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SIZE AND AGE VARIATION OF LARVAL *GYRINOPHILUS PORPHYRITICUS PORPHYRITICUS* IN SYMPATRY WITH *SALVELINUS FONTINALIS*

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by

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ABSTRACT

The Northern Spring Salamander (*Gyrinophilus porphyriticus)* has a larval stage that could extend up to six years, an anomaly unheard of in other plethodontid salamanders. The size of a larva could be larger than an adult of a younger age. A sympatric species, *Salvelinus fontinalis,* the native brook trout, negatively alters the individual mass and survival of *G. p. porphyriticus* larvae. In the absence of trout, the role of *G. porphyriticus* ascends to top predator in a small stream community. Amphibians specifically have proven their role as stream indicators, a position attributed to their porous skin. I attempted to determine the correlation between larval duration and size in streams with or without brook trout, using Skeletochronology as a method for aging. Skeletochronology determined the age of individual salamanders using a cross-section of the femur. Age was determined by counting the lines of arrested growth. Basic methods of capture, measurement, and stream data collecting were implemented at four stream sites in western Pennsylvania from May 2012 to April 2013. Study sites include two streams populated by *Salvelinus fontinalis* and two with no trout with a total of 35 specimens. As expected, a standard growth curve (size vs. age) was found for larvae in streams containing *Salvelinus fontinalis* with a maximum age of 6 years. Larvae in fishless streams exhibited a non-standard growth curve specific to the individual stream attributed to climactic conditions, water quality, resource abundance, competition or other unknown reasons. The maximum age of larvae in fishless streams was 3 years, though all streams had similarly sized larvae. Growth curves between fish and fishless streams were significantly different (P = 0.001). Slow, consistent growth in the streams with trout was probably attributed to lower activity levels caused by risk of predation. Faster, less consistent growth in streams with no trout was probably due to intraspecific competition in addition to the absence of trout. The results of this thesis apply to a species of salamander with a broad range across the eastern United States, as well as the brook trout, an important game fish. My experiment examines the problem at the root, starting with a fundamental life history question of the Northern Spring Salamander.

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

Up to 45% of all salamanders require streams for some part of their life cycle (Lanoo, 2005), and salamanders of the family Plethodontidae show the greatest diversity in the headwater streams of the Appalachian Mountains (Milanovich et al., 2010). Headwater streams are those where water begins in underground springs and flows down slope reaching the surface to begin their descent into larger streams and rivers (Hynes, 1970). Headwater streams provide nutrients for not only the local species, but also cycle and transfer nutrients to downstream ecosystems as well as control floods and sedimentation loads (Peterman and Semlitsch, 2009; Vannote et al., 1980). Species that inhabit these small headwater streams in the eastern United States range from algae and zooplankton, and macroinvertebrates such as crayfish, mayfly nymphs, and insect larvae (Hynes, 1970). These animals provide food for larger predators such as fish and amphibians, including salamanders.

Renowned herpetologist Whit Gibbons (1983) coined the phrase that reptiles and amphibians are the “hidden biodiversity.” In headwater streams, salamanders are the numerically dominant vertebrate and may exceed birds by one order of magnitude in these streams and their terrestrial buffers (Petranka and Smith, 2005). Most large bird and mammal species here are easily seen and documented, and attract public attention. In turn, management practices of these species have been around for decades. The species found in these small stream habitats also exhibit small size and fragile life cycles. Due to the small size of these streams, human development easily alters them by logging, mining, and other sources of habitat destruction and fragmentation. Much of the original forests and streams of the eastern United States, specifically the Appalachian Mountains, have been altered severely in the form of these damages and are only now, in the past 75 years, they are starting to be conserved (Karas, 1997;

Lewis, 1998). The conservation of these small streams can only occur when the species that inhabit their waters are fully understood. This study aims to further understand two relatively common aquatic species of the Appalachians (*Gyrinophilus p. porphyriticus, Salvelinus fontinalis*), and more specifically, the effect one fish species (*S. fontinalis*) has on a salamander species (*G. p. porphyriticus*).

Statement of Problem

The Northern Spring Salamander (Figure 1) ranges in the eastern United States from Maine to Alabama, coinciding with the Appalachian Mountains. Like all amphibians, these salamanders have a larval stage before transforming to a reproducing adult (Hulse et. al., 2001). Most amphibians, especially those in the Eastern United States, have consistent known periods of time for which they are in this larval stage (Hulse et. al., 2001). The larval stage of *G. p. porphyriticus* could last anywhere from two to seven years (Bruce, 1972, 1980; Resetarits, 1995). This high variability in a larval period presents a large ecological gap in not only the life history of this particular species, but for the small streams throughout the Appalachians where it occurs.

Managing ecologically important headwater streams should begin at the bottom of the food chain, which is the common method. Environmental and wildlife management agencies already use macroinvertebrates as a way to assess the health of streams such as in the EPA's Rapid Bioassessment Protocol (Barbour et al., 1999). Can the same be done with amphibian species? The larval stage of the species in question (*G. p. porphyriticus*) preys on some of these macroinvertebrates (Bruce, 1972) and fish prey upon some size classes. Johnson et al. (2009)

found that larval salamanders can indicate hydrologic permanence in headwater streams. Evidence for bottom-up dynamics in detritus based ecosystems has been shown using salamanders as model organisms (Huntsman et al., 2011; Johnson and Wallace, 2005). If the importance of the abundant macroinvertebrates and longer-lived fish plays such a large role in stream health, then why ignore an animal whose abundance rivals fish (Resetarits, 1995), but may live nearly as long as fish in a stream?

One of these fish species, the Brook Trout (*Salvelinus fontinalis*), inhabits these small streams throughout the same Appalachian range as *G. p. porphyriticus*. These fish are the top predators of these streams (Resetarits, 1995) as well as a game species. Their ecological niche shows the importance of this species, which in turn reflects an importance in the species below them in the streams' food web. The Brook Trout are known to negatively affect *G. p. porphyriticus* whether by competitive exclusion or predation (Resetarits, 1995). This leaves *G. p. porphyriticus* as a type of "middle-man" in small stream ecology, acting as predator and prey.

So how can biologists ignore an animal so ecologically important to small streams? It is easy to ignore something biologists don't fully understand. Finding abundant benthic macroinvertebrates or certain size and age classes of fish gives us fine insight to the health of a stream, but what does it mean for a salamander larva that could be a year old or lived in the same stream pool for seven years? First biologists must understand what causes larval *G. p. porphyriticus* to retain this larval stage or transform to an adult and leave the depths of a stream.

Gyrinophilus p. porphyriticus larvae have been the topic of several studies throughout the years. As explained previously, Resetarits (1991, 1995) examined how *G. p. porphyriticus* interacts with its aquatic neighbors. Bruce (1972, 1980) has studied *G. p. porphyriticus,* specifically its larval stage for several years in the Southern Appalachians. Bruce and Castanet

(2006) aged these larvae using skeletochronology, a method to be explained in detail in the methods section. During preliminary experiments, they found that these larvae had no significant relationship of age vs. size (Bruce and Castanet, 2006). The authors then added another species larvae (*Pseudotriton r. ruber*) of the same family, similar size, distribution, and aquatic nature (Hulse et al., 2001) to compare with *G. p. porphyriticus* larvae. They found that *P. r. ruber* exhibited a standard positive growth curve correlating size vs. age. (Bruce and Castanet (2006) attributed this to the more surface dwelling activity of *P. r. ruber* compared to the benthic nature of *G. p. porphyriticus* larvae, which may force *P. r. ruber* into decreased winter activity. Though *S. fontinalis* occurs in the range where this experiment took place, its presence or absence was not noted. Bruce and Castanet (2006) also stated that an error in methods may be at fault, again due to *G. p. porphyriticus* larvae possibly being active year round. Though *G. p. porphyriticus* larvae may be active during winter months in Pennsylvania, harsher winters could drive the species under surface flowing water and possibly decrease their activity (Hulse et al., 2001).

Lowe (2003) studied the dispersal dynamics of *G. p. porphyriticus* larvae and adults in a first order New Hampshire stream. He found that the species exhibited a strong upstream bias of dispersal, attributing to the salamanders moving to more suitable headwater habitat devoid of predators such as fish. This shows that *G. p. porphyriticus* has evolved alongside predators such as *S. fontinalis* and readily attempts to avoid them through dispersing upstream to lay eggs in an area absent of *S. fontinalis* predation (Lowe, 2003).

My study aims to combine the experiments of previous studies done on *G. p. porphyriticus* larvae in an attempt to age the larvae specific to the habitat (trout or no trout) they occupy. Where Bruce and Castanet (2006) set a baseline for aging the larvae and Resetarits

(1991, 1995) set a baseline for the interactions with *S. fontinalis*, there are no studies examining the size and age of *G. p. porphyriticus* larvae in fish streams vs. fishless streams. My study will provide basic data for this specific life history gap so that future studies can better understand an animal frequently encountered in small streams throughout Appalachia. Because *G. p. porphyriticus* larvae interact with a game fish like *S. fontinalis* and several other species of fish and aquatic macroinvertebrates, their importance and ecology related to these species is sure to come into question again if one were to study these other species.

Hypotheses:

1. **Brook Trout affect *G. p. porphyriticus* larval size and age at metamorphosis in fish streams.**

   Salvelinus fontinalis presence negatively affect *G. p. porphyriticus* larval activity and growth, even if these fish are of similar size, such as the fingerlings, and do not directly prey on the larvae (Resetarits, 1991, 1995). *Salvelinus fontinalis* adults preying on larvae further shows *G. p. porphyriticus* larvae can live in a stressed environment. Salamander larvae of ephemeral ponds respond to the rate at which the pond is drying, or metamorphose at a faster rate relative to the rate the pond is drying (Semlitsch and Wilbur, 1988; Sih et al., 2003). If an environmental cue such as pond drying can increase the rate at which a salamander grows to a minimum size to metamorphose, a predation or competition stress could theoretically act in a similar manner. In the presence of *S. fontinalis*, the *G. p. porphyriticus* larvae may not grow as large as in a predator free habitat due to reduced activity (Resetarits, 1995), and have a higher potential to transform faster and escape the negative interactions with *S. fontinalis* and grow into adulthood. Because the presence of *S. fontinalis* in streams where they occur should, in theory, be equal across a population (Karas, 1997), *G. p. porphyriticus* larvae born in these streams should experience the pressure equally, and grow at a rate relatively consistent with this pressure. The size of an individual of a certain age found in a fish stream should be consistent with those occupying other fish streams of similar regions. The use of different microhabitats by *G. p. porphyriticus* larvae, such as the benthic substrate of the stream, should allow them to coexist with *S. fontinalis* long enough to maintain a healthy population (Resetarits, 1995).

2. ***Gyrinophilus p. porphyriticus* larvae in fishless streams transform at high variability in size and age.**

In the absence of *S. fontinalis*, *G. p. porphyriticus* larvae are the most abundant, large aquatic predators of the stream (Lowe, 2005; Resetarits, 1991). Enjoying the status of top predator in the stream should limit selective pressure to leave or transform into adulthood. But this theory could also work in an opposite manner. Streams with less flow are more susceptible to droughts varying year by year, and this could force *G. p. porphyriticus* larvae to transform sooner as in pond-dwelling larvae (Sih et al., 2003; Semlistch and Wilbur, 1988). *Gyrinophilus p. porphyriticus* larvae feed almost exclusively on aquatic invertebrates, while adults feed mainly on other species of salamanders (Bruce, 1972, 1980; Resetarits, 1995). The change in diet from salamanders to invertebrates could cause a variation in the larval period. In the Northeastern U.S., salamanders were eaten less frequently by *G. p. porphyriticus* adults (Bishop, 1941; Burton, 1976). This difference in diet could reflect the decrease in salamander diversity and abundance in the northern part of this species' range (Hulse et al., 2001), and possibly be a reason to delay metamorphosis in fishless streams due to the decreased availability of stage-specific prey items. Cave-dwelling species of the *Gyrinophilus* genus (*G. palleucus, G. subterraneus*) exhibit paedomorphic or neotenic characteristics because of their specific diet (Bruce, 1979). The benefits of living in a cave, i.e. no predators, outweigh the costs of metamorphosis to a more terrestrial adult stage that could have trouble finding abundant prey (Bruce, 1979). If a closely related species of *G. p. porphyriticus* can extend its larval stage in a cave situation devoid of predators, could this occur in a stream small enough or temporary enough to be absent of aquatic predators? Of course, the absence of the aquatic predator *S. fontinalis* in a smaller stream could mean the stream has a higher susceptibility of predation from a terrestrial predator such as a raccoon due to more shallow water depth. There are several variables for which *G. p. porphyriticus* larvae would transform faster or slower in a fishless stream as opposed to a fish stream. The goal of this project is not to pinpoint those variables, but to show that the life history of *G. p. porphyriticus* differs between fish and fishless streams.

CHAPTER 2: SPECIES DESCRIPTIONS

Gyrinophilus porphyriticus porphyriticus

The Northern Spring Salamander (*Gyrinophilus p. porphyriticus),* inhabits the benthic region of cool, well-oxygenated, small streams (Lowe & Bolger, 2002). It is a member of the family Plethodontidae and was first described as a genus in 1869 by Cope, then later by Dunn (1926). All Plethodontids are lungless, a characteristic attributed to the reduction of buoyancy that allows for better movement on the stream bottom (Birchfield and Bruce, 2000; Wilder and

Dunn, 1920). This lifestyle and morphology is the opposite of most ambystomatid salamanders whose larvae are pond dwelling and use lungs to move about the less heavily predated ephemeral water column (Birchfield and Bruce, 2000). Grobman (1959) introduced the idea that the *Gyrinophilus* genus should be not be independent from the genus *Pseudotriton*, based on comparative anterior cranial elements. This concept was refuted by Martof and Rose (1962) noting the difference in nasal bones forming before adult transformation and the fusing of the premaxilla bones. Members of the *Gyrinophilus* genus have longer snouts, are more dorsoventrally flattened, and have shorter, less attenuated limbs (Figures 1 and 2) than other stream salamanders (Birchfield and Bruce, 2000; Martof and Rose, 1962). Martof and Rose (1962) attributed this to an aggressive feeding habit, and Birchfield and Bruce (2000) attributed this as well as the small diameter of the eyes to a non-typical stream-type morphology more adapted to a subterranean lifestyle.

Several other plethodontid species occupy headwater streams during their aquatic larval stages, though most stream dwelling salamanders retain larval characteristics in the egg or transform after 1-2 years (Bishop, 1941; Bruce, 1980). *Gyrinophilus p. porphyriticus* larvae are nocturnal and generalist predators that feed on a variety of benthic macroinvertebrates (Bruce, 1972, 1980; Resetarits, 1995), whereas adults feed almost exclusively on other adult and juvenile salamander species, but will consume other prey including small fish and terrestrial and aquatic invertebrates (Bruce, 1972; Hulse et al., 2001). Adults in Pennsylvania average 92.6mm SVL and typically mature at 77-80mm SVL for males and 82-85mm for females (Hulse et al., 2001). Female reproduction is biennial and egg laying occurs from between April and mid-July, with hatchlings appearing from late July to September (Hulse et al., 2001).

The density of this species was found to be 5-10 individuals per square meter in a southern Appalachian stream (Bruce, 1980). This species shows an extended aquatic larval period that lasts two to seven years (Bruce, 1980; Resetarits, 1995), a period unseen in other stream-dwelling salamanders. The causes in variation of larval age are mostly unknown. Studies have shown (Resetarits 1991, 1995) that larval size varies from 13-65mm snout-vent-length, but adults are found smaller than 65mm. In a Pennsylvania sample of 37 individuals, the smallest transformed individual was 100mm ToL, and the largest larva was 126mm ToL (Hulse et al., 2001).

The causes of this size and age variation could be numerous. Environmental factors such as stream size, latitude, geomorphology, and hydrology (Hulse et al., 2001; Johnson et al., 2009) could affect distribution, which may in turn reflect size and age characteristics. Gian L. Rocco (unpublished) found that *G. p. porphyriticus* were more tolerant of streams affected by acidification than other salamander species in Pennsylvania. Pollutants have been shown to alter duration of larval periods and size at metamorphosis in the pond breeding salamander *Ambystoma barbouri* (Rohr, et al., 2004). Sedimentation negatively affects adult *G. p. porphyriticus* possibly due to the elimination of interstitial spaces used for refugia (Lowe, 2005).

Other influences on size and age variation are that of interactions with the several other species who share the same aquatic habitat as *G. p. porphyriticus* larvae. The smaller, but more abundant Northern Two-Lined Salamander (*Eurycea bislineata*) has been shown to compete with *G. p. porphyriticus* larvae for food items while simultaneously acting as prey in different size cohorts (Rudolf, 2006). *Gyrinophilus p. porphyriticus* larvae are also in competition for resources with crayfish species of the *Cambarus* genus, and could possibly be predated upon by the same species (Resetarits, 1991). Larger *G. p. porphyriticus* larvae may also be cannibalistic toward their smaller conspecifics if given the opportunity (Bruce, 1980; Resetarits, 1991; Rudolf, 2006). Though it has not been documented for this species, the mottled sculpin (*Cottus bairdi*) and darters (*Etheostoma*) could surely compete for the same food items (benthic macroinvertebrates) as *G. p. porphyriticus* larvae but their average size limits them to predation (Eddy and Underhill, 1974). This same idea applies to terrestrial vertebrate predators such as raccoons, opossums, and birds.

Salvelinus fontinalis

Gyrinophilus p. porphyriticus larvae are known to share the resources of these headwater microhabitats in a top predator niche with the native brook trout (*Salvelinus fontinalis*) (Figure 3); even though size variations of at least two orders of magnitude between salamander larvae and fish may occur simultaneously (Resetarits, 1995). Resetarits (1991, 1995) as well as Lowe and Bolger (2002) have shown that population densities of *G. porphyriticus* are lower and mass, survival, and activity are negatively affected by the presence of *S. fontinalis* when compared to fishless streams.

Salvelinus fontinalis differs from *G. p. porphyriticus* larvae in that they are diurnal, but will feed during nights with sufficient moonlight, and are primarily drift feeders (Resetarits, 1995). They are almost exclusively carnivorous, feeding on any type of aquatic invertebrate and vertebrate small enough to consume (Karas, 1997). They are drift feeders, but will wander about during the early morning and late afternoon in search of prey (Karas, 1997). During optimal temperatures, they will eat 50% of their body weight weekly (Karas, 1997). Their optimal water temperature ranges from 55 to 65^0 F with a pH range of 4.1 to 9.5, though neutral pH is optimal

(Karas, 1997). Both sexes usually reach sexual maturity after their first year (Karas, 1997). Brook trout in small mountain streams rarely exceed lengths of 40 cm (Karas, 1997; pers. Obs). *S. fontinalis* occupies a range similar to *G. p. porphyriticus*, or roughly the Appalachian Mountains in the eastern United States from Alabama northward to Quebec (Karas, 1997; Hulse et al., 2001). Though agriculture and deforestation severely damaged *S. fontinalis* populations throughout much of their range in the past 200 years, many populations are re-established by better management practices (Karas, 1997). The stocking of exotic species of trout has also had a negative effect on native populations of brook trout (Karas, 1997). After fisherman noticed brook trout numberbs were declining in streams throughout the Appalachians due to deforestation, the brown trout (*Salmo trutta*), a fish native to Europe, was heavily stocked in the eastern United States as a game fish because of its higher water temperature tolerance (Karas, 1997). Later on, the rainbow trout (*Oncorhynchus mykiss*), a fish native to the western United States were stocked (Karas, 1997). The rainbow trout had a much greater impact on the native brook trout than the brown trout due to their aggressive and competitive nature, forcing brook trout into the smaller streams which it mostly occupies today (Karas, 1997). Though some populations may differ from their genetically native populations to their specific streams, current *S. fontinalis* populations and individuals still exhibit the same phenotypic and ecological characteristics (Karas, 1997). Because *S. fontinalis* feed and reproduce currently the same as historic individuals even after anthropogenic effects, it can be assumed that their interactions with *G. p. porphyriticus* larvae are similar for the purposes of this study.

The lack of documentation on the interactions of other fish sympatric to *G. p. porphyriticus* larvae such as sculpins (*Cottus*), darters (*Etheostoma*) and well documented interactions with *S. fontinalis* (Lowe and Bolger, 2002; Resetarits, 1991, 1995) will, for the purposes of this study, exclude these other fishes from the definition of "fish" and "fishless" streams. Therefore, a "fish" stream will mean a stream which contains a sustainable, wild population of *S. fontinalis*, and a "fishless" stream will contain no populations of or individual *S. fontinalis*, though other smaller fish may be present.

CHAPTER 3: METHODS AND MATERIALS

Study sites

The two species of the experiment are common in headwater to third order streams of the Appalachians (Hulse et al., 2001). Streams were selected throughout western Pennsylvania. Little research has been done on *G. p. porphyriticus* larvae in Pennsylvania, (Hulse et al., 2001) as most studies done on this species were in the southeastern (Bruce, 1972, 1979, 1980; Bruce and Castanet, 2006; Resetarits, 1991, 1995) or northeastern (Lowe, 2003, 2005; Lowe and Bolger, 2002) United States. Streams were standardized by elevation, and water quality. Most Native Brook Trout cannot compete for food resources or space with *Oncorhynchus mykiss* and *Salmo trutta* (Karas, 1997). Years of data have been collected by agencies in Pennsylvania to determine native brook trout populations. The Pennsylvania Fish and Boat Commission classify these streams as "Class A Wild Trout Streams" or "Wilderness Trout Streams." Class A Wild Trout streams are "Streams that support a population of wild (natural reproduction) trout of sufficient size and abundance to support a long-term and rewarding sport fishery. The Commission does not stock these streams (Commonwealth of Pennsylvania, 2012)." Wilderness Trout Streams are "…designed to protect and promote native (brook trout) fisheries, the ecological requirements necessary for natural reproduction of trout and wilderness aesthetics

(Commonwealth of Pennsylvania, 2012)." The Wilderness Trout Streams are classified as having "Exceptional Value" of water or "High Quality Cold Water Fishes."

The actual criteria of Class A streams were determined in 1983 by the PAFBC (http://fishandboat.com/pafish/trout/trout_plan/history.pdf, accessed 11/2012) and are as follows:

a. Total brook trout biomass of at least 30 kg/ha (26.7 lbs/acre)
b. Total biomass of brook trout less than 15 cm (5.9 in.) total length of at least 0.1 kg/ha
c. Brook trout biomass must comprise at least 75% of total trout biomass

Gyrinophilus p. porphyriticus is a common species throughout its range, and throughout Pennsylvania (Hulse et al., 2001). Preliminary searches throughout several streams were conducted to determine *G. p. porphyriticus* presence. I first selected two streams containing brook trout. For the streams without trout, tributaries of these streams were used. The fishless streams were small enough in size, or ephemeral (surface water flowing only part of the year) so that trout cannot survive (Karas, 1997). Using the same watershed for the fish and fishless streams will help to eliminate some cofounding variables such as water quality and riparian vegetation.

Millwood Hollow Run (Figures 4 and 5)

This second order stream flows down the western slope of Chestnut Ridge in Derry Township, Westmoreland County. Though never officially named, the name of the valley this stream flows through is Millwood Hollow according to local knowledge. Under PAFBC data, the stream is referred to as an unnamed tributary to McGee Run and has natural brook trout reproduction. Brook trout of various size classes were caught by line and reel throughout this stream within a period of nine years (pers. Observation). The vegetation is mixed hardwood forest comprised mostly of beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*), black cherry (*Prunus serotina*), and white oak(*Quercus alba*). The land surrounding the stream is part of public-private partnership with the Pennsylvania Game Commission entitled “Trout Run Woods.” This partnership covers roughly 4,000 acres of land on Chestnut Ridge and is open to the public. An access road parallels the stream up and over the ridge with communication towers at the peak. Though signs prohibit motor vehicles, obvious signs and sightings of vehicles are numerously present. The stream flows from an elevation of 550 to 350 meters. The tributaries to the main stem of the stream used for the fishless streams would be considered first order and ephemeral in cases of extreme drought, though they continued to flow year round during my collection year.

Lick Island Run (Figure 6)

Lick Island Run is a second order stream located in Grove Township, Cameron County. It is classified as a “Class A” brook trout stream by the PAFBC. The stream flows down the eastern slope of a high plateau. Vegetation surrounding the stream comprised mostly of eastern hemlock (*Tsuga canadensis*), white oak (*Quercus alba*), and *Rhododendron* sp. The stream is located within Elk State forest, and has a gravel road adjacent to it with sporadic hunting/summer camps along the way. The stream runs through an elevation of 365 to 285 meters, though either side has considerable topographic relief up to 600 meters. The tributaries I planned to use for this stream became too dry to search for use of fishless streams, so a nearby stream was used as the fishless stream. Other tributaries where I could be certain brook trout were absent were too far upstream to be accessed regularly.

Laurel Draft

Laurel Draft is a first-order tributary to Wykoff Run in Grove Township, Cameron County. Both Laurel Draft and Lick Island Run eventually drain into the Driftwood Branch of the Sinnemahoning Creek watershed. Laurel Draft is also located within Elk State Forest and flows down the eastern side of a high plateau, with vegetation similar to Lick Island Run. The stream flows from elevation of 530 to 335 meters and is adjacent to a moderately used hiking trail.

Measurements

I measured streams for pH and water temperature weekly using an Oakton pHtestr 10 and thermometer, both of which influence the distribution of *G. p. porphyriticus* larvae, as they cannot tolerate high levels of acidity (Gian L. Rocco, unpublished; Lindley, unpublished). Plethodontids are sensitive to mortality at a pH less than 4.2 with larvae being even more sensitive than adults (Green and Peloquin, 2008).

Collection

I collected *G. p. porphyriticus* larvae using leaf litterbags (Pauley and Little, 1998). This is done by cutting two 70x70 cm squares of mesh netting (1.9 cm holes), placing one directly on top of the other and placing rocks in the middle of the square (Waldron et al., 2003). Then leaf litter from the stream bank is piled on top of the rocks, and the corners of the netting pulled together in the shape of a bag to be tied together with a cable tie (Waldron et al., 2003). I marked the bag with a bright colored red tape, and placed in the stream in trout habitat until this habitat is unsuitable in the fish streams (Waldron et al., 2003). A pool was designated as an area in the stream that is calm with low flow rate (Shaffer et al., 1994). Placing the bag in a pool area reduces the risk of losing the bag to high water events (personal obs.), but the bag will be tied to nearby vegetation using 0.9 kg test fishing line for reassurance. Also, placing the bag in a pool habitat greatly increases the chance that *S. fontinalis* will occur in the same habitat thus increasing the chance of interaction with *G. p. porphyriticus* larvae. *Gyrinophilus p. porphyriticus* larvae have been shown to be positively associated with riffles and pools and negatively associated with cascades and runs within a stream (Lowe, 2005). The bags were checked every week by swiftly removing them and shaking them into a plastic container for approximately 15 seconds (Waldron et al., 2003). Bags were replenished with fresh leaf litter approximately every 3 weeks. If nothing was found in the bags after several weeks, they were moved to another location. In areas where the stream was too small or has too little flow for a bag to be effective, a dipnet was used at night to collect salamander larvae. Salamander larvae moved into these bags at night to seek refuge from predators, as well as feed on the macroinvertebrates that may already be in the leaf litter, or use the bags as refuge in the same manner as the salamander larvae (Waldron et al., 2003). These bags have little impact on the stream environment and because the animals using the bag are not completely trapped they have the option of escaping through the holes; unwanted mortality should not be an issue. Though other organisms captured in the bags besides *G. p. porphyriticus* larvae were not collected, I identified and recorded them. The bags were checked during the day and a few times during night.

I also used my hands and a dipnet for collection. This method consisted of flipping cover objects (rocks, logs, leaves) and the swift retrieval of the salamanders before they could retreat to a subterranean lair, or be lost in a cloud of silt. The nets used were a Frabill 8" triangular dipnet, and a 5" kitchen strainer. Other equipment included kneepads and patience.

Euthanasia and Measurements

The salamanders collected were euthanized by immersion in MS-222 (buffered aqueous solution with sodium bicarbonate (Cornell, 2012) for 30 minutes (Gentz, 2007), and placed into 70% ethanol solution as recommended by the American Society of Ichthyologists and Herpetologists (2001). Fixation in formalin was unnecessary for my experiment, as it is commonly used to better preserve soft tissue.

Sample size for collection was (n=18) salamander larvae for fish streams, and (n=17) salamander larvae for fishless streams. A total of (n=35) for all streams. A larger sample size would be more ideal, but could be harmful to the populations in each stream. Previous studies (Bruce and Castanet, 2006) used a sample size of 20.

Snout-vent-length is the measurement of the salamander from the tip of the snout to the posterior end of the cloaca. Snout-vent-length is more accurate than total length (ToL) (i.e., measuring from the tip of the snout to the end of the tail)(Werner, 1971). Excluding the tail for the SVL measurement standardizes the length to account for tails being partly or wholly missing due to predation or other unknown causes. Although ToLs data were recorded. Both SVL and ToL data were measured after the specimen was euthanized to ensure accuracy. The *G. p. porphyriticus* larvae were taken back to the Herpetology Lab at Marshall University where further analysis ensued.

Aging

I used skeletochronology to age *G. p. porphyriticus* larvae. This process involves identifying growth marks in bones appearing "…during phases of active osteogenesis, separated by narrower layers that reflect reduced osteogenesis" (Bruce and Castanet, 2006). These narrow layers show slower, or arrested, bone growth also referred to as lines of arrested growth (LAGs) (Bruce and Castanet, 2006; Castanet et al., 1993). LAGs also appear the brightest under polarized light compared to other bone layers (Castanet et al., 1993). The area between LAGs is considered the "growth zone" comprised of relatively pale colored lamellar bone (Leclair et al., 2006). Each line after the hatching line, which the animal is born with, will be seen as one year (Bruce and Castanet, 2006). This method proves very useful on ectotherms of temperate regions due to their stages of slowed activity and growth during winter months (Bruce and Castanet, 2006). Because the *G. p. porphyriticus* larvae usually hatch in summer months, their collection in the summer months can be assumed to have the last LAG mean one full year (Bruce and Castanet, 2006).

Mark and recapture methods for a salamander this small in a stream habitat would not be as accurate as skeletochronology (Russell et al., 1996). An attempt to mark and recapture this species by a previous Marshall Graduate student resulted in only 1 recapture out of 80 marked individuals (Lindley, 1999 unpublished). Capturing and collecting larvae of size classes greater than hatchling size (>12.5mm) greatly increased the accuracy of preparing and sectioning femurs (Bruce, 1980; pers. Obs.).

Preparation

After the specimens were preserved, I removed the femur of each individual using a scalpel and Xacto^{TM} knife (Figures 7 and 8) and placed in 5% nitric acid for 6 h (Bruce and Castanet, 2006). Because the annuli are hypermineralized, this process of acidification demineralizes the area to be viewable (Castanet et al., 1993). The methods and numbers for demineralization were extremely variable throughout the literature according to author and species. Leclair et al. (2006) used the humerus of *Plethodon cinereus* and demineralized for 3 hours in 3% nitric acid then rinsed with tap water for 12 hours. Castanet et al. (1996) used 3% for 1 hour with *Desmognathine* larvae, and 2 hours for adult femurs. Castanet and Bruce (1996) used 3% nitric for 1 hour with an overnight rinse in tap water for several *Desmognathus ochrophaeus, D. quadramaculatus,* and *D. monticola* species. Miaud et al. (1993) washed phalanges of *Triturus cristatus* in distilled water for 1 hour, then demineralized in 3% nitric acid for 20 minutes and rinsed in running tap water for 12 hours.

I used methods outlined by Bruce and Castanet (2006) to age *G. p. porphyriticus* larvae. I originally demineralized several femurs in 1 ml of 5% nitric acid for 12 hours with the thought that extra would do no harm. After demineralization, I pipetted out the nitric acid and added 1 ml of phosphate buffered saline (PBS) solution, then subsequently added and removed 5 ml of

PBS two more times. I then stored the femur in 10 ml of 30% sucrose solution in a refrigerator at 4^{o} C. Femurs were left in the sucrose solution overnight before sectioning. After the 12 hour demineralization, I noticed the femurs were flimsy and lost any rigid bone structure. Many femurs broke in half at the diaphysis (mid-shaft) when I attempted to handle them with forceps. Because of this, I reduced the next set of femurs' demineralization time and concentration to 4 hours using 5% nitric acid. The femurs still seemed flimsy when handled, so I further reduced the time to 3 hours and concentration to 3% nitric acid. Larger specimens (SvL > 40mm) appeared to be rigid at 3 hours so were left in an extra hour. Those smaller than 40mm SvL were taken out at 3 hours.

Sectioning

I poured the entire contents of the 15 ml tube used to store the femur into a 50 ml beaker to avoid damage by dragging it out using forceps. I then placed the femur in a small petri dish with a few drops of sucrose solution for ease of handling by forceps. I then took the femur out of the petri dish and placed upright in a Tissue-Tek OCT Compound freezing medium on a stainless steel chuck. Another drop of the medium was then placed over the exposed femur and allowed to chill at -15 to -19^{o} C for approximately 10 minutes.

I then made cross-sections using a Leica CM 1950 cryostat by first trimming excess medium off at 60 um followed by sectioning at 15 um (Bruce and Castanet, 2006; Castanet et al., 1996). Twenty um sections were used for adult *Desmognathine* species (Castanet et al., 1996) and 24 um sections for adult *Plethodon cinereus* (Leclair et al., 2006), but as stated before the Bruce and Castanet (2006) study provided the best standard for this species and life stage. After sliced, the sections were placed on Fisherbrand Superfrost Plus Microscope Slides with a paintbrush and dried at room temperature overnight. Anywhere from 30-150 individual sections were sliced per femur. The ideal section of the femur for viewing LAGs is the diaphysis or long narrow mid shaft of the bone between the proximal trunk and distal condyles (Bruce and Castanet, 2006; Castanet et al., 1993, 1996). Due to the small femur sizes (no less than 3 mm), it became difficult to tell whether they were standing up straight in the medium and whether I trimmed off too much medium before sectioning. I used a conservative approach and sectioned as much as the bone as I could possibly see including both ends opposite the diaphysis. I stained the slides with Ehrlich's Hematoxylin for 20 minutes and air dried overnight (Bruce and Castanet, 2006). Staining times were also variable in the literature (Castanet et al., 1993, 1996; Bruce and Castanet, 2006; Leclair 2006). I analyzed the cross-sections using a Zeiss Primo Starr light microscope and photographs were taken using a Zeiss AxioVert 200 M.

Analysis

After the samples dried I viewed them under a microscope to observe LAGs. I attempted to eliminate personal bias by having the samples numbered in a random order. Castanet et al. (1993) suggest a "blind analysis" of LAG counting with no prior knowledge of the biology or ecology of the animal in question. Though I could eliminate some bias by not remembering the size of an individually numbered specimen, a completely "blind analysis" was impossible considering I collected and prepared the specimens myself.

Some specimen sections turned out better than others, and to be expected, I got better at sectioning as I went along. I treated each individual section independently and averaged the two viewings as the final count. One method to determine the number of LAGs is to have each author examine the specimen independently once, then again several weeks later and confer with one another (Bruce and Castanet, 2006; Castanet and Bruce, 1996; Castanet et al., 1996). If a deviation of greater or less than 1 LAG was found per specimen, the specimen was not used in analyses. I am the sole author for my experiment so this could not be done exactly. I allowed 1 week to 1 month between the examinations of my specimens in order to have two separate observations.

Because there was a possibility of larvae adding a second ring in a period of slowed growth in the summer months in cases of drought, I used historical weather data to determine this preliminary source of error. National Oceanic and Atmospheric Administration (NOAA) interactive archive data were used using Divisions 7 and 9 in Pennsylvania (southwestern and north-central Pennsylvania respectively). I used the following data parameters: June – August in the years 2009, 2010, 2011, and 2012, average precipitation for each three-month parameter (NOAA Time Series, Accessed June 2013). I used these years to allow for the most rings found in a larva of a fishless stream (3), because these streams could become completely dry while the fish streams have permanent hydrologic flow (pers. obs.). I found that Division 7 received an average precipitation change of 21.84mm above average for these parameters (NOAA Time Series). Division 9 received an average precipitation change of 40.39mm below average for these parameters (NOAA Time Series). This below average precipitation could not be considered drought. The closest value to be labeled a drought was 70.72mm below average precipitation for the three months I used (NRCC Cornell, 2013). The precipitation values presented allow me to assume that a period of drought did not exist in the collection year or the previous three years, and assume that one LAG means one year (Bruce and Castanet, 2006).

I pooled raw data into SVL and age for fish and fishless streams, and then presented as scatterplots. I performed a multiple regression model and an ANCOVA (analysis of covariance) using SAS Version 9.3 for statistical analysis. I used snout-vent-length as the response (dependent) variable. I averaged the two separate ring counts, and then used as the predictor (independent variable), i.e., average age. Average age was then log transformed (Welsh and Ollivier, 1998). I initially tested stream sites for significance before the fish vs. fishless variables were compared. The null hypotheses tested were ($\alpha = 0.05$): (1) There are no significant differences between sites. (2) There are no significant differences between fish and fishless streams. If no difference is found between sites, the first hypothesis will be rejected and a multiple regression model can be performed. If the slopes of the two scatterplots (fish vs. fishless) show a significant interaction, i.e., the difference in size between streams depends on the covariate (average age), a further ANCOVA is unnecessary (Engqvist, 2005). The multiple regression models alone will show whether there is a difference between the two groups.

CHAPTER 4: RESULTS

Collection

I captured 35 *G. p. porphyriticus* larvae during the field season (Table 1). Refugia bags yielded only two individual *G. p. porphyriticus* larvae caught from March 21 to October 14, 2012. One bag in the tributary to Millwood Hollow Run produced the only two individual *G. p.*

porphyriticus larvae on July 29 and August 5, 2012. Some larvae were caught in the bags in the main stem that were too small to identify by the naked eye (< 10mm).

Table 1. Raw data measurements for each specimen. ID refers to the stream name, MH = Millwood Hollow Run, LI = Lick Island Run, LD = Laurel Draft, F = Fish, L = Fishless. Rings 1 and 2 are the LAGs counted the first and second times, respectively.

ID	**SvL (mm)**	**ToL (mm)**	**Rings 1**	**Rings 2**
MHF01	17.75	29	1	1
MHF02	19.9	24.5	0	1
MHF03	22.1	36.4	1	1
MHF04	22	38.5	1	3
MHF05	19.41	29.1	2	1
MHF06	18.35	30.09	2	0
MHF07	27.7	50.04	1	0
MHL01	23.61	42.26	0	1
MHL02	31.69	52.49	2	1
MHL03	33.5	56.2	0	1
MHL04	40.1	67.15	2	2
MHL05	46.15	76.89	1	2
MHL06	40.05	60.3	1	2
MHL07	42.35	47	1	0
MHL08	29	39.71	3	3
MHL09	32.09	52	3	2
MHL10	36.09	51.25	1	1
MHL11	20.5	36.5	3	3

MHL12	50	81	2	2
MHL13	53.25	90.59	2	2
LIF01	19.9	33.5	0	0
LIF02	19.85	31	1	1
LIF03	19.1	34.9	1	1
LIF04	19.1	31.3	0	1
LIF05	54.15	90.1	4	4
LIF06	53.55	82.25	6	6
LIF07	45.15	75.45	4	4
LIF08	39.62	67.69	2	3
LIF09	59.13	94.6	4	5
LIF10	56.41	96.81	3	4
LIF11	9.19	13.92	0	0
LDL01	28.45	50.03	2	2
LDL02	34.83	58.01	2	1
LDL03	45.1	68.89	1	1
LDL04	41.49	68.85	1	1

Other salamander species of all age classes caught in the Millwood Hollow bags included *Desmognathus fuscus, D. monticola, D. ochrophaeus, Eurycea bislineata,* and *Pseudotriton r. ruber*. Invertebrates in the bags included dragonfly nymphs (Odonata), mayflies

(Ephemeroptera), stoneflies (Plecoptera), and crayfish (*Cambarus*). Crayfish were the most common animal caught in the bags, with salamanders the next most common. On only one occasion a bag contained both a salamander and a crayfish.

In Lick Island Run, I found similar results for the refugia bags. On only one occasion did I find a salamander in a bag, which was *E. bislineata*. Invertebrates in the bags included dragonfly nymphs (Odonata), mayflies (Ephemeroptera), stoneflies (Plecoptera), and crayfish (*Cambarus*). Again, crayfish were the most common animal found in the bag. No bags were placed in Laurel Draft.

Because the refugia bags produced so few *G. p. porphyriticus* larvae, the main method of capture was by hand and dipnet. Searches were opportunistic and consisted of walking upstream and flipping rocks within the stream and along the edge of the stream and bank. All *G. p. porphyriticus* larvae except for one individual were caught under rocks at the edge of the stream and stream bank, except for the tributary to Millwood Hollow Run.

Skeletochronology

Ages of specimens found in the fish streams ranged from 0 – 6 years, and 0 – 3 years in the fishless streams. Specimens observed that showed no obvious LAGs after the hatching line were aged as 0 years, and assumed to have hatched either the summer of the collection year (2012) or the previous summer (2011) (Bruce and Castanet, 2006).

Specimen LIF11 was measured at 9.11 mm SVL and 13.92 mm TOL, and was too small for the femur to be dissected and therefore too small for skeletochronology. This specimen was assumed to be a hatchling of the collection year (probably within weeks of collection) and measurements were consistent with historical hatchling data (Bruce, 1980; Hulse et al., 2001), so the age of this specimen was assumed 0 years.

Specimen MHL08 had either an injury or deformity to both its hind legs causing the femur to be too greatly reduced for use of aging. The left front leg was used in place for dissection of the humerus, a bone commonly used in other smaller plethodontid salamanders (Leclair et al., 2006).

Water Data

Table 2. Raw pH and temperatures taken at each study site.

	pH	Temp (^{o}C)
MHL	6.2	16
MHL	6.1	16
MHL	6.2	17
MHL	6.4	16
MHL	6.3	15.5
MHL	6.3	15
MHL	6.4	17
MHF	7	16.5
MHF	7.5	15
MHF	6.9	12
MHF	6.9	17
LIF	6.3	18

LIF	6.3	17
LIF	6.8	14
LIF	6.1	15.5
LIF	6.1	15
LIF	6.3	16
LIF	6.4	16
LDL	6.4	11
LDL	6.4	13
LDL	6.5	13.5

The mean pH for the fish streams was 6.60, and 6.32 for the fishless streams. Both of these values are near neutral (7) and slightly acidic. The difference in pH between fish and fishless streams was not significant (P = 0.0712) using an unpaired t-test. The mean temperature for fish streams was 15.37 and 15.00 for the fishless streams. The difference in temperature between fish and fishless streams was not significant (P = 0.4247) using an unpaired t-test.

Statisical Analysis

The size (SVL) of larvae collected ranged from 9.19 – 59.13 mm with an average of 33.45 mm (Table 1). The average SVL in the fish streams was 30.13 mm and 36.96 mm in the fishless streams, which fall within the range of larvae in Pennsylvania (Hulse et al., 2001). The average age for the fish streams was 2 years and 1.59 for the fishless streams. These averages were tested for normality and all found to be normal except for average age of the fish streams.

These results are probably occurred due to a small sample size and biased toward the extreme ends of young and old individuals (Figure 9).

The first null hypothesis was accepted, meaning there was no difference between the four stream sites (P = 0.4176), using a Type III sum of squares (Table 2). This meant that a multiple regression model could be performed for the factor, fish and fishless streams. A significant interaction term was found between slopes (P = 0.001), using a Type III sum of squares (Table 3), so an ANCOVA was not performed. This shows that there is a significant difference in slopes (size vs. age) between fish and fishless streams, rejecting the second null hypothesis (Figure 11).

Because an ANCOVA assumes homogeneity of slopes, the test for a difference in slopes must be performed first (Engqvist, 2005). This multiple regression model is a statistical analysis test alone, which shows a significant interaction between the slopes. This also shows that the difference between the groups, size (SVL), depends not only on the factor (fish or fishless stream), but the value of the covariate, age (Engqvist, 2005).

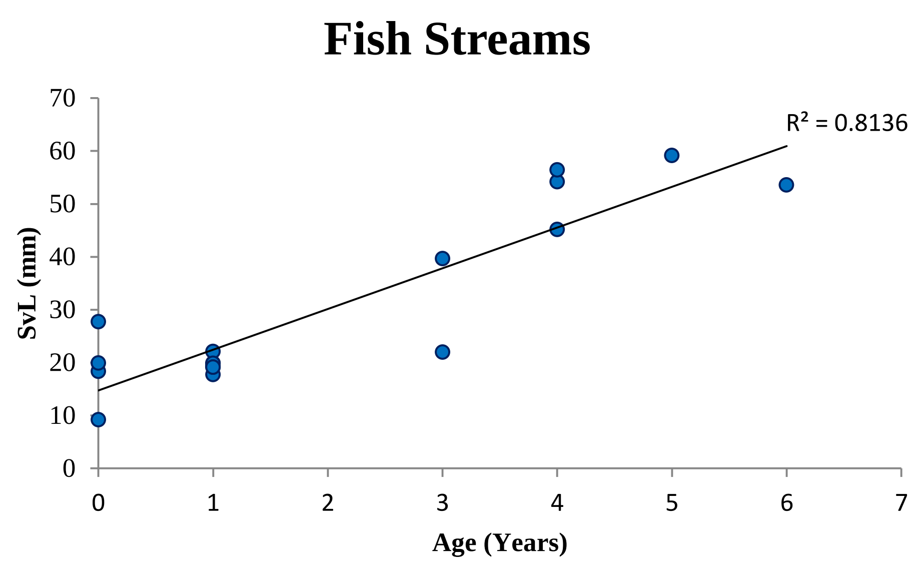


Figure 9. A scatterplot of age versus size for specimens collected in fish streams.

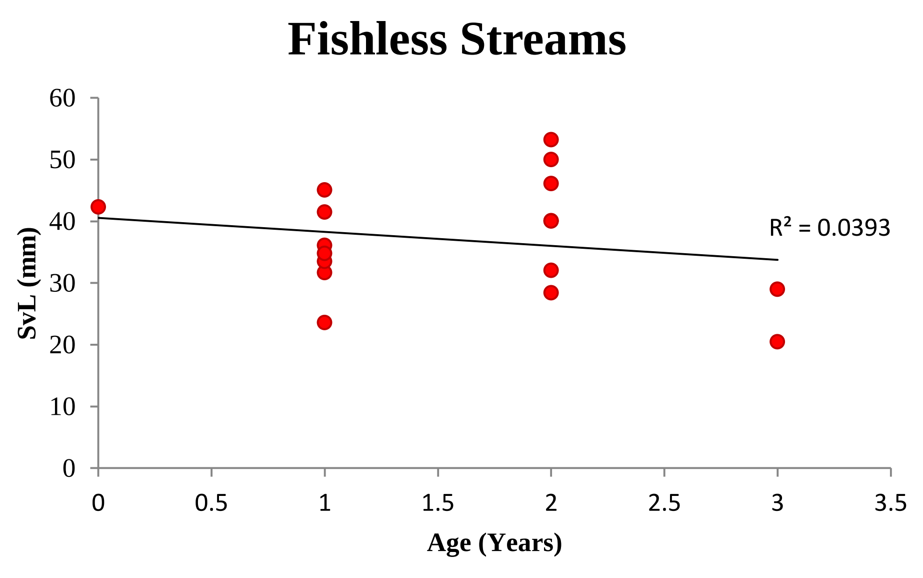


Figure 10. A scatterplot of age versus size for specimens collected in fishless

Source	DF	Type III SS	Mean Square	F Value	Pr > F

Fish	0	0.0000000	.	.	.
logave	1	561.0825152	561.0825152	6.88	0.0142
logave*Fish	1	711.9820353	711.9820353	8.73	0.0064
Site	**2**	**147.0917213**	**73.5458607**	**0.90**	**0.4176**

Table 3. Multiple regression model to test for differences between four stream sites.

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Fish	1	1002.413018	1002.413018	12.38	0.0015
logave	1	888.055109	888.055109	10.97	0.0025
logave*Fish	1	1094.122953	1094.122953	13.51	0.0010

Table 4. Multiple regression model examining differences between slopes of fish and fishless streams.

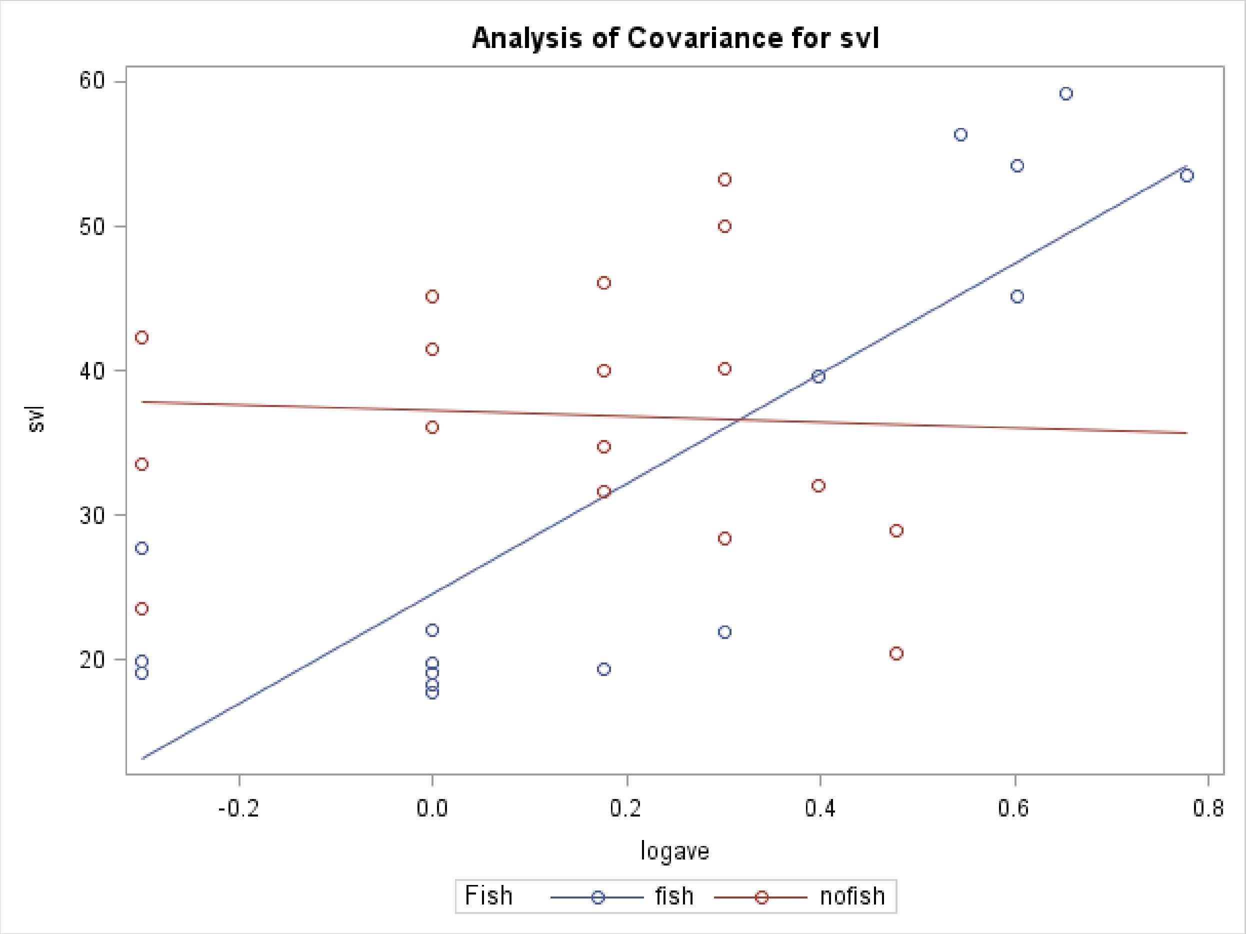


Figure 11. Multiple regression model showing slopes of lines for fish (blue) and fishless (red) streams. Age has been averaged for both ring counts and log transformed.

CHAPTER 5: DISCUSSION

I expected age vs. SVL for the fish streams to have a positive correlation, showing that as *G. p. porphyriticus* larvae increase in age, they increase in SVL at a relatively consistent rate, which they did (Figure 9). For the fishless streams, I expected age vs. SVL to not have a standard relationship (Figure 10). The results showed that there was a significant difference between the slopes of fish and fishless streams, with no significant difference between sites. Though the first null hypothesis was supported, my hypothesis that larvae in the fish stream would transform earlier was not supported. The oldest larva found (6 years) was in the fish stream, and no larvae found in the fishless streams exceeded 3 years (Figures 9 and 10). I based these data on a somewhat small sample size, so it is possible that there are larvae in the fishless streams that are older even though none was found.

There was no difference in temperature and pH (Table 2) between fish and fishless streams, so the effects of these factors in my experiment were not significant. Though temperature has shown to have an effect on metamorphosis relative to elevation or latitude (Hickerson et. al., 2005), my experiment used streams with similar elevation and did not test for these factors. Studies have shown that lower temperature as a result of higher elevation or latitude directly influences larval period and results in a longer larval period, or slower growth (Beachy, 1995; Hickerson et. al., 2005). But Bernardo and Reagan-Wallin (2002) showed that this hypothesis does not always hold true for plethodontids, owing to their lunglessness and fidelity to lotic habitats.

Growth in fish streams

As shown in Figure 9, larvae in the fish streams grew at a relatively consistent rate in comparison to the fishless streams (Figure 10). The negative impacts of the trout on *G. p. porphyriticus* are well documented (Lowe and Bolger 2002; Resetarits, 1991, 1995), but age at metamorphosis has not been shown, and has been suggested to be extremely variable (Bishop, 1941; Bruce, 1980; Bruce and Castanet, 2006). Reasons for this in *G. p. porphyriticus* and other plethodontids have been shown to be lower temperatures as a result of higher latitude or elevation (Adams and Beachy, 2001; Beachy, 1995; Hickerson et al., 2005), time at hatching (Beachy, 1995), and inter and intraspecific competition (Gustafson, 1994; Hileman and Brodie, 1994; Keen, 1982; Lowe, 2005; Ransom and Jaeger, 2008; Rudolf, 2008). But a study by Bernardo and Reagan-Wallin (2002) showed that plethodontids experience complex life histories compared to other salamanders, and temperature is not always a great predictor for growth rate. The older age, larger size, and consist growth of larvae in the fish stream could be attributed to the more consistent hydrology and fish presence of the larger streams. These factors combined with the increased competition and predation could influence a slower rate of development. Although selection should favor larvae that feed and develop faster (Beachy, 1995; Petranka et al., 1987), the increased activity and foraging behavior results in a serious risk of predation (Resetarits, 1995; Sih et al., 2003).

Rudolf (2006) showed in an experiment with two size classes of *G. p. porphyriticus* larvae and *E. cirrigera* larvae (a *G. p. porphyriticus* prey item) that large *G. p. porphyriticus* forced smaller conspecifics into different microhabitats, thus indirectly lowering their predation rates on *E. cirrigera.* These competitive effects could substantially affect the rate of growth and time at metamorphosis in this species by lowering activity levels, and have other varying effects when other species, such as another predator (Brook Trout), occupy a stream. Though the Rudolf (2006) study showed lower mortality of the prey species (*E. cirrigera*), increased growth did not occur. A similar study (Rudolf, 2008) showed an "apparent mutualism" between two stages of one salamander species larvae (*Pseudotriton ruber*) and a prey species (*E. cirrigera*). The study showed that the smaller predator species and prey species had mutually indirect positive interactions when in contact with a larger predator. This theory states that the strength of top-down trophic dynamics are reduced in stream communities with multiple sizes of animals. Another species with high density in the Lick Island Run (fish) study stream was the mottled sculpin (*Cottus bairdi*) (pers. obs.). This fish is of similar size to *G. p. porphyriticus*, as well as nocturnal and has shown to strongly utilize a similar riffle microhabitat and behave similarly (Freeman and Stouder, 1989; Lowe, 2005; Rashleigh and Grossman, 2005). *Cottus bairdi* showed size structure and density dependence similar to *G. p. porphyriticus* (Lowe, 2005; Rashleigh and Grossman, 2005), so it is possible a similar "apparent mutualism" may exist between these species.

Though strong evidence supports size structure in these communities, it has also been shown that gape-limited *S. fontinalis* incapable of consuming salamander larvae larger than themselves still negatively affect larvae growth (Currens et al., 2007; Resetarits, 1995). The voracious behavior of *S. fontinalis* has shown to be a significant factor in regulating the size and age variation in larval *G. p. porphyriticus* in my experiment, consistent with the results of others.

Growth in fishless streams

As predicted, the scatterplot of size versus age for the fishless streams does not represent a standard growth curve (Figure 10), as opposed to the significantly different fish streams (Figure 10). I hypothesized that size and age would vary greatly in the fishless streams, and these results represent a small sample (N=17) of such variation.

No larvae collected were aged over 3 years in fishless streams, as opposed to 6 years for the fish streams, though similarly sized larvae were found in both streams (Figures 9 and 10). The large size at younger ages could be explained by several factors. Due to their smaller size and less stable hydrology, the fishless streams have a greater chance of drying out and becoming unsuitable for larvae. This issue is probably not as significant as on other salamanders; owing to the morphology of *G. p. porphyriticus*, and their adaptability to subterranean habitats (Birchfield et al., 2000; Bruce, 1972). Their specialized morphology (smaller limbs, dorsolaterally flattened body) allows them to retreat underground and find enough water suitable for gas exchange and possibly for foraging activity. In this case, activity and growth can continue and increase.

Many studies have shown that amphibian larvae that inhabit ephemeral ponds have higher activity and develop faster than those in lotic streams (Petranka et al., 1987; Sih et al., 2003; Wells and Harris, 2001). It is theorized that selection is strongly favored toward those individuals that feed and develop fast (Petranka et al., 1987), and evidence shows anuran tadpoles can allocate energy to development due to various selective pressures (Mueller et al., 2012). In the absence of *S. fontinalis* predation, *G. p. porphyriticus* should have the freedom to be active longer, feed more, and develop faster in order to metamorphose to a more semi-aquatic adult stage (Resetarits, 1995).

This absence of *S. fontinalis* may also increase the effects of intraspecific interactions. In the presence of large *G. p. porphyriticus*, smaller conspecifics decreased their activity levels, growth, and changed their use of microhabitats (Rudolf, 2006). Complex intraspecific interactions have been well documented among plethodontids including courtship (Houck, 1988), territorial defense (Keen and Reed, 1985; Mathis, 1990), cannibalism (Rudolf, 2008), and competition (Gustafson, 1994; Lowe, 2005). In general, plethodontids have been shown to have complex life histories (Bernardo and Reagan-Wallin, 2002; Bruce, 2005). *Gyrinophilus p. porphyriticus* larvae together in large densities and different size cohorts in fishless streams could face selective pressure strong enough to force them to metamorphose faster than in fish streams. Larvae of *G. p. porphyriticus* have habitats independent from adults (Lowe, 2005), so metamorphosis to the adult stage could also reduce intraspecific competition and cannibalism (Eitam et al., 2005).

Conclusions

My study showed that there was a significant difference between the size and age of *G. p. porphyriticus* larvae in streams with brook trout versus streams without brook trout. The factors influencing these variations could be solely due to the presence/absence of the trout predator or with other factors not tested in my study.

Consistent and more delayed growth of *G. p. porphyriticus* larvae were found in the fish streams in contrast with highly variable growth in the fishless streams. The consistency of predation or the threat of predation in the fish streams forces *G. p. porphyriticus* larvae further down the trophic food web of the stream. It is my conclusion that predation alone limits larval activity, which reduces growth and development until metamorphosis.

In the absence of *S. fontinalis*, *G. p. porphyriticus* larvae are the top predator in these small headwater streams (Lowe, 2003). This shift in trophic dynamics changes in the variation in size and age from risk of predation to several other factors including hydrology, competition, water quality, and microhabitat use. I conclude that one or more of these factors, as a sole or additive affect, strongly influence size and age variation depending on the stream itself or the microhabitats within a stream. In my study, I believe that intraspecific competition drove growth and development to occur faster than the fishless streams rather than hydrology or water quality. I base this on the non-significant difference in pH and temperature between fish and fishless streams and the annual precipitation in the years these larvae were studied.

Limitations

One potential weakness of this experiment is the low sample size (n=35). More samples would be ideal to drive statistical power, but as stated previously I tried to keep conservation of the species in mind. Another weakness arises in skeletochronology. Bruce and Castanet (2006) found a large variation in LAGs for *G. p. porphyriticus* larvae attributed possibly to this species being more active during winter months than other amphibians due to their benthic nature. My hope is that this variation was not as problematic because where Bruce and Castanet's study was done in the Southern Blue Ridge Mountains (North Carolina, South Carolina, Georgia border), my was done in western Pennsylvania. This change in latitude reflects a colder climate in

Pennsylvania, which could mean *G. p. porphyriticus* larvae are more prone to slower activity and growth in this climate.

The refugia bags present another potential weakness. Salamander larvae living in habitats not in direct interaction with *S. fontinalis* could travel to and occupy the bags. The downside to this method is that I will not know exactly where the larvae came from as opposed to capturing them in direct observation with the trout.

Other species coexist with *G. p. porphyriticus* larvae besides *S. fontinalis* that could negatively influence their growth. Several smaller fish species occur in these streams such as sculpins and darters (Commonwealth of Pennsylvania, 2012), but are probably too small to act as predators, as nothing has been found in the literature to denote otherwise. But even if they do not prey on *G. p. porphyriticus* larvae, competition for habitat and prey must surely exist. To test this variable, these species would have to be captured [probably using electroshocking method (Barbour et al., 1999)] and analyzed for stomach contents.

Experimental streams could also be a useful method, as used by Resetarits (1991, 1995) for studying *G. p. porphyriticus* larvae. Experimental streams could control for climactic factors such as stream drying, and better standardize food and spatial resources as well as observe interspecific interactions. Other possible terrestrial predators could be mammals such as raccoons and opossums, and several species of birds. Aquatic feeding birds such as the Great Blue Heron (*Ardea herodias*) and the Belted Kingfisher (*Megaceryle alcyon*) are known to occur within these streams, though I have seen neither (pers. obs.) in any of the study sites. These birds usually feed in larger streams and rivers rather than smaller headwater streams for which I used (Haas and Burrows, 2005). Though they still may occur, the potential of these birds having a high influence on *G. p. porphyriticus* larvae should be low due to their opposing activity periods (diurnal vs. nocturnal). This leaves nocturnal terrestrial mammals as a question, and a difficult one to answer. A way to test this could be the capture and stomach content analysis of said mammals, or the use of trail cameras.

The knowledge of *S. fontinalis* actually being native to the fish streams being studied also comes into question. Pennsylvania has a long history of deforestation throughout the state that decimated several historic native populations of *S. fontinalis* (Karas, 1997). Whether enough native populations survived or recolonization by stocked Brook Trout occurred in the streams being studied is difficult to know. For this study, it will be assumed that the trout are native, or occurred in that stream for a long enough period of time for *G. p. porphyriticus* larvae to be influenced by them. If the trout were there before an area was logged or affected since Pennsylvania was colonized, and are there now, this gap in time when they possibly were not there should not have that great of an influence on the trout's current interactions with *G. p. porphyriticus* larvae.

Significance

My experiment shows that larvae in fishless regions have a more variable larval period, and variable size, than those where *S. fontinalis* occur. Most salamander studies of this species occur in the southeast United States where salamanders hibernate for shorter periods or not at all (Bruce, 1972, 1980; Resetarits, 1991, 1995). The results of this study will add to the greater understanding of a species that ranges from the southeast, to the northeast, where the ecology of such an ectotherm could change dramatically. A species occupying such a great range should be fully understood across this entire range. Effects of logging, acid mine drainage, and acid precipitation may alter this species morphology as well. Evidence of climate change has already shown to have a negative influence on *G. p. porphyriticus* (Lowe, 2012). This study may set a baseline for these effects if water quality is found to have a significant influence on size and age of *G. p. porphyriticus* larvae.

The unique physiology of this salamander and its possible ability to delay metamorphosis provides interesting questions. It has already been questioned that this species may exhibit a form of paedogenesis or neoteny (Bruce, 1978). Future studies may look to this experiment for a baseline into these questions.

Because salamanders are long-lived and have a philopatric nature, that is occupying and reproducing in the same area through generations, they serve as an indicator for the health of the ecosystem they inhabit (Crawford and Semlitsch, 2007; Johnson et al., 2009; Lowe and Bolger, 2004; Peterman and Semlitsch, 2009; Welsh and Ollivier, 1998). This could even be truer for a species such as *G. p. porphyriticus*, as their larval stage could last several years, as shown by my study. The salamander's porous skin and sensitivity to heavy metals and other pollutants reflects the quality of the stream it occupies, especially a species that has the ability to live several years in a stream (Hulse et al., 2001; Lowe and Bolger, 2002).

The results of these data will help close a simple ecological gap in the natural history of the larval stage of this common salamander species. The information presented applies to not only other Appalachian stream-dwelling salamanders, but species across the globe. Amphibians have shown to be indicator species internationally due to their porous skin and life history (Lowe & Bolger, 2002), and those who represent a long aquatic life stage represent this theory with greater magnitude. Because the native brook trout is the state fish of Pennsylvania, state agencies will always keep a close eye on its abundance. *Salvelinus fontinalis* is also a popular game species in the state as well as other states in its range so its ecology is relevant not only to the scientific community, but the fishing and gaming community. Managing the streams for which the brook trout occur usually begins with assessing the stream for benthic macroinvertebrates (Barbour et al., 1999). I propose that salamander larvae including that of *G. p. porphyriticus* exhibit the same importance in streams and should be looked at closer and included in these bioassessments. But before these amphibians can be included in assessing streams, their complete life history must be understood. Finding a *G. p. porphyriticus* larva in a stream could be meaningless if nothing is known about its size relative to its age.

Literature Cited

Adams, D.C., and C.K. Beachy. 2001. Historical explanations of phenotypic variation in the plethodontid salamander *Gyrinophilus porphyriticus. Herpetologica* 57(3): 353-364.

American Society of Ichthyologists and Herpetologists. 2001. Guidelines for use of live amphibians and reptiles in field research. Web accessed 8 February 2012. http://iacuc.ucsd.edu/pdf_references/

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Beachy, C. K. 1995. Effects of larval growth history on metamorphosis in a stream-dwelling salamander (*Desmognathus ochrophaeus*). *Journal of Herpetology.* 29(3): 375-382.

Bernardo, J., and N. L. Reagan-Wallin. 2002. Plethodontid salamanders do not conform to "general rules" for ectotherm life histories: insights from allocation models about why simple models do not make accurate predictions. *Oikos.* 97: 398-414.

Birchfield, G.L., and R.C. Bruce. 2000. Morphometric variation among larvae of four species of lungless salamanders (Caudata: Plethodontidae). *Herpetologica* 56(3): 332-342.

Bishop, S. C. 1941. The salamanders of New York. New York State Mus. Bull. 324: 1-365.

Bruce, R. C. 1972. Variation in the life cycle of the salamander *Gyrinophilus porphyriticus*. *Herpetologica* 28(3): 230-244.

Bruce, R. C. 1979. Evolution of paedomorphosis in salamanders of the genus *Gyrinophilus. Evolultion* 33(3): 998-1000.

Bruce, R.C. 1980. A model of the larval period of the spring salamander, *Gyrinophilus porphyriticus*, based on size-frequency distributions. *Herpetologica* 36:78-86.

Bruce, R. C. 2003. Ecological distribution of the salamanders *Gyrinophilus* and *Pseudotriton* in a southern Appalachian watershed. *Herpetologica.* 59(3): 301-310.

Bruce, R. C. 2005. Theory of complex life cycles: application in plethodontid salamanders. *Herpetological Monographs.* 19(1): 180-207.

Bruce, R. C., and J. Castanet. 2006. Application of Skeletochronology in Aging Larvae of the Salamanders *Gyrinophilus porphyriticus* and *Pseudotriton ruber. Journal of Herpetology* 40(1): 85-90.

Burton, T. M. 1976. An analysis of the feeding ecology of the salamanders (Amphibia, Urodela) of the Hubbard Brook Experimental Forest, New Hampshire. *Journal of Herpetology.* 10: 187-204.

Castanet, J., Francillon-Vieillot, H., and Bruce, R.C. 1996. Age estimation in *Desmognathine* salamanders assessed by skeletochronology. *Herpetologica* 52(2): 160-171.

Castanet, J., Francillon-Vieillot, H., Meunier, F. J., and Ricqles, A. DE. 1993. Bone and individual aging. *Bone* 7(9): 245-283.

Commonwealth of Pennsylvania. 2012. Pennsylvania Fish and Boat Commission. www.fish.state.pa.us Accessed April 2, 2012.

Cope, E. D. 1869. A review of the species of the Plethodontidae and Desmognathidae. *Proceedings of the Academy of Natural Sciences of Philadelphia.* 21: 93-118.

Cornell University IACUC. 2012. ACUP 110.01 Fish and Amphibian Anesthesia

Crawford, J. A., and Semlitsch, R. D. 2008. Abiotic factors influencing abundance and microhabitat use of stream salamanders in southern Appalachian forests. *Forest Ecology and Management* 255(5-6): 1841-1847.

Currens, C. R., W. J. Liss, and R. L. Hoffman. 2007. Impacts of a gape limited brook trout, *Salvelinus fontinalis*, on larval northwestern salamander, *Ambystoma gracile*, growth: a field enclosure experiment. *Journal of Herpetology*. 41(2): 321-324.

Dunn,E. R. 1926. The salamanders of the family PlethodontidaeNorthampton, MA: Smith College.

Eddy, S., and Underhill, J. C. 1974. Northern Fishes. University of Minnesota Press, Minneapolis. pp. 382-393.

Eitam, A., L. Blaustein, and M. Mangel. 2005. Density and intercohort priority effects on larval *Salamandra salamandra* in temporary ponds. *Oecologia.* 146: 36-42.

Engqvist, L. 2005. The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Animal Behaviour.* 70: 967-971.

Freeman, M.C., and Stouder, D.J. 1989. Intraspecific interactions influence size specific depth distribution in *Cottus bairdi*. *Environmental Biology of Fishes.* 24(3): 231-236.

Gentz, Edward J. 2007. Medicine and Surgery of Amphibians. *ILAR Journal*. 48(3):255-259

Gibbons, W. G. 1983. Their blood runs cold: adventures with reptiles and amphibians. The University of Alabama Press. University, Alabama: 14.

Green, L. E. and J. E. Peloquin. 2008. Acute toxicity of acidity in larvae and adults of four stream salamander species (Plethodontidae). *Environmental Toxicology and Chemistry.* 27(11): 2361-2367.

Grobman, A. B. The anterior cranial elements of the salamanders *Pseudotriton* and *Gyrinophilus*. *Copeia* 1959(1): 60-63.

Gustafson, M. P. 1994. Size-specific interactions among larvae of the plethodontid salamanders *Gyrinophilus porphyriticus* and *Eurycea cirrigera*. *Journal of Herpetology*. 28(4): 470-476.

Haas, F., and Burrows, R. 2005. Birds of Pennsylvania. Lone Pine Publishing International.

Hickerson, C. M., E. L. Barker, and C. K. Beachy. 2005. Determinants of metamorphic timing in the black-bellied salamander, *Desmognathus quadramaculatus. Southeastern Naturalist.* 4(1): 33-50.

Hileman, K. S. and E. D. Brodie, Jr. 1994. Survival strategies of the salamander *Desmognathus ochrophaeus:* interaction of predator-avoidance and anti-predator mechanisms. *Animal Behaviour.* 47: 1-6.

Hulse, A.C., McCoy, J.C., and Censky, E. 2001. Amphibians and Reptiles of Pennsylvania and the Northeast. Cornell University Press: 93-96.

Huntsman, B. M., M. P. Venarsky, J. P. Benstead, and A. D. Huryn. 2011. Effects of organic matter availability on the life history and production of a top vertebrate predator (Plethodontidae: *Gyrinophilus palleucus*) in two cave streams. *Freshwater Biology*. 56: 1746-1760.

Hynes, H.B.N. 1970. The Ecology of Running Waters. Liverpool University Press.

in stream habitats. *Banisteria* 12:32-36.

Johnson, B. R. and J. B. Wallace. 2005. Bottom-up limitation of a stream salamander in a detritus-based food web. *Canadian Journal of Fisheries and Aquatic Sciences.* 62: 301-311.

Johnson, B. R., K. M. Fritz, K. A. Blocksom and D. M. Walters. 2009. Larval salamanders and channel geomorphology are indicators of hydrologic permanence in forested headwater streams. *Ecological Indicators.* 9: 150-159.

Karas, N. 1997. Brook Trout: A thorough look at North America's great native trout – its history, biology, and angling possibilities. The Lyons Press. Guilford, Connecticut.

Keen, W. H. 1982. Habitat selection and interspecific competition in two species of plethodontid salamanders. *Ecology.* 63(1): 94-102.

Lannoo, M.J. (Ed.), 2005. Amphibian Declines: The Conservation Status of United States Species. University of California Press, Berkeley, CA.

Leclair, M. H., Levasseur, M., and Leclair, R. Jr. 2006. Life history traits of *Plethodon cinereus* in the northern parts of its range: Variations in population structure, age, and growth. *Herpetologica* 62(3): 265-282.

Lewis, R. L. 1998. Transforming the Appalachian countryside: railroads, deforestation, and social change in West Virginia, 1880-1920. University of North Carolina Press.

Lowe, W.H. 2003. Linking dispersal to local population dynamics: a case study using a headwater salamander system. *Ecology* 84(8):2145-2154

Lowe, W.H. 2005. Factors affecting stage-specific distribution in the stream salamander *Gyrinophilus porphyriticus. Herpetologica.* 61(2): 135-144.

Lowe, W.H. 2012. Climate change is linked to long-term decline in a stream salamander. *Biological Conservation.* 145: 48-53.

Lowe, W.H. K. H. Nislow, D. T. Bolger. 2004. Stage-specific and interactive effects of sedimentation and trout on a headwater stream salamander. *Ecological Applications* 14(1): 164-172.

Lowe, W.H., and Bolger, D.T. 2002. Local and Landscape-Scale Predictors of Salamander Abundance in New Hampshire Headwater Streams. *Conservation Biology*. 16:183-193. methods of estimation. *Can. J. Zool*. 74: 397 —412.

Martof, B.S., and F.L. Rose. 1962. The comparative osteology of the anterior cranial elements of the salamanders *Gyrinophilus* and *Pseudotriton*. *Copeia* 1962(4): 727-732.

Miaud, C., and P. Joly, J. Castanet. 1993. Variation in age structures in a subdivided population of *Triturus cristatus. Canadian Journal of Zoology* 71(9): 1874-1879.

Milanovich, J.R., Peterman, W.E., Nibbelink, N.P., Maerz, J.C., 2010. Projected loss of a salamander diversity hotspot as a consequence of projected global climate change. Plos One 5, 1–10.

Mueller, C. A., S. Augustine, S. A.L.M. Kooijman, M. R. Kearney and R. S. Seymour. 2012. The trade-off between maturation and growth during accelerated development in frogs. *Comparative Biochemistry and Physiology.* Part A 163: 95-102.

NOAA (National Oceanic and Atmospheric Administration). National Climactic Data Center. Time Series. http://www.ncdc.noaa.gov/cag/time-series/us. Accessed June 2013.

NRCC (Northeast Regional Climate Center). Pennsylvania Drought Periods. http://www.nrcc.cornell.edu/drought/PA_drought_periods.html. Accessed July 2013.

Pauley, T. K., and M. Little. 1998. A new technique to monitor larval and juvenile salamanders. Banisteria 12: 32-36.

Peterman, W. E. and R. D. Semlitsch. 2009. Efficacy of riparian buffers in mitigating local population declines and the effects of even-aged timber harvest on larval salamanders. *Forest Ecology and Management.* 257: 8-14.

Petranka, J. W., L. B. Kats and A. Sih. 1987. Predator-prey interactions among fish and larval amphibians: use of chemical cues to detect predatory fish. *Animal Behaviour.* 35: 426-434.

Petranka, J. W., and C. K. Smith. 2005. A functional analysis of streamside habitat use by southern Appalachian salamanders: implications for riparian forest management. *Forest Ecology and Management.* 210: 443-454.

Ransom, T. S., and R. G. Jaeger. 2008. Intergeneric salamander interactions across an ecotone. *Herpetologica.* 64(1): 20-31.

Rashleigh, B. and G. D. Grossman. 2005. An individual-based simulation model for mottled sculpin (*Cottus bairdi*) in a southern Appalachian stream. *Ecological Modeling.* 187: 247-258.

Resetarits, W.J. Jr. 1991. Ecological interactions among predators in experimental stream communities. *Ecology*. 72(5):1782-1793.

Resetarits, W.J. Jr. 1995. Competitive asymmetry and coexistence in size-structured populations of brook trout and spring salamanders. *Oikos*. 73(2):188-198.

Rohr, J. R., A. A. Elskus, B. S. Shepherd, P. H. Crowley, T. M. McCarthy, J. H. Niedzwiecki, Ty. Sager, A. Sih and B. D. Palmer. 2004. Multiple stressors and salamanders: effects of an herbicide, food limitation, and hydroperiod. *Ecological Applications* , Vol. 14, No. 4 (Aug., 2004), pp. 1028-1040

Rudolf, V. H. 2006. The influence of size-specific indirect interactions in predator-prey systems. *Ecology*. 87(2): 362-371.

Rudolf, V. H. 2008. Impact of cannibalism on predator-prey dynamics: size-structured interactions and apparent mutualism. *Ecology* 89(6): 1650-1660.

Russell, P. Anthony, Powell, Lawrence G., Hall, and Dean R. 1996. Growth and age of Alberta

Semlitsch, R. D., and Wilbur, H. M. 1988. Effects of pond drying time on metamorphosis and survival in the salamander *Ambystoma talpoideum*. *Copeia* 4:978-983.

Shaffer, H. B., Alford, R. A., Woodward, B. D., Richards, S. J., Altig, R. G., and Gascon, C. 1994. Quantitative sampling of amphibian larvae. In: Measuring and Monitoring Biological Diversity. Standard Methods for Amphibians. Washington – London: 130-141.

Sih, A., L. B. Kats and E. F. Maurer. 2003. Behavioural correlations across situations and the evolution of antipredator behavior in a sunfish-salamander system. *Animal Behaviour.* 65: 29-44.

Vannote R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell, and C.E. Cushing: 1980. The River Continuum Concept. *Canadian Journal of Fisheries and Aquatic Sciences.* 37(1): 130-137.

Waldron, J. L., Dodd, C. K. Jr., and Corser, J. D. 2003. Leaf litterbags: Factors affecting capture of stream-dwelling salamanders. *Applied Herpetology* 1:23-36.

Wells, C. S., and R. N. Harris. 2001. Activity level and the tradeoff between growth and survival in the salamanders *Ambystoma jeffersonianum* and *Hemidactylium scutatum. Herpetologica.* 57(1): 116-127.

Welsh, H. H., and L. M. Ollivier. 1998. Stream amphibians as indicators of ecosystem stress: a case study from California's redwoods. *Ecological Applications* 8(4): 1118-1132.

Werner, Y. L. 1971. Some suggestions for the standard expression of measurements. *Systematic Zoology*. 20(2): 249-252.

Wilder, I. W. & Dunn, E. R. 1920. The correlation of lung-lessness in salamanders with a mountain brook habitat. *Copeia* 1920: 63–68.

Figure 1: ***Gyrinophilus p. porphryiticus.*** A typical larva.

Figure 2: ***Gyrinophilus. p. porphyriticus.*** Note the external gills, canthus rostralis, and flattened, shovel-shaped head.

Figure 3: ***Salvelinus fontinalis.*** An average sized native brook trout, caught with a hook and line in Millwood Hollow Run.

Figure 4: Millwood Hollow Run. The main stream section occupied by brook trout.

Figure 5: Millwood Hollow Run. The fishless tributary where the majority of larvae were collected.

Figure 6: Lick Island Run. The main stem occupied by brook trout. Though this picture shows large boulders and cascades, the stream is highly variable in width, depth, and velocity.

Figure 7: Dissected hind leg. A larva's leg removed and ready to have the femur dissected.

Figure 8: Femur. Two separate femurs from two individual larvae. Femurs were placed on a penny to show scale.

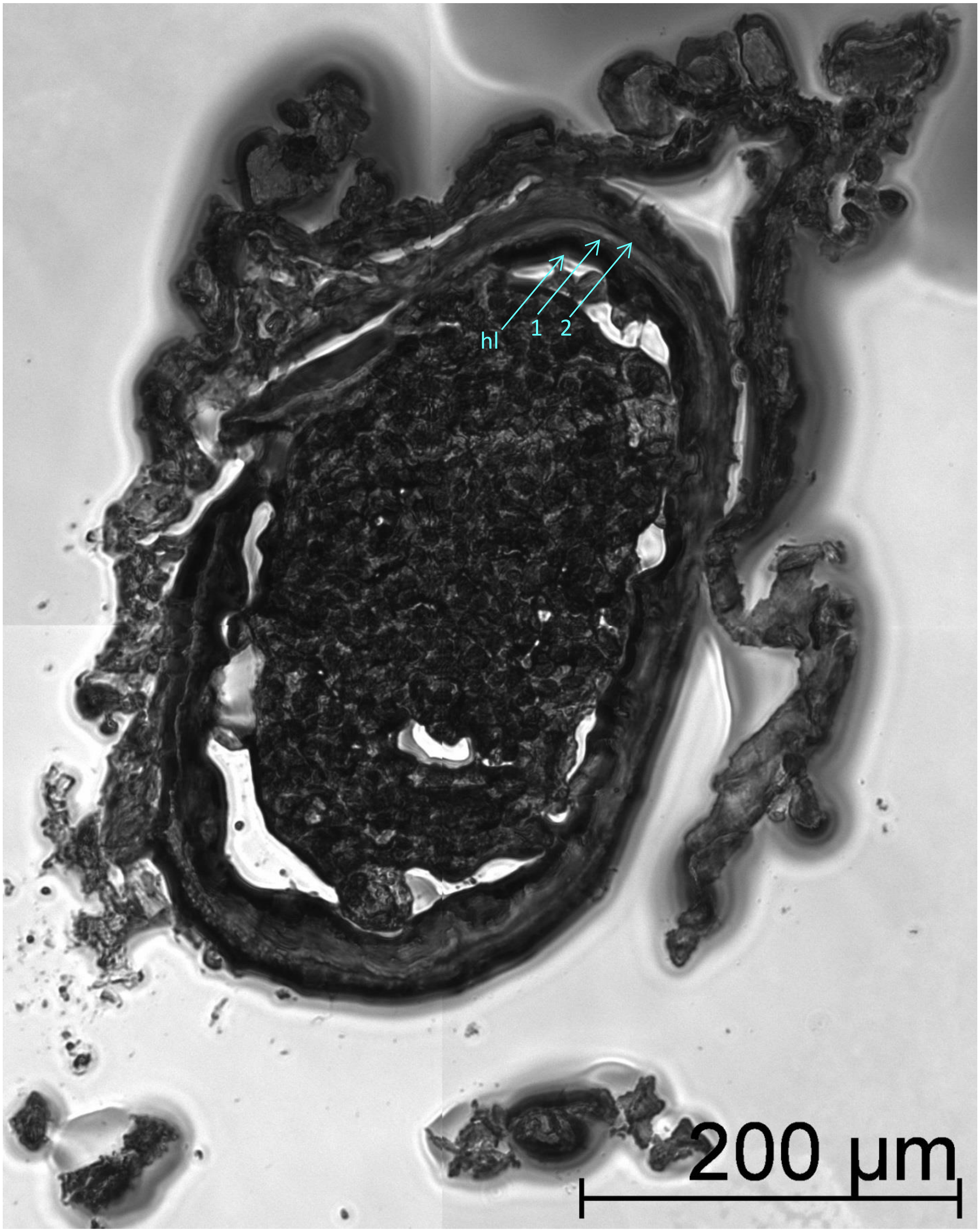


Figure 12: Cross section. The sectioned femur of larva MHL04. 2 rings are present. 40x.

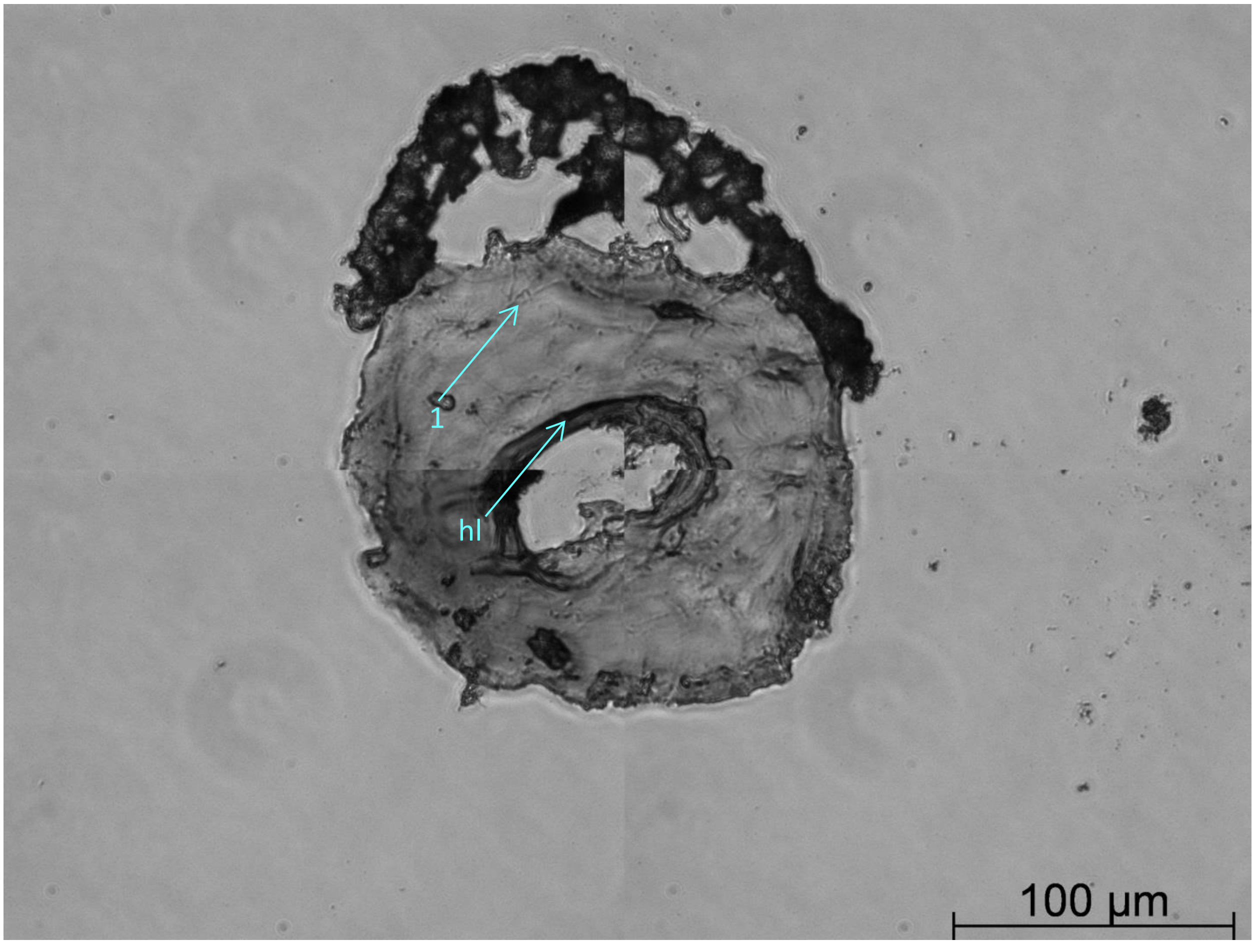
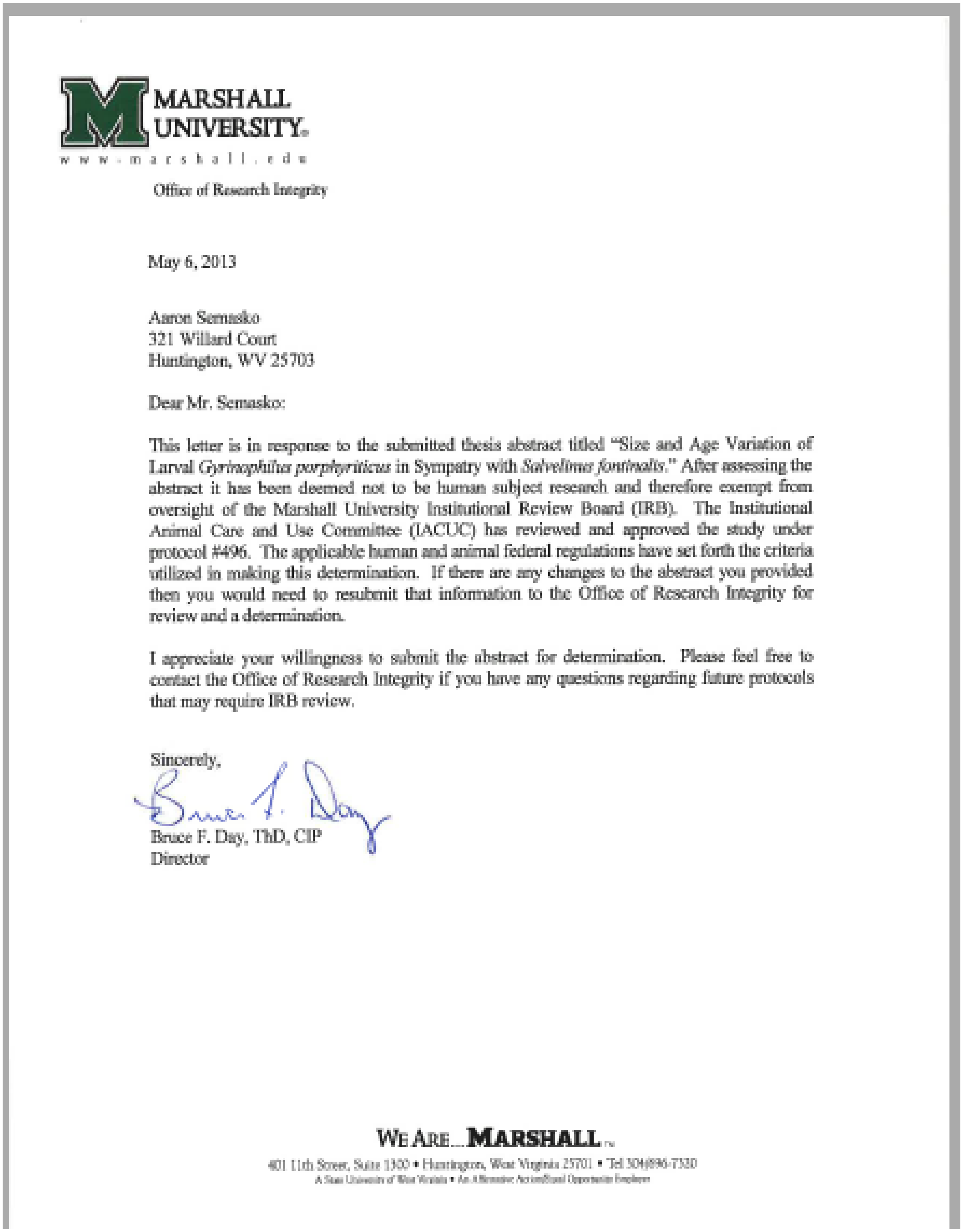


Figure 13: Cross section. Sample LIF02. One ring is present, with the hatching line clearly seen. 40x.

Figure 14: ***Salvelinus fontinalis.*** Me holding a large native brook trout caught with a hook and line.

IRB LETTER



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May 6, 2013

Aaron Semasko
321 Willard Court
Huntington, WV 25703

Dear Mr. Semasko:

This letter is in response to the submitted thesis abstract titled "Size and Age Variation of Larval *Gyrinophilus porphyriticus* in Sympatry with *Salvelinus fontinalis*." After assessing the abstract it has been deemed not to be human subject research and therefore exempt from oversight of the Marshall University Institutional Review Board (IRB). The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the study under protocol #496. The applicable human and animal federal regulations have set forth the criteria utilized in making this determination. If there are any changes to the abstract you provided then you would need to resubmit that information to the Office of Research Integrity for review and a determination.

I appreciate your willingness to submit the abstract for determination. Please feel free to contact the Office of Research Integrity if you have any questions regarding future protocols that may require IRB review.

Sincerely,

Bruce F. Day

Bruce F. Day, ThD, CIP
Director

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