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Mizuki Takahashi
mt027@bucknell.edu

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COMPARISONS IN MORPHOLOGY, REPRODUCTIVE STATUS, AND FEEDING ECOLOGY OF *PLETHODON CINEREUS* AT HIGH AND LOW ELEVATIONS IN WEST VIRGINIA

Thesis submitted to
The Graduate College of
Marshall University

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

by

Mizuki Takahashi
Thomas K. Pauley, Chair person
Charles C. Somerville, PhD
Suzanne G. Strait, PhD

Marshall University

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ABSTRACT

COMPARISONS IN MORPHOLOGY, REPRODUCTIVE STATUS, AND FEEDING ECOLOGY OF *PLETHODON CINEREUS* AT HIGH AND LOW ELEVATIONS IN WEST VIRGINIA

by Mizuki Takahashi

To develop effects of elevation on morphological features, reproductive status, and feeding ecology of *Plethodon cinereus* in West Virginia, overall comparisons between high (>4000 ft) and low (<1260 ft) elevation populations were made. Adult *P. cinereus* from high elevations were smaller in SVL (female: p=0.003, male: p<0.001), but stored larger amounts of fat (female: p=0.041, male: p=0.006) in their tails than from low elevations. Larger amounts of tail fat could be an adaptation to harsh environments at high elevations. It was determined that in West Virginia, whereas females at low elevations oviposit annually, females at high elevations oviposit biennially. Stomach content analysis revealed that salamanders at high elevations were confronted by food shortage. Because of less prey availability and longer winters at high elevations, salamanders at high elevations attained the smaller body size than at low elevations and could not obtain sufficient energy in 1 year to yolk a clutch.

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More than one and a half years have passed since I came here from Japan. The writing of this thesis is a part of the Master's degree that I have spent most of the time in my life in the United States. Until becoming what I am now, I have been struggling and have been helped by many people to complete my education at Marshall. I would like to thank Dr. Thomas K. Pauley for opening the door for me to take the first step toward my dream and, since then, looking after me. My life would be so different if he was not teaching at Marshall and did not accept me as a graduate student. I would also like to thank the other members of my committee, Dr. Charles C. Somerville and Dr. Suzanne G. Strait for their knowledge and advice during the completion of my thesis. I hope that someday I can teach at a college on the model of these faculty. I appreciate the help of Sandy Raimondo. In her busy life, she spent time helping me with stomach content analysis.

I had to change my thesis topic and my previous project also needed help from many people. I would like to thank these people. The field work for the previous project was assisted by Koichi Miyabe, Park Ji Yoon, Naoko Yamada, Seth Myers, Adam Mann, Melissa Obermeyer, Rob Fiorentino, Zach Felix, Andy Johnson, Keith Johnson, Nancy Dickson, and Cody Lockhart. Especially Koichi Miyabe and Park Ji Yoon made a large contribution to 2001 summer field work and I really enjoyed working with them. Jennifer Wykle, Dan Cincotta, and officers at the State Hatchery gave me important information about the salamander that I had searched for.

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CHAPTER I

Review of Literature

1. Introduction

Plethodon cinereus, the Eastern Red-backed Salamander, is a small terrestrial species of the family Plethodontidae. All members of this family are lungless and depend upon the skin and lining of the mouth and throat for gaseous exchange (Green and Pauley 1987). This species has short legs relatively to the body size and has an average of 18-20 costal grooves (Petranka 1998). As in other *Plethodon* species, the tail is rounded in cross section.

The Eastern Red-backed Salamander ranges from southern Quebec and the Maritime Provinces southward to western Minnesota (Petranka 1998), where a geographic isolate occurs (Figure 1.1). In West Virginia, their range (Figure 1.2) is statewide except for the Ohio Valley counties from Wayne north to Pleasants (Green and Pauley 1987).

Two color morphs occur in most populations (Petranka 1998). The "red back" phase has a red, orange-red, or brownish red stripe from the back of the head to the middle of the tail (Figure 1.3); this stripe is bordered on the sides by dark gray or black. The "lead back" phase lacks the red stripe and is entirely dark on the back and sides, sometimes with a faint speckling of lighter color (Harding 1997). Burger (1935) found that both color phases were in the same clutch of eggs, which suggested that there is no evolutionary genetic differences between two phases. In both, the belly is mottled with gray and white in what is often described as a "salt and pepper" pattern. Morphs often have small white spots and brassy flecks on the back, head, and sides of the body (Bishop 1941). In West Virginia, the lead back phase is uncommon except in the border area between Monongalia and Preston counties (Personal communication, Pauley 2002). All specimens analyzed for this study were the red back phase.

Adults inhabit forest litter habitats in deciduous, northern conifer, and mixed deciduous-conifer forests. Populations reach their greatest densities in well-drained, forested habitats. Mature forests with deep soils and scattered logs or rocks provide optimal habitats and permanently wet and boggy places are not populated (Burger 1935). Populations are usually absent or occur at low densities in highly acidic soils, in soils that are perennially wet, and in shallow, rocky soil (Petranka 1998). Primary nocturnal, they frequently forage on the surface on rainy nights and may even climb into shrubs and up tree trunks (Harding 1997). It is difficult to determine mortality of salamanders in the field and even rare to find dead animals in the field, but Hughes et al.(1999) found two dead *P. cinereus* in leaf letter in West Virginia and Pennsylvania although why and how they died was unknown. Pauley (personal communication 2002) has seen several dead *P. cinereus* through years and suggested that they must die of natural causes, such as old age.

Because this species is common throughout its range, *P. cinereus* is one of the most well studied species of salamanders. However, less attention has been placed on elevation effects on their morphological features, reproductive status, and feeding ecology. I am not aware of any studies that focused on elevation effects. Moreover, in West Virginia any studies focusing on reproductive status of *P. cinereus* has not been organized or published. The purpose of this study is to describe effects of elevation on morphological features, reproductive status, and feeding ecology of *P. cinereus* in West Virginia by comparing two different elevation populations.

2. Morphology of *Plethodon cinereus*

Hatchlings measure 1.9-2.5 mm TL and there is no free swimming larval stage. They spend their larval stages in the egg (Pauley 2002 personal communication). Hatchlings and small juveniles resemble miniature adults but have conspicuously shorter tails relative to body size (Bishop 1941) and proportionately broader heads (Maglia 1996). Adults measure 6.5 – 12.5 mm TL (Petranka 1998). The sex ratio of the adult is 1:1 (Burger 1935).

External features unique to sexually active males include greatly swollen nasolabial glands, hedonic glands on the tail, a crescent-shaped mental gland near the apex of the lower jaw, and elongated premaxillary teeth (Dawley and Crowder 1995). In addition to these features, males have papillae in the vent (Pfingsten and Downs 1989).
Sayler (1966) found no statistical sexual dimorphism in SVL-p (snout-vent length; the distance from the tip of the snout to "the posterior angle" of the cloaca) of adult *P. cinereus* in Maryland (males; 39.7 mm, females; 41.2 mm). However, it appears that sexual dimorphism in SVL is weakly developed in adults. In Michigan, Blanchard (1928) reported that the males measured from 35 to 47 mm in SVL-a (the distance from the tip of the snout to "the anterior angle" of the cloaca) and females from 38 to 48 mm. In Ohio, Pfingsten and Downs (1989) reported 40.5 mm for male SVL-a and 41.2 mm for females. In New York, Bishop (1941) found 40.55 mm in SVL-p for males and 43.28 mm for female. Burger (1935) also reported 39 mm in SVL-a for males and 41 mm for females in Pennsylvania and New Jersey, respectively.

I did not find any studies that focused on geographic variation of *P. cinereus* morphology, but it is possible to grasp a trend of morphological variation of this salamander by organizing data collected by different researchers in different regions (Figure 1.4). Nagel (1977) reported that the average SVL of adults in Tennessee was 43.5 mm and Bishop (1941) found 41.86 mm in New York. Burger (1935) reported that 40.0 mm in Pennsylvania and New Jersey and Sayler (1966) found 40.28 mm in Maryland. In Ohio, Pfingsten and Downs (1989) reported 40.79 mm. The former authors (Bishop 1941; Nagel 1977) measured SVL-p as the distance from the tip of the snout to the posterior angle of the cloaca as I measured in this study, but the latter authors (Burger 1935; Pfingsten and Downs 1989; Sayler 1966) measured SVL-a as the distance from the tip of the snout anterior angle of the cloaca. According to Nagel (1977), adult cloacal length in his study was about 2 mm. Figure1.4 was made by adding 2 mm to data collected by the latter authors so that data can be compared. Adding 2 mm to those data

is a rough way and it is not preferable to put data collected by different researchers together because re-measurements by different researchers often end up with slightly different results. However, it seems that the farther north, the smaller body size becomes (Figure 1.4). Pointing to specimens that he collected, Bishop (1941) mentioned "This is one of the smaller salamanders and in New York States does not attain the size reached by individuals farther to the south". Although this trend is not based on reliable scientific data, this might be explained by longer winters and less availability of prey items in northern regions.

It has been known that grass snakes on a small island (Hallands Vadero, close to Swedish coast) are significantly smaller than those on a mainland (Southern Sweden). Madsen and Shine (1993) concluded that prey availability caused marked phenotypical differences in adult body size and the degree of size dimorphism among populations of grass snakes. Adult females on the main land fed mostly on large toads whereas males ate frogs and juvenile toads. On the island, however, neither of these prey types is available (Madsen and Shine 1993). It is likely that prey availability for *P. cinereus* is different at high and low elevations in this study which might cause size dimorphism between the two populations and because of longer winters and less prey availability, populations at high elevation can be expected to be smaller.

From a different view, it is possible to expect that *P. cinereus* would become larger at high elevations where temperatures are lower throughout their lives. Atkinson (1994) organized 109 studies to grasp a trend in temperature and size of ectothermic organisms, which included examples of animals, plants, protists and a bacterium. Those studies showed a significant effect of rearing temperature on size. In 83.5 % of cases, increased temperature led to reduced size at a given stage of development. Only 11.9 % of examples showed a size increase, and 4.6 % showed a mixture of increase and decreases with temperature. This widespread, phenotypically plastic response of body size to rearing temperature can be termed the 'developmental temperature-size rule' and has recently received much attention (Atkinson and Sibly 1997). According to Atkinson (1994), 7 studies targeting amphibians have been done and all of them showed a significant size reduction with increasing temperature. For example, Berven (1982) compared adult body size of wood frog, *Rana sylvatica*, between frogs that bred in 5 mountain ponds (elevation; 865-1100 m) and frogs that bred in 2 lowland ponds (elevation; 43 m) from 1977 to 1980 and concluded that mountain male and female wood frogs were significantly ($p<0.001$) larger (males: average = 55.3 mm; females: average = 64.4 mm) than lowland breeding adults (males: average = 41.7 mm; females: average = 47.7 mm). Temperatures during growing seasons (based on last and first temperature below freezing) averaged 5 °C lower in the mountains that the lowland.

This relationship appears to mirror that found for endotherms by Bergmann as Atkinson (1994) discussed. Bergmann proposed in 1847 his well known adaptive explanation of the increase in body size with latitude observed in endotherms, especially mammals; Bergmann's rule states that selection at low temperatures would favor large individuals with a small ratio of surface are to body mass that should reduce heat loss. This explanation remains controversial when applied to endotherms and is also inappropriate for ectotherms, whose body temperature usually fluctuates rapidly and markedly as environmental temperatures change (Atkinson and Sibly 1997). Although its

adaptive or evolutionary meaning and mechanisms are elusive (Atkinson 1994; Atkinson and Sibly 1997), it seems that scientists reach a consensus on this matter as Van Voorhies (1996) mentioned that in general ectothermic organism grow larger at both lower temperature and higher latitudes. However, in the wild, many factors possibly regulate body size exist, such as competition and food availability. Atkinson and Sibly (1997) carefully concluded that whereas smaller adults emerge if growth is limited by food availability, the reverse is usually observed if growth is limited by temperature. In this study, my expectation and explanation of size dimorphism (SVL in this study) among high and low altitudinal populations are ambiguous and thus is interesting to study.

The main objectives of this section of my thesis are to study sexual dimorphism of *P. cinereus* within populations and to determine size dimorphism among populations located at different altitudes that provide different temperature environments.

3. Reproductive ecology of *Plethodon cinereus*

Most females oviposit in late spring or early summer, but on rare occasion sa female may oviposit in late summer or early autumn (Test and Bingham 1948). Oviposition typically occurs in June in populations in Maryland (Sayler 1966), Pennsylvania and New Jersey (Burger 1935), New York (Bishop 1941) and northeastern Tennessee (Nagel 1977). It seems that there is no significant difference in oviposition season between populations. In several years of field work in elevations above 3,500 feet (1,067 m) in West Virginia, Pauley found several females with eggs from June 6 to August 20 (Unpublished data). The average incubation period is about 6 weeks (Burger 1935). Therefore, most hatching occurs in late summer or early fall. In the case of *Plethodon*, a remarkably favorable start in life is assured by the circumstances that only a few eggs are laid and each egg is well supplied with yolk and the female remains with the eggs until after the larvae are hatched (Burger 1935). Juveniles in an eastern Tennessee population grow an average of 15 mm SVL during their first year of life, but only 8 mm SVL during the second (Nagel 1977). Larvae remain with the female from three to four weeks after hatching and then gradually disperse (Burger 1935). Data from studies in Maryland (Sayler 1966), Michigan (Blanchard 1928; Werner 1971), and Tennessee (Nagel 1977) indicate that juveniles reach sexual maturity about 2 years after hatching. Females probably first deposit follicles 2.5 years after hatchling, when they measure >34-39 mm SVL. Males mature sexually when they reach 32-37 mm SVL (Sayler 1966). Again, it seems that there is no big difference in oviposition season between populations, but I still wonder if there are differences in follicle development and oviposition season between high and low populations because these activities clearly require large amount of energy, which depends on prey availability and length of foraging period in a year.

Since recently-mated females retain the spermatophore for a relatively short period of time (Blanchard 1928) the presence of a spermatophore in the cloaca of the female is the best direct evidence for time of mating. Based on the presence of cloacal spermatophores, the actual mating season in northeastern Tennessee appears to be from December to March (Nagel 1977). Blanchard (1928) found cloacal spermatophores from October to December in Michigan and Sayler (1966) reported cloacal spermatophores

form October to April for Maryland specimens. They have a prolong mating season that seems to start in late fall or winter and lasts until the following spring, but the autumn is indicated as the time when most mating takes place (Blanchard 1928). Werner (1969) studied temperature-photoperiod effects on spermatogenesis in *P. cinereus* and concluded that compared with the groups at 20 °C, all photoperiod groups at 10 °C had an average retarded cycle of 16 days by 26 June. His experiments also indicated that temperatures of 20 °C were needed to initiate the spermatogenic wave (sperm production cycle; detail in Chapter II) in Michigan *P. cinereus*. As I mention in Chapter II, in this study there is 3.4°C of average dairy temperature difference between high and low elevation populations and it is possible that this temperature interval cause difference in spermatogenic wave which can end up with a gap of breeding season between populations at difference elevations.

Males reproduce annually whereas females breed annually or biennially depending on geographic locality. Bishop (1941), Test and Bingham (1948), Sayler (1966) and Pfingsten and Downs (1989) concluded that females laid eggs every other year on the basis of two distinct groups of large females, those with enlarged follicles and those with only small follicles in northern states (New York, Michigan, Maryland, and Ohio respectively). Females probably breed every other year in the Great Lakes Region too, perhaps due to the great amount of energy required to produce and yolk the eggs (Harding 1997). In these locals where biennial oviposition occurs, the growing season is relatively short and females apparently cannot obtain sufficient energy in 1 year to yolk a clutch (Petranka 1998). In contrast, populations in northeastern Tennessee lay eggs every year (Nagel 1977) and possibly in some populations in southwestern Michigan (Werner 1971). Biennial female cycles in the north and annual female cycles in the south have also been reported for *P. glutinosus* (Highton 1962). West Virginia is located between Ohio and Maryland where females reproduce biennially, so it is expected that populations here also deposit follicles every other year. However, elevation effects should not be ignored which provide longer winters in higher regions and might make it difficult for females to obtain sufficient energy for annual oviposition.

The objectives of this section are to develop reproductive status of *P. cinereus* at high and low elevation in West Virginia especially paying attention to how elevation difference affects reproductive strategies.

4. Feeding ecology of *Plethodon cinereus*

Most Plethodontid salamanders are generalists in terms of prey selection and probably feed on the invertebrates that are most common (Pauley 1978c). Stomach content studies conducted two miles west of the Harrison-Doddridge County line in West Virginia revealed that the major prey for *P. cinereus* was ants (Formicidae) (Pauley 1978c). Burton (1976) studied stomach contents of 200 *Plethodon cinereus* in New Hampshire and found that the most abundant prey categories numerically were mites (64.8 %) and 82.5 % of total stomachs contained this prey items. Stomach contents vary depending on geographical areas where different prey communities exist. In Southern Michigan specimens eat their shed skins along with beetles, ants, spiders, snails,

millipedes, bugs, mites, springtails, pseudoscorpions, and miscellaneous invertebrates (Blanchard 1928). In three populations in northern Tennessee, individuals do not shift their diet markedly as they grow. Nonetheless, small prey such as mites and collembolans are more prevalent in juveniles, whereas larger prey such as beetles and hymenopterans occur more frequently in adults (Maglia 1996). They also eat conspecific eggs or juveniles in both the laboratory (Highton and Savage 1961) and the field (Burger 1935; Burton 1976; Heatwole and Test 1961).

The study conducted in West Virginia revealed that 160 of 169 (95.0 %) salamanders had ingested some food item (Pauley 1978c). This would indicate that food was abundant and available to all individuals throughout the study area (Pauley 1978c). As I discussed previously, it has been known that grass snakes on a small island (Hallands Vadero, close to Swedish coast) are significantly smaller than those on a mainland (Southern Sweden). The key that explained this was prey availability. Adult females on main land fed mostly on large toads whereas males ate frogs and juvenile toads. On the island, however, neither of these prey types are available (Madsen and Shine 1993).

Jaeger (Jaeger 1972) determined that under low moisture conditions, food may become a limited resource for *P. cinereus* and *P. richmondi shenandoah* due to restrictions in the movements therefore limiting foraging opportunities. Fraser (1976) showed that during times of low moisture, *P. cinereus* and *P. hoffmani* probably do not forage.

There are two factors that regulate energy uptake of salamanders; prey availability and restrictions in their movement. Both are controlled by environmental factors, and in any case, stomach content analysis must play the major role to explain differences in morphology and reproductive status between high and low elevation populations.

The objectives of this section are to simply demonstrate food items of *P. cinereus* and, furthermore, to determine the difference in prey types and availability between populations at high and low elevations.

5. Conclusion

Plethodon cinereus is a common terrestrial species in the Eastern United States, hence, well studied. However, less attention has been placed on elevation effects that must have strong influences on their life cycle. The objectives of this study are to demonstrate elevation effects on their morphology and reproductive status and, furthermore, to explain differences of morphological features and reproductive status if there are any by focusing on food items and availability.

CHAPTER II

Materials and Methods

1. Salamander collection

Plethodon cinereus collected in 1993 and 1994 and stored in 80% ethanol in the West Virginia Biological Survey Museum, Marshall University, Huntington, West Virginia were used for this study. Specimens are classified into two groups, one from high elevations and the other from low elevations. Specimens from high elevation [above 4000 ft (1219 m)] were collected in 1993 by Dr. Thomas K. Pauley in Pocahontas County. Low elevation specimens were captured and preserved in 1994 by Peter A. Kramer (Kramer 1996) and those were from Harrison and Doddridge Counties. Information of specimens is listed in Table 2.1 and Figure 2.1 and temperature and precipitation data of low and high elevation sites were also listed (Table 2.2). I analyzed only specimens that showed adult animal's feature (presence of testes or ovaries). At high elevations, it is hard to collect specimens in March and April because of long winters and collateral lingering snows (personal communication, Pauley 2002). On the other hand, dry summers make it difficult to find salamanders in Harrison and Doddridge Counties because they stay under the ground to avoid summer heat and dry conditions (personal communication, Pauley 2002). Nagel (1977) also reported difficulty in finding animals in summer in Tenessee and suggested that by June, individuals of *P. cinereus* were apparently retreating into subsurface refugia to avoid the heat of summer in Tennessee; Taub (1961) agreed with this. For these reasons, specimens at low and high elevations could not be collected throughout the year.

2. Morphological analysis

Snout-vent length (SVL), cranial width (CW), and tail circumference (TC) of each specimen were measured to the nearest 0.01 mm with a digital caliper (PRO-MAX Series, PREDV. FOWLER Co., INC.) and recorded with its gender and museum identification number. SVL is the length from the posterior lip of the cloaca to the tip of the snout. CW is width behind the eyes and TC is circumference at the posterior lip of the cloaca. Associations among these values were determined by linear regression test. Comparisons in these measurements between male and female within the same population and between two different altitudinal populations were made.

3. Reproductive analysis and dissecting procedure

Each specimen was dissected and excess fat was removed. Stomachs were removed and placed in labeled tubes filled with 80% ethanol for stomach content analysis. Stomachs from low elevation populations were removed previously by Kramer (1996) for stomach content analysis. Therefore, I only processed specimens from high elevations. To assess percent of tail fat, the first anterior 18 mm of tail of each specimen was removed and put into a labeled tube along with remainder of the tail. Adult males were separated from immature males on the basis of pigmentation of the testes and vasa deferentia (Sayler 1966). I especially emphasized pigmentation of vasa deferentia. Adult males had clear black vasa deferentia and immature males had invisible vasa deferentia. All mature females were distinguished from immature females by their enlarged oviducts, which were partly covered by a pigmented mesotubarium (Sayler 1966). The largest oviducts were found in spent females. Their oviducts were nearly straight and their muscle fibers were separated into distinct bands (probably due to stretching by the recent passage of large eggs). Oviducts of females that possessed maturing ovarian eggs were large and convoluted, while those of mature females with small follicles were thin and almost straight. When females were mature, the presence of follicles was observed with the naked eye and by a binocular dissecting scope. Mature females were classified into three groups, 1) spent, 2) visible follicles with the naked eye, and 3) immature follicles that were uncountable or invisible with the naked eye, but countable or visible by a dissecting scope. Females with follicles that were 1.3 mm or greater in diameter are capable of mating (Sayler 1966). All visible follicles were equal to (the ones collected in November from low elevations) or lager than 1.3 mm in diameter. Contrastively invisible follicles were smaller than 1.3 mm in diameter. These specimens will deposit follicles the following year (Sayler 1966). Therefore, these individuals were classified into a different group than females with visible follicles with naked eye. When follicles were visible with the naked eye, number of follicles was counted and total volume of both ovaries with follicles were determined by immersing ovaries in 10ml water in a graduated flask and removing displaced water with a graduated syringe. Ovaries were preserved in 80% ethanol.

In the case of males, testes and vasa deferentia were removed from the body. Anterior half and posterior half of testes, anterior end and posterior end of vasa deferentia were placed separately on microscope slide with a drop of Wright's stain. Therefore, 8 sections were prepared from one mature male. A plastic cover slide was placed on them and I mashed them (tissue with Wright's stain) by pushing down on the cover slide thoroughly. To determine the sperm wave, the presence of sperm in each section was examined and numbers of reproductive sections that contained sperm were recorded. After here, this number is referred as 'sperm wave number' in this study. When spermatozoa (sperm) develop from spermatids, testes become enlarged and the surfaces of testes consist of many small divisions, called testicular ampulla. Each testicular ampulla usually contains cells in the same developmental stage and there is a posterior to anterior sequence of stages throughout the length of the testes (Werner 1969). In other word, sperm is first mature in the posterior parts of testes and gradually sperm production wave moves toward the anterior part of testes. In this stage, sperm wave number is 4

(posterior and anterior parts of both testes). Sperm cells then move from the region of the testes where they develop through the efferent ductules the proximal region of the vasa deferentia which is adjacent to the testes, through the distal region of the vasa deferentia (sperm wave number = 8; full), and finally move into the cloaca before deposition (Canterbury and Pauley 1994). In case of *P. cinereus*, breeding starts in fall and lasts until next spring as males evacuate sperm. As evacuation proceeds, sperm wave number decreases to zero in late spring or early summer. As a result, the sperm wave number fluctuates from 0 to 8 and stands for reproductive status of males. The method that I adopted here is similar to Canterbury and Pauley (1994) who studied reproductive status of green salamanders (*Aneides aeneus*) in West Virginia.

4. Tail fat analysis

In collecting data for tail fat percentage, I referred to Pauley (1999). Anterior 18 mm of the tail of each specimen was removed and put into a labeled tube along with the remainder of tail. Tubes were placed in oven at 35°C for about 8 hours or until dry. After that, dry weight of 18 mm part and remainder were recorded separately. Dried parts were placed into labeled tubes again and filled with petroleum ether. These tubes were sealed and left for 12 hours. Petroleum ether was poured into wasted bottle, and the tubes with tails were placed in the oven again at 35°C for 8 hours or until dry. Each lean part was weighed (lean weight) and recorded. Tail fat percentage was calculated by the following equation:

$$\frac{DryWeight - LeanWeight}{DryWeight} \times 100$$

Comparisons in tail fat percentage between males and females within the same population and between two different altitudinal populations were made.

5. Stomach content analysis

Stomachs of animals from low elevation sites (Harrison and Doddridge Counties) had been analyzed by Kramer (1996) as mentioned previously. Therefore, I only analyzed stomachs of salamanders from high elevations. Stomachs were placed in labeled tubes filled with 80% ethanol and examined under a dissecting scope. Stomach contents were identified to order level (Kramer 1996; Raimondo 1999). Frequency of prey and percent frequency of prey were calculated. Percentage of stomachs that contained each prey category was also calculated. Feeding patterns between two different altitudinal populations were compared. Dietary overlap was calculated using the similarity index:

$$D = \left(1.0 - 0.5\sum \left| Px,i - Py,i \right|\right) \times 100$$

where D is the percentage of overlap and $P_{x,i}$ and $P_{y,i}$ are the proportions of the number of items species x and y utilized in resource category i (Holomuzki 1980; Rathcke 1976; Schoener 1970). Comparisons of food overlap between males and females within the same altitudinal populations and between two different altitudinal populations were made. Because the only prey item from high elevations large enough to measure for were mites, prey volume analysis was not performed.

6. Data analysis

I performed all statistical analyses with SigmaStat version 2.00 (Jandel Scientific Software). To compare two groups, t-test or Mann-Whitney Rank Sum Tests were adopted depending on results of normality and equal variance tests.

To demonstrate association of two groups, linear regression test was used to predict a trend in data, or predict the value of a variable from the value of another variable, by fitting a straight line through the data. In using linear regression, the independent variable has to be known. For example, SVL is independent variable when an association between SVL and CW is determined. When the independent variable (SVL) is varied, it produces a corresponding value (CW) for the dependent, or response, variable. Linear regression test also give a regression equation with the correlation coefficient (R) for a straight line to predict values of dependent variable;

$$y = b + ax$$

where y is the dependent variable, x is the independent variable, b is the constant, or intercept (value of the dependent variable when x=0, the point where the regression line intersects the y axis), and a is the slope (increase in the value of y per unit increase in x). R (correlation coefficient) is a measure of how well the regression model describes the data. R values near 1 indicate that the straight line is a good description of the relation between the independent and dependent variable (© Copyright 1992-1995 Jandel Corporation).

CHAPTER III

Results

1. Morphological and tail fat analyses

A total of 156 specimens (84 from high elevation and 72 from low elevation) were measured and data of SVL (snout-vent length), CW (cranial width), and TC (tail circumference) analyzed. Tail fat percentage was also calculated for each specimen.

Comparison within populations

i. Populations at high elevation

Eighty four specimens from high elevations were analyzed, of which, 43 were females and 41 were males. Of all females, 30 were adults (sexually mature) and 13 were subadults (sexually immature) (Figure 3.1). The adult females ranged in SVL from 48.00 to 37.45 mm and subadult females ranged 42.66 to 32.06 mm. Of all males, 36 were identified as adults ranging in SVL from 44.97 to 38.17 mm and 4 subadults were confirmed ranging from 38.17 mm to 32.57 mm (Figure 3.2). Linear regression tests showed that there were significant associations between SVL and both CW and TC of females ($p<0.001$and $p<0.001$, respectively; Figure3.3) and regression equations of CW and TC were:

$$CW = 1.697 + (0.0804 \times SVL) \quad (R = 0.786)$$

$$TC = 3.172 + (0.149 \times SVL) \quad (R = 0.664)$$

where CW and TC are the dependent variables, SVL is the independent variable. R is a correlation coefficient and a measure of how well the regression model describes the data. R values near 1 indicate that the straight line is a good description of the relation between the independent and dependent variable. Both CW and TC tend to increased as SVL value became larger. Tail fat percentage of female animals also increased as SVL became larger (Figure 3.4) and their association was also significant ($p=0.022$). Regression equation of tail fat percentage was expressed by:

$$\text{Tail fat (\%)} = -6.696 + (0.381 \times SVL) \quad (R= 0.352)$$

Compared with those of CW and TC, tail fat equation does not describe the data well (R=0.352) although the correlation was significant ($p=0.022$). TC and tail fat percentage of females showed strong association ($p<0.001$) and tail fat percentage increases as TC becomes larger (Figure 3.5). Regression equation for this association was:

Tail fat (%) = -14.168 + (2.497 x TC) (R = 0.507)

As results of linear regression tests, male *Plethodon cinereus* from high elevation showed the same tendency as that of females except for SVL – tail fat association (Figure 3.6 and 3.7). SVL and both of CW and TC showed significant associations ($p<0.001$ and $p<0.001$, respectively), but the association between SVL and tail fat percentage did not reach significant level ($p=0.054$). Tail fat percentage was associated with TC significantly ($p=0.003$; Figure 3.8). These equations were:

CW = 1.162 + (0.0961 x SVL) (R = 0.628)

TC = 4.115 + (0.126 x SVL) (R = 0.489)

Tail fat (%) = -5.987 + (0.319 x SVL) (R = 0.304; not significant)

Tail fat (%) = -9.973 + (1.832 x TC) (R = 0.450)

Comparisons in SVL, CW, TC, and tail fat between adult females and males from high elevations were shown (Table 3.1). Adult female was significantly larger in SVL than adult males ($p<0.001$) and stored significantly larger amounts of fat in their tails than males ($p=0.024$). There were no significant difference in CW and TC ($p=0.321$ and $p=0.061$, respectively).

ii. Populations at low elevation

Seventy-two specimens from low elevations were analyzed, of which, 46 were females and 26 were males. Female SVL distribution is shown in Figure 3.9 and 23 out of 46 were adults ranging in SVL from 40.33 to 53.30 mm 23 specimens were subadults ranging from 34.58 to 43.94 mm. Of all males, 25 were adult whose SVL ranged from 35.26 to 51.57 mm and only one subadult (SVL=38.38 mm) was found (Figure 3.10). Scattered plots showing correlations among SVL, CW, TC, and tail fat are displayed in Figure 3.11, 3.12, and 3.13. Linear regression tests resulted that there were significant associations between SVL and both CW and TC of adult females from low elevations ($p<0.001$ and $p<0.001$, respectively; Figure 3.11), but not between SVL and tail fat ($p=0.051$; Figure 3.12). There was also strong significant associations between TC and tail fat ($p<0.001$; Figure 3.13). Regression equations for these associations were:

CW = 1.762 + (0.0708 x SVL) (R=0.811)

TC = 2.690 + (0.182 x SVL) (R=0.690)

Tail fat (%) = -1.713 + (0.181 x SVL) (R=0.290; not significant)

Tail fat (%) = -7.632 + (1.310 x TC) (R=0.552)

Overall, these results showed exactly the same tendency as adult males from high elevations. CW and TC of adult females from low elevations tended to increase together with SVL, but tail fat did not follow the same pattern. Although there was no direct significant association between SVL and tail fat, tail fat increased as TC became larger and TC tended to increased as SVL became larger. Therefore, there was indirect association between SVL and tail fat.

Results of males from low elevations showed the same pattern as females from low elevations. As shown in Figure 3.14, values of SVL can be used to predict CW and TC (p<0.001 and p=0.009, respectively) by fitting straight lines. An association between SVL and tail fat was not strong enough to predict a trend in tail fat from SVL (p=0.403; Figure 3.15) but the trend in tail fat was able to be predicted by TC (p=0.004; Figure 3.16). These regression equations were:

CW = 1.427 + (0.0847 x SVL) (R=0.829)

TC = 5.188 + (0.121 x SVL) (R=0.503)

Tail fat (%) = 0.612 + (0.103 x SVL) (R=0.171; not significant)

Tail fat (%) = -9.133 + (1.361 x TC) (R=0.543)

Comparisons in average SVL, CW, TC, and tail fat between adult females and males from low elevations were shown in Table 3.2. As adult females from high elevations were significantly larger in SVL than males (Table 3.1), adult females from low elevations were also significantly larger in SVL than males. There were no significant differences in the other measurements (CW, TC, and tail fat).

Comparisons between high and low populations

Comparisons in average SVL, CW, TC, and tail fat (%) between females from high and low elevations were made (Table 3.3). Females from low elevations were significantly larger in SVL and TC (p=0.003 and p<0.001, respectively), but significantly smaller in CW (p=0.020) than females from high elevations. Although females from low elevation had thick tails, they stored significantly less fat on their tail (p=0.041). In other words, females from high elevation had significantly larger amount of tail fat than females from low elevations.

Comparisons between males from high and low elevations showed the similar tendency (Table 3.4). Males from low elevations were significantly larger in SVL and TC (p<0.001 and p<0.001, respectively), but had significantly less fat on their tail than males from high elevations (p=0.006). There was no significant difference in CW.

In summary, *P. cinereus* from low elevations was significantly larger in SVL and TC than animals from high elevations. However, salamanders from high elevations stored significantly larger amount of fat in their tails.

2. Reproductive analysis

Male

It is known that males reproduce every year (Petranka 1998) and in this study, it was also confirmed that all adult males were in the process of sperm development. Some males had sperm in both testes and vasa deferentia (Figure 3.17and Figure3.18; details are discussed in Chapter II) and some did not contain any sperm. Although some males did not contain any sperm in their reproductive organs, it was found that next spermatogenic wave was beginning as indicated by the presence of spermatids in testes (Figure 3.19). Spermatids are the haploid products of second meiotic division in spermatogenesis that differentiates into mature spermatozoa (sperm).

In Figure 3.20, sperm waves of males form high and low elevations were shown. Numbers beside dots represent numbers of specimens included in those dots. All males from low elevations collected in March contained sperm in some sections of their reproductive organs. One male on 3/28 was packed with sperm (sperm observed in 7 sections out of 8). Two males collected on 4/30 and two collected on 5/6 from low elevation did not have any sperm. Two males also collected on 5/6 from low elevations contained sperm. At high elevations, two on 5/20, five on 5/26, and 7 specimens on 6/16 contained no sperm and the rest of all contained sperm in at least two reproductive sections out of eight. The one collected on 8/24 from high elevations had sperm in six sections out of eight. Because no specimens were collected from 6/16 to 7/21, sperm wave of high populations during that period could not be determined.

Female

Adult females were separated from subadult females by structures of oviducts (Discussed in Chapter II). Immature females (subadult) had very thin and straight oviducts along kidneys (Figure 3.21) whereas mature females had enlarged and waved oviducts because of past egg depositions (Figure 3.22) as (Sayler 1966). All of subadult animals had tiny follicles which apparently showed that they were going to be mature in the next breeding season. Adult females were classified into three groups, females with mature eggs (>1.3 mm in diameter; Figure 3.23), spent females (Figure 3.24), and females with immature eggs (<1.3 mm in diameter; Figure 3.22). All mature females (n=17) from low elevations hold mature eggs (Figure 3.25, Table 3.5) while only two females from high females had mature eggs in their ovaries (Figure 3.26, Table 3.5). Six females were collected in November 1994 from low elevations, but these were excluded from Figure 3.25 and Table3 .5 because these were developing follicles for the next

breeding season and Figure 3.25, Figure 3.26, and Table 3.5 were targeted on their breeding status in that year. Eleven adult females from high elevations were found holding immature eggs from entire SVL classes except for class 36-38 mm and 17 spent females from high elevations were distributed among all classes but class 38-40 mm (Figure 3.26 and Table 3.5).

There was a significant association between SVL and egg numbers of females from low elevations (p=0.001; Figure 3.27) and regression equation was:

$$\text{Egg number} = -7.398 + (0.373 \times \text{SVL}) \quad (R=0.630)$$

As a result, larger females tended to have more eggs. Because only two females from high elevation had mature eggs, this association could not be developed for high populations. Average clutch size was 9.83 for low populations (n=23) and 9 for high populations (n=2). Average clutch size of all females was 9.76.

Egg developments were analyzed in diameter (Figure 3.28) and volume (Figure 3.29). Two females collected on 4/9 and 5/6, 1994 from low elevation had eggs larger than 3.0 mm in diameter and were about to lay eggs (Figure 3.28). The rest of females collected in spring 1994 from low elevations (low female 1 in Figure 3.28) held eggs ranging from 1.75 and 2.88 mm in diameter and it seemed that they were going to be laid at most within two months. Six females were collected on 11/6 1994 from low elevations (low female2 in Figure 3.28) and all of them had relatively small eggs ranging from 1.25 to 1.7 in diameter and these eggs were going to be laid in the following spring or summer. As mentioned previously, only two females from high elevations had mature eggs and one female collected on 5/26 from high elevations had eggs larger than 3.0 mm in diameter and the other on 5/20 was 2.89 mm in diameter. They were also about to be laid. Since the sample size was not big enough, it was impossible to compare egg development between females from high and low elevations. Egg development in volume per egg (Figure 3.29) showed the similar tendency to that of egg development in size per egg, but one female collected on 5/6 1994 from low elevation had comparatively larger eggs (0.019 per egg) and the other female collected on the same day contained relatively smaller eggs (0.009 per egg). Sizes in diameter per egg of these two females from high elevations were similar (Figure 3.28), but their volumes per egg were different (Figure 3.29).

3. Stomach content analysis

Stomach contents of 79 animals collected from high elevation during 1993 were analyzed, of which, 41 were female and 38 were male. Out of 79, 25 empty stomachs were recorded and this occupied 31.65 % of all stomachs. The most abundantt prey categories numerically were springtails (75.63 %) and mites (16.65 %) for populations at high elevation (Table 3.6). The rest of all prey categories occupied very small percentages ranging 0.1 % to 3.91 % (Table 3.6). Prey items found in the highest proportion of their stomachs were mites (44.3 %). The second highest prey items were

ants and wasps (27.85 %), flies (16.46 %), and beetles (10.13 %). The reason for high frequency of springtails and low proportion of stomachs containing springtails was because three animals collected on 20th May, 1993 contained more than 100 tiny springtails up to 500 per animal in their stomachs. These were calculated as 250 each because it was incredibly difficult to count those numbers. Another calculation was also made excluding these three (Table 3.7) because those cases were rare (Personal communication, Raimondo 2002) and there was a possibility to prevent suitable data interpretations. In Table3.7 that excludes those rare cases, numerically mites were by far the most abundant prey category (66.40 % of all items found in the stomachs). The second most abundant prey category was ants and wasps accounting for 15.6 % of all items found in the stomachs. The rest of all categories occupied only small portion ranging from 0.4 % to 6 % of all items found in the stomachs. Prey items found in the highest proportion of their stomachs were mites (46.05 %) again. The next highest prey items were ants and wasps (28.95 %), flies (17.11 %), and beetles (10.53 %). In any case, proportions of prey categories that salamanders at high elevations utilized were one-sided.

Kramer (1996) studied stomach contents of 102 animals (56 females, 26 males, and 20 juveniles) from low elevation. Seven empty stomachs were found and this occupied 6.86 % of all stomachs. The most abundant prey categories numerically were springtails (18.81 %), ants and wasps (18.33 %), mites (13.45 %), Isopods (10.95 %) and beetle larvae (10.83 %). Prey items found in the highest proportion of their stomachs were mites (48.04 %), ants and wasps (47.06 %), springtails (46.08 %), beetles (42.16 %), and beetle larvae (40.20 %). Compared with those of animals collected at high elevations, proportions of prey categories that animals at low elevation utilized were well balanced.

Dietary overlap (similarity index: D) between high and low populations was 39.57 % including the 3 salamanders that contained large numbers of springtail and 41.20 % if they were excluded (Table 3.8). Dietary overlaps between females and males from the same elevations were 83.79 % for high populations and 81.40 % for low populations.

CHAPTER IV

Discussions and Conclusions

1. Morphological and tail fat analyses

Regression equations of SVL-CW, SVL-TC, SVL-tail fat, and TC-tail fat

There are 4 subsamples of *Plethodon cinereus* collected for this study, high elevation males (n=41) and females (n=43), and low elevation males (n=26) and females (n=46).

In all 4 subsamples, there were significant associations between SVL (snout-vent length) and both CW (cranial width) and TC (tail circumference) which meant that both CW and TC tended to increase as SVL value became larger. This result was understandable and showed that all the measurements were reliable. I summarized the slope values and correlation coefficients of these regression equations (Table 4.1) and found that the slope values of TC were significantly larger than those of CW (p=0.006). This means that TC increases more rapidly than CW as SVL becomes larger. However, correlation coefficients of TC were significantly smaller than those of CW (p=0.045) which mean that SVL-TC equations do not describe their associations as well as SVL-CW equations do. This is probably because whereas SVL and CW reflect body frame directly, TC values are influenced by both of the size of tail bones and fat around them. It is possible to predict the values of CW and TC from SVL if appropriate equations for the populations are given.

Only high elevation females showed a significant association between SVL and tail fat and this association in other subsamples did not reach statistically significant level. On the other hand, all subsamples showed significant associations between TC and tail fat. Summary of the slope values and correlation coefficients for the equations is shown in Table 4.2. Comparisons of the slopes and correlation coefficients between SVL-fat and TC-fat were not made because in three of four subsamples SVL-fat associations did not reach significant levels and comparisons would be pointless under these conditions. Although statistical analyses were not done, it is noted that the slope values of both SVL-fat and TC-fat relationships are quite different between high (the SVL-fat slopes: 0.381 for female and 0.319 for male; the TC-fat slopes: 2.497 for female and 1.832 for male) and low (the SVL-fat slopes: 0.181 for female and 0.103 for male; the TC-fat slopes: 1.31 for female and 1.361 for male) elevations (Table 4.2). It seems that each population has each unique regression equation for both SVL-fat and TC-fat although this tendency was not observed for SVL-CW and SVL-TC (Table 4.1). In general it is not appropriate to predict tail fat from SVL values, but it can be allowed to predict tail fat from TC values if regression equations for the certain populations are known. Since tail fat analysis consumes time and needs toxic chemical (petroleum ether), measurements of tail circumferences can be easy and adequate way to grasp tail fat trends in case that there is not enough time and proper facilities. However, the tail fat trends that can be predicted by TC have to be insistently relative values and it is not

appropriate to predict absolute values from tail fat without precise equations since each population has each equation for it and it varies greatly as the results showed.

In conclusion, there were significant associations between SVL (snout-vent length) and both CW (cranial width) and TC (tail circumference) in all of 4 subsamples (males and females in high and low elevations). Regression equations for SVL-CW described their associations significantly better than regression equations for SVL-CW (p=0.045). This is probably because whereas SVL and CW reflect body frame directly, TC values are influenced by both of the size of tail bones and fat around them. It is possible to predict the values of CW and TC from SVL if appropriate equations for the populations are given. Whereas only females at high elevations showed significant association between SVL and tail fat, all subsamples showed significant associations between TC and tail fat. Tail fat can be predicted by TC but not SVL and TC can be a good indicator of the energy in the tail.

Sexual dimorphism

As most researcher (Bishop 1941; Blanchard 1928; Burger 1935; Pfingsten and Downs 1989) reported, sexual dimorphism in SVL was observed in this study (Table 3.1 and Table 3.2). In high elevations, adult females (average SVL = 43.48 mm) were significantly (p<0.001) larger than adult males (average SVL = 40.99 mm). In low elevations, adult females (average SVL = 46.16 mm) also were significantly larger (p=0.041) than adult males (average SVL = 44.03 mm). Differences between females and males were 2.49 mm in high elevations and 2.13 mm in low elevations which were similar to 2.73 mm in New York (Bishop 1941) and 2 mm in Pennsylvania and New Jersey (Burger 1935), but quite difference from 0.7 mm in Ohio (Pfingsten and Downs 1989). As far as I know, only one researcher (Sayler 1966) reported no sexual dimorphism in Maryland (males; 39.7 mm, females; 41.2 mm). In West Virginia, regardless of elevations, sexual dimorphism in SVL seems to exist.

There were no significant differences in CW and TC between males and females in both high and low elevations (Table 3.1 and Table 3.2). I could not compare these values with other studies because less attention has been placed on these measurements, especially on TC and these are not listed in papers. The reason why CW measurements are not reported is probably because this value does not have a wide range (3.65 – 5.79 mm in this study) and is not an accurate indicator for growth and sexual or size dimorphism. It is also difficult to measure TC while animals are alive, which might make researchers avoid taking this measurement. However, TC can be a good indicator for the energy on tails as mentioned previously and its measurement is recommended.

Sexual dimorphism in tail fat percentage was found in high elevations (Table 3.1). In high elevation populations, females stored significantly larger amounts of fat in their tails (9.28 %) than males (7.24 %) (p=0.024). On the other hand, no sexual dimorphism in tail fat percentage was detected in low elevations (Table 3.2). Females in low elevations seemed to stored more fat in their tail (6.80 %) than males (5.21 %), but this difference did not reach statistical significance (p=0.18). Tail fat percentage is an interesting indicator for energy storage and the result here can be explained by energy

consuming process of female reproduction. As discussed in reproductive analysis of this chapter, females at high elevations mostly oviposit biennially whereas females at low elevations do so annually in West Virginia. Biennial ovipositions were observed in northern states, such as New York (Bishop 1941), Michigan (Test and Bingham 1948), Maryland (Sayler 1966), and Ohio (Pfingsten and Downs 1989). Petranka (1998) pointed out that in these location where biennial oviposition occurs, the growing season is relatively short and females apparently cannot obtain sufficient energy in 1 year to yolk a clutch. Moreover, stomach content analysis showed 31.65 % of all stomachs from high elevation populations were empty while only 6.86 % empty stomachs were found from low elevation populations (Chapter III). This result shows that prey items are less available in high elevations. Females in high elevations in West Virginia take two years to obtain enough energy for oviposition while males always reproduce every year. Possibly this is the reason why there was sexual dimorphism of tail fat in high elevation populations, but not in low elevation populations.

In conclusion, in West Virginia, sexual dimorphism in SVL exists regardless of elevations. Females were significantly larger than males in both high ($p<0.001$) and low ($p=0.041$) elevation populations. Females from high elevations stored significantly larger amounts of fat in their tails than from low elevations ($p=0.024$) while there was no difference in low elevations ($p=0.18$). This is probably due to the fact that high elevation females reproduce every other year (they have two years to store energy in their tails) whereas females in low elevations do so every year.

Size dimorphism between high and low altitudinal populations

There were two theories that could predict size dimorphism between high and low elevation populations as discussed in Chapter I. The first theory was that populations at high elevations could be smaller due to longer winters and less prey availability. The other one was that populations at high elevations could be larger following the 'developmental temperature-size rule'; developmental temperature-size rule states that in general ectothermic organism grow larger at both lower temperature and higher latitudes (Atkinson and Sibly 1997; Van Voorhies 1996).

Adult *P. cinereus* from high elevations was significantly smaller in SVL and TC than from low elevations (SVL: $p=0.003$ for female and $p<0.001$ for male, TC: $p<0.001$ for female and $p<0.001$ for male). This result seemed to be explained by the first theory, but this must be explained by trade off of those two theories as Atkinson and Sibly (1997) carefully concluded that whereas smaller adults emerge if growth is limited by food availability, the reverse is usually observed if growth is limited by temperature. According to Atkinson (1994), 7 studies that focused on rearing temperature and size of amphibians have been published and all of them showed a significant size reduction with increasing temperature. At high elevations, 31.65 % of salamanders had empty stomachs whereas only 6.86 % empty stomachs were recorded at low elevations. Clearly less prey availability in high elevations played a major role to cause size dimorphism in SVL and TC between high and low elevation populations.

An opposite result was gained in tail fat. Salamanders from high elevations stored significantly larger amounts of fat in their tails than from low elevations (p=0.041 for female and p=0.006 for male). This was interesting because lower tail fat in high altitudinal populations was expected as SVL and TC results showed. If only females from high elevations showed significantly more tail fat percentage than from low elevations, this would be explained by biennial reproduction of high elevation females. They take 2 years to develop follicles and deposit them while females at low elevations oviposit every year. Therefore, they have 2 years to store energy which would store more fat in their tails than low elevation females that reproduce annually. However, both females and males at high elevations had significantly larger amounts of fat in their tails. The one possibility to explain this result was adaptation to colder environments. Because they live in harsh environments, they have to save more energy despite less prey availability and individuals that do not save enough energy would die during long winters. It cannot be concluded if it is phenotypic or genotypic.

In conclusion, adult *P. cinereus* from high elevations was significantly smaller in SVL and TC than from low elevations (SVL: p=0.003 for female and p<0.001 for male, TC: p<0.001 for female and p<0.001 for male) and this result was explained by less prey availability and shorter growing seasons at high elevations. Contrastively salamanders from high elevations stored significantly larger amounts of fat in their tails than from low elevations (p=0.041 for female and p=0.006 for male) and this could be an adaptation toward harsh environments at high elevations.

2. Reproductive analysis

Males

Werner (1969) studied temperature-photoperiod effects on spermatogenesis in *P. cinereus* and concluded that compared with the groups at 20 °C, all photoperiod groups at 10 °C had an average retarded cycle of 16 days by 26 June. His experiments also indicated that temperatures of 20 °C were needed to initiate the spermatogenic wave (sperm production cycle; detail in Chapter II) in Michigan *P. cinereus*. As I mention in Chapter II, in this study there was 3.4°C of dairy temperature difference between high and low elevation populations and it is possible that this temperature interval causes difference in spermatogenic wave which can result in with slightly different breeding season between populations at different elevations.

In comparison of sperm evacuation season between males from high and low elevations, there seemed to be no differences in spring evacuation of sperm between males from high and low elevations. This is based on two individuals collected on 5/6, 1994 from low elevation that still had sperms in at least half of their reproductive organs and two specimens from high elevation that had already finished evacuation by 5/17 (Figure 3.20). However, because of different sampling periods as discussed in Chapter II, it was impossible to appropriately compare spring sperm evacuation of male animals between high and low populations and was difficult to grasp tendencies of the sperm waves of both elevation populations.

When data from high and low elevations were combined, it was possible to see a general trend of the sperm wave which corresponded to reported sperm wave (Werner 1969) and breeding seasons (Blanchard 1928; Nagel 1977; Sayler 1966) in the literature. In July and August, all males collected from high elevation started producing sperm cells in their testes for the coming fall, the beginning of mating season (Figure 3.20). They start evacuating sperms right after filling the testes and vasa deferentia with sperm in the fall. Three males on 3/28 were packed with sperm (sperm observed in 7 parts out of 8 in one specimen, sperm observed in 6 parts out of 8 in two specimens) which showed that breeding season lasted into late March. The end of sperm evacuation corresponds to the end of breeding season, generally in May. In Figure 3.20, the plot on 6/16 representing 7 males from high elevations marked sperm wave number 0 indicating no sperm in any parts of their reproductive organs. Therefore, it seemed that by at most 6th June, the breeding season is finished.

In conclusion, it was impossible to appropriately compare spring sperm evacuation of male animals between high and low populations because of different sampling periods. However, when data from high and low elevations were combined, it was possible to see a general trend of the sperm wave which corresponded to reported sperm wave (Werner 1969) and breeding seasons (Blanchard 1928; Nagel 1977; Sayler 1966).

Females

Juveniles reach sexual maturity about 2 years after hatching (Blanchard 1928; Nagel 1977; Sayler 1966) and, therefore, there are three groups, juveniles (1st year), immature adult or subadult (2nd year), and adults (2 years old or more). Immature females (2nd year) contained ovarian eggs usually less than 0.5 mm in diameter and had thin, straight oviducts (Sayler 1966). All of non-adult females that I found in this study contained tiny (<0.5 mm) follicles and that indicated they were immature females (2nd year) and preparing for the next oviposition season. However, it was noted that in high elevations, the overlap range in size of immature females and mature females were 5.21 mm (immature: 32.06 to 42.66 mm in SVL, mature: 37.45 to 48.00 mm) while in low elevations it was 3.61 mm (immature: 34.58 to 43.94 mm in SVL, mature: 40.33 to 53.30 mm). This is probably because in high elevations, the majority of females are able to lay eggs their third summer, but a small percentage may be unable to do so because of retarded growth caused by harsh environments of high elevations and less prey availability. As a result, some females at high elevations probably take 3 years to be mature while the majority takes two years.

Blanchard (1928) found no correspondence between the size of the female and the total number of follicles produced, but he did not statistically analyze this relationship. Nagel (1977) calculated a regression line with 0.023 – 0.256 slope and suggested a weak relationship. However, both authors did not make it clear whether an association between SVL and follicle number was significant. In this study, there was a significant association between SVL and egg numbers of females from low elevations (p=0.001) and a regression line with 0.373 slope was given (Chapter III). Therefore, the general trend

that larger females produce more follicles was corroborated by this study. Average clutch size of all females in this study was 9.76 and this is similar to 8-10 in New York populations (Bishop 1941), 9-10 in Michigan populations (Blanchard 1928; Nagel 1977), and 8.4 in Tennessee populations (Nagel 1977).

I wondered if there are differences in follicle development and oviposition seasons between high and low populations because these activities apparently require large amount of energy, which depends on prey availability and length of foraging period in a year. However, because only two females held clutches in high elevations while 15 females were gravid in low populations, this comparison could not be made.

Biennial female reproduction cycle for northern populations of *P. cinereus* have been reported (Bishop 1941; Pfingsten and Downs 1989; Sayler 1966; Test and Bingham 1948). These authors concluded that females laid eggs every other year on the basis of two distinct groups of large females (adults), those with enlarged follicles and those with only tiny follicles. On the other hand, Nagel (1977) found only one group of adult females and concluded annual oviposition in Tennessee. In this study, whereas one group with enlarged follicles was found in low elevations, two groups, those with enlarged follicles or spent (63 % of all females) and those with only tiny follicles (37 % of all females) were found in high elevations. Females with mature follicles (enlarged) were animals that were ready for oviposition that year and spent females were ones that had already laid eggs that year. Females with immature follicles (tiny follicles) were animals that were not ready to lay eggs that year, but following year. This result gave us the interesting fact that in West Virginia, females deposit follicles annually at low elevations, but biennially at high elevations. At high elevations females apparently cannot obtain sufficient energy in 1 year to yolk a clutch because of longer winters and less prey availability. The reason why there was difference in occurrence percentage (16%) between two groups in high elevations is probably because some females (13 %) that are well fed in high elevations oviposit every year. Larger adult females could be expected as these healthy specimens, but those two female groups (ones with enlarge follicles and ones with tiny follicles) in high elevations were equally distributed in size which denied the possibility that larger females were especially well fed. Moreover, as mentioned previously, 3 out of 4 subsamples (except females in high elevations) did not show significant associations between SVL and tail fat percentage. Therefore, females that possibly lay eggs every year at high elevations do not have to be larger individuals.

In conclusion, it was implied that some females at high elevations take 3 years to be mature while the majority at high elevations and all females at low elevations take 2 years. There was a significant association between SVL and follicle numbers and the general trend that larger females produce more follicles was supported by this study. Average clutch size of all females in this study was 9.76 similar to 8-10 in New York populations (Bishop 1941), 9-10 in Michigan populations (Blanchard 1928; Nagel 1977), and 8.4 in Tennessee populations (Nagel 1977). In West Virginia, females oviposit annually or biennially depending on elevations. Females at low elevations showed annual reproductive cycle while females at high elevations showed biennial reproductive cycle. At high elevations females apparently cannot obtain sufficient energy in 1 year to yolk a clutch because of longer winters and less prey availability.

3. Stomach content analysis

Salamanders at high elevations tended to depend on a few prey categories while populations at low elevations ate balanced food. In high elevation populations, the most abundant prey categories numerically were springtails (75.63 %) and mites (16.65 %) and other categories occupied less than 5 % of all items found. As mentioned in Chapter III, it was found that three salamanders from high elevations contained more than 100 tiny springtails up to 500 per each and although these aberrations were excluded, high altitudinal populations still relied mainly on two categories, mites (66.4%) and ants & wasps (15.6 %). On the other hand, food for low altitudinal populations consisted of 5 main categories, springtails (18.81 %), ants and wasps (18.33 %), mites (13.45 %), isopods (10.95 %), and beetle larvae (10.83 %). The similarity index also reflected these results with about 40 % dietary overlap between high and low elevation populations and about 80 % within each population (between males and females) and indicated that prey communities were considerably different between high and low elevation sites. Because salamanders are opportunistic feeders, populations at high elevations apparently do not have as many chances to come across various kinds of prey as at low elevations. Stomach content studies conducted two miles west of the Harrison-Doddridge County line in West Virginia (close to low elevation sites) revealed *P. cinereus* depended on two major prey items, ants (48.8 %) and mites (25.9 %) (Pauley 1978c). This result is quite different from the result from low elevations in this study and indicates that prey communities vary greatly depending on geographical and environmental factors.

It was noted that whereas 31.65 % of all stomachs from high elevations were empty, only 6.86 % from low elevations were empty. Pauley (1978c) concluded that 160 of 169 (95.0 %) salamanders (*P. cinereus*) had ingested some food item and concluded that food was abundant and available to all individuals throughout the study area. It is pointed out that foods were crucially less available for populations at high elevations than at low elevations. Put these results together with one sided food items at high elevations, it is more manifest that populations at high elevations are confronted by food shortages.

In conclusion, populations at high elevations are confronted by food shortages while foods are available enough for all individuals at low elevations.

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Appendix-I

Figures

Figure 1.1. Range of *Plethodon cinereus* (Cited from Petranka, 1998).

Figure 1.2. Distribution map of *Plethodon cinereus* in West Virginia (Adapted form Green and Pauley 1987).

Figure 1.3. *Plethodon cinereus*, the red back phase. This picture was taken in Pocahontas county, West Virginia by Mizuki Takahashi.

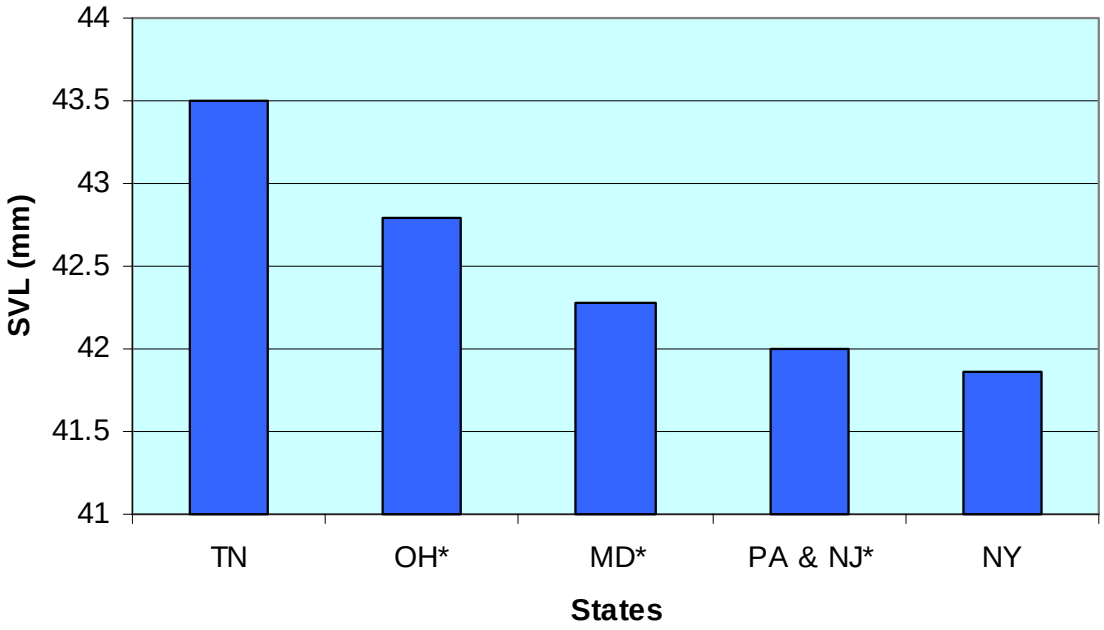

Figure 1.4. Snout-vent length (SVL) variation of *Plethodon cinereus* in different states (Date sources: TN; Nagel 1977, OH; Pfingsten and Downs 1989, MD; Sayler 1966, PA & NJ; Burger 1935, NY; Bishop 1941). Authors who studied in states with * measured SVL as the distance from the tip of snout to the anterior end of cloaca. Therefore, 2 mm was added to these measurements according to Nagel (1977) in order to be able to compare with others.

Figure 2.1. Range of *Plethodon cinereus* in WV with sampling sites. Numbers besides sites black dots corresponded to site numbers in Table2.1.

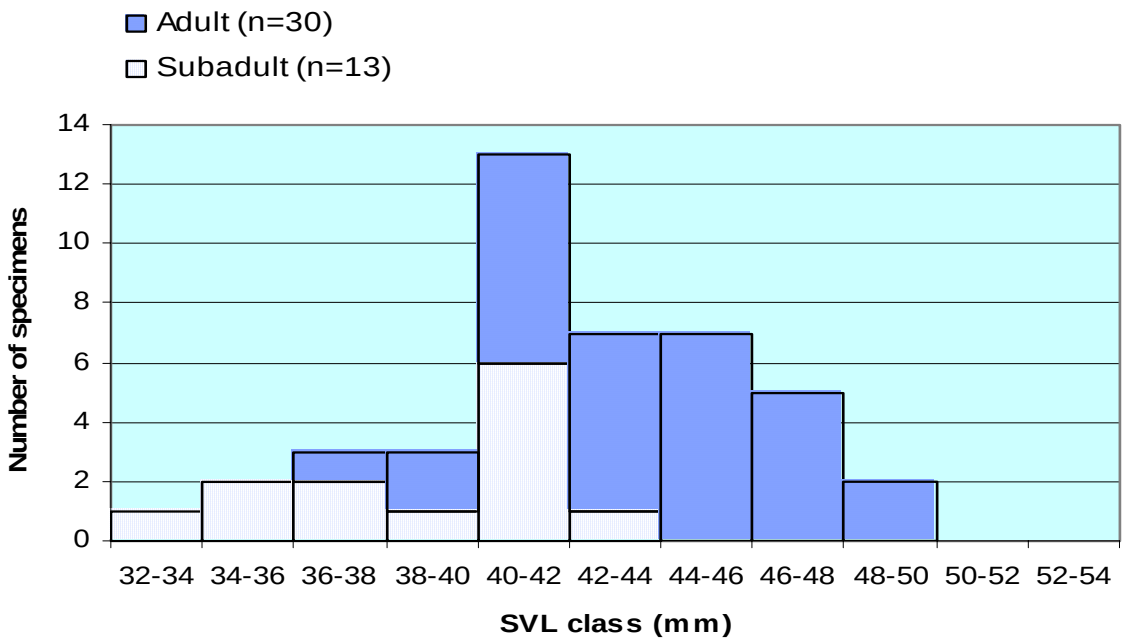

Figure 3.1. SVL (snout-vent length) distribution of female *Plethodon cinereus* from high elevations.

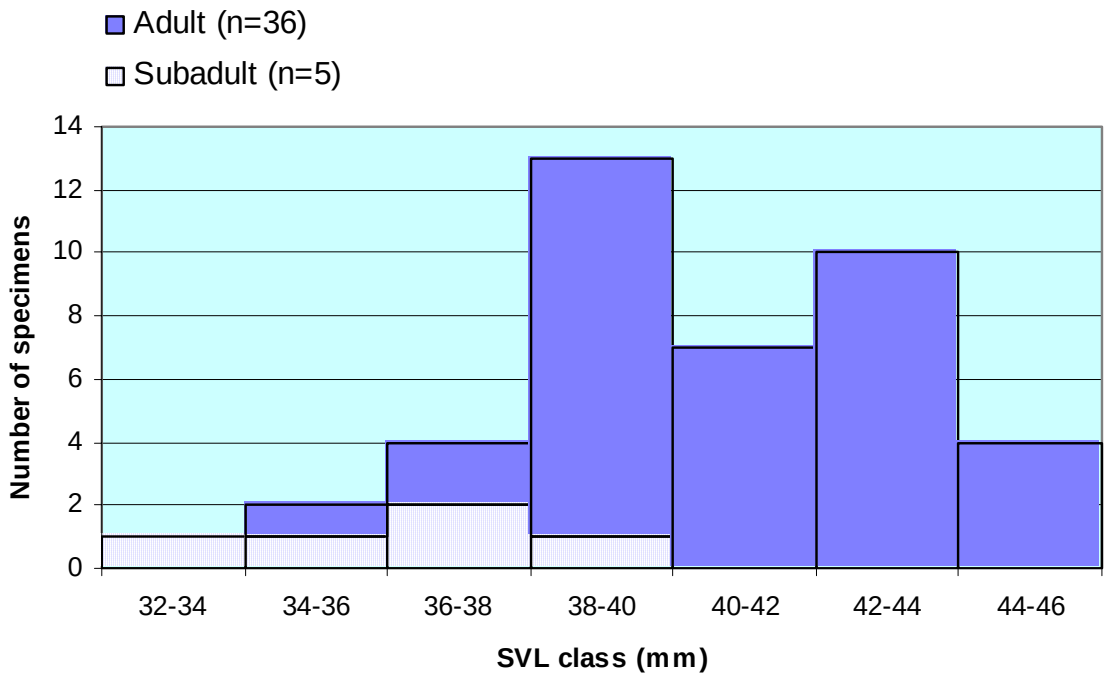

Figure 3.2. SVL (snout-vent length) distribution of male *Plethodon cinereus* from high elevations.

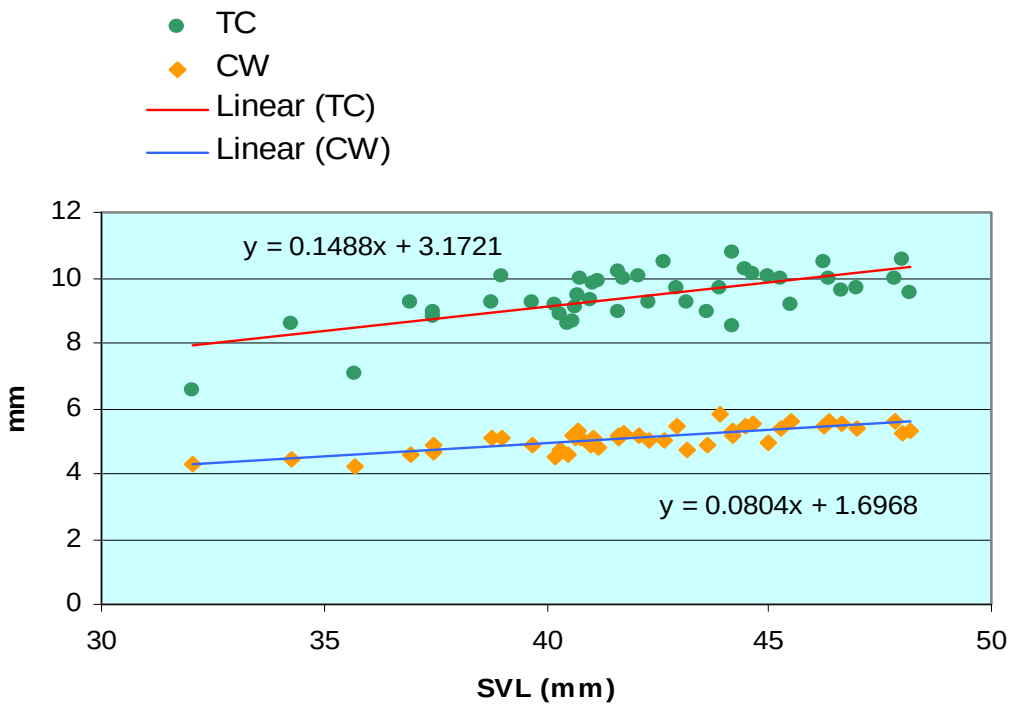

Figure 3.3. Scatter plot comparing SVL (snout-vent length), and CW (cranial width) and TC (tail circumference) of female *Plethodon cinereus* from high elevations. Estimated regression lines are shown (SVL-CW: $p<0.001$), SVL-TC: $p<0.001$).

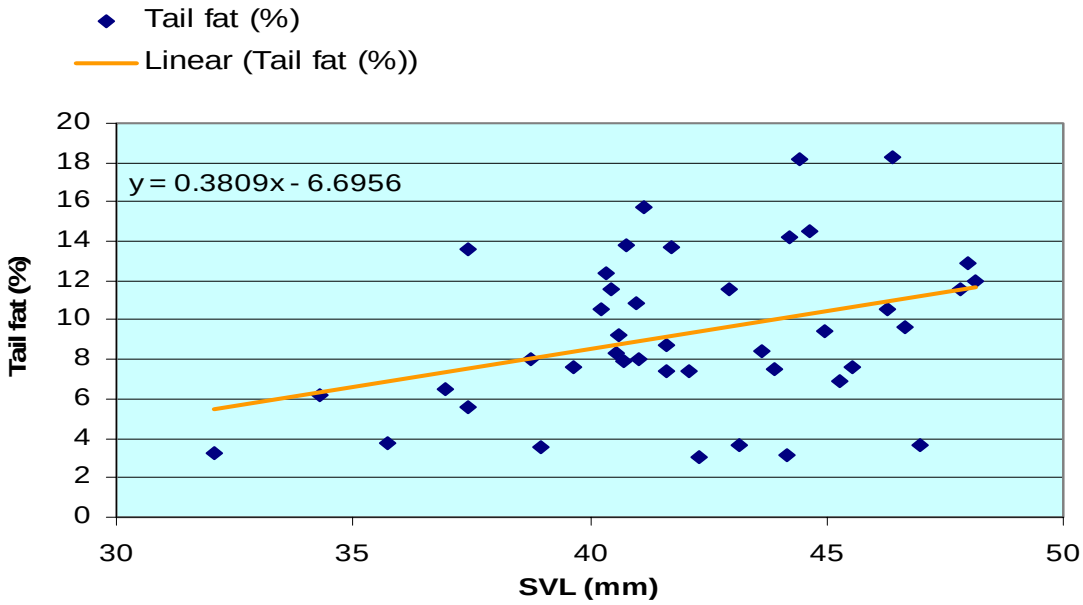

Figure 3.4. Scatter plot comparing SVL (snout-vent length) and tail fat of female *Plethodon cinereus* from high elevations. Estimated regression line is shown (p=0.022).

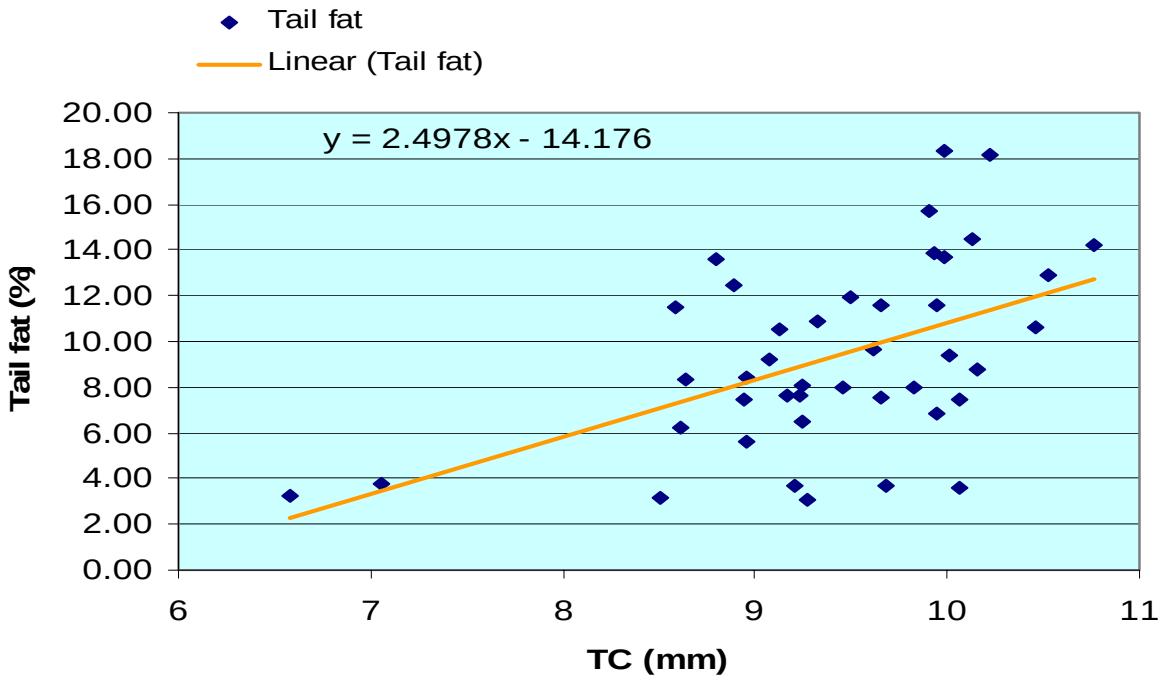

Figure 3.5. Scatter plot comparing TC (tail circumference) and tail fat of female *Plethodon cinereus* from high elevations. Estimated regression line is shown (P<0.001).

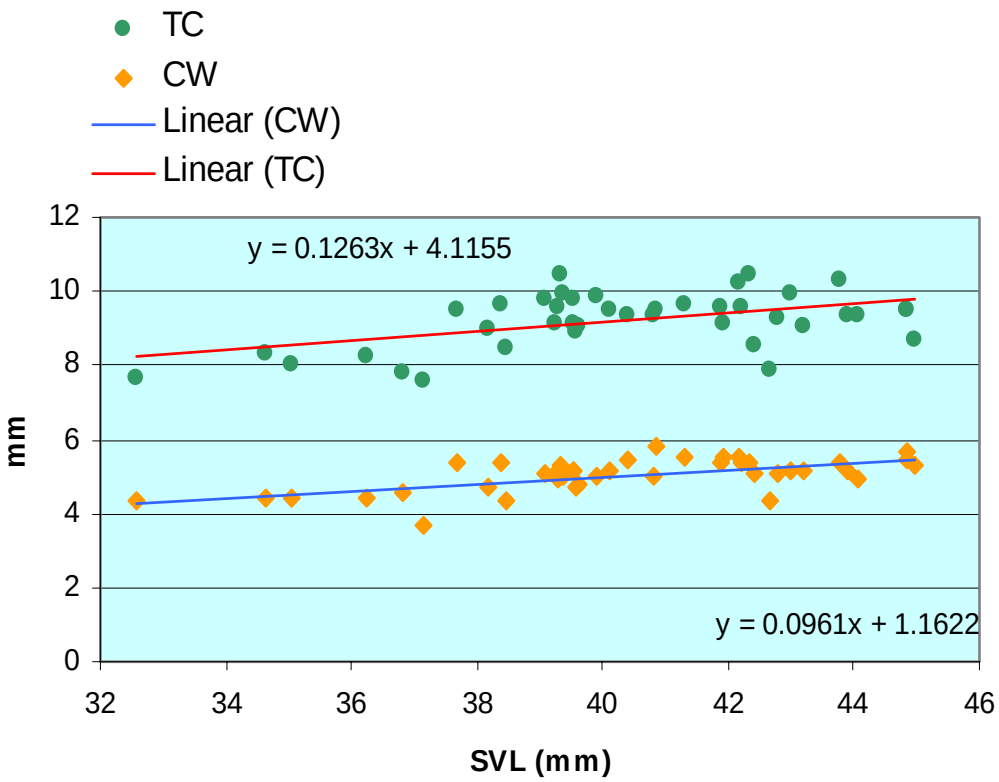

Figure 3.6. Scatter plot comparing SVL (snout-vent length), and CW (cranial width) and TC (tail circumference) of male *Plethodon cinereus* from high elevations. Estimated regression lines are shown (SVL-CW: $p<0.001$, SVL-TC: $p<0.001$).

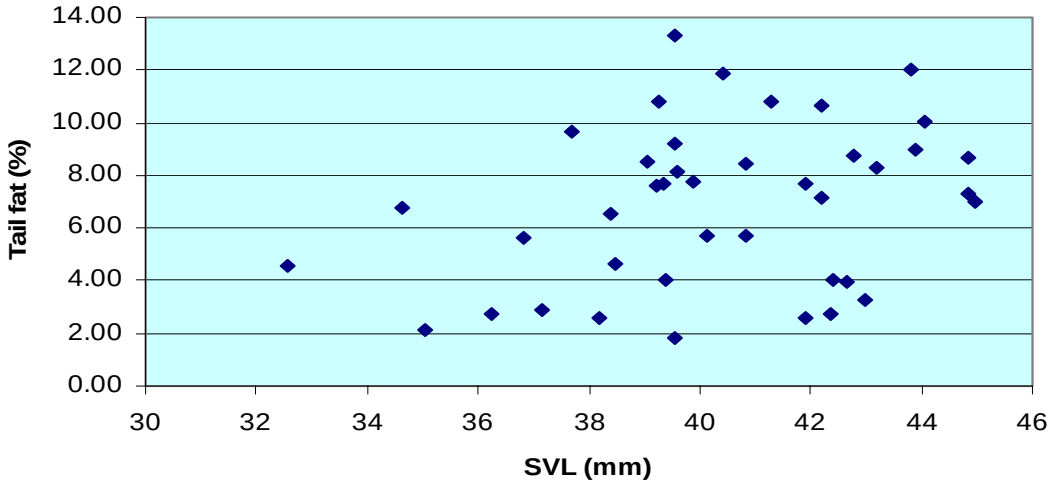

Figure 3.7. Scatter plot comparing SVL (snout-vent length) and tail fat of male *Plethodon cinereus* from high elevations. Regression line is not shown because an association between SVL and tail fat was not significant (p=0.054).

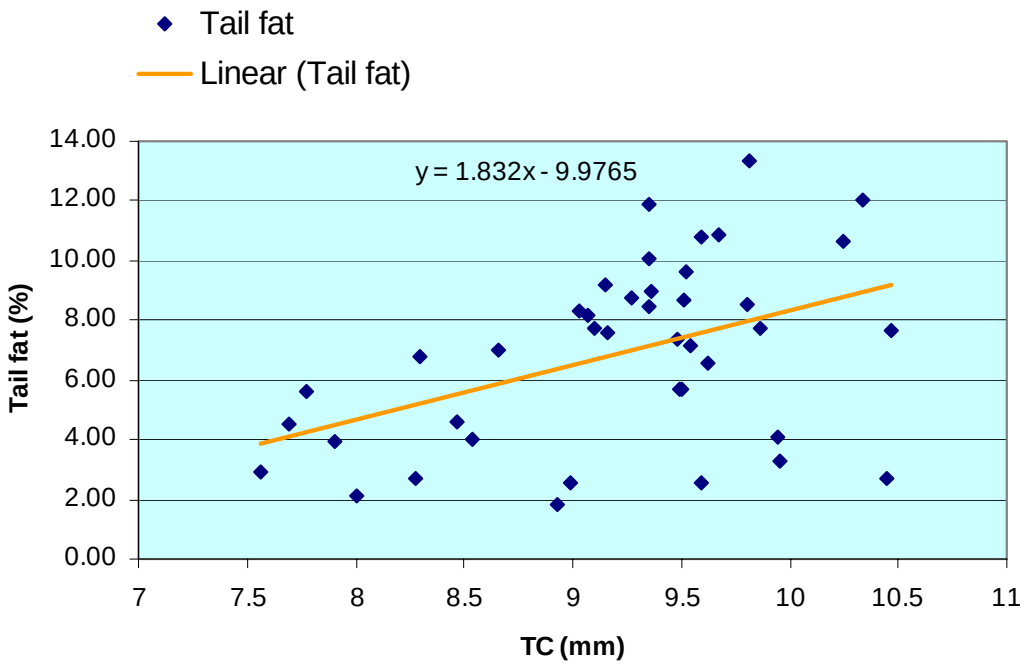

Figure 3.8. Scatter plot comparing TC (tail circumference) and tail fat of male *Plethodon cinereus* from high elevations. Estimated regression line is shown (p=0.003).

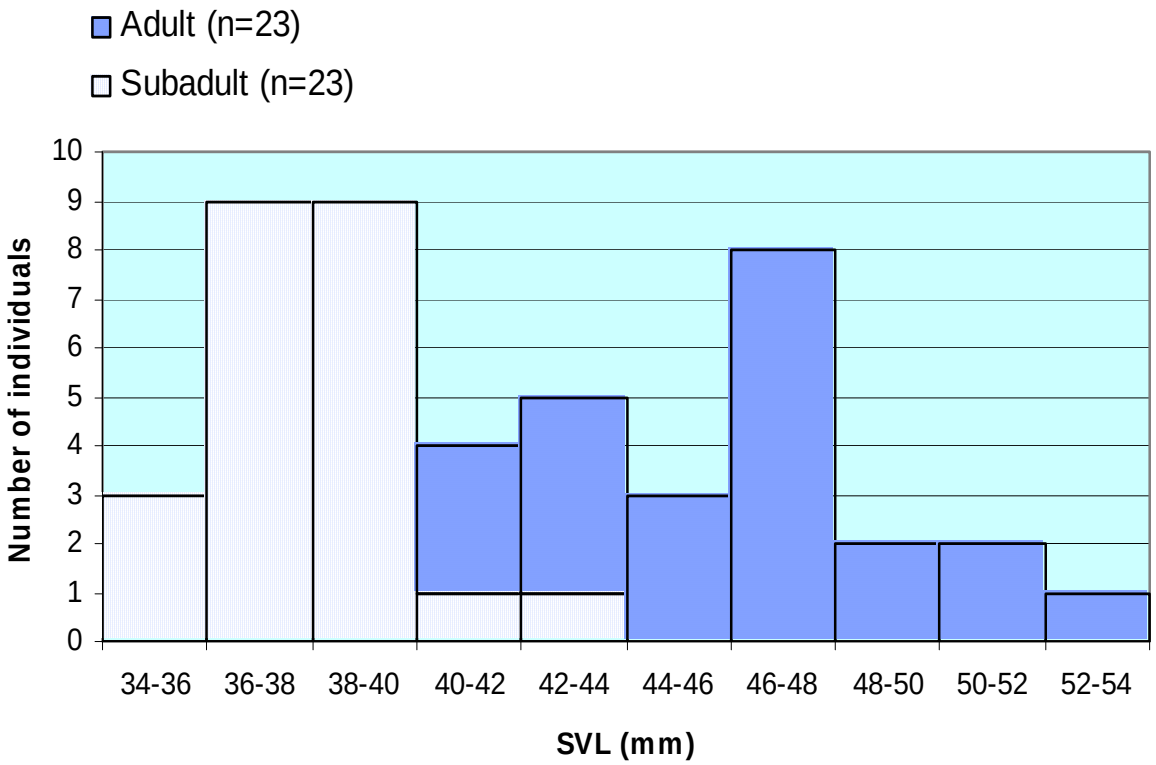

Figure 3.9. SVL (snout-vent length) distribution of female *Plethodon cinereus* from low elevations.

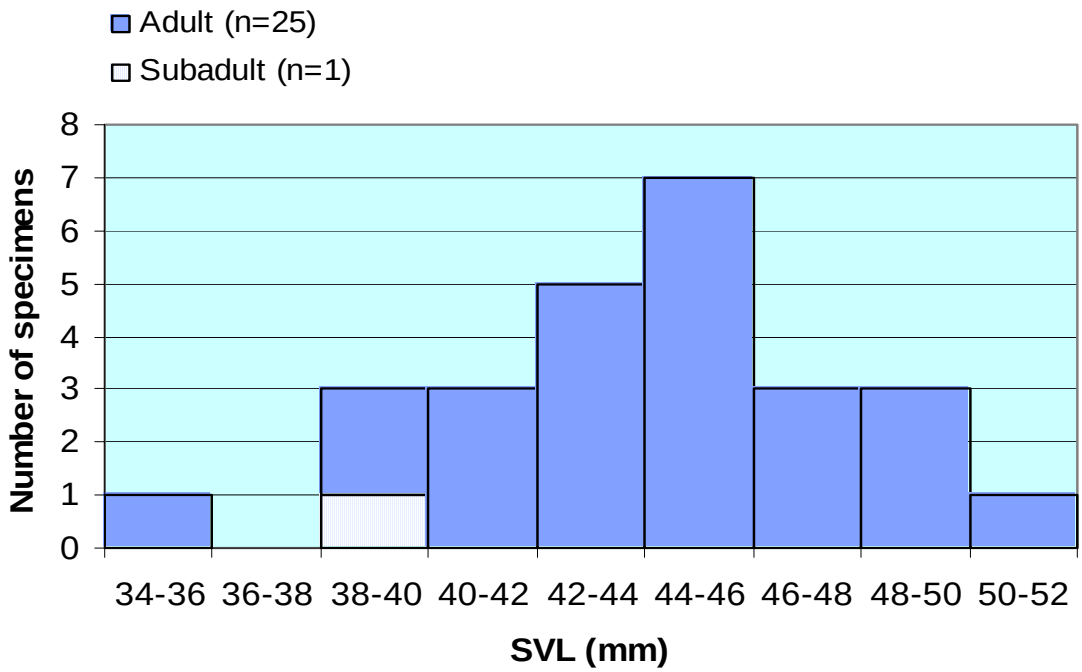

Figure 3.10. SVL (snout-vent length) distribution of male *Plethodon cinereus* from low elevations.

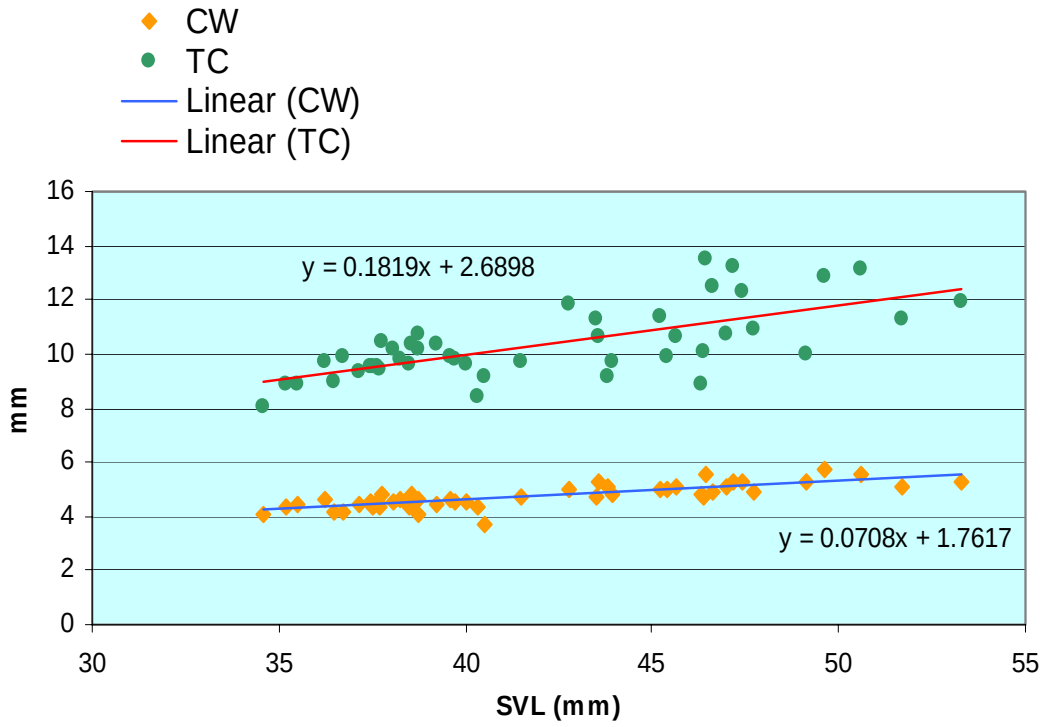

Figure 3.11. Scatter plot comparing SVL (snout-vent length) and CW (cranial width) and TC (tail circumference) of female *Plethodon cinereus* from low elevations. Estimated regression lines are shown (SVL-CW: $p<0.001$, SVL-TC: $p<0.001$).

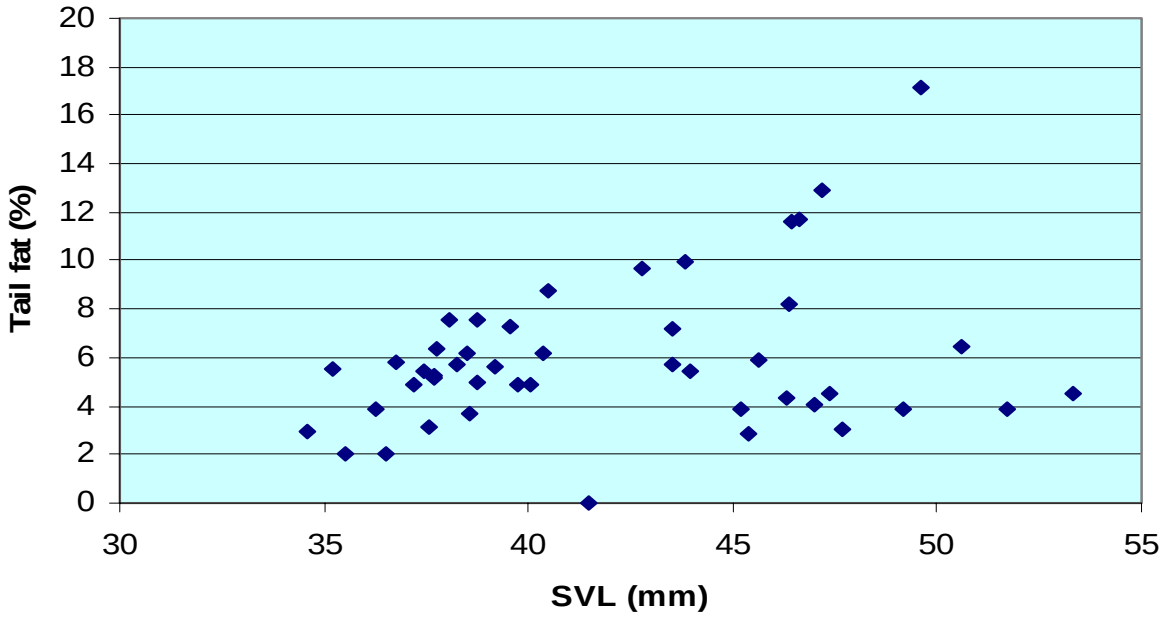

Figure 3.12. Scatter plot comparing SVL (snout-vent length) and tail fat of female *Plethodon cinereus* from low elevations. Regression line is not shown because an association between SVL and tail fat was not significant (p=0.051).

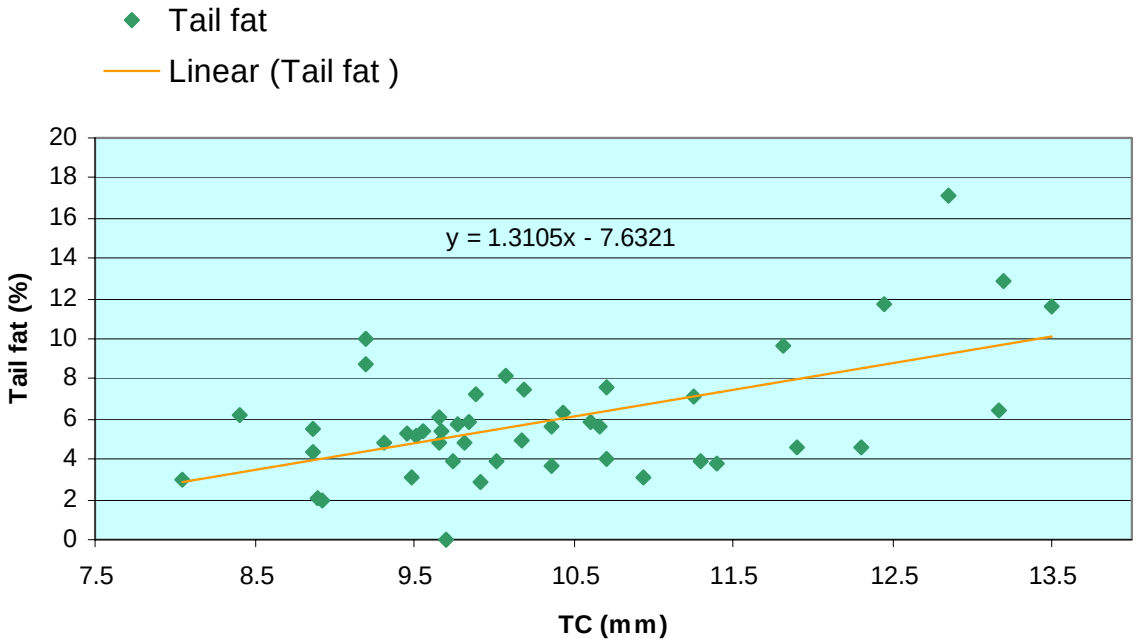

Figure 3.13. Scatter plot comparing TC (tail circumference) and tail fat of female *Plethodon cinereus* from low elevations. Estimated regression line is shown ($p<0.001$).

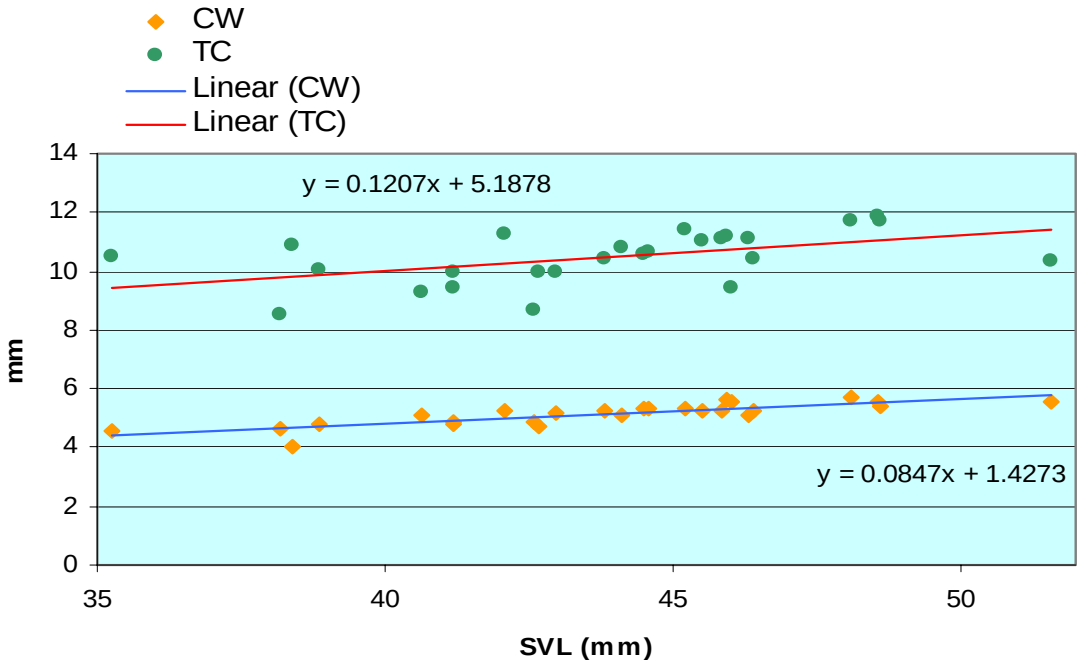

Figure 3.14. Scatter plot comparing SVL (snout-vent length) and CW (cranial width) and TC (tail circumference) of male *Plethodon cinereus* from low elevations. Estimated regression lines are shown (SVL-CW: $p<0.001$, SVL-TC: $p=0.009$).

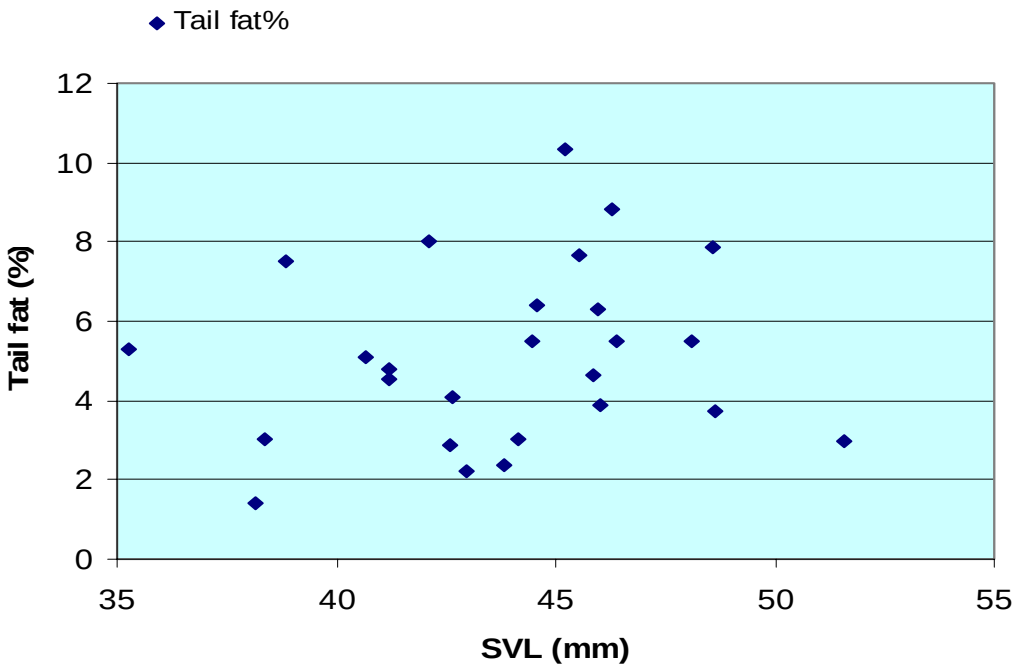

Figure 3.15. Scatter plot comparing SVL (snout-vent length) and tail fat of male *Plethodon cinereus* from low elevations. Regression line is not shown because an association between SVL and tail fat was not significant (p=0.403).

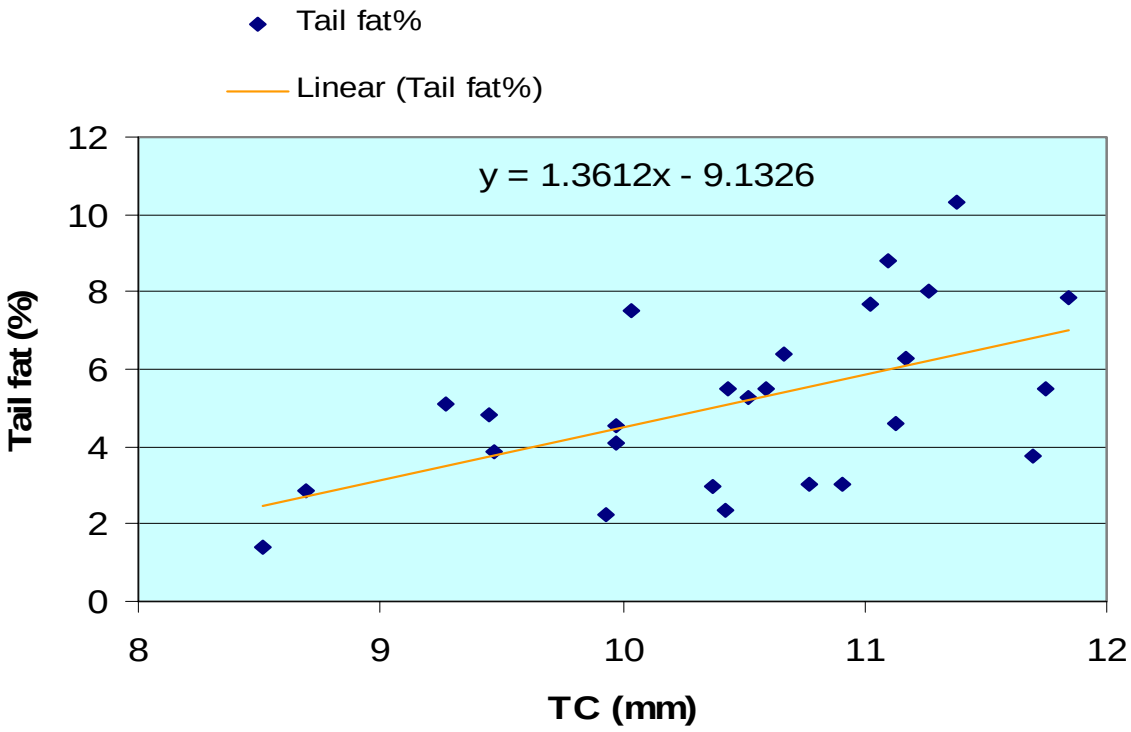

Figure 3.16. Scatter plot comparing TC (tail circumference) and tail fat of male *Plethodon cinereus* from low elevations. Estimated regression line is shown (p=0.004).

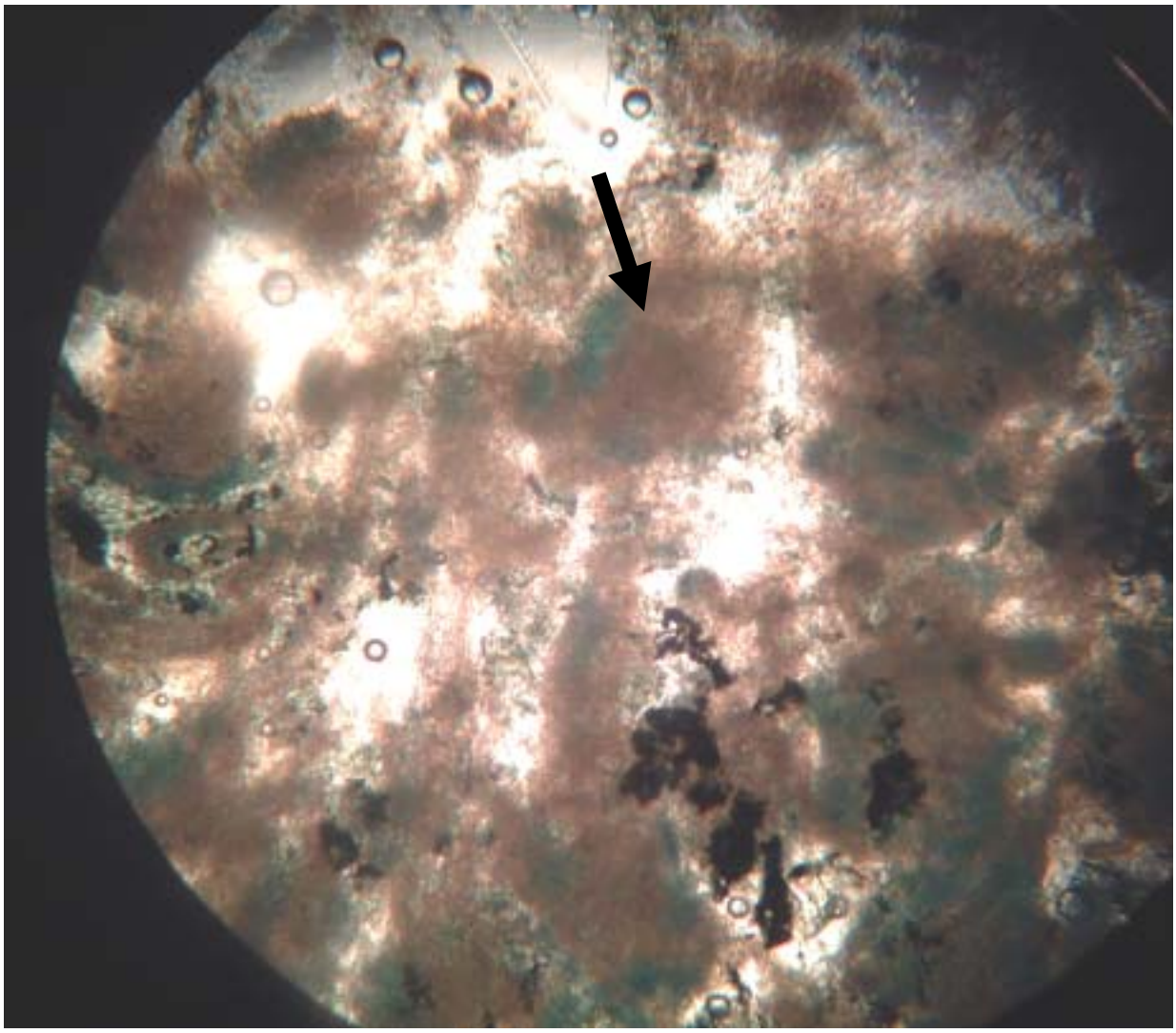

Figure 3.17. Sperm (spermatozoa) of Plethodon cinereus observed in testes (Scale: x100). This test is full of sperm that look tangled thread.

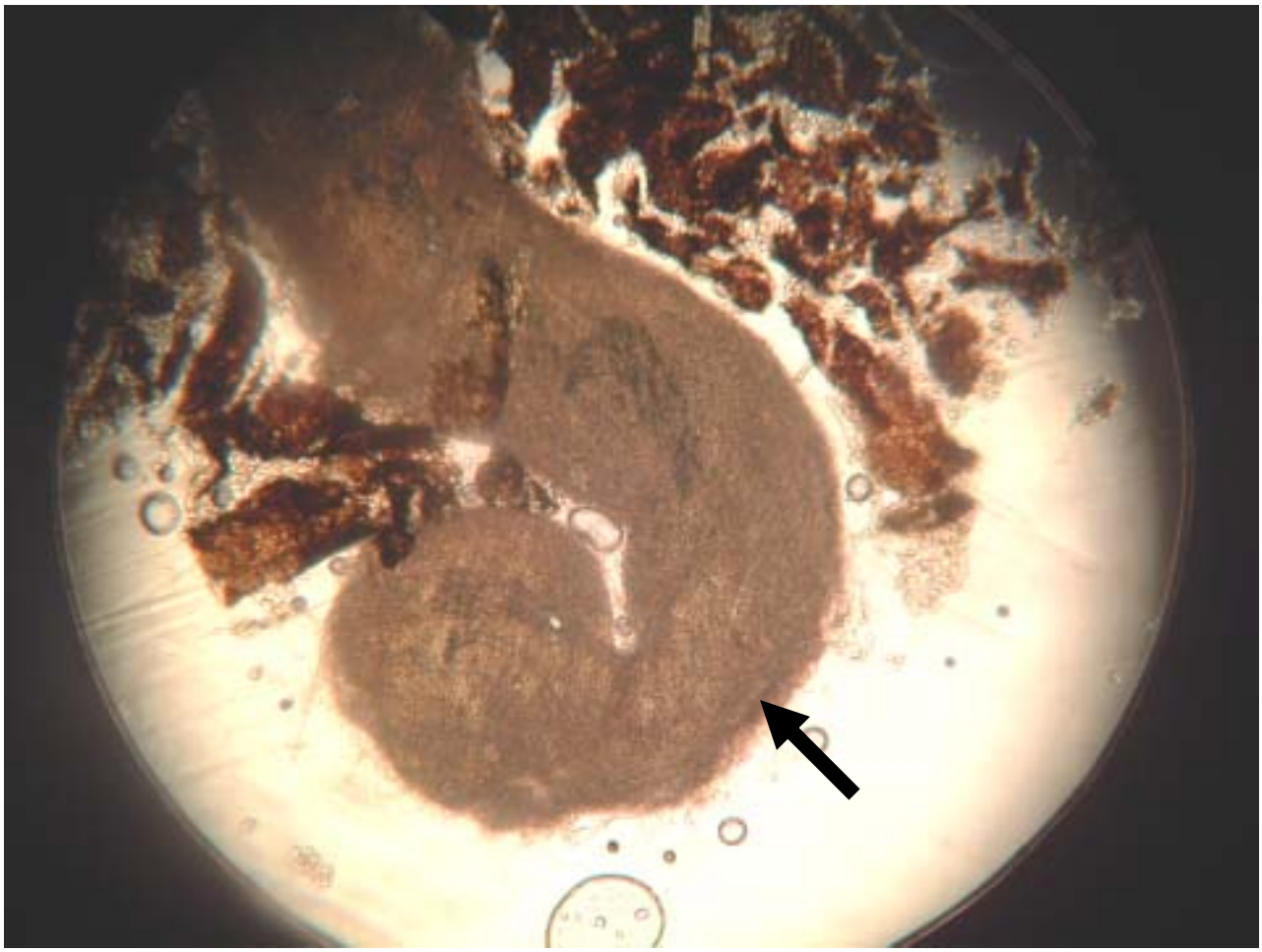

Figure 3.18. Sperm (spermatozoa) of Plethodon cinereus observed in vasa deferentia (Scale: x100). Uncountable sperm forms stream shape in vasa deferentia. Black pieces are smashed tissue of vasa deferentia.

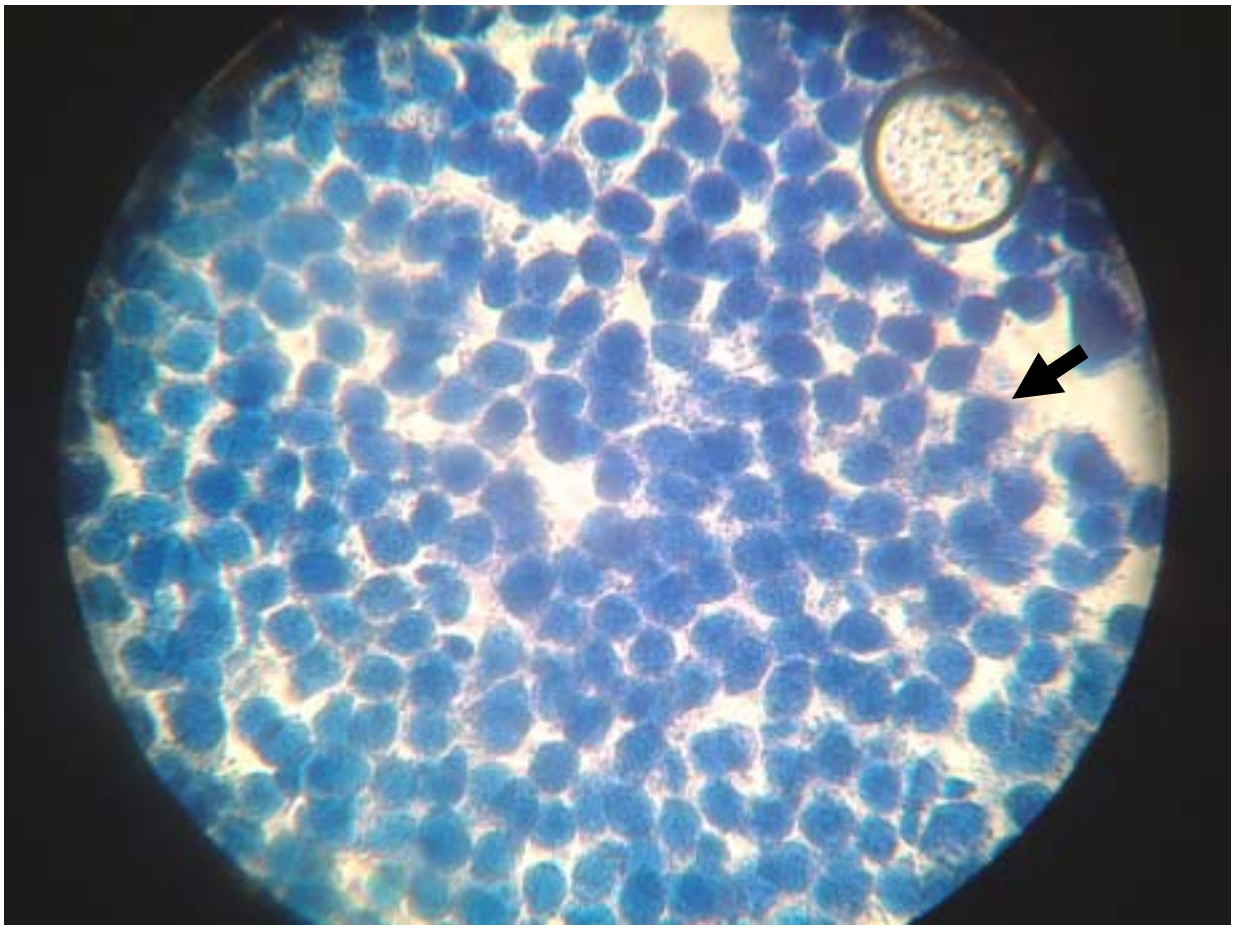

Figure 3.19. Spermatids of *Plethodon cinereus* observed in testes in early stage of spermatogenetic cycle (Scale: x100). Blue round things are spermatids.

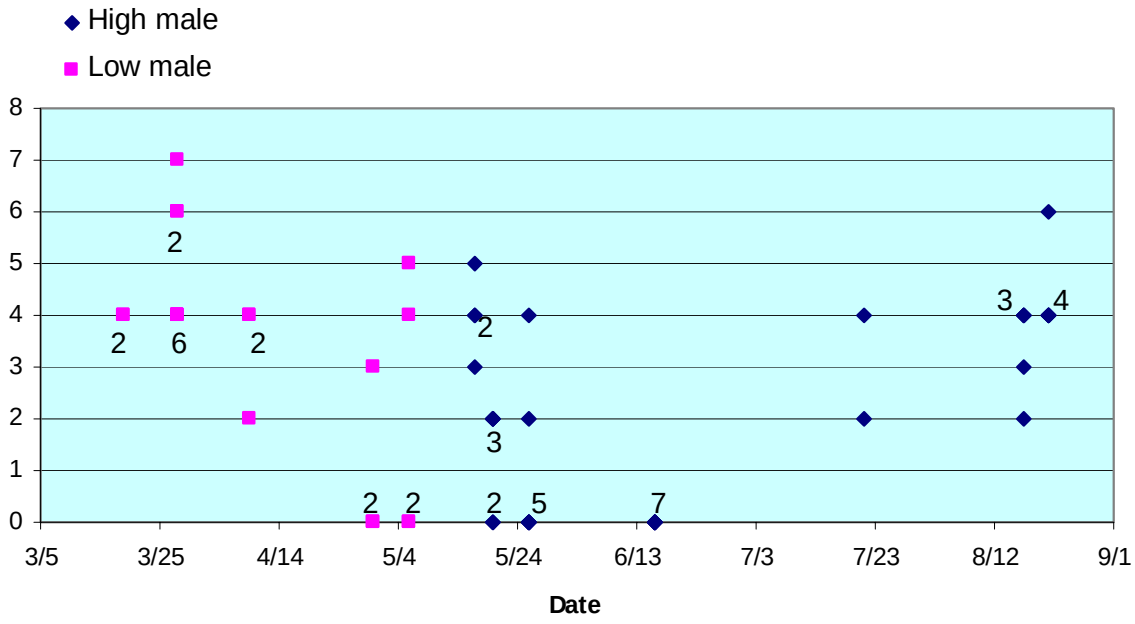

Figure 3.20. Reproductive (sperm) wave of male *Plethodon cinereus* from high and low elevations. Y axis represents number of reproductive parts that contain sperms. The numbers besides plot are numbers of specimens that one plot represents.

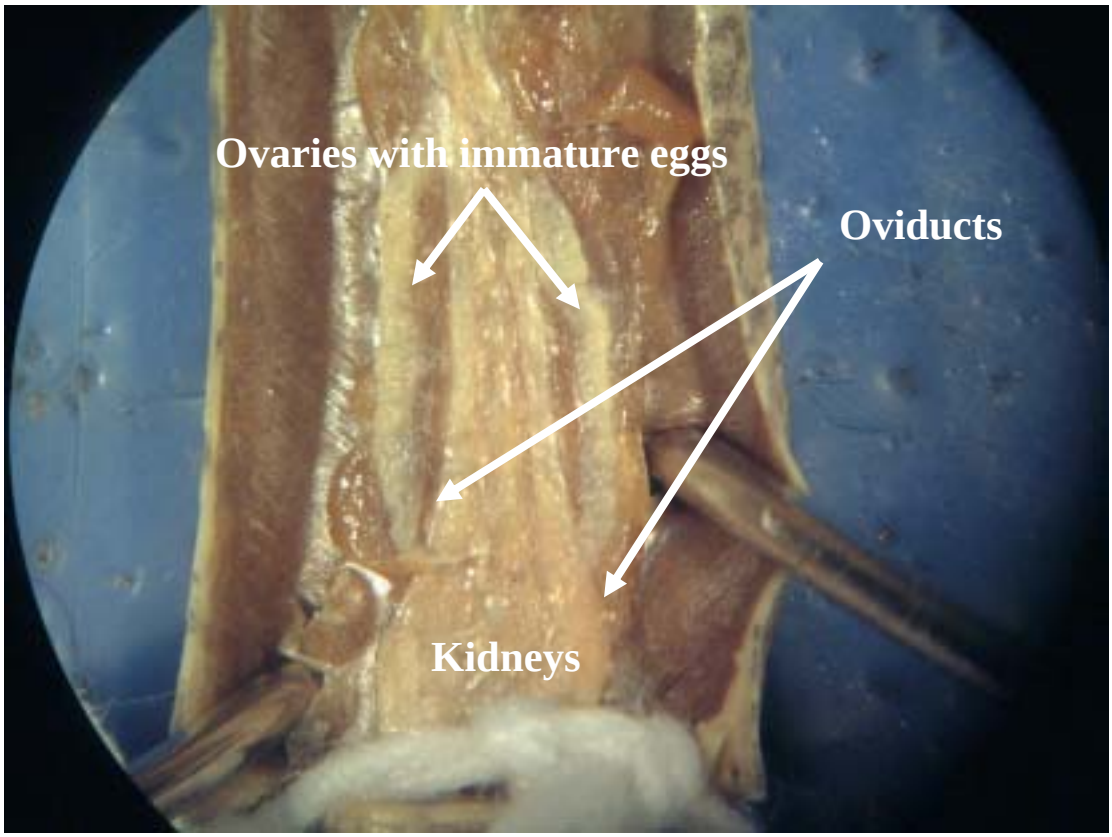

Figure 3.21. Reproductive organs of typical immature female with immature eggs of *Plethodon cinereus* (Scale: x15). They have thin and straight oviduct along their kidneys.

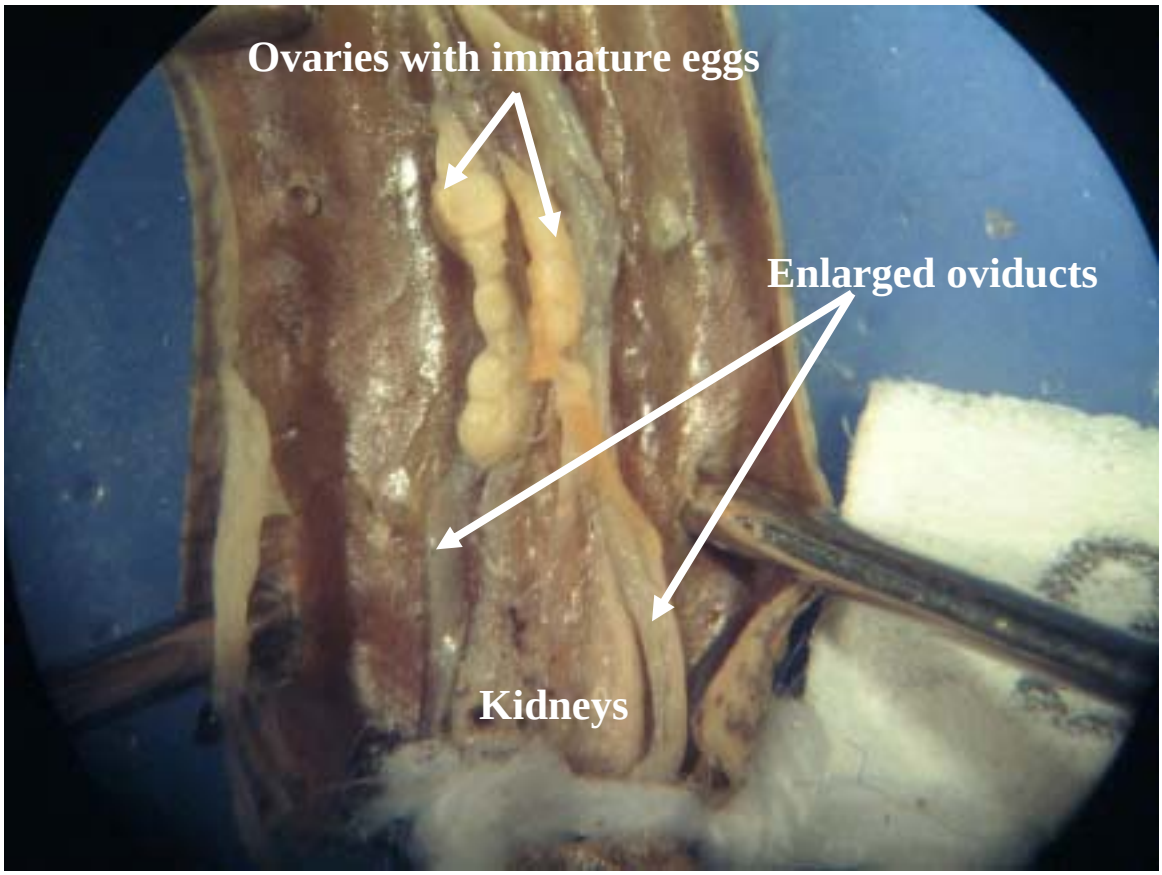

Figure 3.22. Reproductive organs of typical mature female with immature eggs of *Plethodon cinereus* (Scale: x15). They have enlarged and waved oviducts along kidneys.

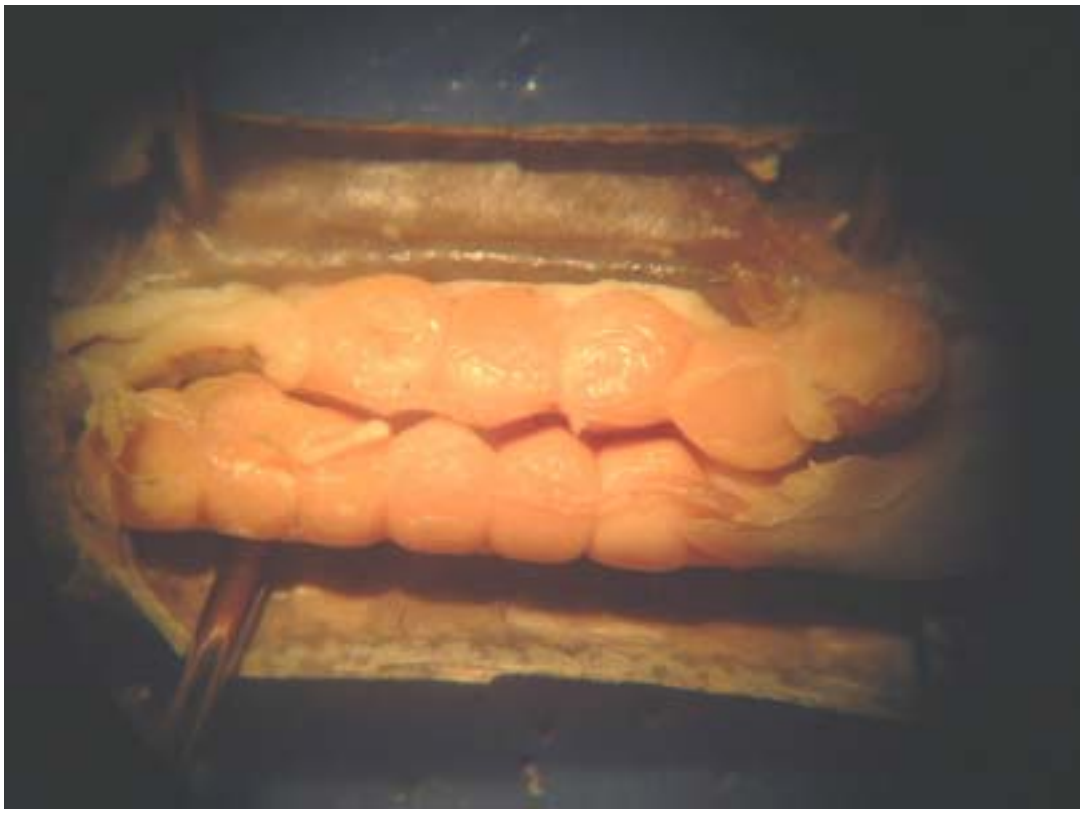

Figure 3.23. Reproductive organs of typical mature female with mature eggs of *Plethodon cinereus* (Scale: x15).

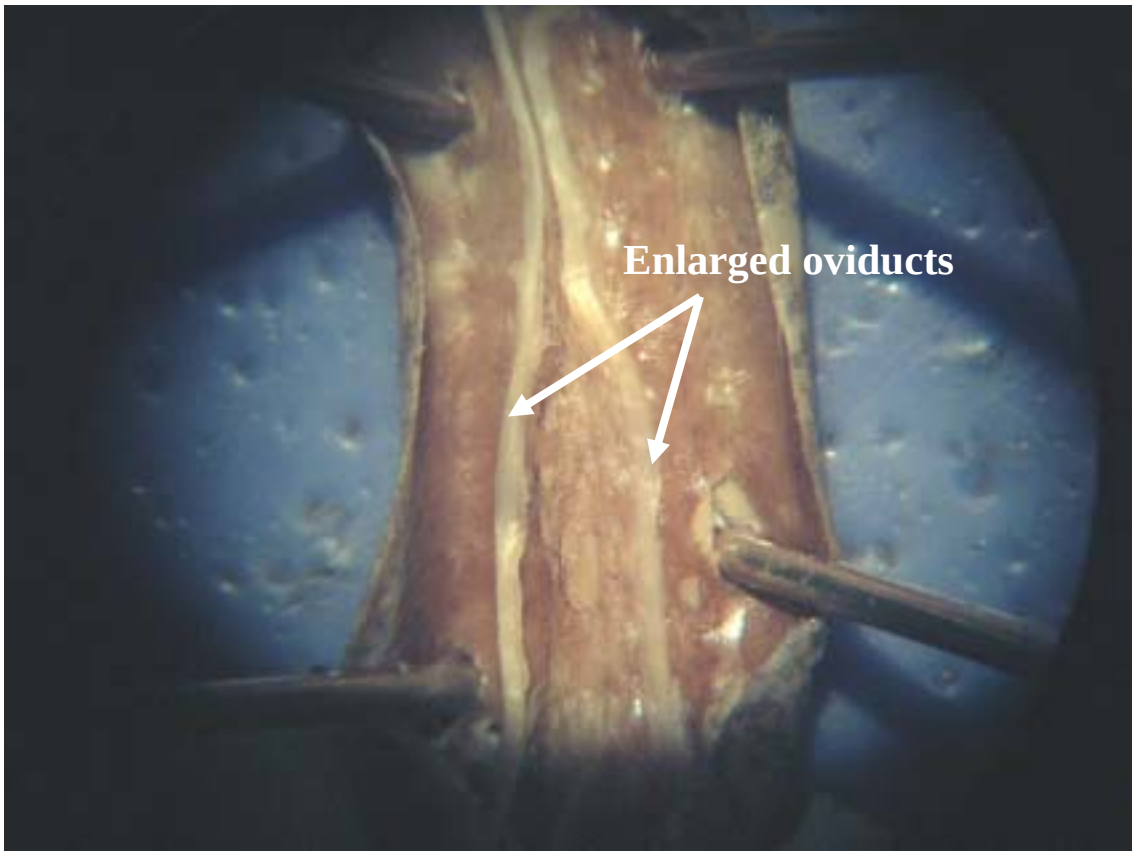

Figure 3.24. Reproductive organs of typical spent female of *Plethodon cinereus* (Scale: x15).

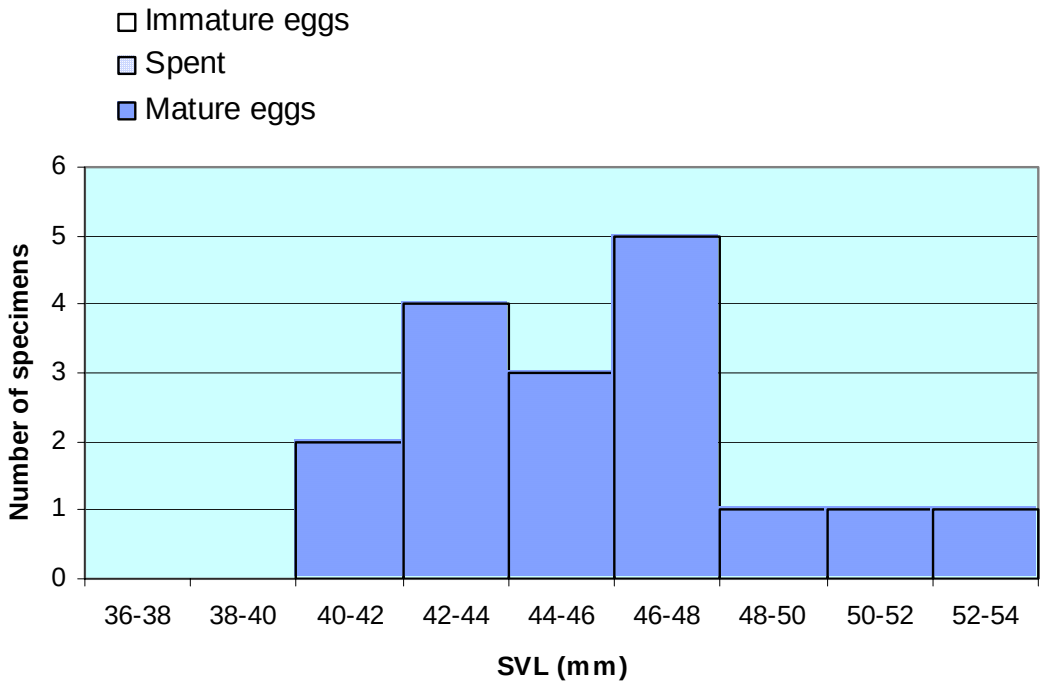

Figure 3.25. SVL (snout-vent length) distribution of adult female *Plethodon cinereus* collected in spring and summer 1994 from low elevations classified into three categories, females with mature follicles, spent females, and females with immature follicles. This figure did not include animals collected in November because those females were for the next breeding season.

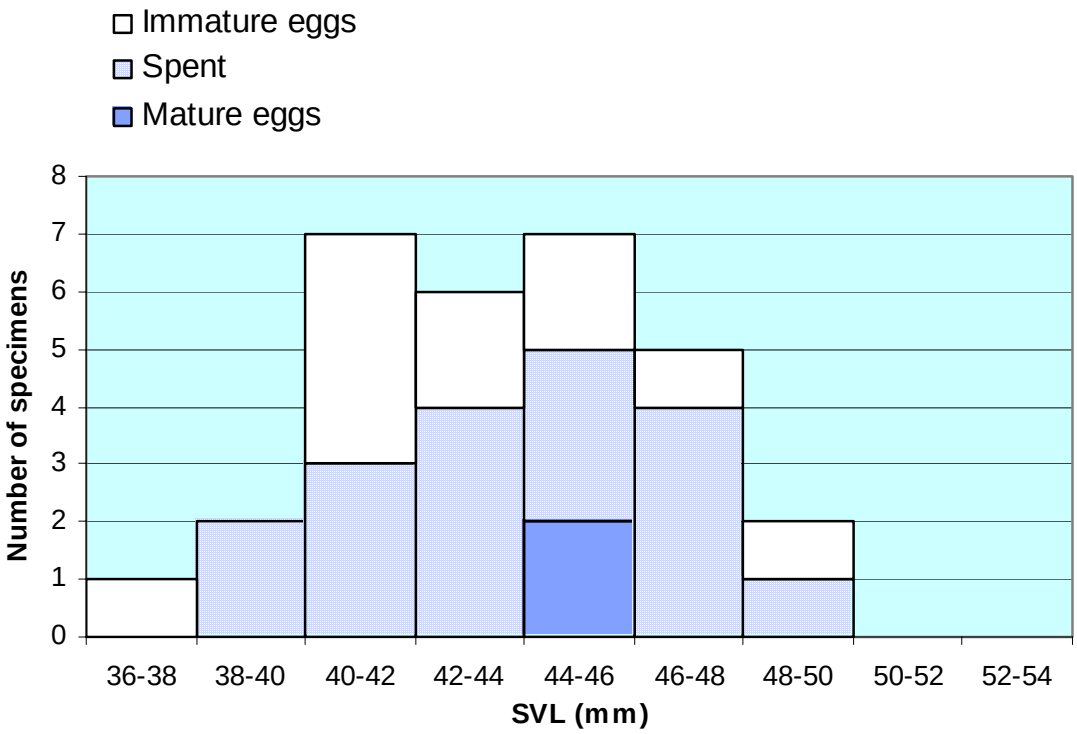

Figure 3.26. SVL (snout-vent length) distribution of adult female *Plethodon cinereus* collected in spring and summer 1993 from high elevations classified into three categories, females with mature follicles, spent females, and females with immature follicles.

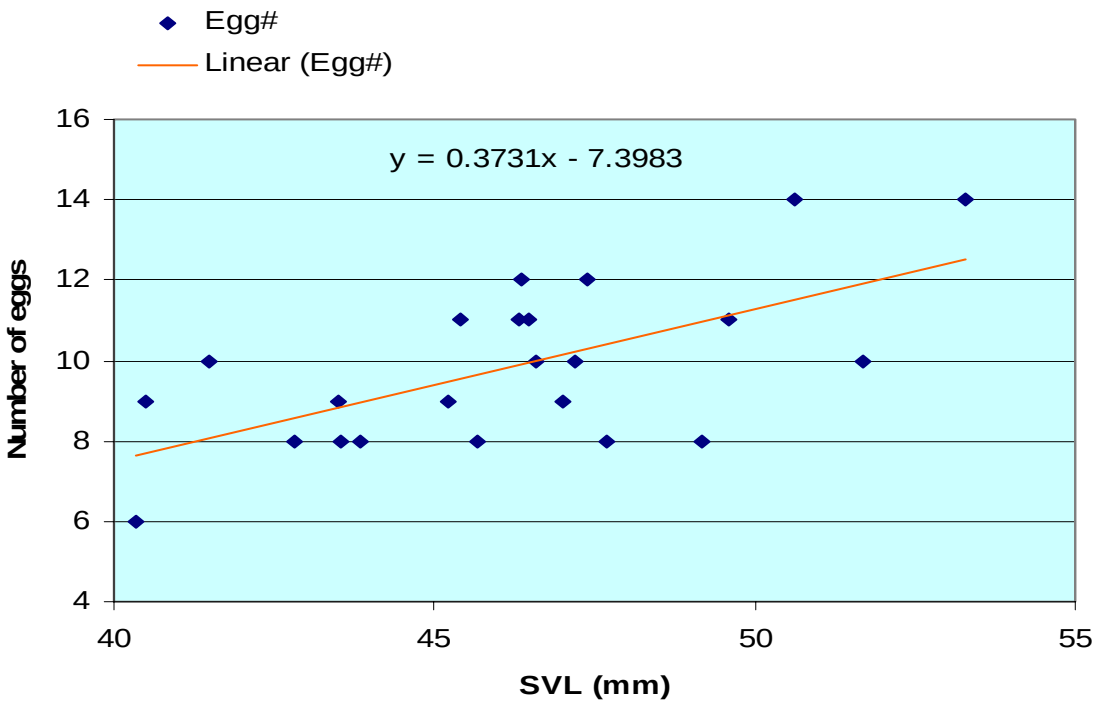

Figure 3.27. Scatter plot comparing SVL (snout-vent length) and number of eggs of female *Plethodon cinereus* from high elevations. Estimated regression line is shown (p=0.001).

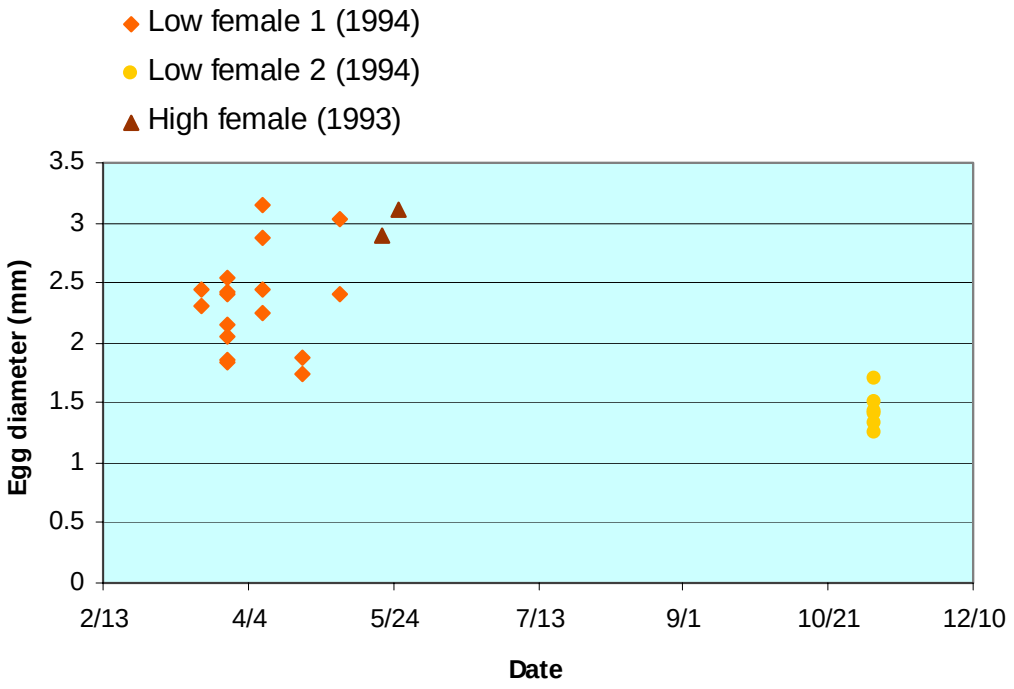

Figure 3.28. Scatter plot showing egg development of *Plethodon cinereus* in diameter. 'Low female 1' represents specimens at low elevations laying eggs that year and 'low female 2' represents ones at low elevations laying eggs the following year. Only two clutches were found in females from high elevations. The numbers in parentheses were collection years.

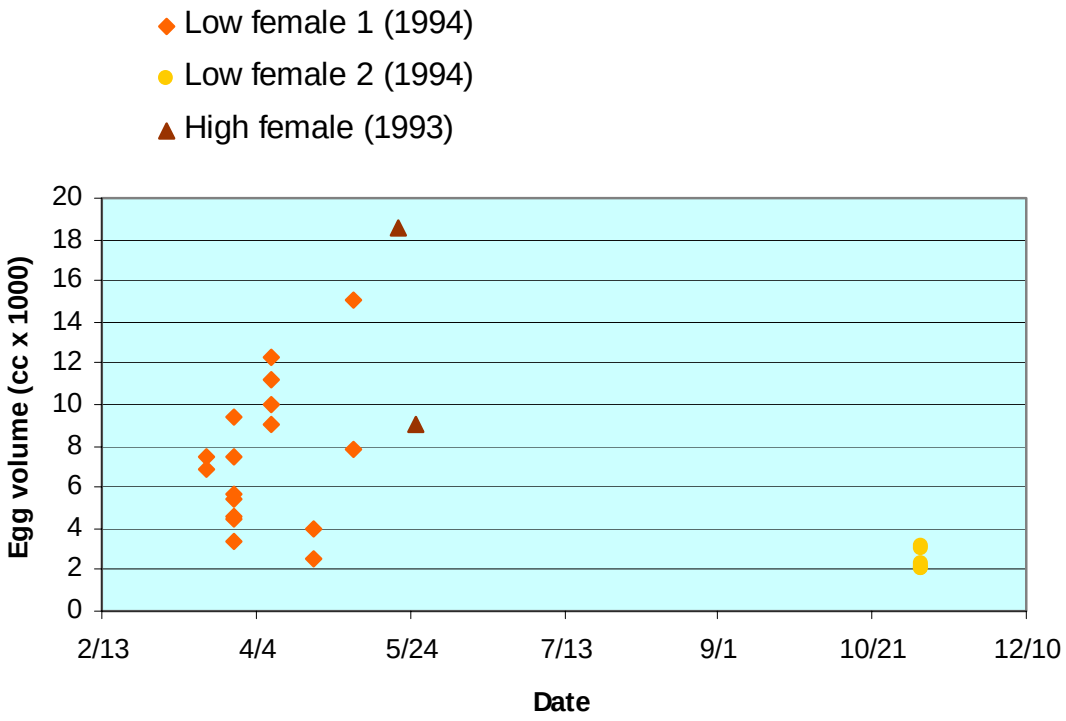

Figure 3.29. Scatter plot showing egg development of *Plethodon cinereus* in volume. 'Low female 1' represents specimens at low elevations laying eggs that year and 'low female 2' represents ones at low elevations laying eggs the following year. Only two clutches were found in females from high elevations. The numbers in parentheses were collection years

Appendix-II

Tables

Table 2.1. Collection date, locations, elevations, and numbers of specimens studied.

Collection date	Site	Site No.	County	Elevation (ft.)	Number of Specimens studied		
					Male	Female	Total
High elevation							
5/17/1993	Cloverlick	1	Pocahontas	4200	5	3	8
5/20/1993	Green Bank	2	Pocahontas	4400	5	10	15
5/26/1993	Bald Knob	3	Pocahontas	4410	7	7	14
6/16/1993	Turkey Mtn.	4	Pocahontas	4020	8	7	15
7/21/1993	Tea creek Mtn.	5	Pocahontas	4220	6	7	13
8/17/1993	Cloverlick	1	Pocahontas	4200	5	4	9
8/21/1993	Flat Ridge	6	Pocahontas	4180	5	5	10
Total					41	43	84
Low elevation							
3/19/1994	Salem College	7	Harrison	1250	2	11	13
3/28/1994	Long Run	8	Doddridge	1050	9	8	17
4/9/1994	Salem College	7	Harrison	1250	3	9	12
4/23/1994	Salem College	7	Harrison	1250	0	3	3
4/30/1994	Salem College	7	Harrison	1250	1	2	3
4/30/1994	Long Run	8	Doddridge	1050	2	1	3
5/6/1994	Salem College	7	Harrison	1250	2	3	5
5/6/1994	Long Run	8	Doddridge	1050	3	3	6
11/6/1994	Long Run	8	Doddridge	1050	3	2	5
11/6/1994	Salem College	7	Harrison	1250	1	4	5
Total					26	46	72

Table 2.2. Environmental data of high and low elevations. High elevation data were from National Climatic Weather Station at Snowshoe, WV (4763.9 ft; 1452.4m) and low elevation data from National Climatic Weather Station at Clarksburg (Clarksburg Benedum Airport), WV (1202.8 ft; 366.7m).

	Low elevation site		High elevation site	
	Daily average temperature (C°)	Annual precipitation (mm)	Daily average temperature (C°)	Annual precipitation (mm)
1993	11.2	1078.5	6.5	1457.5
1994	11.0	1457.5	6.8	1461.8
Average	11.1	1268.0	6.7	1459.6

Table 3.1. Comparisons in average SVL (snout-vent length), CW (cranial width), TC (tail circumference), and tail fat (%) between adult females and males from high elevations. *P* values from t-test (normal distribution) or Mann-Whitney Rank Sum Test (not normal distribution) are also listed.

	n	SVL	CW	TC	Tail fat (%)
		***			*
Female	30	43.48	5.24	9.65	9.28
Male	36	40.99	5.12	9.35	7.24
p value		<0.001	0.321	0.061	0.024

*Significantly different ($p<0.05$)

**Significantly different ($p<0.01$)

***Significantly different ($p<0.001$)

Table 3.2. Comparisons in average SVL (snout-vent length), CW (cranial width), TC (tail circumference), and tail fat (%) between adult females and males from low elevations. *P* values from t-test (normal distribution) or Mann-Whitney Rank Sum Test (not normal distribution) are also listed.

	n	SVL	CW	TC	Tail fat
		*			
Female	23	46.16	5.01	11.02	6.80
Male	25	44.03	5.18	10.46	5.21
p value		0.041	0.114	0.173	0.18

*Significantly different ($p<0.05$)

**Significantly different ($p<0.01$)

***Significantly different ($p<0.001$)

Table 3.3. Comparisons in average SVL (snout-vent length), CW (cranial width), TC (tail circumference), and tail fat (%) between adult females from high and low elevations. *P* values from t-test (normal distribution) or Mann-Whitney Rank Sum Test (not normal distribution) are also listed.

		n	SVL	CW	TC	Tail fat (%)
			**	*	***	*
Adult female	High elevation	30	43.48	5.24	9.65	8.40
	Low elevation	23	46.16	5.01	11.02	5.88
	p value		0.003	0.020	<0.001	0.041

*Significantly different ($p<0.05$)

**Significantly different ($p<0.01$)

***Significantly different ($p<0.001$)

Table 3.4. Comparisons in average SVL (snout-vent length), CW (cranial width), TC (tail circumference), and tail fat (%) between adult males from high and low elevations. *P* values from t-test (normal distribution) or Mann-Whitney Rank Sum Test (not normal distribution) are also listed.

		n	SVL	CW	TC	Tail fat (%)
			***		***	**
Adult male	High elevation	36	40.99	5.17	9.35	7.24
	Low elevation	25	44.03	5.24	10.46	5.21
	p value		<0.001	0.747	<0.001	0.006

*Significantly different ($p<0.05$)

**Significantly different ($p<0.01$)

***Significantly different ($p<0.001$)

Table 3.5. Occurrences (%) of three categories of adult females, one with mature follicles, spent, and ones with immature follicles.

	Mature eggs	Spent	Immature eggs
High female	6.7 (2)*	56.7 (17)*	36.7 (11)*
Low female	100 (17)*	0	0

*Parentheses represent actual numbers of specimens
- Low elevation females did not include animals collected in November because those females were for next breeding season.

Table 3.6. Comparison of prey item data found in *Plethodon cinereus* from high and low elevations - version I. Three animals collected from high elevation held 100 - 500 springtails per animal. These were calculated as 250 each. Data of low populations were cited from Kramer (1996).

Taxa		High elevations (n=79)			Low elevations (n=102)		
Scientific name	Common name	Frequency of Prey	Percent frequency of prey	Percent of stomachs containing prey	Frequency of Prey	Percent frequency of prey	Percent of stomachs containing prey
Acari	Mites	166	16.65	44.30	113	13.45	48.04
Araneae	Spiders	5	0.50	6.33	30	3.57	22.55
Chilopoda	Centipedes	1	0.10	1.27	6	0.71	5.88
Coleoptera	Beetles	11	1.10	10.13	76	9.05	42.16
Coleoptera larvae	Beetle larvae	2	0.20	2.53	91	10.83	40.20
Collembola	Springtails	754	75.63	6.33	158	18.81	46.08
Diplopoda	Millipedes	0	0	0	3	0.36	2.94
Diptera	Flies	15	1.50	16.46	10	1.19	9.80
Gastroptera	Snails	1	0.10	1.27	36	4.29	25.49
Hemiptera	True bugs	1	0.10	1.27	3	0.36	1.96
Homoptera	Plant hoppers	1	0.10	1.27	0	0	0
Hymenoptera	Ant s& Wasps	39	3.91	27.85	154	18.33	47.06
Isopoda	Isopods	0	0	0	92	10.95	24.51
Lepidoptera	Caterpillars	1	0.10	1.27	7	0.83	5.88
Oligochaeta	Segmented worms	0	0	0	46	5.48	27.45
Orthoptera	Grasshoppers & Crickets	0	0	0	3	0.36	2.94
Pseudoscorpiones	Pseudoscorpions	0	0	0	7	0.83	5.88
Psocoptera	Booklice	0	0	0	5	0.60	2.97

Table 3.7. Comparison of prey items data found in *Plethodon cinereus* from high and low elevations - version II. Three animals collected from high elevation held more than 100 - 500 springtails per animal. This Table excludes these animals because those were rare cases and could result in inadequate data for comparisons. Data of low populations were cited from Kramer (1996).

Taxa		High elevations (n=76)			Low elevations (n=102)		
Sientific name	Common name	Frequency of Prey	Percent frequency of prey	Percent of stomachs containing prey	Frequency of Prey	Percent frequency of prey	Percent of stomachs containing prey
Acari	Mites	166	66.40	46.05	113	13.45	48.04
Araneae	Spiders	5	2.00	6.58	30	3.57	22.55
Chilopoda	Centipedes	1	0.40	1.32	6	0.71	5.88
Coleoptera	Beetles	11	4.40	10.53	76	9.05	42.16
Coleoptera larvae	Beetle larvae	2	0.80	2.63	91	10.83	40.20
Collembola	Springtails	4	1.60	2.63	158	18.81	46.08
Diplo[oda	Millipedes	0	0	0	3	0.36	2.94
Diptera	Flies	15	6.00	17.11	10	1.19	9.80
Gastroptera	Snails	1	0.40	1.32	36	4.29	25.49
Hemiptera	True bugs	1	0.40	1.32	3	0.36	1.96
Homoptera	Plant hoppers	1	0.40	1.32	0	0.00	0
Hymenoptera	Ant s& Wasps	39	15.60	28.95	154	18.33	47.06
Isopoda	Isopods	0	0	0	92	10.95	24.51
Lepidoptera	Caterpillars	1	0.40	1.32	7	0.83	5.88
Oligochaeta	Segmented worms	0	0	0	46	5.48	27.45
Orthoptera	Grasshoppers & Crickets	0	0	0	3	0.36	2.94
Pseudoscorpiones	Pseudoscorpions	0	0	0	7	0.83	5.88
Psocoptera	Booklice	0	0	0	5	0.60	2.97

Table 3.8. Percentage overlap in diet (similarity index: D) for intra- and inter-population comparisons. Letter "n" indicates numbers of individuals in each subset respectively. Three animals collected from high elevations contained 100 - 500 springtails per animal. These were calculated as 250 each (High elev. populations / Low elev. populations –I). Another calculation was made for High / Low (High elev. populations / Low elev. populations –II) excluding these animals (see Table3.7.) Data of low population were cited from Kramer (1996).

Comparison	n	Dietary overlap
High elev. populations / Low elev. populations -I	79/102	39.57%
High elev. populations / Low elev. populations -II	76/102	41.20%
High elev. females / High elev. Males	41/38	83.79%
Low elev. females / Low elev. males	55/26	81.40%

Table 4.1. Summary of slope values and correlation coefficients of SVL (snout-vent length) – CW (cranial width) and SVL – TC (tail circumference) regression equations.

	High elevation		Low elevation			
	Female (n=43)	Male (n=41)	Female (n=46)	Male (n=26)	Average	
CW Slope	0.0804	0.0961	0.0708	0.0847	0.0830	*
TC Slope	0.149	0.126	0.182	0.121	0.145	*
CW Correlation coefficient	0.786	0.628	0.811	0.829	0.7635	**
TC Correlation coefficient	0.664	0.489	0.690	0.503	0.587	**

*Significantly different (p=0.006)
**Significantly different (p=0.045)

Table 4.2. Summary of slope values and correlation coefficients of SVL (snout-vent length) – tail fat and TC (tail circumference) – tail fat regression equations.

	High elevations		Low elevations		
	Female (n=43)	Male (n=41)	Female (n=46)	Male (n=26)	Average
SVL-tail fat Slope	0.381	0.319 (NS)	0.181 (NS)	0.103 (NS)	0.2460
TC-tail fat Slope	2.497	1.832	1.31	1.361	1.750
SVL-fat Correlation coefficient	0.352	0.304 (NS)	0.290 (NS)	0.171 (NS)	0.2793
TC-fat Correlation coefficient	0.507	0.450	0.552	0.543	0.513

Curriculum Vitae

Mizuki Takahashi

EDUCATION:

2000.8 – Present MARSHALL UNIVERSITY, HUNTINGTON, WEST VIRGINIA
-Master of Science; Major: Biology

1998.7 – 1998.10 UNIVERSITY OF ARKANSAS AT LITTLE ROCK
-Intensive English Language Program

1996.4 – 1998.3 UNIVERSITY OF TOKYO, TOKYO, JAPAN
-Master of Science; Major: Forestry

1992.4 – 1996.3 UNIVERSITY OF TSUKUBA, IBARAGI, JAPAN
-Bachelor of Science; Major: Agriculture

RESEARCH EXPERIENCE:

2001.8 – Present COMPARISONS IN MORPHOLOGY, REPRODUCTIVE STATUS, AND FEEDING ECOLOGY OF *PLETHODON CINEREUS* AT HIGH AND LOW ELEVATIONS IN WEST VIRGINIA
-Marshall University, Department of Biological Sciences
-Master's thesis

2000.11 – Present DISTRIBUTION AND NATURAL HISTORY OF *NECTURUS M. MACULOSUS* IN WEST VIRGINIA
-Marshall University, Department of Biological Sciences
-Funded by West Virginia Division of Natural Resources

2000.8 – Present GRADUATE RESEARCH ASSISTANT
-Marshall University, Department of Biological Sciences, Huntington, WV 25755
-Supervisor: Thomas K. Pauley, Ph.D. (304)696-2376
-Duties include:

1. Inventories for Amphibians, Reptiles, Birds, and Mammals in the Gauley River Recreation Area funded by NPS
2. West Virginia Herpetological Atlas funded by WVDNR
3. Non-target Impacts from Insecticide Applications and Gypsy Moth Defoliation on Terrestrial and Aquatic Salamanders funded by USDA-FS

1996.4 – 1998.3 ALLOZYME VARIATIONS OF META-POPULATIONS OF TODO FIR, *ABIES SACHALINENSIS* ALONG VERTICAL GRADIENTS
-Master's thesis at the University of Tokyo
-Presented at the 109th Annual Meeting of Japanese Forestry

Society at the University of Utsunomiya, Tochigi, Japan, 1998.4

1996.4 – 1998.3 GENETIC STRUCTURES OF A SELECTION-CUT FOREST AND A NATURAL FOREST OF TODO FIR, *ABIES SACHALINENSIS*

-Master's thesis at the University of Tokyo
-Published in Hokkaido no Rinboku Ikusyu (Breeding of Forest Trees in Hokkaido) 42(2): 11- 14 1999 (in Japanese)

1995.4 – 1996.1 MITOCHONDRIAL (MT) DNA VARIATION IN JAPANESE WHITE BEECH, *FAGUS CRENATA*

-Bachelor's thesis at the University of Tsukuba
-Presented by Yoshihiko Tsumura at the International symposium "Diversity and Adaptation in Forest Ecosystem in A Changing World" at University of British Columbia in Vancouver, Canada
-Published in American Journal of Botany 85(5): 629-639 1998

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Takahashi, M. and T.K. Pauley. 2002. Reproductive status of *Plethodon cinereus* along vertical gradients in West Virginia. 63th Annual Meeting of the Association of Southeastern Biologists, Appalachian State University, Boone, North Carolina

Takahashi, M., H. Kisanuki, and U. Ide. 1998. Allozyme variations of meta-populations of Todo fir, *Abies sachalinensis*. 109th Annual Meeting of Japanese Forestry Society, University of Utsunomiya, Tochigi, Japan