ABSTRACT

The salamander family Plethodontidae is known for being an indicator of environmental health and has been labeled as the "canary in the coal mine" of forest ecosystems (Urban et al. 2014). One of the most widely distributed and commonly studied plethodontid species is the red-backed salamander (RBS, *Plethodon cinereus*). Populations of *P. cinereus* will be influenced by different variables within the environment including temperature, humidity, and leaf litter. Fundamental data on populations of *P. cinereus* must be gathered before we can understand how these intercorrelated variables will influence *P. cinereus* distribution and demographics. In this study I aimed to gather foundational data on the reproductive ecology of female red-backed salamanders in Stokes State Forest, Sussex County, New Jersey. Reproductive data were collected on 366 clutches from April 2017 to October 2019. I analyzed yearly and seasonal data as well as measured the effects of body size on clutch size. Results showed a positive correlation between body size and clutch size of *P. cinereus*. When seasons were analyzed separately, there were significant differences in clutch size among years for both the fall and the spring. Additionally, I manipulated leaf litter quantity and measured its impact on the clutch size of *P. cinereus*. I found that clutch size was positively associated with the quantity of leaf litter and decreased as leaf litter was removed from the study sites. This investigation provides a foundational analysis on the clutch size of *P. cinereus* in Stokes State Forest, providing significant information necessary for monitoring this population.

MONTCLAIR STATE UNIVERSITY

FACTORS AFFECTING CLUTCH SIZE IN A POPULATION OF RED-BACKED

SALAMANDER (*Plethodon cinereus*) IN NORTHWESTERN NEW JERSEY

by

VERONICA LYNN PUZA

A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of

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College/School: College of Science and
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INTRODUCTION

The salamander family Plethodontidae is known for being an indicator of environmental health and has been labeled as the "canary in the coal mine" of forest ecosystems (Urban et al. 2014). The family's unique characteristics of high site fidelity, small territory size, low sampling costs, and sensitivity to natural and anthropogenic disturbances make it an excellent model for monitoring forest ecosystems (Welsh and Droege 2001). The most distinguishing physiological feature of Plethodontids is the lack of lungs. Respiration takes place exclusively through cutaneous gas exchange and the linings of the mouth (Feder 1983). It allowed some members of this family to be freed from the constraints of breeding in water, which facilitated radiation from their origins in the streams of Appalachia to their colonization of moist forest habitats throughout the United States and lastly, their dispersal into the New World tropics (Welsh and Droege 2001).

Plethodontid species can be easily studied because of their widespread distribution, and their physiological constraints within an environment demonstrate their importance as bioindicators. Although numbers of plethodontid species will fluctuate as a result of different environmental stressors, it is imperative to consider their physiological constraints relative to leaf litter, soil moisture, and temperature (Feder 1983; Welsh and Droege 2001). One of the most widely distributed and commonly studied plethodontid species is the red-backed salamander (RBS, *Plethodon cinereus*). The RBS has an extensive range throughout much of Eastern North America extending from Southern Quebec and the Maritime Provinces into North Carolina and westward into Minnesota (Petranka 1998). RBS inhabit deciduous, northern conifer, and mixed deciduous-conifer forests (Petranka 1998). *P. cinereus* is one of the smaller salamander species in the family Plethodontidae. The average total length for adults is 65-127 mm while the average

total length for juveniles is 19-25 mm (Petranka 1998). Two color morphs occur in most

populations with the exception of the erythristic morph (Petranka 1998). The striped morph is

distinguished by an orangish red stripe running down the length of the individual whereas the

unstriped morph or lead-back phase is distinguished by its dark gray coloring and lack of stripe

on its dorsal region. Both morphs' ventral area is distinguished by a mottled pattern, which

yields a salt and pepper appearance.

Male and female individuals of RBS reach sexual maturity after their second or third

summer (Sayler 1966; Nagel 1977). Depending on geographic location and age, males will breed

annually whereas females breed biennially (Petranka 1998). Most females will oviposit every

other year and only breed during the second year of the egg maturation cycle when eggs are >1.3

mm in diameter (Sayler 1966). Oviposition will take place in June with the largest eggs found

from April to June (Sayler 1966). Clutch size will vary by local population but usually falls

between 6 to 9 eggs per individual (Petranka 1998). Outside of location, other variables within

the environment have been found to affect clutch size of *P. cinereus*. Yurewicz and Wilbur

(2004) found that brooding status and tail condition affected future reproductive potential and

clutch size of RBS. While brooding, females generally do not leave their eggs to forage but

rather rely upon their fat reserves. Reduced foraging results in depleted fat reserves and a loss in

mass. Growth affects future reproductive success in this species because there are positive

correlations between female body size and number of mature ova in several populations (Nagel

1977; Lotter 1978; & Fraser 1980). Therefore, brooding status is a credible explanation for

differences in clutch size within populations. Yurewicz and Wilbur (2004) also found females

that had almost all of their tail length removed were unable to yolk as many new ova, which is

likely because of the loss of lipid reserves.

P. cinereus are habitat generalists; however, their sensitivity to environmental fluctuations influences their survival. As a result, specific environmental conditions are required and determine their distribution in the ecosystem. Moisture and cover availability (e.g., rocks and logs) are essential to the survival of *P. cinereus* because they rely on moisture for cutaneous respiration. Previous research on the influence of cover availability and moisture levels on abundances of *P. cinereus* determined that cover and moisture availability is linked to the abundance of *P. cinereus* in the ecosystem (Grover 1998). Thus, *P. cinereus* will be restricted in its distribution and movement based on these dependent factors. Cover objects are essential to maintaining evaporative water loss while also providing refugia to rehydrate during times of unfavorable conditions (Feder 1983). Additionally, cover objects ensure that an individual will have a place to forage when surface activity is limited. Surface activity can be limiting when moisture levels in the leaf litter are low and risk of dehydration is increased. As demonstrated by Heatwole and Lim (1961), moisture levels are linked to evaporative water loss and dehydration of *P. cinereus*. The 'absorption threshold' is the level of substrate moisture above which there is net gain in body water by dehydrated salamanders and beneath which there is a net loss (Heatwole and Lim 1961). *P. cinereus* will adjust itself within the microtopography of its home range by utilizing cover objects in the environment to remain above the 'absorption threshold' (Heatwole and Lim 1961). This response provides evidence that *P. cinereus* are sensitive to some processes of the forest ecosystem, which in turn exemplifies its use as a bioindicator of ecosystem stressors.

Building on the needs for moisture in the forest floor, leaf litter also holds significance in identifying the presence of *P. cinereus* and other plethodontid species in the environment. *P. cinereus* forages in the leaf litter of the forest floor during times of heavy rainfall when risk of

desiccation is low (Fraser 1976). Evidence suggests that plethodontids can forage under cover objects during periods where rainfall is sparse but are unable to forage effectively in burrows (Fraser 1976; Jaeger 1980). Since territorial defenses center around a cover object, disturbances that affect the depth of leaf litter could affect the availability and quality of microhabitats (Jaeger et al., 1982; O'Donnell et al. 2014). In turn, this response may increase competition between individuals for natural cover objects, which could then lead to changes in distribution and density of populations (Grover; 1998; O'Donnell et al. 2014). Evidence shows that leaf litter is an integral component for *P. cinereus* in the ecosystem and its presence may affect other ecosystem processes. One possible effect on forest ecosystems comes from experiments conducted by Wyman (2003), which suggests that *P. cinereus* is an important link to longstanding retention of nitrogen compounds from leaf litter and is a potential regulator of the carbon-nitrogen cycles in forests. As salamanders reduce the number of invertebrates that prefer feeding on leaf sections with elevated nutrient content, predation by salamanders may allow for longer retention of nutrients in the soil (Wyman 2003). This is but one example of how slight modifications in the microhabitat of a forest can directly impact the ecosystem.

Temperature is another factor to consider when determining the presence and distribution of *P. cinereus*. Due to their thin, permeable layer of skin, amphibians are vulnerable to variations in temperature. Temperature influences every aspect of amphibian physiology including metabolism, growth, digestion, and locomotion (Homyack et al. 2010). Studies have shown that there is a positive correlation between an increase in environmental temperature and metabolic rates of *P. cinereus (*Homyack et al. 2010*).* This knowledge is essential for understanding the energy budget, ecological trade-offs, and ultimately on fitness to natural or anthropogenic impacts. Muñoz et al. (2016) addressed the adaptive potential of *P. cinereus* to climate change.

RBS are most active on the surface during the spring and autumn seasons and remain active throughout the summer and winter seasons but spend most of their time underground to avoid the physiological pressures brought on by fluctuating temperatures. (Taub, 1961; Grover, 1998; Petranka 1998*). Two vital facets of *P. cinereus* ecology occur on the surface: foraging and courtship (Jaeger 1980; Petranka 1998). Research by Muñoz et al. (2016) found that intensive seasonal shifts to warmer and drier conditions may curb opportunities for surface activity of *P. cinereus*. What's more, they found that increasing temperature would greatly reduce autumn growth, which is the most productive season for *P. cinereus* (Muñoz et al. 2016). Consequently, as a result of reduced autumn growth and surface activity it could take longer for salamanders to become sexually mature (Nagel 1977; Sayler 1966). Reduced growth and a delay in reproduction rates may have an impact on the population dynamics of RBS (Homyack et al. 2010; Muñoz et al. 2016). This research highlights the importance of studying a common amphibian as a model system for investigating population processes (Homyack et al. 2010; Muñoz et al. 2016).

It is challenging to separate the effects of intercorrelated habitat variables to determine how they will exert their influence on salamander populations. At the fundamental level we must first gather information on the population dynamics of species. In this study I aimed to gather foundational data on the clutch sizes of female *P. cinereus* in Stokes State Forest. I gathered yearly and seasonal data on clutch size and measured the effects of body size on clutch size. Additionally, I measured changes to leaf litter quantity and its impact on the clutch size of *P. cinereus*. With this data, I sought to answer several questions on *P. cinereus* such as: (1) Is there a correlation between body size and clutch size of *P. cinereus* in Stokes State Forest? (2) Are there yearly differences in the clutch size of *P. cinereus*? (3) Are there seasonal differences in the clutch size of *P. cinereus* and (4) How is clutch size affected by different quantities of leaf litter?

METHODS

Study Area

This study was conducted at the New Jersey School of Conservation (NJSOC) in Sandyston, NJ. The NJSOC is located in Stokes State Forest, which is managed by the New Jersey Division of Parks and Forestry, a division belonging to the New Jersey Department of Environmental Protection. The NJSOC encompasses 240 acres of the 15,000 plus that make up Stokes State Forest (NJDEP 2003). Little research has been done on RBS within Stokes State Forest and thus, two experimental study sites were established within the forest.

Stand composition of the two sites is mixed oak-hardwood with hemlock also present. Overstory of the two sites were similar, made up of oaks (*Quercus alba; Q. rubra*), Eastern hemlock (*Tsuga canadensis*), American beech (*Fagus grandifolia*), hickory (*Carya ovata*), and maple (*Acer rubrum.*) Understory consists of blueberry (*Vaccinium* spp.) and ferns.

Experimental Design

The study at the NJSOC is an ongoing collaboration with the Salamander Population and Adaptation Research Collaborative Network (SPARCnet; coordinated by Penn State University and the United States Geological Service – Northeast Amphibian and Research Monitoring Initiative). Thus, the experimental design utilized for this project follows the protocol supplied by the network (SPARCnet 2016).

During the Fall of 2015 artificial coverboard object (ACO) arrays were set out into the forest to establish the framework needed to carry out this research. ACOs are a common sampling method used in herpetological studies for species that are dependent on natural cover and are especially useful in detecting RBS within the environment because of the animals'

indispensable need for cover whether that be artificial or natural (Fellers and Drost 1994; Grover 1998).

The array of ACOs follows a replicated plot study design, which include two different sites at the NJSOC. Each study site contains three separate ACO plots, which are spaced a minimum of 20 m apart. Within each ACO array there are a total of 50 individual coverboards, which comprises a 5 m x 10 m array. Individual coverboards are approximately 30 cm x 30 cm squares of 1 in thick plywood and spaced 1 m apart (Muñoz et al. 2016; Sutherland et al. 2016). Rows of coverboards are labeled A-E and columns are labeled 1-10. Rows and columns of labeled coverboards facilitate the mark-recapture element needed to carry out this research.

Field Sampling Procedures

Sampling of ACO plots began in April of 2017 and concluded in the Fall of 2019 (Table 1.)

	Season	
Year	**Spring**	**Fall**
2017 (pre-treatment)	April 17 - June 12	October 9 - October 31
2018 (pre-treatment	March 29 - May 18	October 5 - November 9
2019	April 18 - May 9	October 8 - October 31

Table 1. Survey periods for the spring and the fall during the years of 2017-2019. Leaf litter treatments were initiated at the beginning of the Spring 2019 survey period.

Each site was surveyed a minimum of three times during the spring and fall seasons. Surveys take place in the spring and fall as this is when RBS are most active on the surface (Petranka 1998). RBS continue to remain active during the summer and winter season but spend most of their time underground to avoid the physiological pressures brought on by increased and

decreased temperature respectively (Taub, 1961; Grover, 1998). Throughout the three years of surveys each site was sampled three times per season excluding the spring of 2018 where each site was sampled four times.

On the day of the survey, both environmental data and capture of salamanders took place. Environmental data collection consists of Julian day, air temperature, soil temperature from two different areas of the plot, and conditions for wind, sky, and rain in the past twenty-four hours. Additional information collected consists of observers present during the time of collection, start and end time of the sampling period, and additional information that may be of importance (e.g. non-target species present under an ACO).

Upon collection of environmental data each ACO in the plot was checked for the presence of RBS. If present under an ACO, an individual RBS was captured by hand and placed into a sandwich size resealable plastic bag. Once secured, the bag was labeled according to the ACO the salamander was found under (e.g. row E, column 10) and then placed into a cooler. At the conclusion of field sampling, collected salamanders were then brought back to the lab at the NJSOC to be assessed for length, sex, and assignment of uniquely identifying markers.

Lab Procedures

Salamanders were measured in duplicate by two investigators to avoid identification and observation inaccuracies. Snout-vent length and total length were measured in millimeters. Sex was determined through candling techniques. Candling serves as a common and simple practice to determine the sex of red-backed salamanders (Gillette and Peterson, 2001). Presence of vas deferens indicated that the individual was male whereas presence of oviducts or eggs indicated

that the individual was female. If neither reproductive organ was visible the individual was assigned as "unknown".

After both observers determined length and sex, each individual salamander was given a uniquely identifying marker using visible implant elastomer. Visible implant elastomer is a liquid polymer that is injected into the subcutaneous layer of skin which results in an identifying marker in particular locations of the body (Heemeyer et al. 2007). This method is a safe, efficient way to mark amphibians and has proven to be an effective practice compared to other traditional marking techniques (e.g. toe clipping and PIT tagging) (Heemeyer et al. 2007). Upon completion of marking procedures at the end of the day, salamanders were then returned to the original ACO they were found under.

Leaf Litter Treatments

Treatments began at the end of the first survey day in April of 2019 (Table 1). Treatment assignments consisted of a control, leaf litter removed, and leaf litter added. A separate, individual treatment occurred at each of the three plots across the two sites. The first plot at each of the two sites was assigned as the leaf litter removal treatment. Leaf litter was removed from each plot through temporary displacement of the ACO array. Each ACO array at the two sites was moved so that leaves within the plot could be raked and gathered into piles before being placed into large garbage bags. After raking was completed, the ACO array was arranged to its original position. The second plot at each of the two sites was assigned as the leaf litter added treatment. The leaves from the first plot were brought to the second plot and spread evenly over top of the ACO array at each site. The third plot at each of the two sites was assigned as the control and left untouched.

Statistical Analysis

The statistical software package used for assessing the relationships between yearly and seasonal clutch size, body size, and leaf litter treatments was JMPPRO version 14.2. ANOVAs were used to test for differences in means for clutch size when analyzing clutch size over the years and seasons, clutch size in comparison to body size, and clutch size in comparison to the leaf litter treatments. Where ANOVA results were significant, a Tukey-HSD was performed to test for differences among means. The significance level for all statistical tests was set at α=0.05.

RESULTS

Demography

Over the six field seasons of the study, we had 1,551 captures during 33 sampling occasions of *P. cinereus*. We captured 413 salamanders in 2017; 582 salamanders in 2018; and 556 salamanders in 2019. Throughout the three-year sampling period we captured 905 females (58.35% of total); 551 males (35.53% of total); and 95 salamanders where sex could not be determined (6.13% of total) (Table 2.1).

	Sex		
Year	**Female**	**Male**	**Unknown**
2017	206	160	47
2018	250	207	25
2019	349	184	23
N	905	551	95
% of Total	58.35%	35.53%	6.13%

Table 2.1 Capture numbers for female, male, and undetermined red-back salamanders from April 2017-October 2019.

Of the 1,551 captures, 1,201 (77.48% of total) salamanders were striped morphs. The remaining 349 (22.52% of total) salamanders were lead-back morphs (Table 2.2). Striped morph numbers totaled at 668 females; 441 males; and 72 unknown individuals. Lead-back morph numbers totaled at 217 females; 110 males; and 22 unknown individuals.

	Morph	
Sex	**Lead-back**	**Striped**
Female	217	688
Male	110	441
Unknown	22	72
N	349	1201
% of Total	22.52%	77.48%

Table 2.2 Capture numbers by morph for female, male, and undetermined red-back salamanders from April 2017-October 2019.

Over the course of the study, 781 unique individuals were captured, and an additional 770 recaptures were made of those individuals (Table 2.3). These results show an almost 50:50 ratio of new and recaptured individuals. Numbers of new and recaptured individuals vary by sex. New individuals of female salamanders total at 475; male salamanders at 216; and unknown salamanders at 90 individuals. Recaptured female salamanders total at 430; male salamanders at 335; and unknown salamanders at 5 individuals.

	Year				
	2017	**2018**	**2019**	**N**	**% of Total**
New	304	264	213	781	50.35%
Recaptures	109	318	343	770	49.65%

Table 2.3 Total number of new and recaptured individuals from April 2017-October 2019.

Clutch Size Characteristics

The number of eggs per female varied from 1 to 16. The majority (94.8%) of eggs per female fell between 3 and 10 (Figure 1).
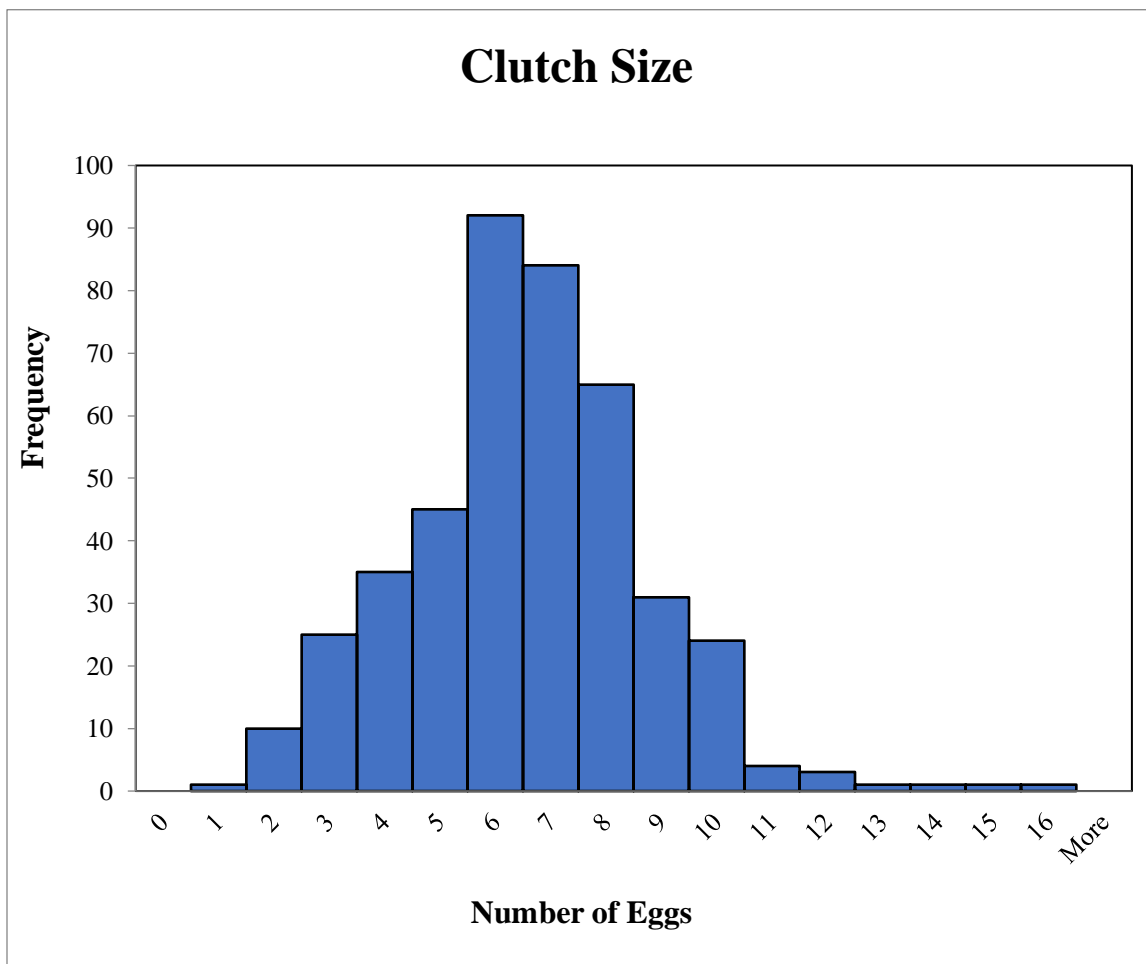

Figure 1. Frequency distribution for number of eggs for *P. cinereus*.

Body Size of P. cinereus

There is a significant correlation between snout-vent length and total length for males, females, and unknown of *P. cinereus* (Figure 2.1).
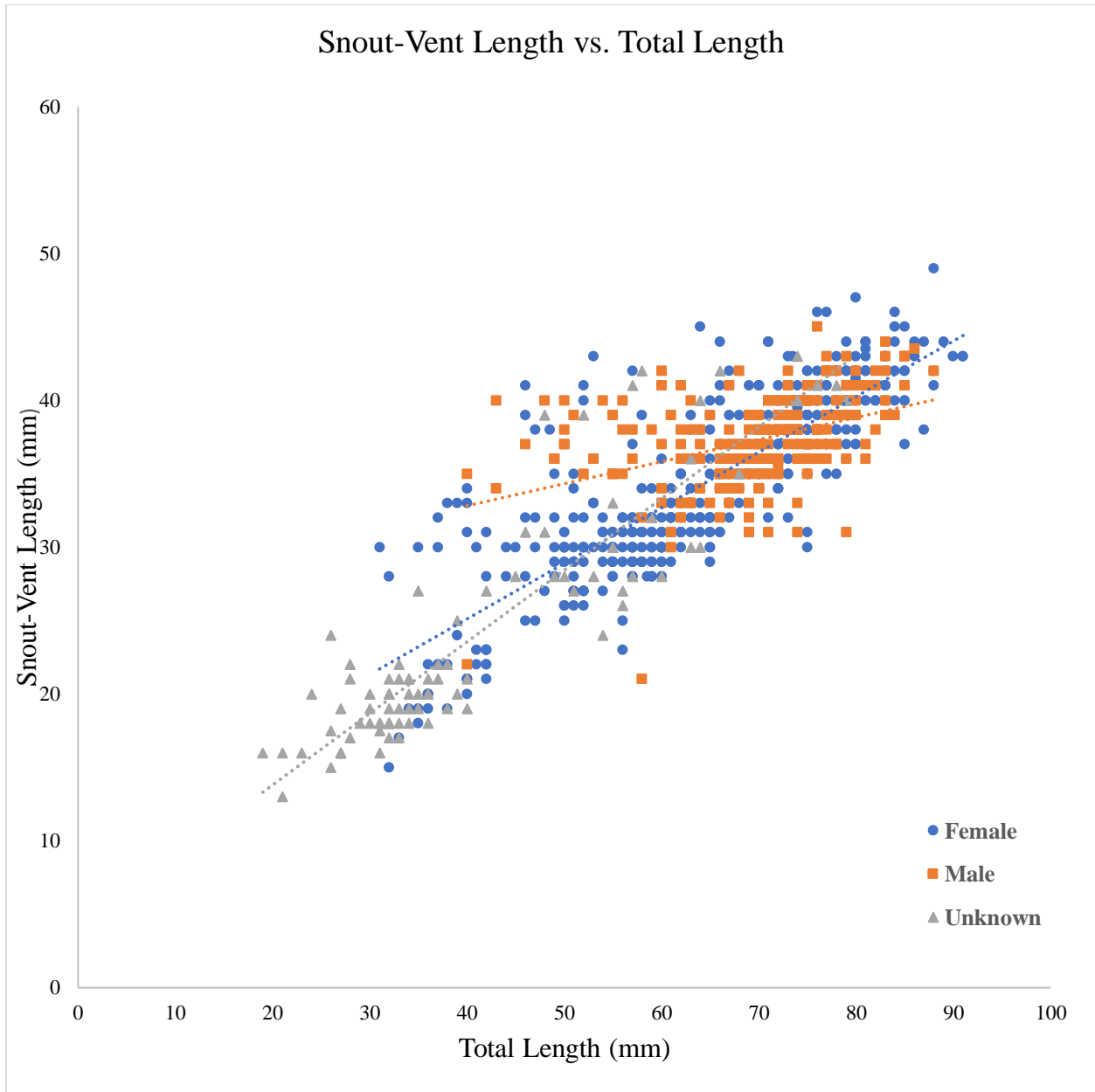
Figure 2.1 Snout-vent length and total lengths of male, female, and unknown *P. cinereus* from April 2017-October 2019. Females: y = 0.3785x + 9.9768 (R^2 = 0.6944, p < 0.0001). Males: y = 0.1503x + 26.812 (R^2 = 0.1748, p < 0.0001). Unknowns: y = 0.4878x + 4.0445 (R^2 = 0.8169, p < 0.0001).

In addition to correlations between snout-vent length and total length of individuals there are also correlations seen when comparing tail length to snout-vent length (Figure 2.2).
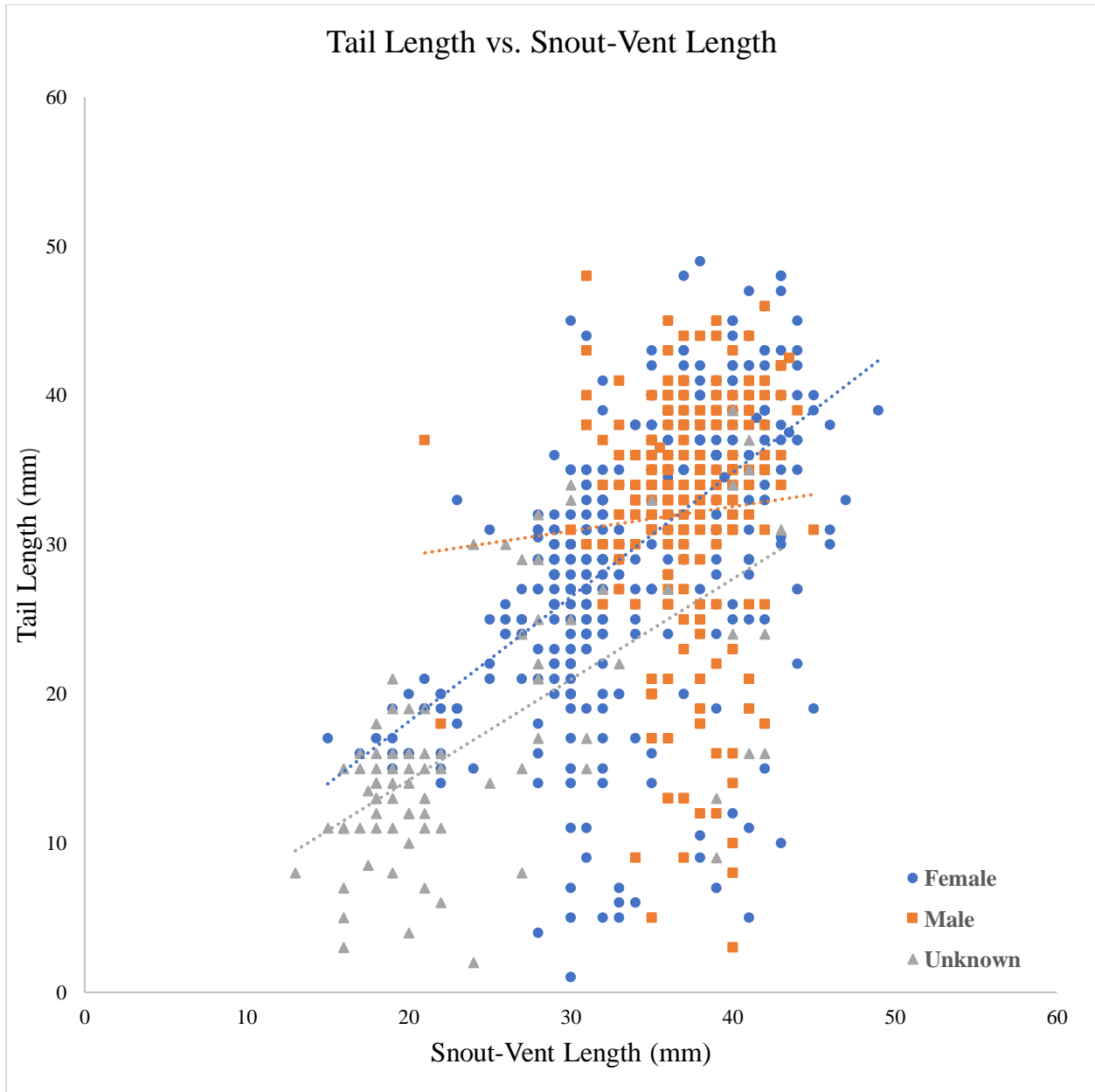
Figure 2.2 Tail length and snout-vent length of male, female, and unknown *P. cinereus* from April 2017 to October 2019. Females: y = 0.8346x + 1.4563 (R^2 = 0.3199, p < 0.0001). Males: y = 0.163x + 26.037 (R^2 = 0.0041, p = 0.3464). Unknown: y = 0.6747x + 0.7218 (R^2 = 0.42, p < 0.0001).

Clutch Size Effects

There is a significant correlation between clutch size and body size of *P. cinereus*. Total length, snout-vent length, tail length, and tail length as percent of snout-vent length were the measurements considered when assessing body size and its correlation to clutch size. Total length and snout-vent length correlations were strongest in regard to body size and clutch size (Figure 3.1 and 3.2).
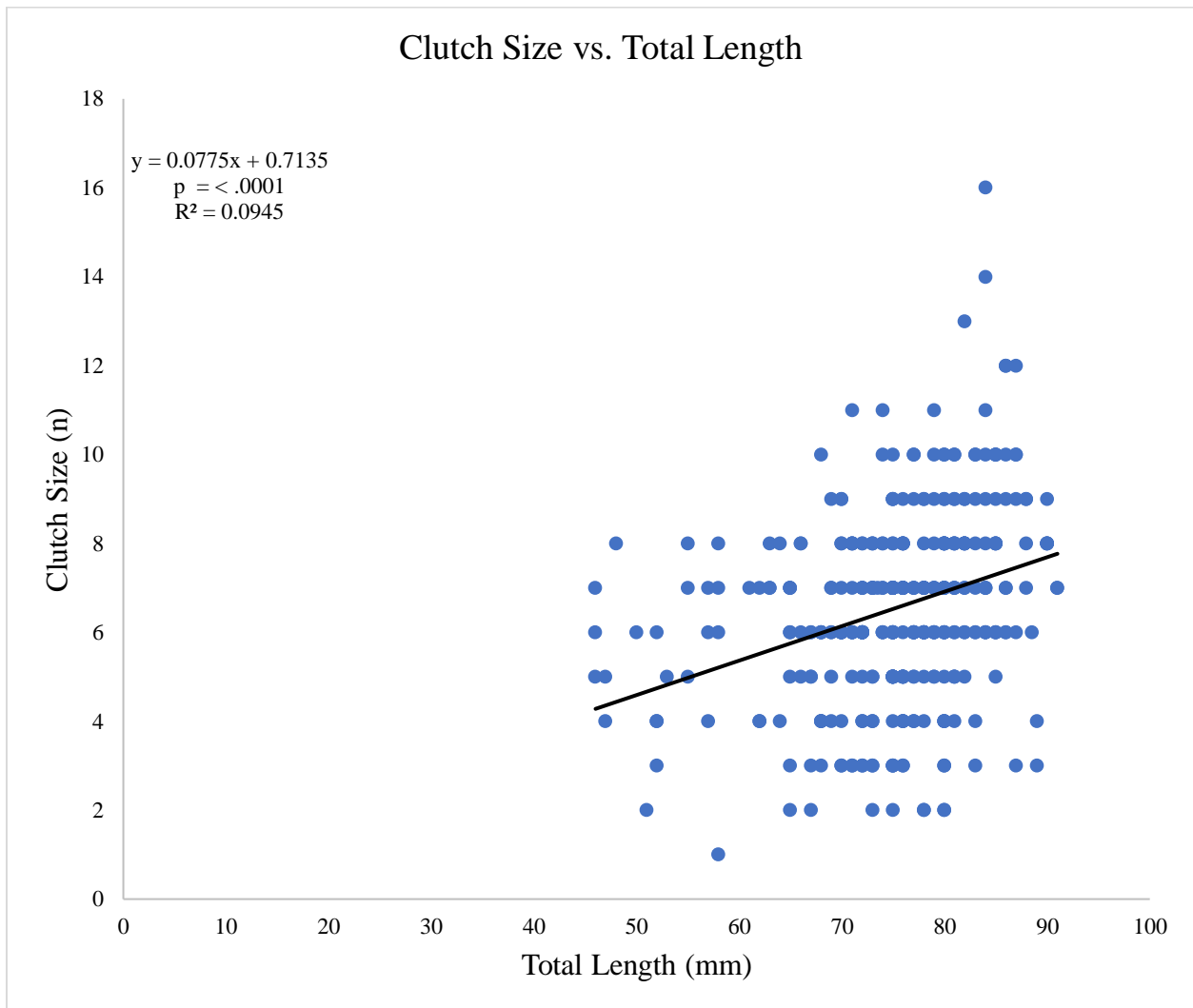

Figure 3.1 Clutch size and total lengths of female *P. cinereus* from April 2017-October 2019.
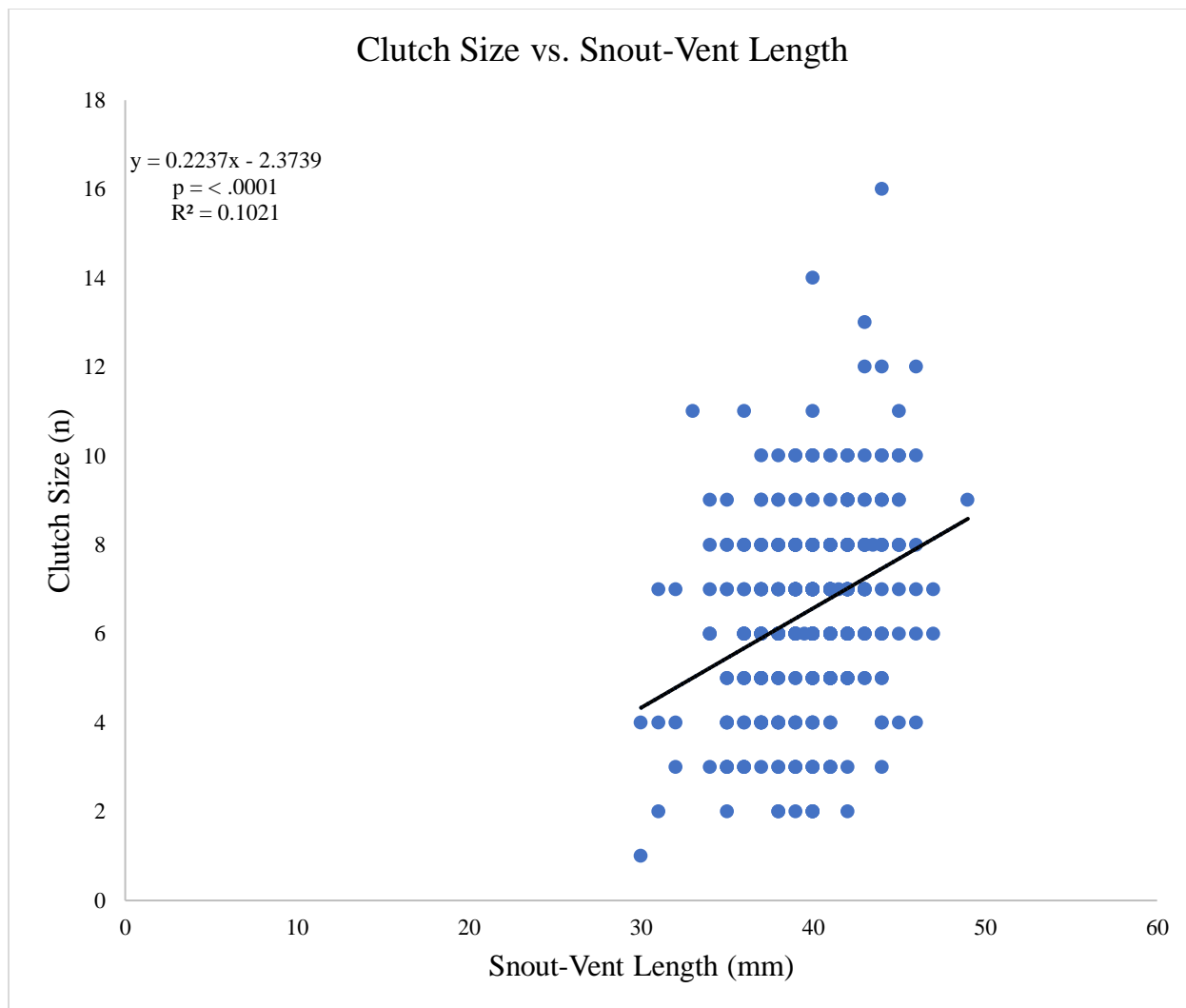
Figure 3.2 Clutch size and snout-vent lengths of *P. cinereus* from April 2017-October 2019.

Yearly and Seasonal Data

Yearly data were analyzed to determine whether there was a significant difference in average clutch size across the three years of study. It was found there were no significant differences (ANOVA: $F_{2,363} = 0.9249$, $p = 0.3975$) in the average clutch size of salamanders from 2017-2019.

Yearly data were then analyzed to determine whether there was a significant difference in average clutch size by season across the three years of study. Analyses concluded when seasons

were analyzed separately, there were significant differences among years in both the fall

(ANOVA: $F_{2,186} = 6.3692$, p = 0.0021) and the spring (ANOVA: $F_{2,174} = 4.6225$, p = 0.0111).

Significance was not exclusive to all years of the study but varied throughout seasons and years

(Table 3).

Year	Spring	Fall
2017	5.92 ± 0.36**b**	6.79 ± 0.27**a**
2018	6.68 ± 0.19**ab**	6.69 ± 0.29**a**
2019	7.24 ± 0.25**a**	5.48 ± 0.29**b**

Table 3. Means ± S.E. for clutch size of female *P. cinereus* during the spring and fall seasons of 2017-2019. Means sharing the same superscript are not significantly different from each other.

Yearly Data for Leaf Litter Treatments

      Leaf litter treatments were not performed until the third year of the study and thus, the initial two years of the study act as a control.

      There were no significant differences of average clutch size in both the control (ANOVA: $F_{2,104} = 0.0972$, p = 0.9075) and leaf litter added (ANOVA: $F_{2,131} = 0.3310$, p = 0.7188) treatments across the three years of study. Females produced a smaller clutch size over the three years of study in regard to the leaf litter removed treatment (Table 4; ANOVA: $F_{2,122} = 4.6799$, P = 0.0110).

Year	Control	Leaf Litter Added	Leaf Litter Removed
2017 (pre-treatment, Control Year)	6.45 ± 0.38	6.70 ± 0.35	6.48 ± 1.29**ab**
2018 (pre-treatment, Control Year)	6.36 ± 0.29	6.51 ± 0.31	7.15 ± 2.20**a**
2019	6.21 ± 0.38	6.88 ± 0.34	5.84 ± 2.37**b**

Table 4. Means ± S.E. for clutch size of female *P. cinereus* by leaf litter treatments from 2017-2019. Means sharing the same superscript are not significantly different from each other.

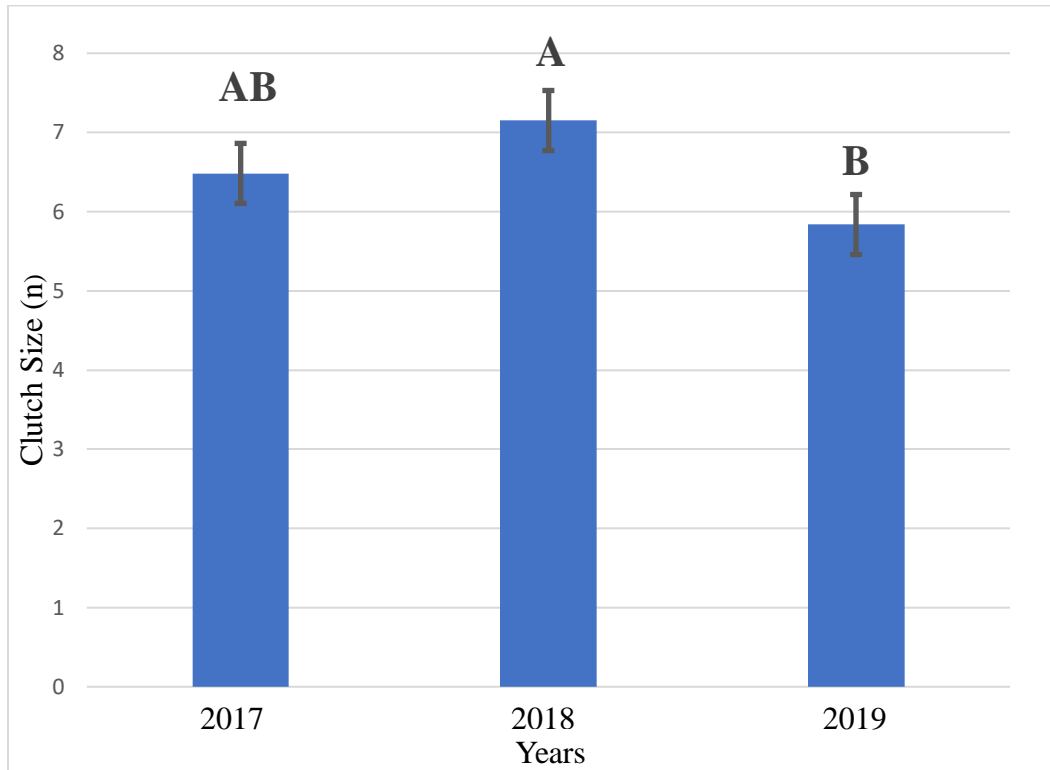In particular, the years of 2018 and 2019 differed significantly from one another (Tukey-HSD test, Figure 4).

Figure 4. Average clutch size of female *P. cinereus* for the leaf litter removed treatments (mean ± S.E.) from 2017-2019. ANOVA: $F_{2,122} = 4.6799$, p = 0.0110 and Tukey-HSD test. Years that share the same letter did not differ. N = 125.

Seasonal Data for Leaf Litter Treatments

Under the control treatments, no significant differences were found among the years when split out by season. Significant differences were found among the years when split out by season for the spring leaf litter added treatments (Table 5; ANOVA: $F_{2,58} = 5.5032$, p = 0.0065).

Year	Spring; LL Added
2017	5.78 ± 0.64**b**
2018	6.40 ± 0.35**b**
2019	7.91 ± 0.41**a**

Table 5. Means ± S.E. for clutch size of female *P. cinereus* under the leaf litter treatments for spring 2017-2019. Within season, means sharing the same superscript are not significantly different from each other.

Analyses found that average clutch size was significantly larger in 2019 when compared to 2017 and 2018 (Tukey-HSD, Figure 5). Although differences were found under the leaf litter added treatments during the spring seasons, analyses failed to find significant differences under the leaf litter added treatments for the fall seasons.
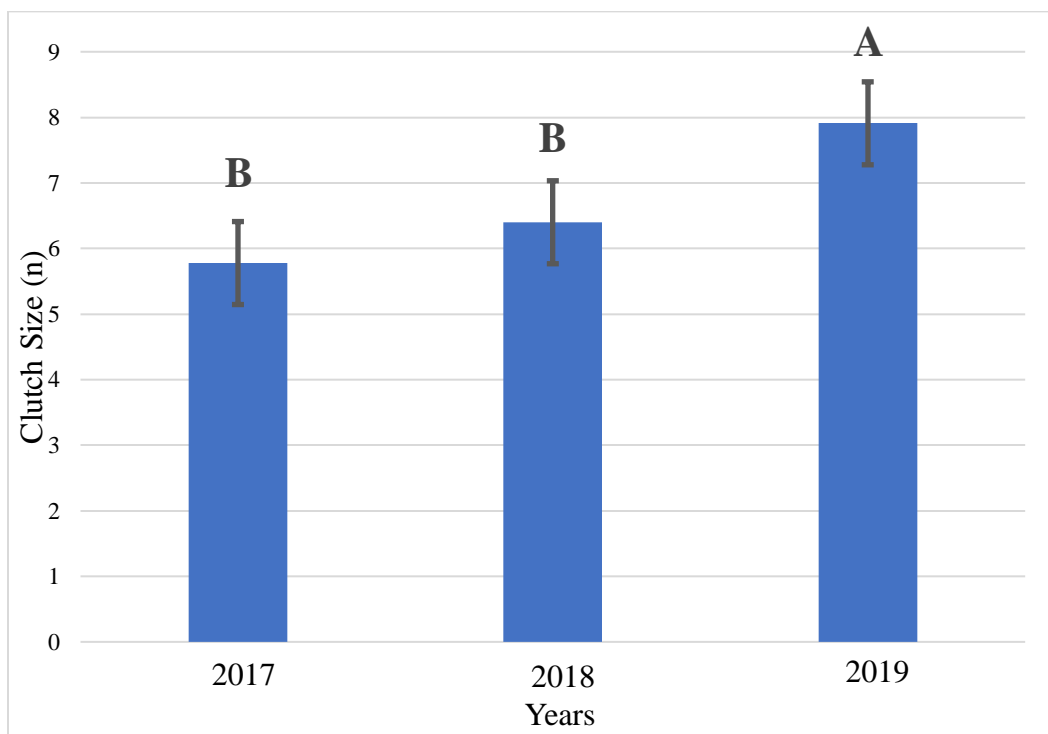

Figure 5. Average clutch size of female *P. cinereus* for the leaf litter added treatments (mean ± S.E.) from the spring seasons of 2017-2019. ANOVA: $F_{2,58} = 5.5032$, $p = 0.0065$ and Tukey-HSD test. Years that share the same letter did not differ. $N = 61$.

Lastly, significant differences were found under the leaf litter removed treatments for the fall seasons (Table 6; ANOVA; $F_{2,59} = 4.7445$, $p = 0.0123$).

Year	Fall; LL Removed
2017	6.62 ± 0.51**ab**
2018	7.06 ± 0.57**a**
2019	4.96 ± 0.48**b**

Table 6. Means ± S.E. for clutch size of female *P. cinereus* under the leaf litter treatments for fall 2017-2019. Within season, means sharing the same superscript are not significantly different from each other.

Analyses found that average clutch size was significantly smaller in 2019 when compared to 2018 (Tukey-HSD, Figure 6). Analyses failed to find any significant difference amongst the three years of spring data from the leaf litter removed treatments.
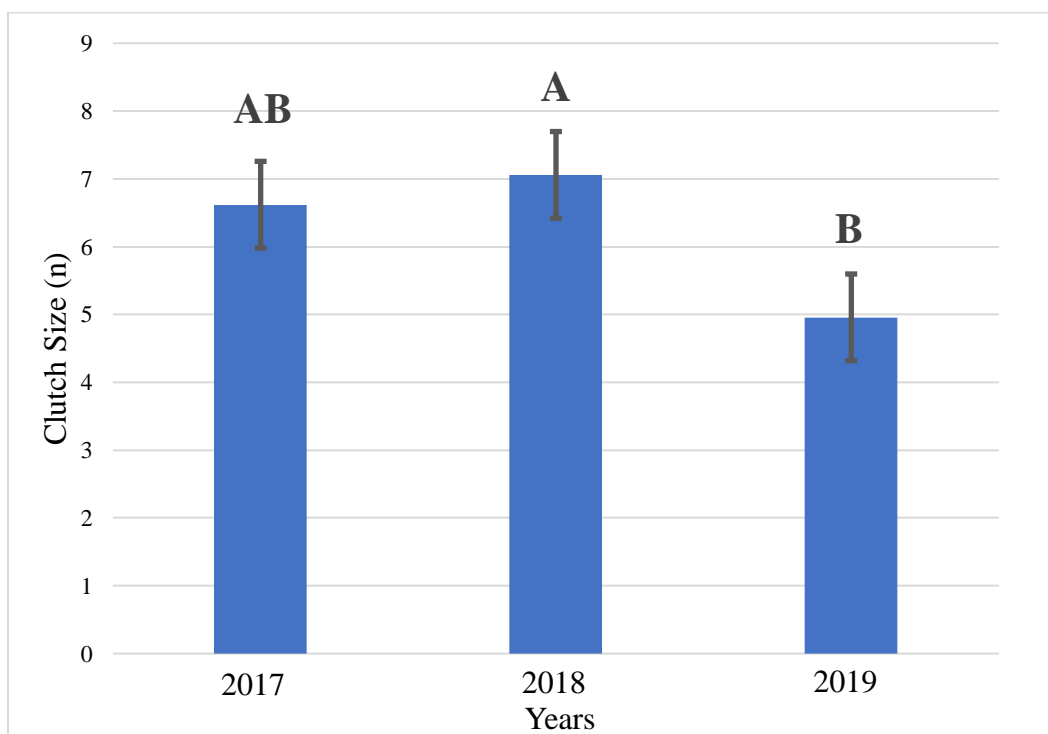

Figure 6. Average clutch size of female *P. cinereus* for the leaf litter removed treatments (mean ± S.E.) from the fall seasons of 2017-2019. ANOVA; $F_{2,59} = 4.7445$, $p = 0.0123$ and Tukey-HSD test. Years that share the same letter did not differ. N = 61.

DISCUSSION and CONCLUSION

Body Size

Our findings suggest that females do not reach reproductive maturity until they measure > 30 mm snout-vent length (Figure 3.2). Previous research by Sayler (1966) supports our findings which suggests that females first oviposit 3.5 years after hatching, when they measure > 34-39 mm snout-vent length. Additional findings by Lotter (1978) found that Connecticut females of *P. cinereus* reach sexual maturity when they measure 34-38 mm snout-vent length.

Our results indicate that body size of female *P. cinereus* is positively correlated to clutch size (Figure 3.1 and 3.2). Information reported by Lotter (1978) and Nagel (1977) also suggest that the number of mature ova is positively correlated with snout-vent length.

Yearly and Seasonal Data

I found there were no significant differences in the average clutch size of salamanders from 2017-2019 but did find there were differences among the years when split out by season (Table 3).

The average clutch size of females in the spring of 2019 was significantly larger than the average clutch size of females during the spring of 2017 (Table 3). Alternatively, during the fall of 2019 the average clutch size of females was significantly lower when compared to the fall years of 2017 and 2018 (Table 3).

There are several possible explanations for why we saw these results. These explanations are based on the variation of individuals within the population and specifically on body condition of individuals. Studies conducted by Yurewicz and Wilbur (2004) found that fecundity of female *P. cinereus* varies with body condition (i.e. amount of fat reserves). Differences among

individual fat reserves during brooding may be related to resource availability and physiology but may also be linked to a behavioral hypothesis – speculating that females demonstrate behavioral plasticity and differentiate investment of fat reserves each breeding season (Fleming 2018). Under this hypothesis, female *P. cinereus* may adopt one of two reproductive 'strategies' (Fleming 2018). (1). A female's investment into more fat reserves to increase survival rate of neonates. This strategy favors the female's current clutch. Nonetheless, the female's depleted fat reserves delay the development of ova for the following breeding season. In turn, this may impact the brooding success of the following clutch. (2). A female will invest fewer fat reserves, and her current clutch will have reduced survival. In spite of this, the female will be in better condition post-brooding, and this increases the rate of new ova production. These individuals have a 'head-start' in development of ova and highly developed ova may boost survival of the following clutch.

Influencers of female choice in reproductive strategy in a given breeding season are otherwise unknown. Females may choose to bypass breeding seasons under poor conditions (e.g. low rainfall), and instead invest in developing ova for future clutches. On the other hand, because size is correlated with age, it is reasonable that some females will skip brooding until reaching a certain size (Sayler 1966).

Leaf Litter Treatments

There were no significant differences among clutch size in the control and leaf litter added treatments across the three years of study, but females did produce a significantly smaller clutch when leaf litter was removed (Table 4). In particular, the years of 2018 and 2019 differed significantly from one another (Tukey-HSD test, Figure 4).

Additional analyses were run to determine if there were significant differences among the years when split out by season and leaf litter treatments. Under the control treatments, no significant differences were found among the years when split out by season. Alternatively, when leaf litter was added, significant differences were found for the spring seasons. Analyses found that average clutch size was significantly larger in 2019 when compared to 2017 and 2018 (Tukey-HSD test, Figure 5). Although differences were found when leaf litter was added during the spring seasons, analyses failed to find significant differences when leaf litter was added for the fall seasons. Lastly, significant differences were found when leaf litter was removed for the fall seasons. Analyses found that average clutch size was significantly smaller in 2019 when compared to 2018 (Tukey-HSD test, Figure 6). Analyses failed to find any significant difference amongst the three years of spring data from the leaf litter removed treatments.

The results answer our question on whether changes to leaf litter quantity will influence clutch size but not without some consideration to the reproductive ecology of *P. cinereus*. I found that clutch size increased when leaf litter was added to a plot whereas clutch size decreased when leaf litter was removed from a plot. Our findings suggest that manipulation of leaf litter quantity will increase or decrease clutch size respectively yet it is important to consider the timeline for this study and more specifically, the reproductive timeline for female *P. cinereus*. Most females will oviposit every other year and only breed during the second year of the egg maturation cycle (Sayler 1966). By the time leaf litter treatments began in April of 2019, females within the population were either depositing eggs or developing ova to deposit during the spring of 2020. Therefore, it would be unlikely that our leaf litter treatments could be directly linked to the increased clutch sizes seen during the spring of 2019 and decreased clutch sizes seen during the fall of 2019.

Numerous variables will exert influence over *P. cinereus* distribution and demographics. In this study, I investigated one of the most important influencers of RBS ecology; leaf litter. Disturbances that affect the depth of leaf litter could affect the availability and quality of microhabitats (Fraser 1976; Jaeger 1980). Considering *P. cinereus* are territorial, changes to microhabitat quality and availability could then impact foraging and courtship. Foraging and courtship are two crucial components of *P. cinereus* reproductive ecology as they both happen within the leaf litter. Therefore, changes to leaf litter quantity and depth have the potential to impact local populations of *P. cinereus*, which may then lead to changes in the clutch size of females.

There have been few studies on how changes to leaf litter quantity will influence populations of *P. cinereus*. I hope this research provides valuable information on the reproductive ecology of *P. cinereus* as well as provide insight to the research community on how an intercorrelated variable will influence the distribution and demographics of a species.

Suggestions for Further Research

The sample in this research consisted of individuals that are part of a capture-mark-recapture study. For the purpose of this study we did not incorporate females that were caught multiple times in a season and thus, data points were lost in the process. Further research is encouraged to utilize the individual data that has been recorded for each female. Mark-recapture data provides the ability to dive deeper into each female's reproductive ecology. I hope in the future this information can be utilized to gain a better understanding of individual variation within the species and its effect on clutch size.

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