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The effects of soil acidity on the age structure and age at sexual maturity of eastern red-backed salamanders (*Plethodon cinereus*) in hardwood forests of New Hampshire and Vermont

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ABSTRACT

Acidic deposition resulting from emissions of sulfur and nitrogen has negatively impacted the hardwood forests of the northeastern United States, causing depletion of key nutrients such as calcium and chronic acidification of forest soil habitats. Strongly acidic habitats (pH < 3.5) have long been considered lethal to eastern red-backed salamanders (*Plethodon cinereus*), but recent studies found that *P. cinereus* were abundant in hardwood forests with soil pH as low as 2.7 – a condition resulting from anthropogenic acid inputs. Although abundance of *P. cinereus* does not appear to be constrained by soil pH, I hypothesized that very acidic habitats would negatively impact the demographics of *P. cinereus* populations, including age distribution, growth rates, and age at sexual maturity. I analyzed demographic parameters of extant *P. cinereus* populations that were sampled in 2012 at four hardwood forests in NH and VT (USA) that ranged in soil/forest floor pH from 2.7 – 3.7. I determined the age of each *P. cinereus* using skeletochronology techniques to estimate population age structure, estimated growth curves for each population using the von Bertalanffy equation and Chapman's method, and evaluated mean age at sexual maturity for each population. Overall, soil pH did not appear to strongly affect *P. cinereus* populations. However, the most acidic site (pH 2.7) had a greater proportion of juveniles to adults, suggesting that fewer juveniles survive to adulthood at soil pH < 3.0. The mean age of sexually mature individuals was significantly higher at the most acidic site compared to least acidic site, but was not significantly different from the sites with intermediate pH sites. My results suggest that it is possible that *P. cinereus* populations have locally adapted to very acidic soils, but that demographic differences may reveal sensitivity of populations to this stressor. Further study of habitat pH and *P. cinereus* is warranted because these salamanders comprise a large portion of forest faunal biomass and play an key ecological role in nutrient cycling

TABLE OF CONTENTS

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INTRODUCTION

Many organisms that are sensitive to low pH levels are stressed by acid deposition, which is the transfer of air pollutant emissions of nitrogen oxides, sulfur dioxide, and ammonia to the Earth's surface as strong acids (Driscoll et al. 2001). These air pollutants contribute to the formation acid rain, which contains high concentrations of sulfuric and nitric acids (Schindler 1988). In areas of high rainfall, base cations, such as calcium, magnesium, sodium, and potassium are leached, and are unable to neutralize these acids (Ball 1999, Hamburg et al. 2003). Acid deposition has altered terrestrial and aquatic landscapes in several countries, including the United States of America, where the first indication of acid rain was at the Hubbard Brook Experimental Forest (HBEF) in the White Mountains of New Hampshire (Driscoll et al. 2001).

The Clean Air Act Amendment in 1970 led to a reduction in SO_x emissions, but hasn't reduced the acidity of rain in eastern North America (DeHayes et al. 1999, DeHayes et al. 1999). Vermont mountain fog events, for example, have had a lower average cloud water pH of 2.8 compared to the cloud water pH of similar elevations in New York (Scherbatskoy et al. 1999). Northeastern soils are particularly acid-sensitive because the cations in the soil have been severely depleted (Driscoll et al. 2001). Calcium cations in at the HBEF of New Hampshire diminished considerably during periods of high acid deposition in the 20^{th} century (DeHayes et al. 1999, Driscoll et al. 2001). Cations are also leached from northeastern soils naturally due to the decomposition of organic matter in forests that forms organic acids (Driscoll et al. 2001). Aluminum undergoes podzolization, where it is mobilized and deposited into the soil and surface waters by organic acids (Driscoll et al. 2001). Due to acid deposition, this non-toxic, natural level of aluminum has been increased to toxic levels that further degrade the ecosystem (Driscoll

et al. 2001). Organisms that inhabit neutral soils typically cannot tolerate the decrease in pH associated with acid deposition (Driscoll et al. 2001).

Acid deposition impacts the survival of both terrestrial and aquatic organisms in the northeastern United States because many of these species are not adapted to these low pH conditions. Red spruce trees, for instance, are more susceptible to freezing injury when they have low foliar calcium concentrations (DeHayes et al. 1999). DeHayes et al. (1999) found that acid mists of pH 3 leach more calcium from northeastern red spruce needles than acid mists of pH 5. The increase in acid deposition may contribute to the decrease of red spruce populations in these northeastern forests (DeHayes et al. 1999). Northeastern streams are particularly impacted by acid rain during high flow, which is called episodic acidification (Lawrence 2002). Fish populations that are sensitive to high acidity have been severely diminished due to low pH and high aluminum concentrations during relatively short high flow episodes of the northeastern streams (Baker et al. 1996, Lawrence 2002).

Amphibians are significantly impacted by acid deposition due their permeable skin, sensitive embryological stage, energetically costly metamorphosis, and their placement in the food chain (Pierce 1993). Declines in amphibian populations reduce the biodiversity and functionality of an ecosystem. They are unique and crucial components of both terrestrial and aquatic habitats as a source of nutrient flow. Salamanders, for instance, comprise a significant portion of terrestrial animal biomass (Pierce 1993). Many amphibians use ephemeral pools, which are primarily precipitation fed, with limited surface and ground flow. Therefore, these ponds are highly influenced by acid rain and low calcium levels (Pierce 1993). Understanding the impact of acid deposition on amphibians gives insight on the long lasting effects of pollution on the ecosystem.

Certain amphibian species, such as the moor frog (*Rana arvalis*), have adapted to inhabit naturally acidic ecosystems. Moor frog embryos collected from ponds with pH of 4.0 and 4.5 and raised in experimentally low pH conditions have higher survival rates and less growth impairments compared to moor frog embryos collected from ponds with a pH of 7.0 and raised in these same low pH conditions (Räsänen et al. 2003). Although this frog species undergoes local adaptation, there are relatively few indications of amphibians adapting to current anthropogenic influences, particularly acid deposition.

Many studies demonstrate a correlation between amphibian decline and low pond pH, but studies on the effects of decreased soil pH on amphibian populations are limited (Pierce 1993). In the early literature, it was demonstrated that most terrestrial salamander species prefer basic pH soil levels and that soil acidity negatively impacts their survival (Frisbie and Wyman 1991). Soil pH between 2.5 and 3.0 was demarcated as the acutely lethal pH range for the eastern red-backed salamander (*Plethodon cinereus)* and between 3.0 and 4.0 pH was considered chronically lethal (Frisbie and Wyman 1991, Moore and Wyman 2009). The eastern red-backed salamander is one of the most abundant terrestrial vertebrate in northeastern North America and has been used as a bioindicator to monitor these forests for high acidity (Moore and Wyman 2009).

Contrary to previous conceptions, *P. cinereus* are present in forest soils with high acidity (Bondi et al. 2016, Moore and Wyman 2009). The Lake Clair Watershed near Quebec, Canada has soil pH ranging from 3.1 to 5.2 with a mean pH of 3.7 (Moore and Wyman 2009). In fact, 82% of *P. cinereus* in this study were located in soil with a pH less than 3.8 (Moore and Wyman 2009). The abundance and size of *P. cinereus* in acidic sites compared to other less acidic sites indicates that high acidity may not adversely affect their populations (Moore and Wyman 2009). Bondi et al. (2016) assessed 34 hardwood forests in northeastern United States and also found

that *P. cinereus* is prevalent in low soil pH, below 3.0. Soil pH did not impact *P. cinereus* abundance, body size, or health condition in these northeastern populations (Bondi et al. 2016). Despite the fact that recent studies have found an abundance of *P. cinereus* in low soil pH, further research on the effects of high soil acidity on salamander survival and age demographics is crucial for a greater understanding on the effects of acid deposition.

I investigated the effects of acidic soils on the health of *P. cinereus* populations in terms of age structure, growth rate, and age at sexual maturity at four of the sites studied by Bondi, in New Hampshire and Vermont (Bondi et al. 2016). Age was determined using skeletochronology of the *P. cinereus* femur bone, which is a method in which lines of arrested growth (LAG) on the bone cross section are counted (Ento and Matsui 2002). These LAGs can be viewed when the cross section of the bone is stained because bone deposition is increased during the warm season of the year (Boyle 2012). Skeletochronology has been used to determine age structure and growth of amphibians for over 70 years (Boyle 2012). It is generally regarded as an accurate method to age amphibians, however, the age count can be slightly skewed by one extra LAG (double-LAG) or one less LAG due to resorption of the endosteal (layer of cells with calcium that the salamander can absorb if needed) (Boyle 2012). In this study, I examined each of the four eastern red-backed salamander populations using the skeletochronology technique, so the results would be comparable for assessment.

I evaluated the age structure of each *P. cinereus* population and predicted that the age distribution would differ most from a normal, or bell-curve, distribution in sites with low pH, particularly site VTBC01 (pH 2.73), because young individuals may not be surviving to adulthood in these poor conditions. I also predicted that *P. cinereus* populations at low pH sites would have reduced growth rates compared to populations at higher pH sites. Furthermore, I

hypothesized that the mean age of eastern red-backed salamanders would be highest at the site of highest pH, which was NHSC01 (pH 3.89), and lowest at the site of lowest pH, which was VTBC01 (pH 2.73). Lastly, I predicted that it would take more time for *P. cinereus* individuals to reach sexual maturity in acidic soils due to the harsher conditions, so I hypothesized that *P. cinereus* individuals' mean age at sexual maturity would be higher in low pH soil compared to higher pH.

METHODS

Study sites and previously collected data

I analyzed *P. cinereus* individuals collected by Bondi et al. (2016) in 2012, at two sites in the White Mountain National Forest in New Hampshire (NHCP05 & NHSC01) and two sites in the Green Mountain National Forest in Vermont (VTBC01 & VTEQ02).

The White and Green Mountain regions contain heterogeneous soil and rock classifications which contributes to the diverse soil acidity across the landscape (Bondi et al. 2016). Spodosols, or Podzols, which are acidic soils with high concentrations of humus, aluminum oxides and iron oxides are the dominating soil type of these regions (Bondi et al. 2016, McDaniel n.d.). These four mature hardwood forest sites with elevations ranging from 250 m to 589 m are dominated by sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), and yellow birch (*Betula alleghaniensis*), as well as red spruce (*Picea rubens*) and balsam fir (*Abies balsamea*) at higher elevations (Bondi et al. 2016).

From late June to early July in 2012, eastern red-backed salamanders were randomly collected at each site and their snout-vent length (SVL) and body mass were measured (Bondi et al. 2016). Soil pH data was previously calculated for each site using a 0.01 mol L^{-1} $CaCl_2$ extraction and using three soil pits from the Oa/A horizon (Bondi et al. 2016). Soil pH at each

salamander location was measured using the top 10 cm of the Oa/A horizon, along with a pair soil core that was 10 m from the location (Bondi et al. 2016). I considered the soil pH and number of collected specimens to select these four sites from the research of Bondi et al. (2016) which were VTBC01 (pH 2.73), NHCP05 (3.30 pH), VTEQ02 (3.68 pH), and NHSC01 (3.89 pH) (Table 1).

Sexual maturity assessment

Each eastern red-backed salamander specimen collected from the field was dissected and images were taken of the reproductive tract (Bondi *unpublished data*). Using these images, I determined the sex of each individual based on the presence of ovaries or testes (see examples in Figure 1). Males were considered sexually mature if their testes and vasa deferentia had dark/black pigmentation (Takahashi 2002). Immature males had a clear, or invisible, vasa deferentia (Takahashi 2002). Sexually mature females were determined based on their enlarged oviducts and also their large mature ovarian eggs, if they were not already spent (Figure 2, Takahashi 2002). If neither the testes, nor the ovaries could be distinguished or viewed in the image, the sex and sexual maturity of the eastern red-backed salamander were recorded as unknown. If the testes or ovaries could be distinguished, but the image was not clear enough to determine sexual maturity, the sexual maturity was recorded as unknown.

Skeletochronology procedure

After collecting each *P. cinereus* from the field, one femur was removed from each individual and stored in closed vial with 70% ethanol solution. I fixed each *P. cinereus* femur (89 total) in formalin for the skeletochronoloy procedure (Castanet et al. 1996). After the femurs were in formalin for 24 hours, they were rinsed with water and transferred back to the 70% ethanol solution for storage in the closed vials. These untrimmed eastern red-backed salamander femurs were sent in vials to Mass Histology Service for decalcification and paraffin embedding,

which solidifies the samples in order to be cut and mounted on to a slide (Castanet et al. 1996). 15 µm cross sections of each femur bone at the midshaft of the diaphysis were created and stained with hematoxylin in order to visualize and count the LAGs of each individual (Figure 3, Castanet et al. 1996, Ento and Matsui 2002, Ash et al. 2003).

To reduce the bias of counting the LAGs as an individual observer, another observer and myself counted the LAGs of each eastern red-backed salamander femur cross section separately and recorded both counts afterwards (Boyle 2012). If the number of LAGs that one observer counted was two more or two less than the count of the other observer, the age was recorded as undetermined for that salamander. If the number of LAGs was only one more or one less than the other observers LAG count, the mean of each observer's count was calculated to determine the estimated age of the salamander. LAGs were counted where they appeared most recognizable and clear to the observer. If the LAGs were too difficult to count, the age count of the salamander remained undetermined and not applicable. Using a high power microscope and built-in camera, I took pictures of each slide and altered the brightness, contrast, and other image variables, in order to increase certainty of the correct LAG counts.

Analysis

I created histograms of the number of eastern red-backed salamanders at each age per site to display the age structure. Growth curves for each site were generated based on the von Bertalanffy equation: $SVL_t = SVL_{max}(1\text{-}e^{(-k(t-t_0))})$ and using the Chapman's method (Sparre and Venema 1998, Lima et al. 2001, Miaud et al. 2001). SVL_t is the average snout vent length at a given age of t. SVL_{max} is the maximum snout vent length reached in the population (asymptotic size). The value k is the growth coefficient, or the speed at which the maximum SVL is approached. The value t_0 is the theoretical age where SVL is equal to zero, which is often a negative value or zero. If there were no salamanders at a site for a specific age, I used the SVL

measurement of the closest aged salamander of a 0.5 year difference. If there were no SVL measurements of salamanders that were 0.5 years older or younger than the age required to create the growth curve, the SVL measurement for that age was estimated by creating a slope of the two salamanders closest to that age. This was only necessary for computation at three ages, which were all older ages where the growth was already approaching maximum SVL. I also applied ANOVA tests to compare the mean age of salamanders at each site, the mean age of sexually mature individuals at each site, and the mean age of immature individuals at each site. I used a Tukey's HSD test for multiple comparisons. The alpha level for each test was 0.05.

RESULTS

Based on the histogram of the age structure at site VTBC01 (pH 2.73), eastern red-backed salamander abundance peaks at age 1, with six salamanders, and steadily decreases, until rising again with two salamanders at age 8, and one salamander at age 9.5 (Figure 4). This site's age structure does not appear to have a normal distribution (Figure 4). At site NHCP05 (pH 3.3), salamander abundance peaks at age 5, with five salamanders and then decreases, having a more normal distribution (Figure 5). VTEQ02 (pH 3.68) has a wider distribution of salamanders at various ages compared to NHCP05, but the abundance of salamanders also peaks at age 5, with five salamanders (Figure 6). The age structure at site NHSC01 (pH 3.89) also has a relatively normal age distribution, but the peak in abundance of salamanders is shifted left at age 3, with six salamanders (Figure 7).

The hypothesis that the growth rate of *P. cinereus* would be the lowest at the most acidic site did not appear to be supported by the von Bertalanffy growth models of each population. The lowest growth rate, or k coefficient, of *P. cinereus* was observed at VTEQ02 (pH 3.68) and

the mean maximum SVL was also the highest at this site (k = 0.216/year, L_{max} = 47.59 mm; Figure 10). The most basic site, NHSC01 (pH 3.89) had the third lowest growth rate of *P. cinereus* (k = 0.595/year, L_{max} = 38.83 mm; Figure 11). The most acidic site, VTBC01 (pH 2.73), had the second highest growth rate and lowest mean maximum SVL compared to the other sites (k = 0.781, L_{max} = 33.48 mm; Figure 8). The site NHCP05 (pH 3.30) had the highest growth rate of *P. cinereus* compared to the other sites and a relatively similar mean maximum SVL to NHSC01 (k = 1.220/year, L_{max} = 40.94 mm; Figure 9).

Based on the ANOVA and Tukey HSD test (see Table 1 for n values), the mean age of individuals at the most acidic site VTBC01 (pH 2.73), which was 3.13 (± 2.6) years, was significantly lower than the mean age of individuals at NHCP05 (pH 3.30), which was 4.98 (± 1.8) years (p = 0.019; Figure 12). The mean age of individuals at the least acidic site, NHSC01 (pH 3.89), which was 3.26 (± 1.6) years, was also significantly lower than the mean age of individuals at NHCP05 (pH 3.30) (p = 0.038; Figure 12).

The mean age of sexually mature individuals at the most acidic site VTBC01 (pH 2.73) of 6.10 (± 3.5) years was significantly greater than the mean age of sexual mature individuals at the most basic site NHSC01 (pH 3.89) of 4.00 (± 1.7) years based on the ANOVA and Tukey HSD test (p = 0.04; Figure 13). The mean age of sexually immature individuals did not significantly differ among sites (p = 0.07; Figure 14).

DISCUSSION

Initial research indicated that habitats with a pH < 3.5 were lethal to *P. cinereus* (Frisbie and Wyman 1991), but more recent studies have revealed that *P. cinereus* are abundant in forest soils well below this pH threshold (Moore and Wyman 2009, Bondi et al. 2016). In this study, *P.*

cinereus were abundant in the four sites with soil pH of 2.73, 3.30, 3.68, and 3.89. Recent studies have also found an abundance of *P. cinereus* in other conditions that were previously thought to be unsuitable for their survival. For instance, *P. cinereus* were found in high densities of non-forested fields in West Virginia, which were previously considered to be inhospitable to salamanders due to their permeable skin that cannot endure desiccation (Riedel et al. 2012). However, the age structures indicated that juvenile *P. cinereus* were less abundant compared to adult *P. cinereus* in non-forested habitats (Riedel et al. 2012). Therefore, reproduction and survival of young to adulthood may be negatively affected by these disturbed field sites (Riedel et al. 2012). *Plethodon cinereus* populations were also abundant in higher latitudes of Canada, but they had a lower growth rate and larger minimum size at sexual maturity compared to lower latitudes of Maryland (Leclair et al. 2008). A shorter favorable foraging season in these higher latitudes of Canada were most likely the cause of these lower growth rates (Leclair et al. 2008).

In this study, I found less evidence that high soil acidity had similar demographic effects on *P. cinereus* populations, relative to the above studies (Riedel et al. 2012, Leclair et al. 2008). The normal age distribution of the three *P. cinereus* populations at low soil pH of 3.30, 3.68, and 3.89 indicates that low acidity may not negatively impact their survival. However, juveniles may be adversely effected by the high soil acidity of pH 2.73 and not be likely to survive to adulthood, which would impact the continuation of the population. Although the growth rate of *P. cinereus* appears relatively high in the two most acidic sites with a pH of 2.73 and 3.30, the individuals of the most acidic site (pH 2.73) grow to a smaller maximum SVL compared to the other sites. Juveniles of age one and two may grow quickly because they are not impacted by high soil acidity and adults may grow slowly because they allocate energy to becoming sexually mature, or developing eggs. It is also possible that growth curves indicate high growth rates in

young eastern red-backed salamanders because individuals that grow quickly as larvae and as juveniles may outcompete smaller individuals of the same age in these highly acidic sites.

Salamanders that reach sexual maturity at a later age are typically larger and lay larger eggs, which increases the survival of their larval offspring (Ento and Matsui 2002). It may be possible that *P. cinereus* in acidic sites reach sexual maturity later and wait longer to lay their eggs because their offspring may be more likely to survive in these stressful acidic conditions when they are large. This scenario is consistent with my observation of faster growth rates of young individuals. However, adults may be more likely to reach sexual maturity in acidic sites at a later age because these conditions negatively affect their growth and maturation process. However, in this study, there appears to be a weak effect of soil pH on eastern red-backed salamander age at sexual maturity.

More replications and including other environmental factors could increase the ability to make substantial conclusions on the effects of acidity on the age demographics, growth rates, and age at sexual maturity of eastern red-backed salamanders. Small sample sizes may have affected the outcome of this study because only four populations were sampled from. Replicating of each soil pH would significantly improve the sample size and statistical analysis of this study. Including replications of higher soil pH would also improve the significance of this study. Furthermore, the mean age at sexual maturity at the site with lowest acidity may not have been significantly higher compared to the sites of soil pH 3.30 and 3.68 due to low sample sizes (Table 1). The difference in mean age at sexual maturity of *P. cinereus* between the most acidic site of soil pH 2.73 and the most basic site of soil pH 3.89 was significant, but may require a larger sample size because n=3 does not support an accurate trend. Several individuals could not be evaluated for their age because the LAGs were unable to be distinguished, which lowered the

sample size for the purpose of age structure comparisons. Overall, this preliminary study indicates some potentially important effects of soil pH on *P. cinereus* demographics, which helps to interpret the findings of Bondi et al. (2016) but further study is required.

Eastern red-backed salamanders could be adapting to the pressures of acid deposition because they are abundant in acidic sites and their growth and survival does not appear to be excessively compromised by this high acidity. Several amphibians that naturally inhabit slightly acidic habitats have locally adapted to anthropogenic increases in acidity, such as *Rana arvalis* (Räsänen et al. 2003). Various salamander species have been able to locally adapt to other types of anthropogenic pollutants, such as spotted salamanders (*Ambystoma maculatum*) that are able to live in ponds with high roadside salt concentrations (Brady 2012). Future research on the effects of acid deposition on *P. cinereus* age demographics, growth rates, and age at sexual maturity will increase our understanding on their ability to adapt to conditions with high acidity. It is important to study and conserve eastern red-backed salamander populations because they play a crucial role in nutrient cycling, especially in soils of very low pH that may be inhospitable to other organisms with a similar ecological role.

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LIST OF FIGURES

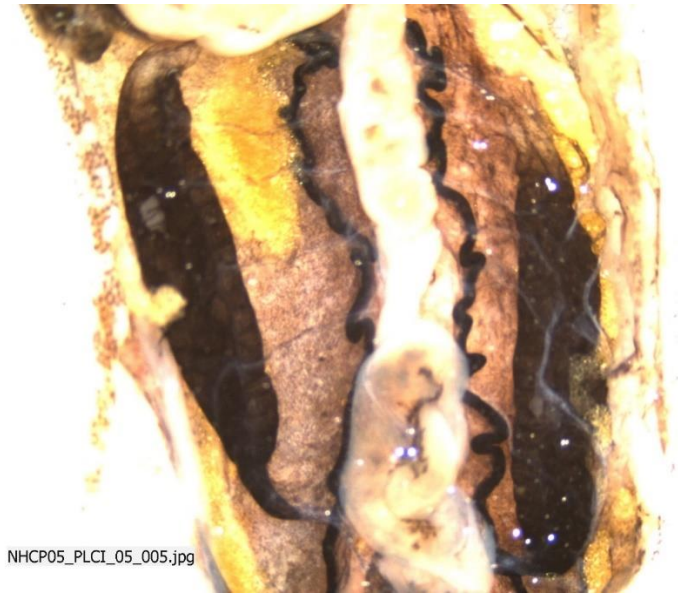

Figure 1. Dissection of a sexually mature eastern red-backed salamander male displaying darkened/black testes and vas deferentia.

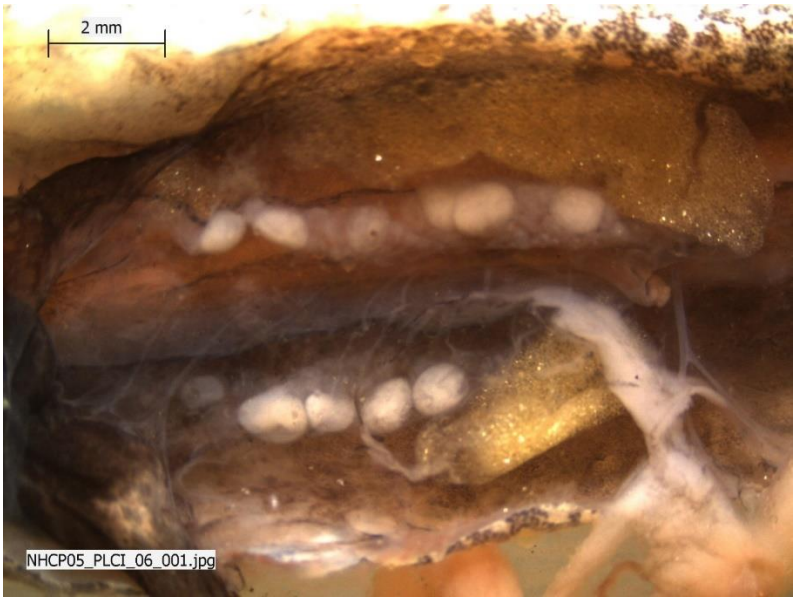

Figure 2. Dissection of a sexually mature eastern red-backed salamander female with maturing eggs in her ovaries.

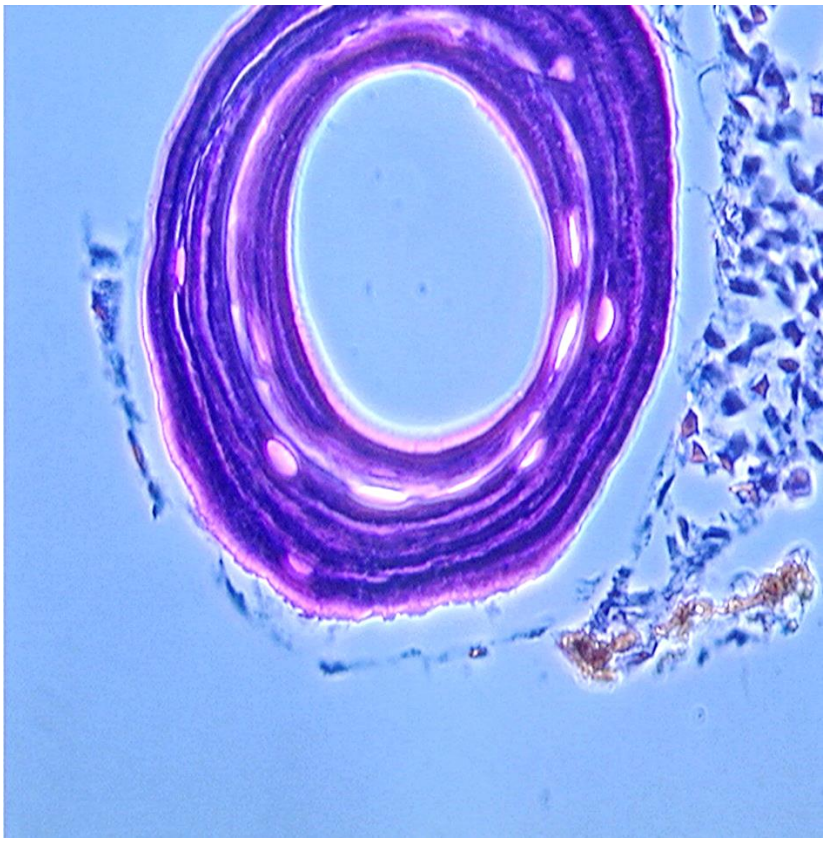

Figure 3. Cross section of an eastern red-backed salamander femur showing lines of arrested growth (LAGs)

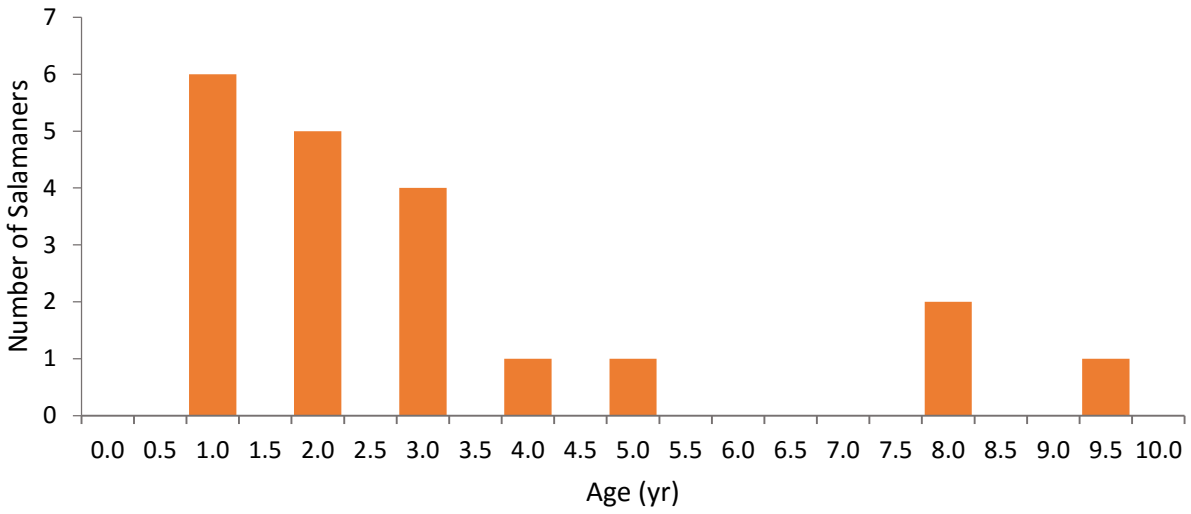

Figure 4. Age distribution and abundance of *P. cinereus* at site VTBC01 with a pH of 2.73.

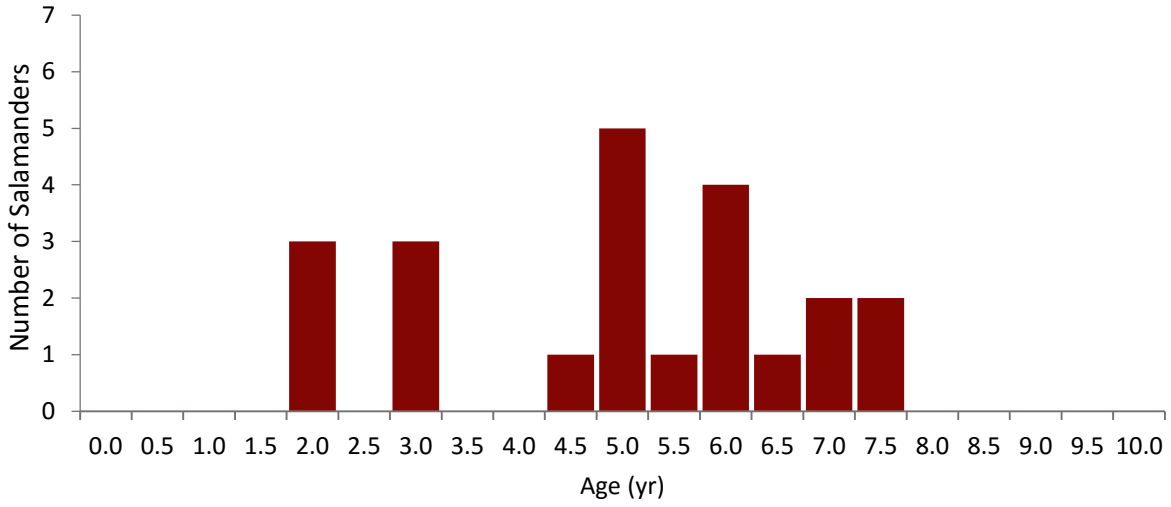

Figure 5. Age distribution and abundance of *P. cinereus* at site NHCP05 with a pH of 3.30.

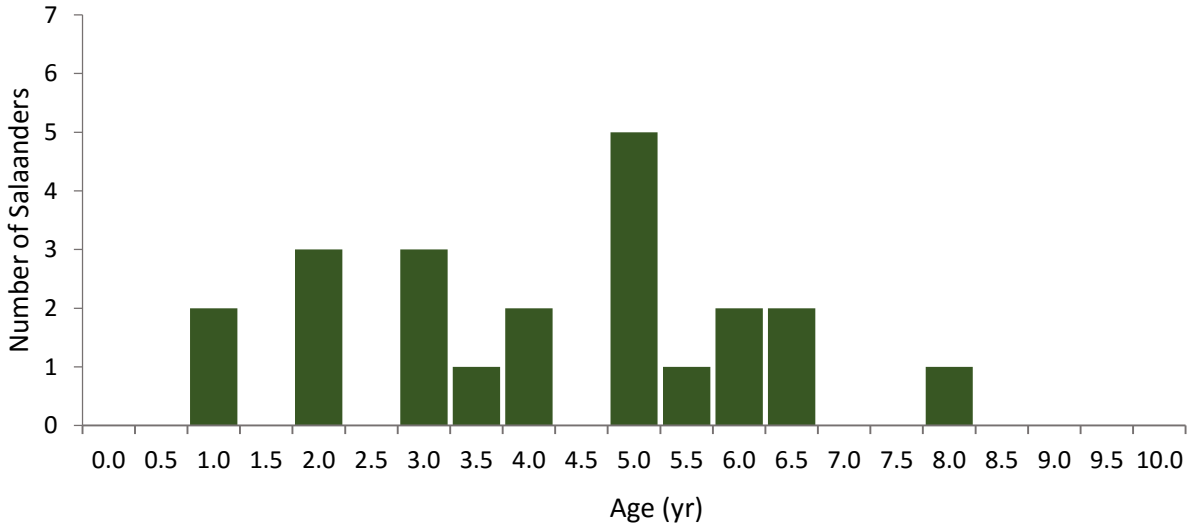

Figure 6. Age distribution and abundance of *P. cinereus* at site VTEQ02 with a pH of 3.68.

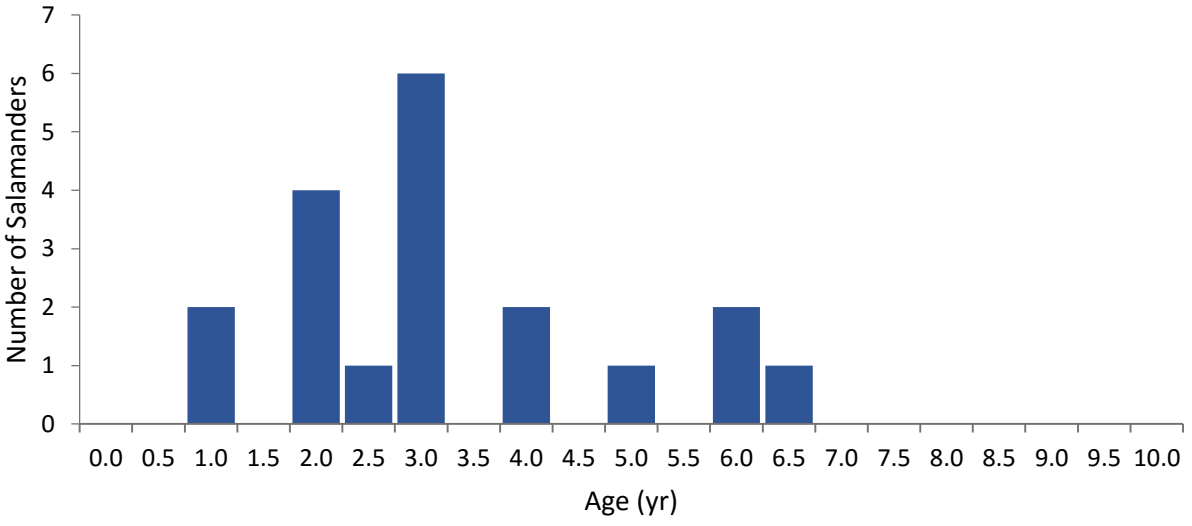

Figure 7. Age distribution and abundance of *P. cinereus* at site NHSC01 with a pH of 3.89

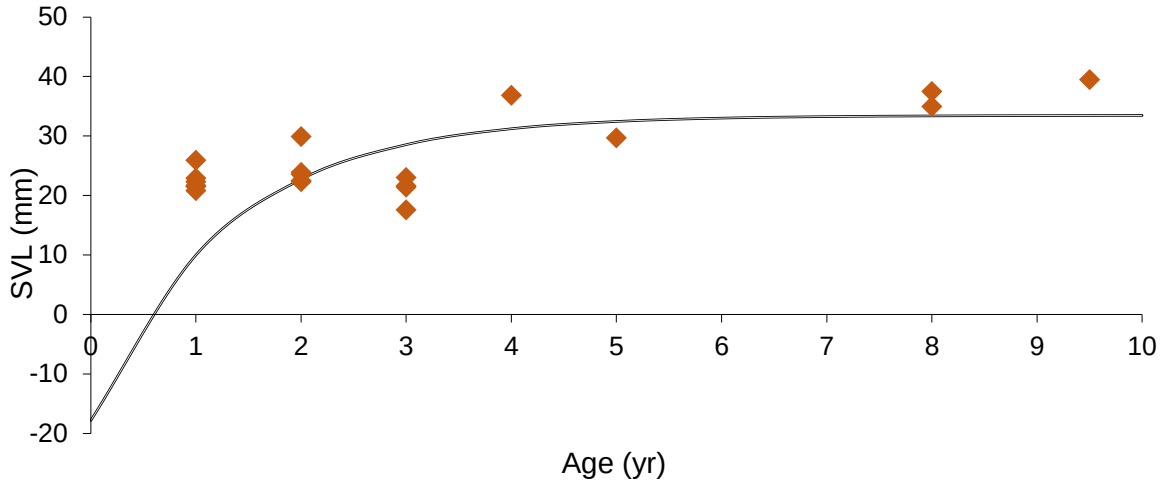

Figure 8. The von Bertalanffy growth curve of *P. cinereus* population at site VTBC01 (pH 2.73) with the equation: $L_t = 33.48\ (1 - e^{(-0.781\ (t - 0.546))})$. The growth rate, or k coefficient is 0.781/year. The mean maximum SVL, or L_{max} and sometimes referred to as L_∞, is 33.48 mm.

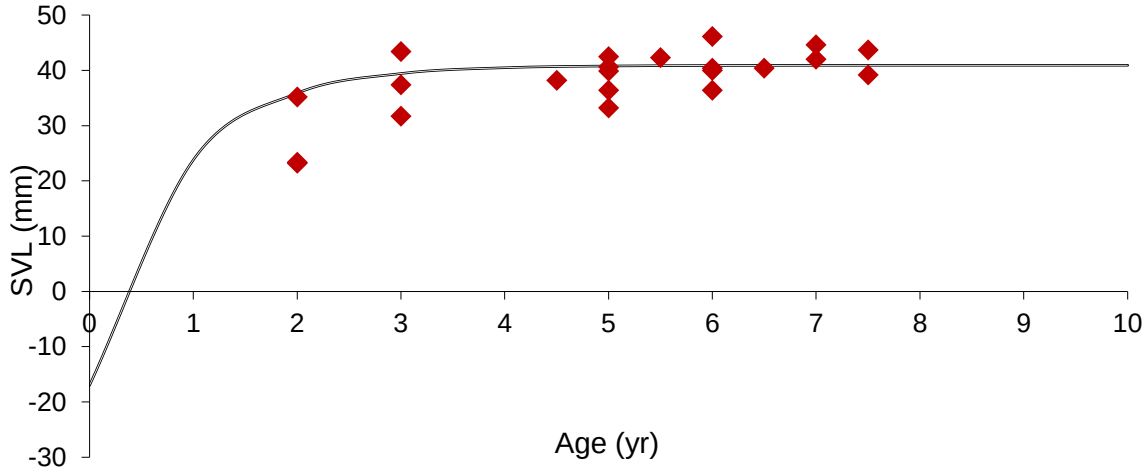

Figure 9. The von Bertalanffy growth curve of *P. cinereus* population at site NHCP05 (pH 3.30) with the equation: $L_t = 40.94\ (1 - e^{(-1.220\ (t - 0.285))})$.The growth rate, or k coefficient is 1.220/year. The mean maximum SVL, or L_{max} and sometimes referred to as L_∞, is 40.94 mm.

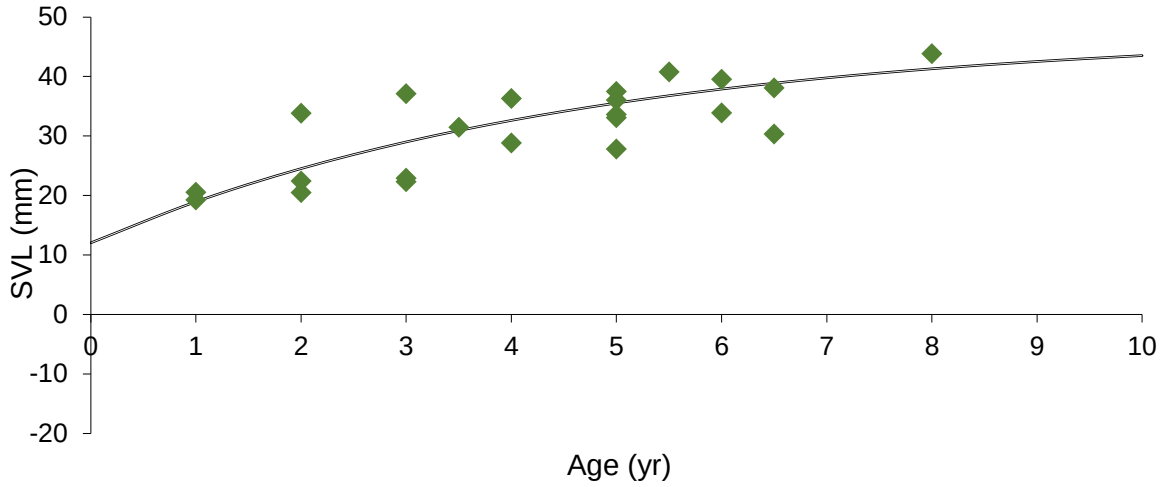

Figure 10. The von Bertalanffy growth curve of *P. cinereus* population at site VTEQ02 (pH 3.68) with the equation: $L_t = 47.59\ (1 - e^{(-0.216\ (t + 1.355))})$. The growth rate, or k coefficient is 0.216/year. The mean maximum SVL, or L_{max} and also referred to as L_∞, is 47.59 mm.

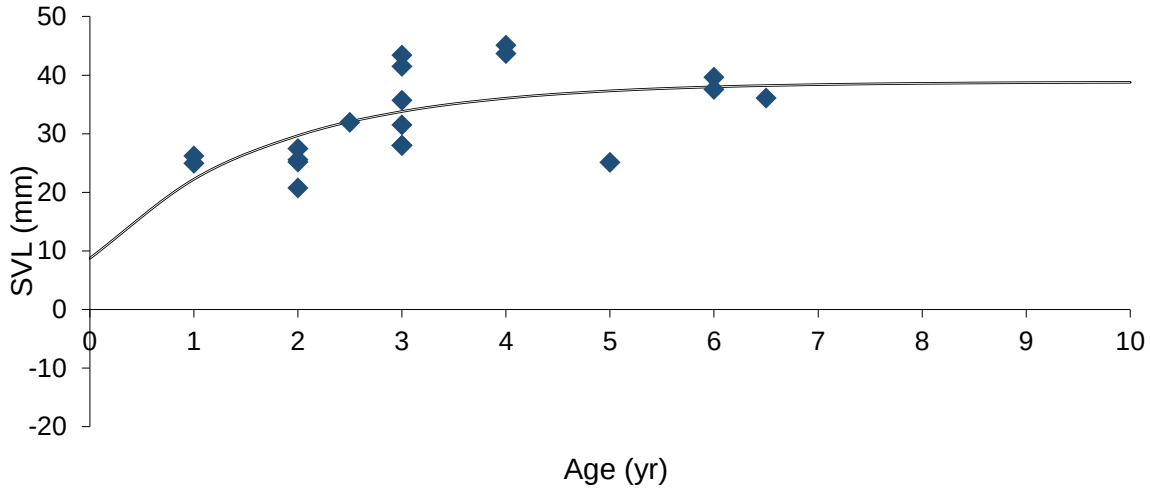

Figure 11. The von Bertalanffy growth curve of *P. cinereus* population at site NHSC01 (pH 3.89) with the equation: $L_t = 38.83\ (1 - e^{(-0.595\ (t + 0.430))})$. The growth rate, or k coefficient is 0.595/year. The mean maximum SVL, or L_{max} and also referred to as L_∞, is 38.83 mm.

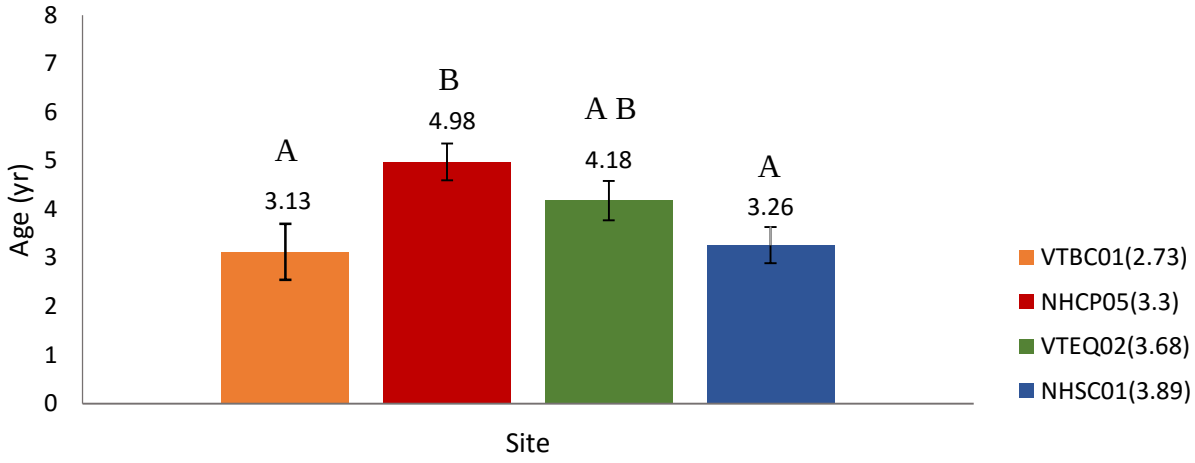

Figure 12. Mean age in years of *P. cinereus* individuals at each site with standard error bars. The same letter represents means that are not significantly different (ANOVA, F = 3.92, p = 0.01).

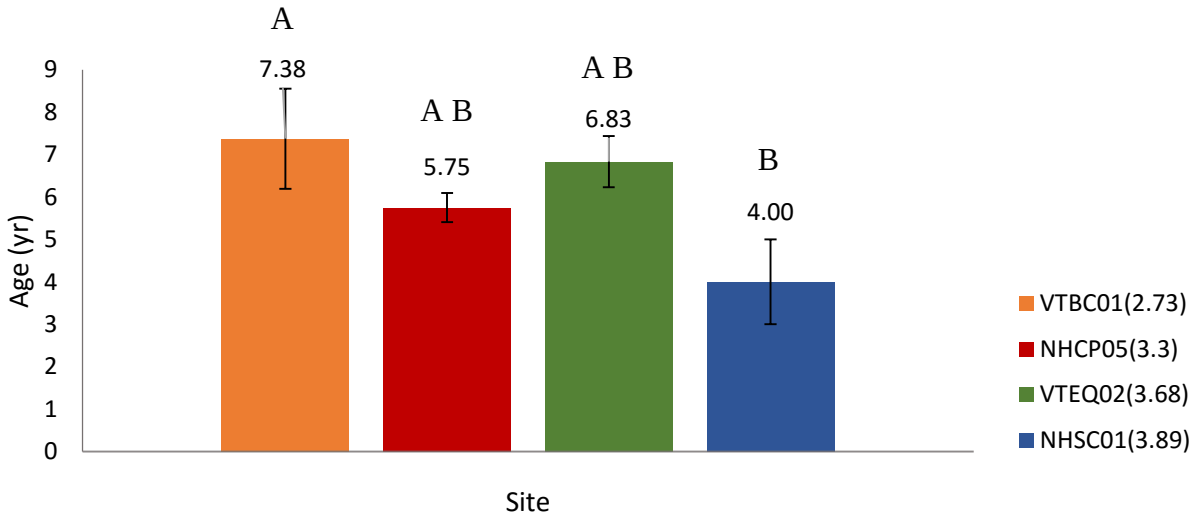

Figure 13. Mean age in years of sexually mature *P. cinereus* individuals at each site with standard error bars. The same letter represents means that are not significantly different (ANOVA, F = 3.23, p = 0.04).

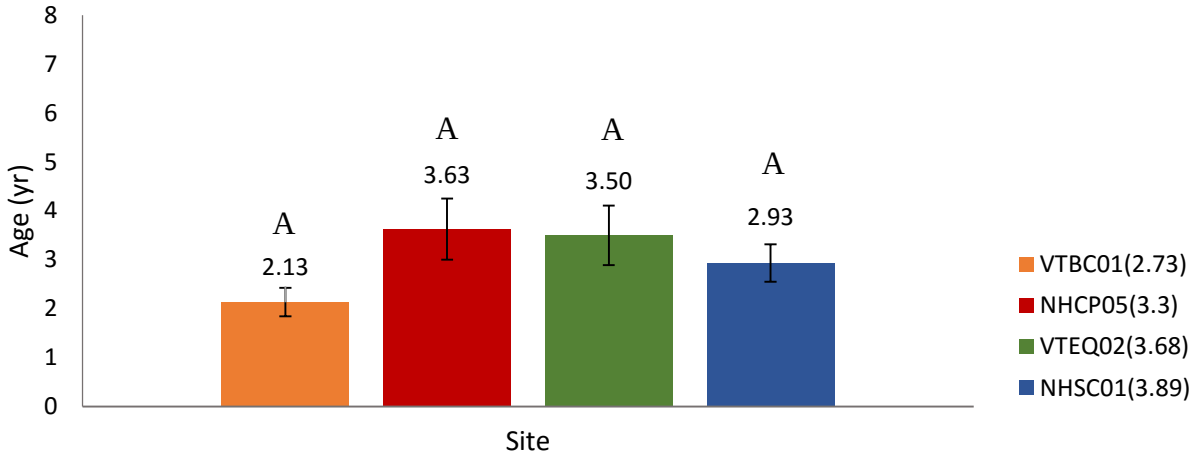

Figure 14. Mean age in years of sexually immature *P. cinereus* individuals at each site with standard error bars. The same letter represents means that are not significantly different (ANOVA, F = 2.84, p = 0.07)

LIST OF TABLES

Table 1. The mean, standard deviation, and confidence interval (α=0.05) of mean SVL and mean age of *P. cinereus* at each site including the means of immature and mature individuals. The minimum and maximum values for each populations SVL and estimated year are also recorded.

	SVL (mm)				Estimated Age (years)			
	N	**Mean ± SD**	**(Min, Max)**	**Confidence interval (α=0.05)**	**N**	**Mean ± SD**	**(Min, Max)**	**Confidence interval (α=0.05)**
VTBC01(2.73)	21	25.76 ± 6.34	(17.60, 39.50)	(23.04, 28.47)	20	3.13 ± 2.57	(1.00, 9.50)	(2.00, 4.25)
immature	16	23.16 ± 3.11	(17.60, 29.90)	(21.64, 24.68)	15	2.13 ± 1.13	(1.00, 5.00)	(1.56, 2.70)
mature	4	37.20 ± 1.87	(34.97, 39.50)	(35.37, 39.05)	5	6.10 ± 3.51	(4.00, 9.50)	(3.03, 9.17)
NHCP05(3.30)	24	38.08 ± 5.76	(23.20, 46.10)	(35.77, 40.38)	22	4.98 ± 1.78	(2.00, 7.50)	(4.24, 5.72)
immature	8	32.56 ± 6.25	(23.20, 40.00)	(28.23, 36.89)	8	3.63 ± 1.77	(2.00, 6.00)	(2.40, 4.85)
mature	15	41.11 ± 2.77	(36.40, 46.10)	(39.71, 42.51)	14	5.75 ± 1.28	(3.00, 7.50)	(5.08, 6.42)
VTEQ02(3.68)	22	31.37 ± 7.33	(19.27, 43.83)	(28.30, 34.43)	22	4.18 ± 1.91	(1.00, 8.00)	(3.39, 4.98)
immature	6	29.93 ± 8.06	(19.27, 40.80)	(23.48, 36.38)	6	3.50 ± 1.48	(1.00, 5.50)	(2.31, 4.69)
mature	3	38.60 ± 5.00	(33.86, 43.83)	(32.94, 44.26)	3	6.83 ± 1.04	(6.00, 8.00)	(5.66, 8.01)
NHSC01(3.89)	22	32.80 ± 7.20	(20.73, 45.10)	(29.79, 35.80)	19	3.26 ± 1.62	(1.00, 6.50)	(2.54, 3.99)
immature	17	30.66 ± 6.72	(20.73, 45.10)	(27.46, 33.85)	15	2.93 ± 1.47	(1.00, 6.50)	(2.19, 3.68)
mature	4	40.18 ± 2.78	(37.60, 43.43)	(37.45, 42.90)	3	4.00 ± 1.73	(3.00, 6.50)	(2.04, 5.96)