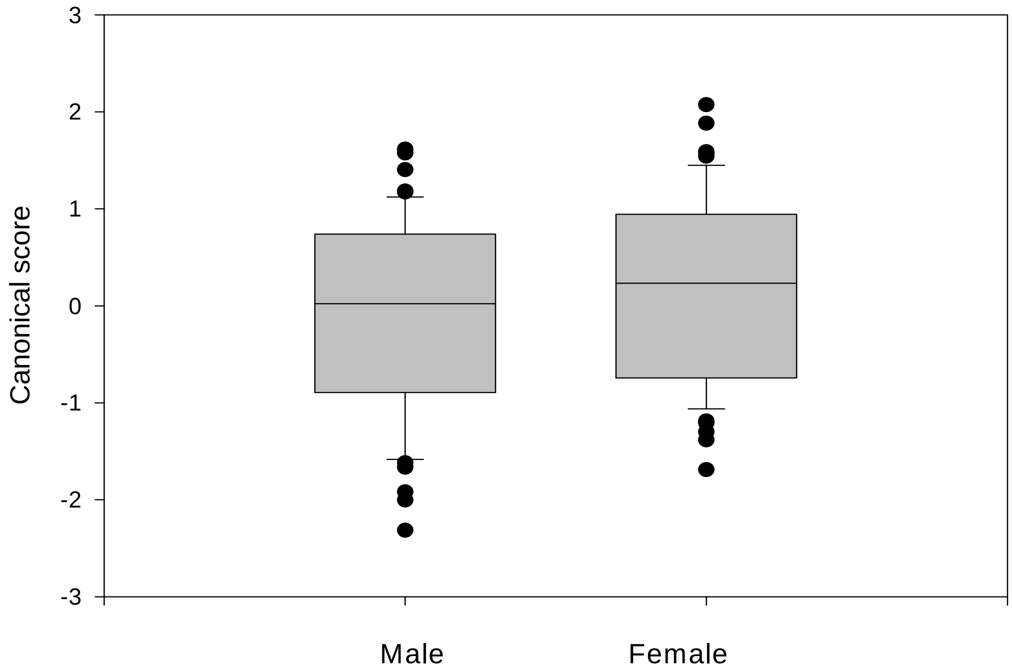

Figure 27. How males and females compared in their canonical scores for standard ratios.

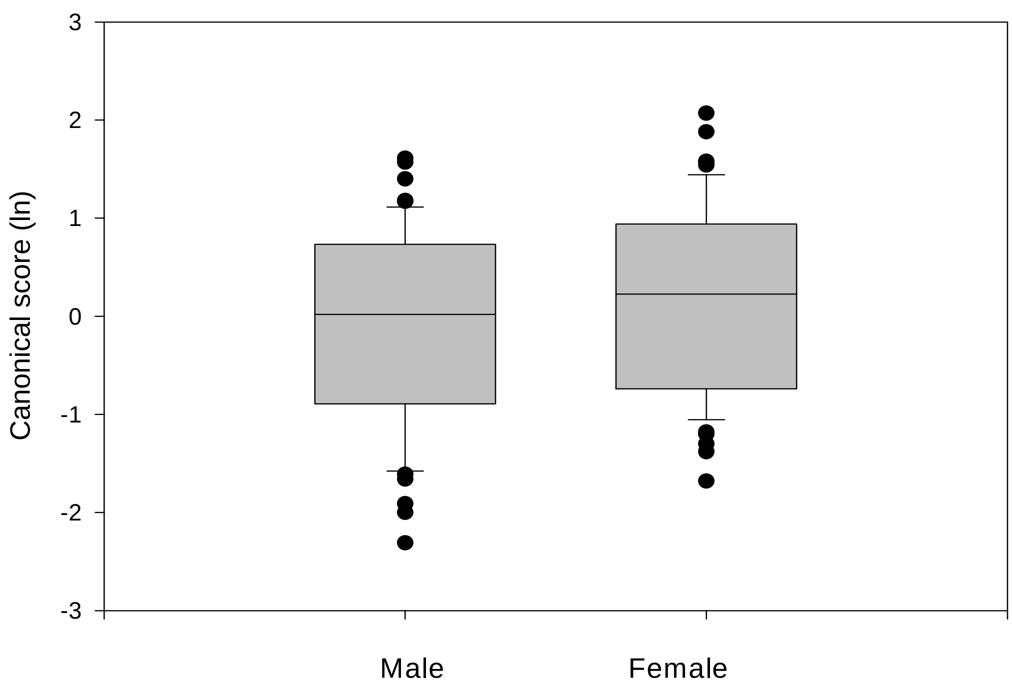

Figure 28. How males and females compared in their canonical scores for log transformed ratios.

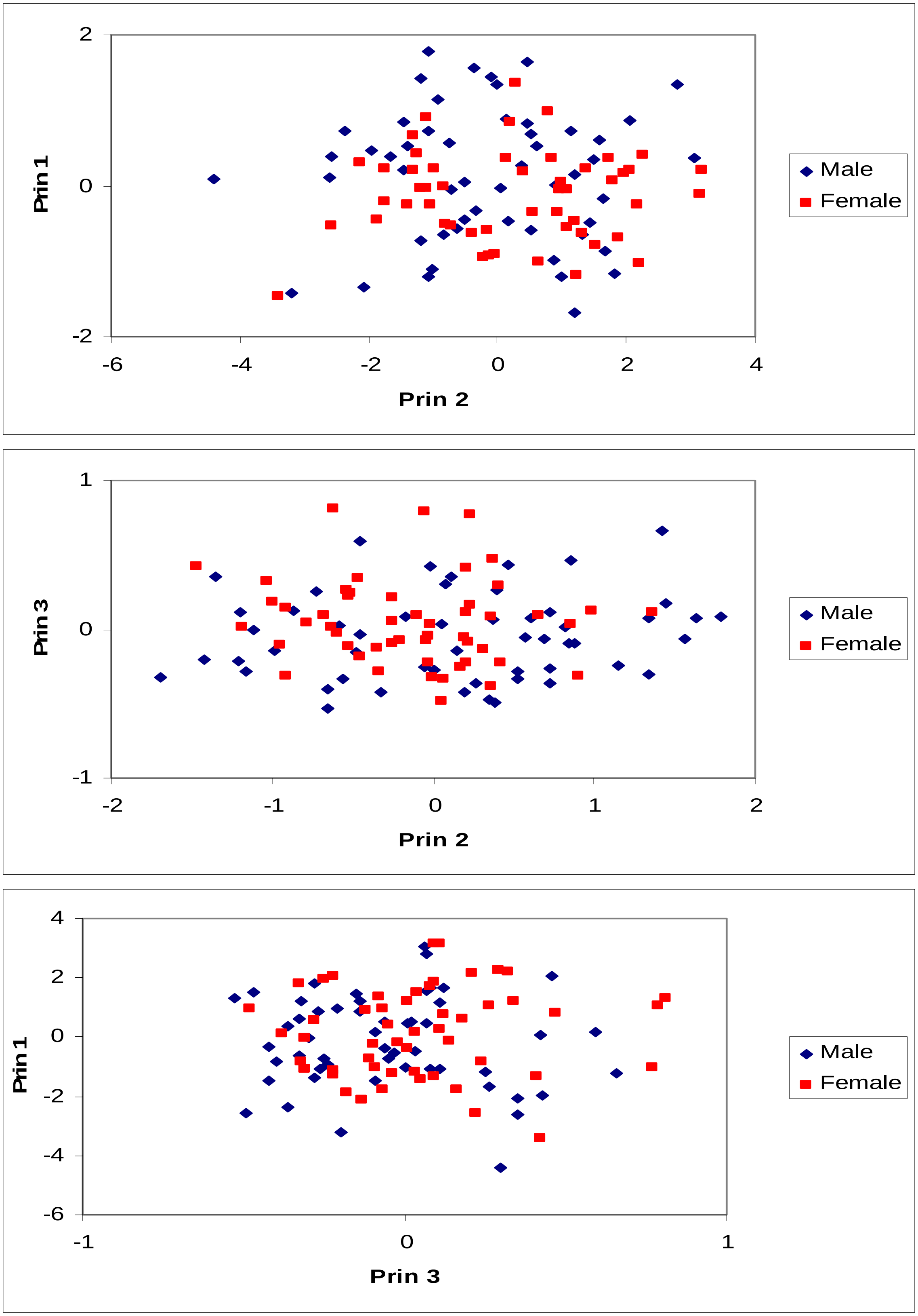

Figure 29. Separation of males and females using PCA analysis on standard ratios.

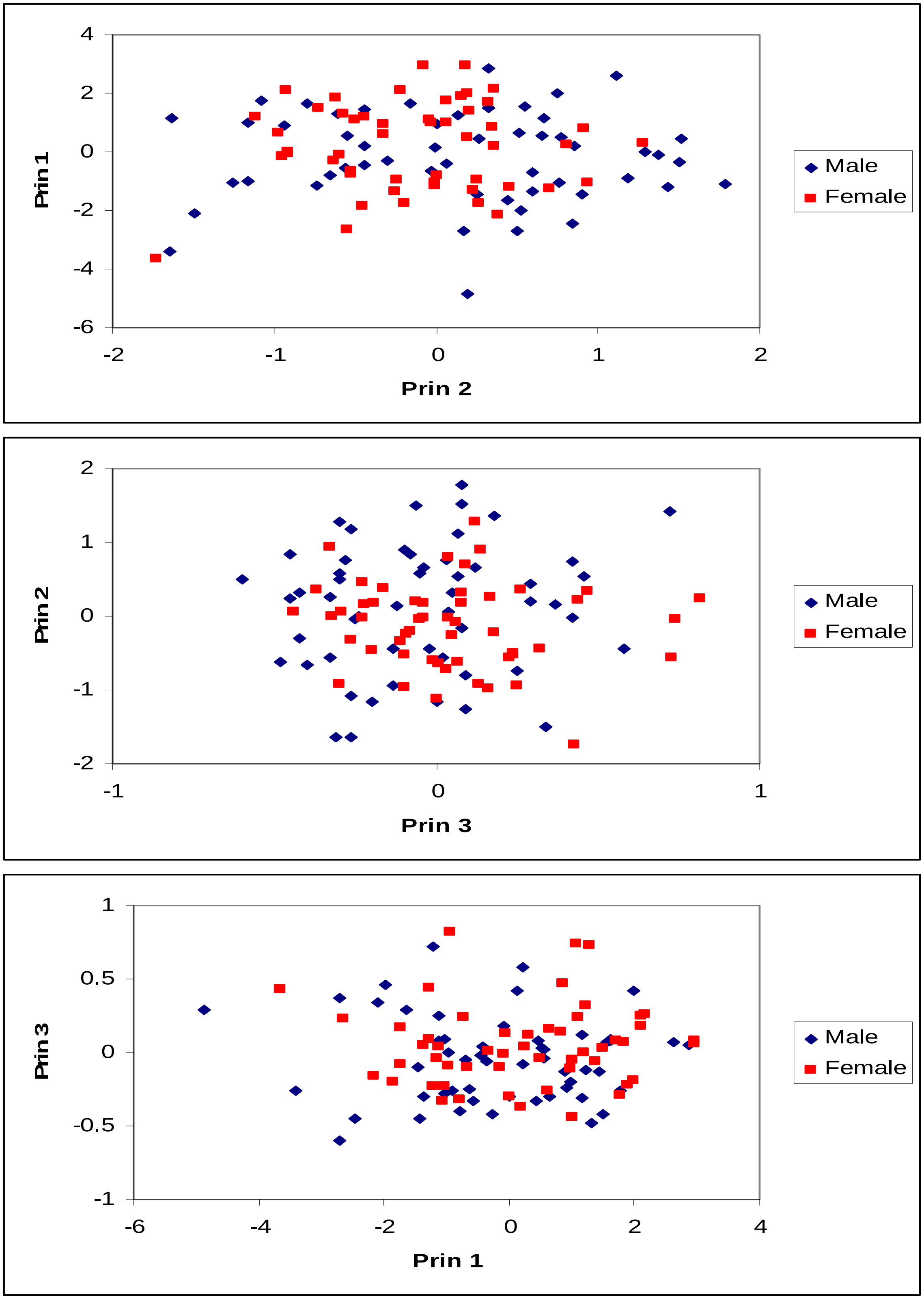

Figure 30. Separation of males and females using PCA analysis on log transformed data.

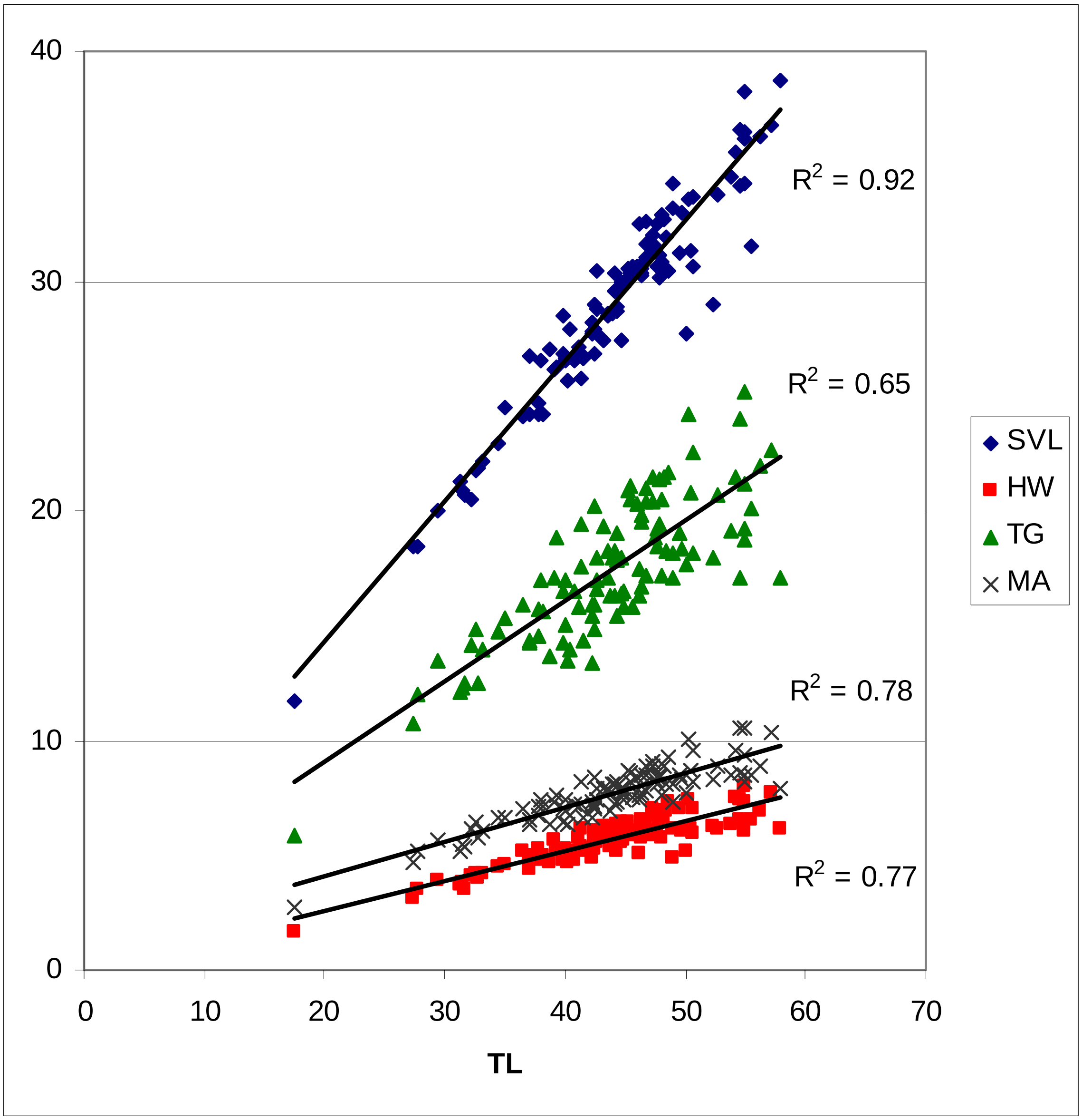

Figure 31. Four linear regressions all using TL as the dependent variable for both genders.

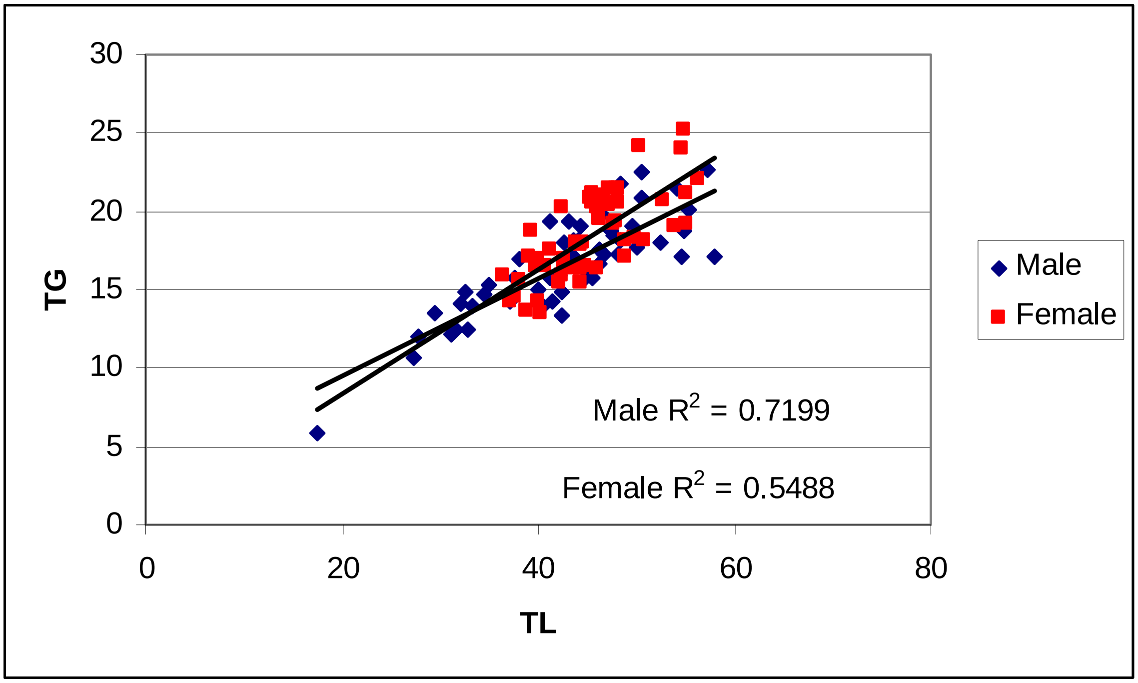

Figure 32. Linear regression of TG and TL for males and females. Together, R^2= .65

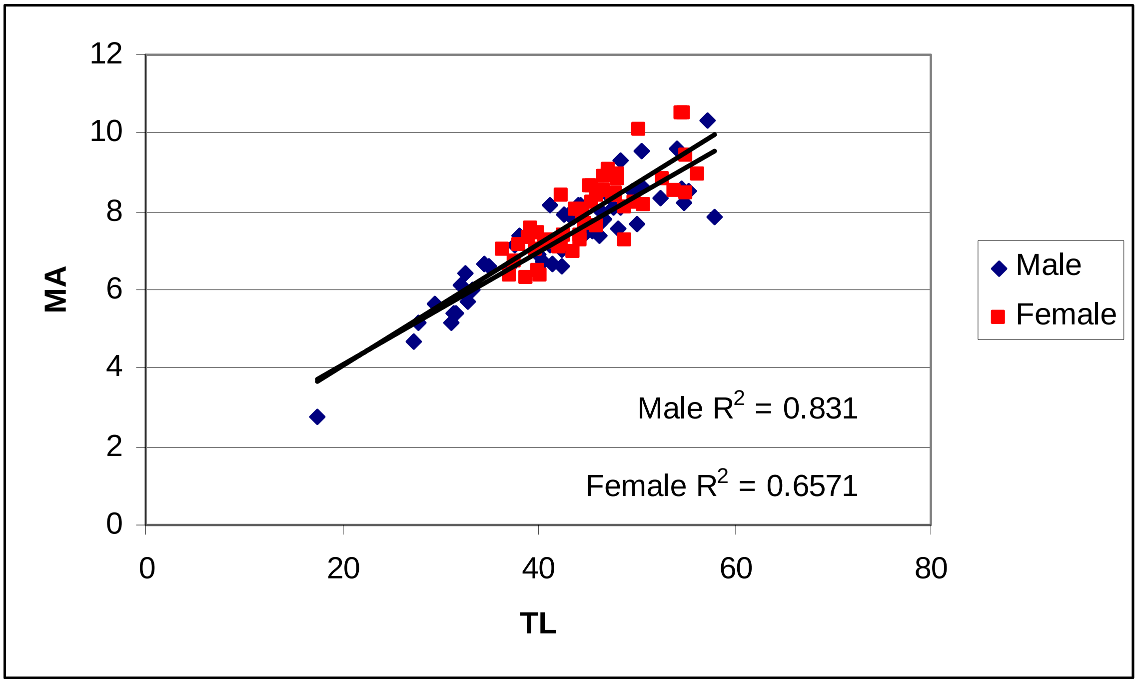

Figure 33. Linear regression of MA and TL for males and females. Together, R^2= .78

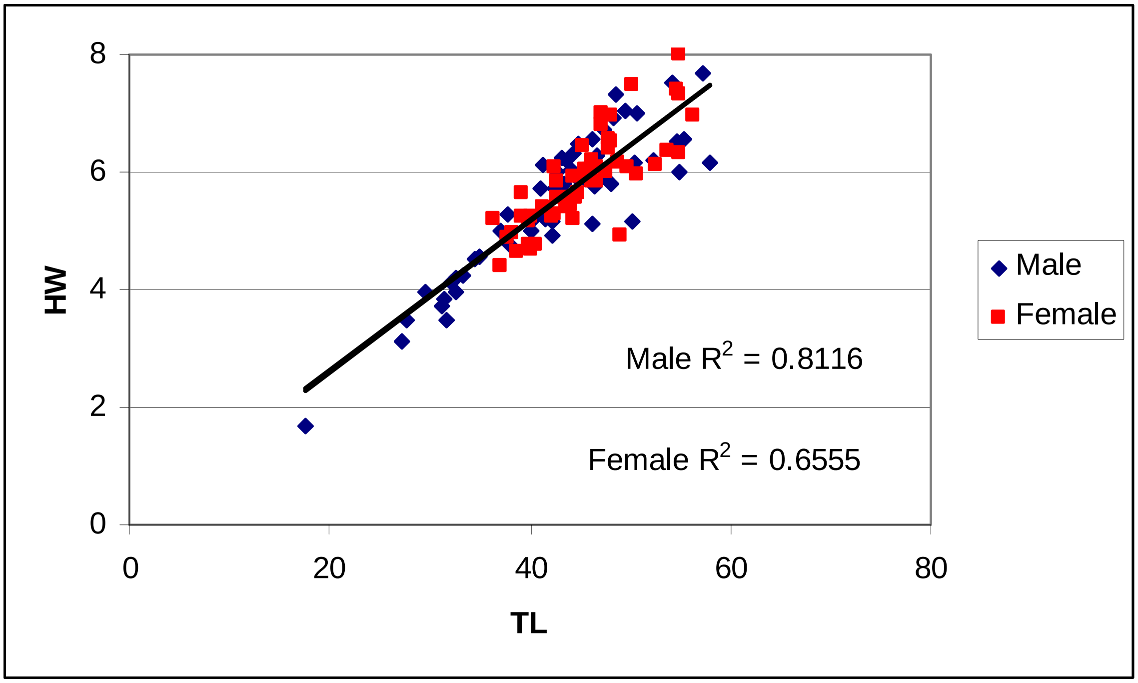

Figure 34. Linear regression of HW and TL for males and females. Together, R^2= .77

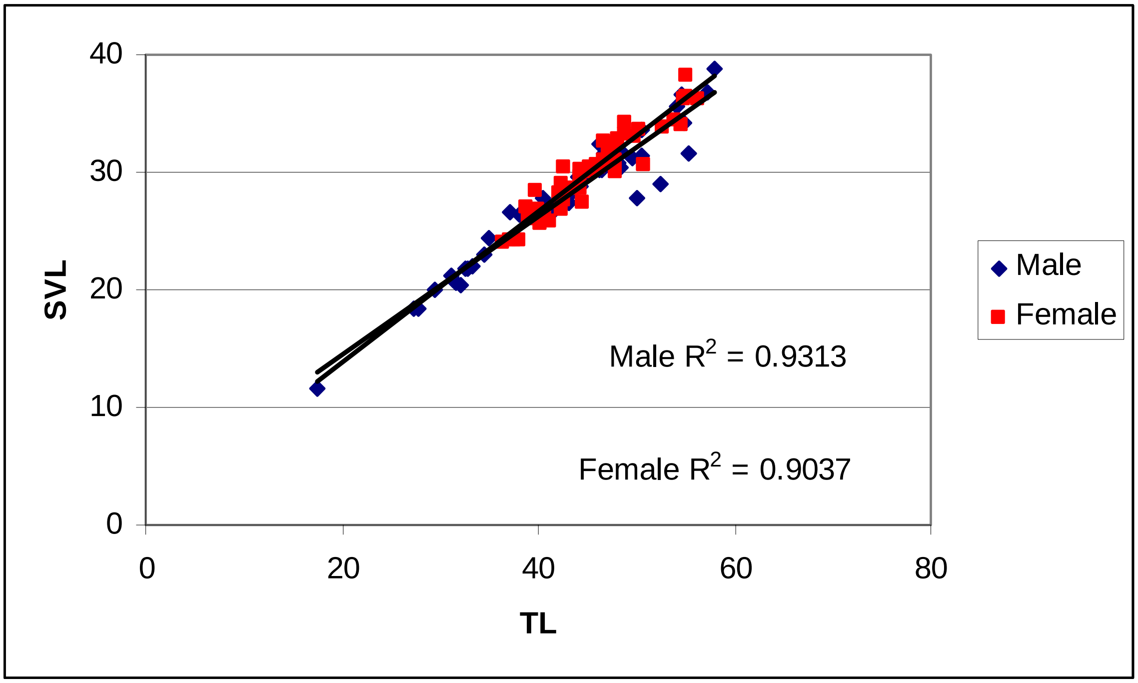

Figure 35. Linear regression of SVL and TL for males and females. Together, R^2= .92.

Appendix-II

Tables

Table 1. Sites visited during 2003, date of search, method(s) of search, time of day these searches were conducted, and life stage found (A=adult, L=larvae, E=egg.) Some streams were visited multiple times and at multiple locations.

Site	Found	Day	Night	Wading	Diving/ Snorkel	Shocking
Bells Creek						
26 June, 2003	No	X		X		
Buckhannon River						
6 August, 2003	No	X		X		
Buffalo Creek						
9 July, 2003	No	X		X		
Bull Run						
7 August, 2003	No	X		X		
Cherry River						
10 September, 2003	No	X		X		
17 September, 2003	No		X	X	X	
Cranberry River						
19 July, 2003	A	X	X	X	X	
4 August, 2003	L	X		X		
Elk River						
1 June, 2003	No	X		X		
	L	X			X	
Fish Creek						
8 July, 2003	No		X	X	X	
Gauley River						
20 July, 2003	No	X		X	X	
Holly River						
23 September, 2002	E	X	X	X		
26 June, 2003	No	X		X		
14 July, 2003	No	X		X		
Howard’s Creek						
	No	X				X

Site	Found	Day	Night	Wading	Diving/ Snorkel	Shocking
Hominy Creek						
	No	X				X
Kanawha River						
10 June, 2003	No	X			X	
Laurel Fork						
26 June, 2003	No	X		X		
Little Coal River						
10 September, 2002	No	X	X	X		
Little River						
23 May, 2003	No		X	X		
Meadow River						
21 July, 2003	No	X		X	X	
Middle Fork River						
6 August, 2003	No	X		X		
6 September, 2003	No	X		X		
Mud River						
4 May, 2003	No	X		X		
N. Fork of Cherry R.						
16 September, 2003	No	X	X	X	X	
New River						
10 June, 2003	No	X			X	
Paint Creek						
24 September, 2002	No	X		X		
Peters Creek						
26 June, 2003	No	X		X		
Upper Pond Lick						
25 May, 2003	No	X		X		
Potts Creek						
	No	X				X
Shavers Fork						
25 May, 2003	No	X		X		
29 September, 2003	No	X		X		
Slaty Fork						
24 May, 2003	No	X		X		
Tea Creek						
23 May, 2004	No	X		X		

Site	Found	Day	Night	Wading	Diving/ Snorkel	Shocking
Twelve Pole Creek						
1 May, 2003	No	X		X		
22 May, 2003	No	X		X		
30 May, 2003	No		X	X		
31 May, 3003	No		X	X		
8 June, 2003	No		X	X		
11 July, 2003	No		X	X		
Tygart Valley River						
5 May, 2003	No	X	X	X	X	
5 August, 2003	No	X		X		
W. Fork of Greenbrier						
23 May, 2003	No		X	X		
24 May, 2003	No	X	X	X		
25 May, 2003	No	X		X		
29 September, 2003	No	X		X		
Williams River						
23 May, 2003	No	X		X		
	No	X				X

Table 2. Daily streamflow (ft^3/ s) statistics for rivers searched during this study and the dates when peak streamflow was attained. Data provided by the USGS water resources divisions.

Station name	Years of discharge data	Mean daily stream flow	Percent of average	Peak stream flow (2003)	Date of peak stream flow
Bluestone River near Pipestem	53	773	164 %	14,100	Feb 22
Buckhannon River at Hall	88	793	132 %	9,430	Feb 23
Buffalo Creek at Barrackville	80	178	106 %	4,220	Feb 23
Cranberry River near Richwood	44	309	133 %	4,120	Sep 04
East fork Twelve Pole Creek near Dunlow	39	81.1	157 %	4,770	Jun 17
Elk River below Webster Springs	43	922	133 %	12,600	May 10
Gauley River above Belva	75	4048	141 %	26,500	Feb 22
Kanawha River at Kanawha Falls	126	19,960	165 %	123,000	Feb 23
Meadow River near Mount Lookout	35	1,055	144 %	9,370	Feb 23
Middle Fork River at Audra	52	486	138 %	6,300	Sept 02
New River at Thurmond	22	16,600	167 %	96,600	Feb 23
Shavers Fork below Bowden	14	637	144 %	13,100	May 10
Tygart Valley River near Elkins	59	747	143 %	8,470	Feb 23
Mean increase in flow for 2003			144%		

Table 3. Specimens and their measurements used in the dimorphism study.

Specimen #	Locality	M/F	Total length	Snout-vent len.	Head width	Thoracic girth	Mass	Tail length
230	WV	F	50.72	30.66	5.974	18.1	543.7	20.06
1554	PE	F	38.2	24.2	4.957	15.6	366	14
1556	PE	F	44.4	29.7	5.936	15.4	397.4	14.7
1557	PE	F	41.3	25.8	5.413	17.6	382.4	15.5
2454	WV	F	48.93	34.2	4.919	17.1	385.1	14.73
2659	WV	F	52.62	33.72	6.108	20.7	690.1	18.9
2836	PE	F	44.6	27.4	5.55	18	500.3	17.2
2929	WV	F	55.01	38.25	6.328	19.2	605.5	16.76
3984	WV	F	44.39	28.7	5.188	17.9	516.7	15.69
5884	PE	F	40.1	26.5	5.257	17	407.8	13.6
5939	PE	F	45.5	30.3	6.029	21.1	550.1	15.2
6260	PE	F	37.7	24.2	4.893	14.5	303.5	13.5
9805	PE	F	46	30.6	6.039	20.3	597.5	15.4
9813	PE	F	47.9	30.1	6.574	19.4	603.7	17.8
9815	PE	F	47.2	31.4	6.988	21.5	707.6	15.8
12287	WV	F	38.7	27	4.652	13.7	251.6	11.7
19384	PE	F	47.8	31.1	6.397	21.4	598	16.7
25801	PE	F	56.3	36.3	6.963	22	710.3	20
29203	PE	F	40.2	25.7	4.675	13.5	254.4	14.5
112202	PE	F	55	36.5	8.016	21.2	831.2	18.5
114273	VI	F	49.8	33	6.095	18.3	561	16.8
126883	VI	F	53.8	34.5	6.357	19.1	616.1	19.3
127805	VI	F	39.2	26.2	5.235	18.8	437.1	13
127876	VI	F	45.2	30.5	6.448	20.9	646.9	14.7
128722	VI	F	45.5	30.3	5.978	20.5	642.8	15.2
128723	VI	F	43.9	28.6	5.579	18	521.6	15.3
128725	VI	F	47.2	32	6.789	20.4	747.6	15.2
128727	VI	F	48.1	32.9	6.955	20.5	715.5	15.2

Tab. 3 cont.								
Specimen #	Locality	M/F	Total length	Snout-vent len.	Head width	Thoracic girth	Mass	Tail length
128728	VI	F	50.3	33.6	7.461	24.2	1016.2	16.7
129245	VI	F	48.2	32.7	6.533	21.5	688.3	15.5
129246	VI	F	46.3	30.5	6.214	19.5	614.4	15.8
148164	VI	F	37.1	24.2	4.385	14.3	259.3	12.9
148165	VI	F	46.8	31	5.842	21	693.74	15.8
148166	VI	F	39.8	28.5	5.15	16.5	343.1	11.3
148168	VI	F	54.9	36.2	7.311	25.2	1165.9	18.7
148169	VI	F	54.6	34.1	7.395	24	1165.7	20.5
148173	VI	F	40.7	26.5	4.745	16.5	377.7	14.2
37480 D	PE	F	46.8	32.6	6.072	20.4	617.94	14.2
37480 F	PE	F	43.7	28.6	5.382	16.3	338.28	15.1
37480 G	PE	F	42.6	27.7	5.839	16.6	399.8	14.9
37480 H	PE	F	42.2	28.2	5.228	15.4	357.71	14
37480 J	PE	F	44.8	30	5.635	16.5	450.57	14.8
37480 L	PE	F	44.2	30.3	5.442	16.3	380.15	13.9
37480 M	PE	F	42.6	30.4	5.554	17	406.48	12.2
37480 P	PE	F	48.9	33.2	6.146	18.1	525.57	15.7
37480 S	PE	F	46.1	30.6	5.851	16.3	442.24	15.5
37480 T	PE	F	47.6	32.5	6.018	19.2	587	15.1
37480 U	PE	F	39.9	26.8	4.745	14.2	271	13.1
4138 A	PE	F	42.4	26.8	6.085	20.2	597	15.6
5884 A	PE	F	36.4	24.1	5.192	15.9	343.8	12.3
5884 C	PE	F	42.5	29	5.3	15.9	366	13.5
5884 E	PE	F	39.1	26.1	5.659	17.1	388.9	13
567	WV	M	34.42	22.95	4.51	14.7	293	11.47
793	WV	M	17.54	11.66	1.697	5.9	20.5	5.88
794	WV	M	27.76	18.48	3.477	12	139.7	9.28
1086	WV	M	27.33	18.4	3.123	10.7	101.58	8.93
1559	PE	M	33.2	22.1	4.234	14	217	11.1

Tab. 3 cont.								
Specimen #	Locality	M/F	Total length	Snout-vent len.	Head width	Thoracic girth	Mass	Tail length
2198	WV	M	57.98	38.78	6.162	17.1	489.1	19.2
2658	WV	M	52.4	29.02	6.217	18	574.4	23.38
2688	WV	M	50.49	31.33	6.166	20.8	649.2	19.16
2810	WV	M	50.15	27.71	5.171	17.7	452.4	22.44
2928	WV	M	46.2	32.48	5.112	17.5	402.8	13.72
3651	WV	M	54.93	34.26	6.002	18.7	557.8	20.67
3977	WV	M	55.43	31.52	6.569	20.1	617	23.91
3981	WV	M	46.41	30.22	5.777	19.8	511.5	16.19
5940	PE	M	44.3	28.9	6.339	19	547.7	15.4
6032	PE	M	49.5	31.2	7.033	19	614.3	18.3
6218	PE	M	31.2	21.3	3.721	12.1	137.9	9.9
6220	PE	M	31.4	20.9	3.851	12.3	159.1	10.5
9814	PE	M	43.1	27.4	6.246	19.3	499	15.7
9816	PE	M	43.6	28.5	6.198	18.2	486.1	15.1
9817	PE	M	38	26.5	4.763	17	400.3	11.5
17172	PE	M	29.5	20	3.945	13.5	177.2	9.5
30305	PE	M	32.2	20.5	4.118	14.1	228.2	11.7
36604	PE	M	57.2	36.8	7.694	22.6	1108.66	20.4
36651	PE	M	37.7	24.7	5.284	15.7	368.1	13
113385	PE	M	50.6	33.7	7.002	22.5	861.7	16.9
114214	VI	M	31.7	20.7	3.479	12.5	155.3	11
126882	VI	M	54.1	35.6	7.515	21.5	878.1	18.5
128724	VI	M	46.3	30.3	6.563	16.7	458.3	16
128726	VI	M	35	24.5	4.547	15.3	291	10.5
129244	VI	M	42.7	28.8	6.009	18	496.4	13.9
129247	VI	M	44.1	29.6	6.025	18.2	539.2	14.5
129248	VI	M	32.5	21.8	4.218	14.8	261	10.7
143509	PE	M	41.3	26.8	6.124	19.4	542.64	14.5
148167	VI	M	44.7	30	6.482	16.4	459.74	14.7

Tab. 3 cont.								
Specimen #	Locality	M/F	Total length	Snout-vent len.	Head width	Thoracic girth	Mass	Tail length
148170	VI	M	47.7	30.6	5.888	18.4	535.7	17.1
148171	VI	M	42.2	27.7	4.924	15.9	397	14.5
148172	VI	M	43.5	28.6	5.8	17.1	482.8	14.9
37480 A	PE	M	46.7	31.6	6.264	17.2	476	15.1
37480 B	PE	M	42.3	27.8	5.165	13.4	290.18	14.5
37480 C	PE	M	45.6	30.6	5.863	15.8	420.3	15
37480 E	PE	M	37.1	26.7	4.998	14.2	284.3	10.4
37480 I	PE	M	42.5	27.9	5.724	14.8	346.2	14.6
37480 K	PE	M	44.9	29.8	5.881	15.8	414.6	15.1
37480 N	PE	M	48.1	30.8	5.787	17.2	434.11	17.3
37480 O	PE	M	40.4	27.9	5.182	14	303.2	12.5
37480 Q	PE	M	40.1	26.6	5.011	15	326.1	13.5
37480 R	PE	M	54.6	36.6	6.532	17.1	631.1	18
37480 V	PE	M	41.1	27.1	5.715	15.8	364.2	14
37480 W	PE	M	47.4	31.5	6.702	18.8	575.06	15.9
37480 X	PE	M	41.5	26.6	5.212	14.3	296.4	14.9
37480 Y	PE	M	32.7	21.9	3.965	12.5	187.35	10.8
4138 B	PE	M	48.5	30.4	7.333	21.7	804.1	18.1
5884 B	PE	M	48.4	31.9	6.937	18.2	527.4	16.5
1555	PE		32.1	20.9	3.967	11.9	168.4	11.2
6221	PE		35.5	24.1	4.762	14	279.7	11.4
28632	PE		36.7	25.7	5.291	12.9	214.7	11
29204	PE		35.3	23	4.026	12	177.2	12.3

Table 4. Other measurements from WV specimens and whether t-tests found significant differences between sexes. HH=head height, EE=eye to eye, EN=eye to nostril, EXN=eye to cross nostril, NN=nostril to nostril, TH= tail height, 1T=first toe, 3T=third toe, AG=axillary to groin.

WVBS #	Sex	HH	EE	EN	EXN	NN	TH	1T	3T	AG
2929	F	28.54	34.72	19.62	30.56	13.38	59	10.57	16.7	174
2659	F	33.35	34.25	18.63	29.58	15.72	59	11.59	16.49	176
230	F	25.21	31.03	16.84	27.4	13.43	48	10.28	15.99	177
2454	F	25.8	27.85	16.31	24.98	12.79	41	7.41	12.71	139
3984	F	28.76	29.9	16.4	27.83	14.82	51	10.3	16.14	164
2198	M	25.13	35.86	20.49	31.6	15.85	41.5	8.98	13.89	193
3977	M	31.75	34.77	20.56	31.41	11.65	51	12.08	16.9	153
3651	M	31.81	33.11	16.48	26.06	12.42	39.5	9.01	15.23	150
2658	M	29.27	35.53	19.36	28.28	13.71	58	10.74	17.67	150
2688	M	32.48	35.11	19.69	30.66	15.29	49	10.36	18.7	174
2810	M	27.3	28.87	17.16	27.84	14.96	48	10.99	18.23	165
3981	M	29.15	31.68	18.67	29.1	13.16	48	9.68	12.88	161
2928	M	25.43	29.98	15.95	26.21	12.8	54	10.95	15.09	143
567	M	22.66	26.3	15.33	24.22	11.21	34.5	6.83	14.33	140
794	M	17.45	20.14	11.86	18.03	9.42	30.5	6.32	10.68	107
1086	M	13.43	17.91	11	16.39	8.65	28	6.64	11.02	96
Sig?		No	No	No	No	No	No	No	No	No

Table 5. Mean values of males and females for each measurement and ratio.

Ratio	Male mean	Std. Dev.	Female mean	Std. Dev.	Normality
Total length (TL)	42.46	8.59	45.46	5.11	Passed
Snout-vent Length (SVL)	27.74	5.24	30.09	3.45	Passed
Head width (HW)	5.48	1.23	5.88	.80	Passed
Thoracic girth (TG)	16.48	3.18	18.46	2.74	Passed
Mass	430.04	208.72	536.31	206.13	Passed
Tail Length (Tail)	14.72	3.80	15.37	2.12	Failed
TG/ TL	.390	.041	.406	.040	Passed
TG/ SVL	.596	.064	.614	.065	Passed
Mass/ TL	.173	.014	.176	.012	Passed
Mass/ SVL	.265	.021	.267	.022	Passed
HW/ TL	.129	.012	.129	.010	Passed
HW/ SVL	.196	.019	.195	.016	Passed
Tail/ TL	.344	.031	.338	.023	Failed
SVL/ TL	.656	.031	.662	.023	Failed

Table 6. How each ratio scored in t-tests comparing males and females and whether or not the ratio was used in PCA for both standard values and log transformed values.

Measurement or Ratio	Significant	Used in PCA?	LN Significance	Used in PCA?
Total length (TL)	No	---	---	---
Snout-vent length (SVL)	.Yes	---	---	---
Head width (HW)	No	---	---	---
Thoracic girth (TG)	Yes	---	---	---
Mass	Yes	---	---	---
Tail Length (Tail)	No	---	---	---
TG/ TL	**Yes**	Yes	**Yes**	Yes
TG/ SVL	No	No	No	No
Mass/ TL	No	Yes	No	Yes
Mass/ SVL	No	No	No	No
HW/ TL	No	No	No	No
HW/ SVL	No	Yes	No	Yes
Tail / TL	No	No	No	No
SVL/ TL	No	No	No	No

Table 7. Eigenvectors SAS assigned to compute PCA for both standard ratios and log transformed ratios.

	Prin 1	Prin 2	Prin 3	LN Prin 1	LN Prin 2	LN Prin 3
Mass/TL	..623	-.286	-.728	.623	-.280	-.730
HW/SVL	.491	.867	.081	.494	.865	.090
TG/TL	.608	-.408	.681	.606	-.417	.677

Table 8. How much specimens shrank after being preserved in percentage and the significance level for both sexes combined. (*P* at least less than .05)

	Mean of females	Mean of males	Mean of both genders	Sig.?	Difference between sexes	Sig.?
Mass	12.7%	13.7%	13.1%	Yes	1%	No
SVL	6.2%	4.5%	5.5%	Yes	1.7%	No
TL	6.9%	4.1%	5.6%	Yes	2.8%	No

Table 9. Conditions when different search methods are affective.

Method	Day	Night	Fast flow	Slow flow	Comments
Wading		X		X	Only affective during spring and fall
Snorkel	X	X		X	Combined with flipping it is affective during the day
SCUBA	X	X		X	The only method suitable in deep waters
Flipping	X		X	X	Requires two people if rocks are large
Trapping	X	X	X	X	Efficacy is not yet determined. Bait ?
Shocking	X		X	X	Possible negative side affects. Not recommended

CURRICULUM VITAE

Robert Makowsky

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SUMMARY STATEMENT

A masters student with a planned graduation of May, 2004. I am a field biologist with interests in herpetology and evolution. I am self-motivated, willing and able to find my own financial support, and goal oriented. I aspire to obtain a Ph.D working with reptiles and amphibians while studying some aspect of evolutionary sciences.

EDUCATION

Marshall University, advisor Dr. Thomas K. Pauley

M.S., Biological Sciences, 4.0

Thesis: Natural History and Sexual Dimorphism of the Eastern Hellbender

May, 2001, Emory University, Decatur, GA

B.S., Biology and Neurobehavioral Biology, 3.0

PROFESSIONAL EXPERIENCE

Museum Curator, 2003-2004

Marshall University, Huntington, WV

Worked in the West Virginia Amphibian and Reptile Museum. Responsible for collections, specimen loans, ordering of supplies, and copying all written information about specimens into electronic form. Funded by the WVDNR

Field Assistant, 2002-2004

Marshall University, Huntington, WV

Took part in field surveys of Mud River in Milton, WV. This was funded by the USGS. We were responsible for frog call surveys, documentation of individuals, and submitting a report of findings.

Field Coordinator, 2002-2004

Marshall University, Huntington, WV

Was coordinator for surveys of the Gauley River Scenic Recreation Area. Responsibilities included planning trips, obtaining equipment, conducting various searches (including SCUBA,) and entering data. Funded by the NPS.

Field Assistant, 2002-2004

Marshall University, Huntington, WV

Participated in stream salamander surveys throughout West Virginia. This research was funded by the USGS and EPA.

Teaching Assistant, 2003

Marshall University, Huntington, WV

Participated in coordination all lab activities for Herpetology including setup, maintenance of specimens, teaching, administration of lab particles, and grading.

Field specialist: Diver, 2003

Marshall University, Huntington, WV

Conducted various collections and counts of fresh water mussels in Ohio near Dresden. Research funded by Dominion Power

Publication Coordinator, 2002-2003

Marshall University, Huntington, WV

Compiled all findings and produced a 450 page report concerning three years of field work in the Bluestone National Recreation Area

Amphibian and Reptile Keeper, 2001-2002

Finn’s Aquatics, Atlanta, GA

Responsible for cleaning, housing, breeding, and care of animal inventory. Experienced hundreds of species and hundreds of thousands of specimens

Teaching Assistant, 2000

Emory University, Decatur, GA

Responsible for Vertebrate Population Biology lab activities including setup, working with students, collection of live specimens, administration of lab particles, and grading

Key Employee, 1996-2001

T.J. Applebees, Stone Mountain, GA

Responsibilities included cooking, ordering food and supplies, overseeing other employees, and scheduling.

PAPERS PRESENTED

ASB, 2004. Sexual Dimorphism in the Eastern Hellbender

Hellbender Symposium, 2003. Sexual Dimorphism in the Eastern Hellbender

ASB, 2001. Diet of the Eastern Hellbender

POSTERS PRESENTED

ASB, 2004. Amphibian and reptile surveys in the Gauley River National Recreation Area in West Virginia

ASB, 2004. Status of the West Virginia state collection of amphibians and reptiles

GRANTS/AWARDS

May, 2003. Received an $8,000.00 grant to study the Eastern Hellbender in West Virginia by the WVDNR

July, 2003. Received a $500.00 award to supplement above studies from the Marshall University College of Science

April, 2001. Received $100.00 award to cover travel expenses for undergraduate research

PROFESSIONAL AFFILIATIONS

Member of Society for the Study of Amphibians and Reptiles and Society for the Study of Evolution

SKILLS

Proficient in MS Access, Word, Excel, SAS, SIGMA STAT and SYSTAT

Certified Open Water Diver and Equipment Specialist

Certified Emergency Responder