LIFE HISTORY OF THE COASTAL TAILED FROG (*ASCAPHUS TRUEI*) ACROSS AN ELEVATIONAL GRADIENT IN NORTHERN CALIFORNIA

By

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A Thesis Presented to
The Faculty of Humboldt State University
In Partial Fulfillment of the Requirements for the Degree
Master of Science in Biology

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December 2019

ABSTRACT

LIFE HISTORY OF THE COASTAL TAILED FROG (*ASCAPHUS* TRUEI) ACROSS AN ELEVATIONAL GRADIENT IN NORTHERN CALIFORNIA

Adrian D. Macedo

The life history of a species is described in terms of its growth, longevity, and reproduction. Unsurprisingly, life history traits are known to vary in many taxa across environmental gradients. In the case of amphibians, species at high elevations and latitudes tend to have shorter breeding seasons, shorter activity periods, longer larval periods, reach sexual maturity at older ages, and produce fewer and larger clutches per year.

The Coastal Tailed Frog (*Ascaphus truei*) is an ideal species for the study of geographic variation in life history because it ranges across most of the Pacific Northwest from northern California into British Columbia, and along its range it varies geographically in larval period and morphology. During a California Department of Fish and Wildlife restoration project in the Trinity Alps Wilderness, I had incidental captures of Coastal Tailed Frog larvae and adults. To date, no population across the species' range has been described above 2000m. These populations in the Trinity Alps range from 150m to over 2100m in elevation, and those that are in the higher part of the range are likely living at the species' maximum elevational limit.

In this study, I examined size, growth, larval period, size at sexual maturity, and longevity of *A. truei* across populations along an elevational gradient in the Klamath Mountains of northern California. I calculated growth rates and movement by individually marking tadpoles and post-metamorphic frogs with visual implant elastomer (VIE), then tracking them from May through October of 2018. I described the length of the larval period using length-density histograms to visualize larval cohorts, I determined size at sexual maturity using secondary sexual characteristics of post-metamorphic frogs, and I determined longevity using skeletochronology.

I found that the larval period of *A. truei* in the Klamath Mountains of northern California ranges from two years in low and mid-elevations, to at least three years in high elevations. I also found decreased body size and increased growth rates of tadpoles with increasing elevation. Post-metamorphic frogs grew at similar rates as previously described coastal California populations. There was high site fidelity and significantly greater movement during the months of June and August in post-metamorphic animals. Frogs in the high elevations are capable of great longevity, with a maximum observed age estimated at eight years post-metamorphosis.

The high elevation populations described here have the longest larval period documented in California. This study also provides the first field estimates of larval growth rates and the first longevity estimates of post metamorphic frogs in California. Future laboratory experiments will be necessary to separate phenotypic plasticity of life history traits from true genetic differences between *A. truei* populations in the Klamath Mountains of northern California, as potential explanations for the variation seen.

ACKNOWLEDGEMENTS

I would like to first and foremost thank my parents: Richard and Suzanna Macedo. I was fortunate enough to have been raised by two excellent scientists and naturalists, my father a biologist for the California Department of Fish and Wildlife (CDFW), my mother a high school science teacher. I also had the privilege to have been raised in the mountains of northern California and had a perennial creek and forested country behind my house that would inspire me to dedicate my life to the understanding and preservation of nature. At an early age, I would spend hours in the forest catching frogs, salamanders, picking flowers, and I would bring them to my parents. My parents to this day continue to support and nurture my passion for the natural world, and I owe them the greatest acknowledgements in that regard.

I would like to acknowledge Justin Garwood who first hired me as a technician in the backcountry of the Trinity Alps Wilderness to remove non-native fish and study amphibians and reptiles. It was there where I started catching Coastal Tailed Frogs (*Ascaphus truei*), and it was Justin Garwood who encouraged me to pursue their study. He also provided me with skeletochronology slides from the East van Matre Creek and some data from the Canyon Creek *A. truei* populations.

I would like to thank Justin Demianew—a fellow alpsman and invaluable colleague in the roughest field conditions and the most complicated statistical analyses. In the field with him by my side I felt like no snowstorm, lack of funding, flood events, or forest fire could stop us from collecting data and producing novel scientific work. He aided me immensely and is an excellent teacher.

I must not forget to acknowledge Mary Carlquist, my field technician, for her bravery and toughness in the field. She was able to help me collect data day and night for 70 days in the wilderness, she learned how to drive stick shift in one single afternoon, and she was able bounce back to work after serious injury. Mary was one of those rare assistants who, along with giving me a hand, also thought deeply about the purpose of the study and always had good thoughts and questions.

To all the undergraduate assistants in the laboratory: Julie Trejo, Daisy Ceja, Ryan Aberg, Robyn Botsch, Syndey Gerstenberg, Bailey Andrews, Monica Jarquin, and Mario Vasquez. I would like to thank you for your dedication and company. As well as others whom helped me in the field: Chelsea Stewart, Ethan Snee, Shannon Hedge, James Bettaso, Kyle Orr, and Adam Mohr.

To the thank the folks in the Dr. John Reiss/Dr. Mihai Tomescu lab: Kelly Pfeiler, Kyle Orr, Jaclyn Patmore, Megan Nibbelink, Shayda Abidi, and Dr. Allison Bronson for support in the forms of good company in the lab, taco Tuesdays, and listening to my complaints about R and scientific writing. I would also like to thank some of my other graduate student colleagues who supported me friends for the past two years such as Skye Salanek, Ashley Abitz, and Jason Holmes.

I would like to thank my funders for financial support: CSU’s Research, Scholarship, and Creative Activities Program, CDFW State Wildlife Grants, and Biology Graduate Student Association. I would like to thank Susan Wright in the HSU Biological

Sciences Stockroom, David Baston in the CNRS CORE, and Colin Wingfield in the Wildlife Stockroom for material support.

I would like to thank my graduate advisor Dr. John Reiss, who accepted my proposal for this project, met with me countless times, and read and edited countless drafts of writing and presentations all with a smile and friendly face. John's kindness towards me and help was a breath of fresh air. I would like to thank Dr. Karen Kiemnec-Tyburczy for assistance and funding to do the *A. truei* genetic work, and Dr. R. Bruce Bury for advice and guidance. Lastly, I would like to thank my committee members: Dr. Karen Pope, Dr. Sharyn Marks, and Dr. Daniel Barton for their valuable insight and helping me improve my scientific writing.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER ONE: LARVAL LIFE HISTORY OF THE COASTAL TAILED FROG (*ASCAPHUS TRUEI*) ACROSS AN ELEVATIONAL GRADIENT IN NORTHERN CALIFORNIA

Introduction

The life history of a species is described in terms of its growth, longevity, and reproduction. Unsurprisingly, life history traits are known to vary in many taxa across environmental gradients (Begon et al. 1990). For example, one well known ecogeographical rule is Bergmann's rule, which states that within endothermic vertebrates, there is a general intra- and interspecific trend towards larger body size in cooler environments (Bergmann 1847). Amphibian adults, although ectotherms, also follow Bergmann's rule, with 67% of species overall, and 62% of anuran species, having larger body size at high latitudes or elevations (Ashton 2002). Intraspecifically, amphibian populations at high elevations and latitudes tend to have shorter breeding seasons, shorter activity periods, longer larval periods, reach sexual maturity later, and produce fewer and larger clutches per year relative to those at lower elevations (Baraquet et al., Bears et al. 2009, Lindgren and Laurila 2005, Miaud et al. 1999, Moore 1949, and Morrison and Hero 2003). Here I will focus on the larval phase of the life cycle; in particular, larval period and larval size variation.

Such variation has been documented in a number of species. For example, Green Frog (*Rana clamitans*) larvae in montane populations overwinter twice before completing metamorphosis, while lowland populations metamorphose within the same year (Berven et al. 1979). Interestingly, with respect to Bergmann's rule, larvae of Western Spotted Frogs (*Rana pretiosa*) have a pattern that contrasts with that described for other species: larvae are significantly larger in lowland populations than in highland populations; this pattern is thought to be due to genetic differences between the populations (Licht 1975).

Phenotypic plasticity in growth and development rates may occur in response to differing environmental conditions. Variation in growth and development at the proximate level can lead to differences in life history traits like longevity and maturity across latitudinal and elevational gradients (Lindgren and Laurila 2005, Licht 1975).

Reznick (1990) developed two models to understand the proximate origins for variation in age and length at maturity of guppies (*Poecilia reticulata*). The first model used constant values for the body length that initiates maturation and the time interval between initiation and completion of maturation. In this way, the model assumes that development (size, growth rate, minimum size to initiate maturation, and maximum size at metamorphosis) does not respond to variation in the environment, meaning there is no plasticity in their development across different environmental conditions. The second model allowed for plasticity in development in response to variation in the environment.

Using these two models, Reznick (1990), found that guppies in favorable conditions (more food) could grow faster and initiate the process of maturation earlier or at a larger size. In unfavorable conditions (less food), individuals grow slower and reach maturity later, or at a smaller size. This suggested that growth rates are plastic in guppies; however, guppies were not flexible in the amount of time it takes to complete maturation once the process was initiated (Reznick 1990). These findings suggest that flexible growth rates could produce variation in body size and time to reach maturity in guppies (Reznick 1990).

These same models were applied to the post-metamorphic Common Frog (*Rana esculenta*) with similar results – in environments with a shorter activity period, post-metamorphic frogs grew more slowly and reached sexual maturity later. However, adult Common Frogs at high elevations eventually attained a greater final length (Miaud et al. 1999). This was because they also had greater longevity than individuals in populations with a longer activity period (Miaud et al. 1999).

By contrast, when comparing populations from high and low latitudes using a common garden experiment, individuals collected from higher latitudes actually had increased growth rates compared to individuals from lower latitudes (Lindgren and Laurila 2005). This is an example of countergradient variation with respect to growth rates. Countergradient variation is a geographical pattern of genotypes (with respect to environments) in which genetic influences on a trait oppose environmental influences, thereby minimizing phenotypic change along the gradient (Conover et al. 1995). This increase in growth was not due to a high food intake rate, but higher growth efficiency in high latitude populations (Lindgren and Laurila 2005). Increased growth rates in high elevation populations have also been described for Western Spotted Frogs, *Rana pretiosa* (Licht 1975). However, in natural environments, countergradient variation means that environmental conditions can dampen or mask phenotypic variation such as higher growth efficiency in high elevations, so that observed growth rates may look the same or appear lower in high elevations. While these lasting effects have been documented for post-metamorphic anurans, they remain undocumented for larvae. Measuring variation in growth and development rates in larvae can help explain why life history traits such as larval size and larval period differ among populations.

The Coastal Tailed Frog (*Ascaphus truei*) is an ideal species for the study of geographic and elevational variation in life history, because it ranges across most of the Pacific Northwest from northern California into British Columbia, and from near sea level to high elevations in interior mountains. Along that range, *A. truei* varies widely in larval period (Bury and Adams 1999, Metter 1967, and Wallace and Diller 1998), with a range from one to at least three years across a south-north latitudinal gradient (Bury and Adams 1999). Populations in the mountains of northwestern Washington are thought to have a four-year larval period (Brown 1990).

Little is known with regards to growth rates of Coastal Tailed Frogs across their range. Studies assessing growth rates of tadpoles (using body mass measurements) in coastal British Columbia, Canada suggested that decreased light, nutrients, and increased consumer density all had a negative influence on growth (Kiffney and Richardson 2001, Mallory and Richardson 2005). No studies to date have calculated growth rates for tadpoles in California.

During the summers of 2016-2018, I worked for the California Department of Fish and Wildlife (CDFW) on a restoration project in the Trinity Alps Wilderness and encountered Coastal Tailed Frogs while removing fish from a stream at 2,100m elevation. In California, *A. truei* are commonly found in headwater streams of coastal forests, and no population across the species' entire range has been described above ~2,000m (Bury 1968). I was immediately intrigued by this high elevation population and sought to learn about how the species survives in the vastly different conditions of coastal and mountain streams. Coastal Tailed Frogs are cold, headwater stream specialists, yet appear to be elevational generalists. The discovery of these populations extends the range from the coast to the slopes of the highest peaks in the Klamath-Siskiyou Mountains. Coastal Tailed Frogs have clearly been able to find the specific habitats to meet their unique life history needs at a range of elevations. However, the broad climatic differences across elevations (e.g., snow at the high elevations) likely influence life history traits such as size, growth rates, longevity, age at sexual maturity and length of larval development period. Here I examine these for larval animals; I report on post-metamorphic animals in Chapter Two, below.

Research objectives and predictions

My objective was to describe whether life history traits of larval *A. truei* in northern California differ across an elevational gradient, and if larval *A. truei* follow Bergmann's rule along the same gradient. My predictions were as follows:

Growth and development of larvae

I proposed three alternative predictions for the variation of growth of *A. truei* tadpoles across an elevational gradient:

1. No difference: Growth rates do not differ across elevations.

2. Growth rates of tadpoles are greater in populations at higher elevations, as reported for *Rana pretiosa* (Licht 1975) and *R. temporaria* (Lindgren and Laurila 2005).
3. Growth rates are lower in higher elevation populations, as reported for *Poecilia reticulata* (Reznick 1990), *Ascaphus truei* (Kiffney and Richardson 2001, Mallory and Richardson 2005) and *Rana temporaria* (Miaud et al. 1999).

<u>Length of the larval period</u>

I proposed two alternative predictions for the length of the larval period across an elevational gradient:

1. Null hypothesis: Age at metamorphosis is the same across an elevational gradient in northern California and is two years (the maximum described currently for California) (Bury and Adams 1999 and Wallace and Diller 1998).
2. Age at metamorphosis is one to two years in lowland populations, as was described in coastal California (Wallace and Diller 1998) and up to four years in higher elevations, as was described for populations in the mountains of northwestern Washington (Brown 1990).

<u>Bergmann’s rule</u>

I developed three alternative predictions for larvae with regards to Bergmann’s rule (Figure1):

1. There is no difference in body size (at a given developmental stage) across an elevational gradient.

2. Larval body size (at a given developmental stage) increases along an increasing elevational gradient.
3. Tadpoles (at a given developmental stage) are smaller in higher elevation populations, as described for Western Spotted Frogs (Licht 1975).

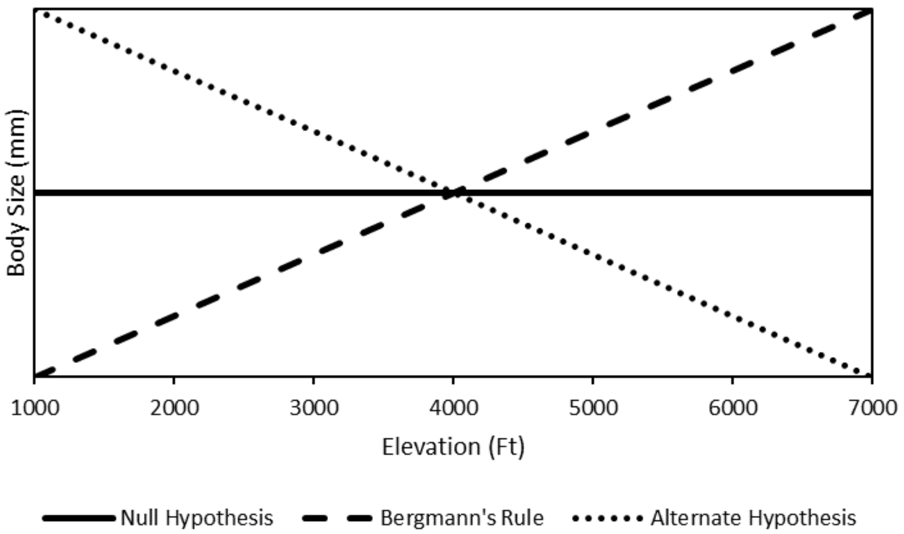


Figure 1. Alternative predictions of the relationship of body size to elevation of Coastal Tailed Frogs in northern California, USA.

Importance and need

Most of the research on Coastal Tailed Frogs in California has focused on how the species may respond to human impact and climate change and has been conducted in coastal areas. Only a few studies consider the life history variation across California (Bury and Adams 1999, and Wallace and Diller 1998). The Coastal Tailed Frog is a Species of Special Concern in California, more information is needed regarding population dynamics, and life history studies could aid in determining which life history stages limit population growth (Thomson et al. 2016). This chapter describes new localities and information on life history traits of larval coastal tailed frog populations that will help inform how populations can respond to disturbance and climate change based on their life history strategy and plasticity in strategy.

Methods

Study area

This study was conducted along an elevational gradient from the upper reaches of the Trinity River in Siskiyou and Trinity Counties, CA (Decimal Degrees, WGS84: 40.929834, -122.889931) down to the lower reaches of the Trinity River, Humboldt County, CA (Decimal Degrees, WGS84: 40.89425, -123.69389, Figure 2) with elevations ranging from ~150 m to ~2100 m.

All the study sites lie within the Klamath Mountain bioregion, which ranges from northern California to south-eastern Oregon (Skinner et al. 2006). In California, this bioregion lies between the northern Californian coast on the west and the southern Cascades range to the east and contains the Klamath and Trinity River systems.

The climate is Mediterranean, and is characterized by wet, cool winters and dry, warm summers. The proximity to the Pacific Ocean causes a moisture and temperature gradient that leads to characteristic patterns of precipitation via orographic effects. Average precipitation over the entire region is 101.2 cm annually with most rainfall from October to April (Skinner et al. 2006). The summer months are typically warm and dry, and infrequent precipitation events during this time of year occur in the form of thunderstorms (Ferlatte 1974). The average April 1st snowpack is 259 cm at an elevation of 2,042 m (Skinner et al. 2006), and in the highest elevations 5m of snow was measured in mid-May of 2017 (personal observation). Snowpack does not persist more than a month in the summer at low and mid-elevation sites and habitat is usually clear of snow by May (personal observation). Summer air temperatures at the lower elevations sites frequently reach 38°C or more, whereas temperatures in the high elevations sites rarely reach 32°C.

Site selection

I selected four high elevation study sites (~1500m to ~2100m), seven mid-elevation sites (~600 to ~1500m), and five low elevation sites (~150m to ~600m) (Table 1). Sites were selected from a combination of previous explorations, discussions with herpetologists, and locations reported from museum collections. High elevation sites were all in the Trinity Alps Wilderness and included the Echo Lake, Siligo Creek, and Canyon Creek Basins, while the mid- and low-elevation sites extended along the lower Trinity River watershed, near the towns of Helena, Burnt Ranch, and Willow Creek, (Figure 2; Table 1; for site photos, see Appendices E-G).

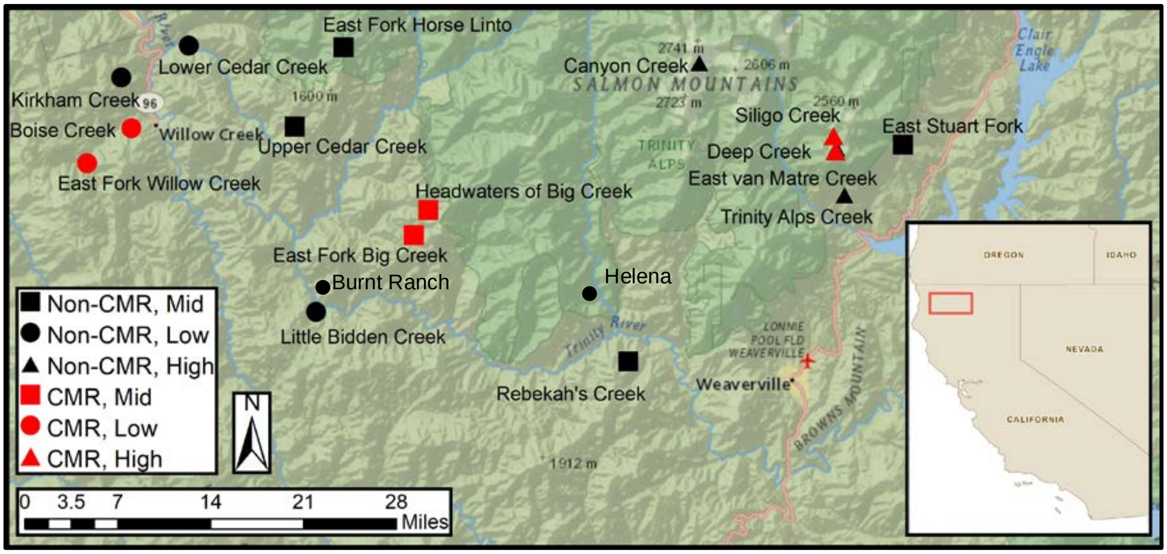


Figure 2. Capture mark-recapture (CMR) and ancillary (non-CMR) study sites across low, mid, and high elevations within the Trinity River watershed in northern California.

Stream sampling design

At ten of the study sites, I conducted a minimum of one survey to collect data on larval period and age structure. I also selected two high, two mid, and two low elevation streams for a rigorous capture-mark-recapture (CMR) study to examine growth rates (Table 1). All CMR sites were sampled every other week from May 2018-August 2018. Each stream was surveyed along a 200m stretch or more.

Table 1. Capture mark-recapture (CMR) and ancillary (non-CMR) study sites across an elevational gradient in the Trinity Alps, CA, USA

CMR Site Names	Elevation (m)	Elevation Category	UTM Easting (WGS 84)	UTM Northing (WGS 84)
Boise Creek	244	Low	444527	4531930
East Willow Creek	399	Low	440409	4528759
Headwaters of Big Creek	1009	Mid	471706	4524290
East Fork Big Creek	1082	Mid	470372	4522006
East van Matre Creek	2040	High	509101	4529635
Silago Creek	2076	High	508940	4531005

Non-CMR Site Names	Elevation (m)	Elevation Category	UTM Easting (WGS 84)	UTM Northing (WGS 84)
Lower Cedar Creek	152	Low	449840	4539431
Kirkham Creek	591	Low	443631	4536574
Little Bidden Creek	610	Low	461357	4515026
Rebekah's Creek	930	Mid	490063	4510414
Upper Cedar Creek	994	Mid	459495	4531987
East Horse Linto	1339	Mid	463995	4539212
East Stuart Fork	1361	Mid	515316	4530237
Trinity Alps Creek	1487	Mid	509946	4525589
Deep Creek	1969	High	509189	4529848
Canyon Creek	2131	High	496607	4537647

Larval capture and immobilization

I captured tadpoles using a combination of backpack electrofishing with a long-handled dip net and rubble-rousing (light touch). I used rubble-rousing when the backpack electrofisher batteries died or when stream connectivity was too low. I set backpack electrofishers at 300-500V with low pulse set channel at three pulses per second at 25% duty cycle, and a high pulse set at 30 pulses per second at 23% duty cycle. I adjusted the voltage and pulse up or down as necessitated by stream conductivity. Rubble-rousing in sensitive areas may alter stream bed conditions, so I minimized this by using the light touch approach and spacing surveys at least two weeks apart. Once I captured an animal or animals, they were transferred to plastic bags with cool stream water and the site of capture was recorded.

Measurements

For larvae, I made the following measurements:

Snout-vent length (SVL): Measured from the tip of the snout to the posterior end of the anal flap.

Total length: from the tip of snout to tip of tail.

Developmental stage: I scored the developmental stage with a staging table (modified from Gosner 1960) for *Ascaphus,* modified for use in the field by John Reiss and me (Appendix A). I measured hind-limb bud length through the tadpole's anal flap when hindlimb buds were visible. Because some stages were difficult to distinguish in the field, I grouped them into a series of stages; for example, between stages 28 and 34 toe differentiation and development is obscured by the anal flap, but the length of the limb bud can be measured. Therefore, any tadpole that had a limb bud length less than 0.7mm I reported as stage 28-33, and any tadpole with limb bud greater than 0.7mm I reported as stage 34-36 (Appendix A). This inability to distinguish stages 29 through 33 is reflected as a gap in the developmental stage histograms (Appendix K).

Immobilization and marking

I anesthetized tadpoles in the field with MS-222 (300 mg/L in stream water, buffered to pH 7.0 with 600mg/L of baking soda) to immobilize them prior to marking and measurement. I used the same solution for all larvae from the same stream. One at a time, I placed individuals in the solution, and they were removed once they were determined to be unresponsive (i.e., failed to exhibit a righting reflex). Once removed, I rinsed them with stream water to prevent overdose. I followed anesthetizing protocols used previously for Coastal Tailed Frogs (Chelgren and Adams 2017).

I marked tadpoles using visual implant elastomer (VIE) (Northwest Marine Technology Inc. Seattle, Washington). I marked each animal for individual recognition using a combination of four possible colors (yellow, orange, red, and blue). I made the marks just under the skin, 3-5mm long and sometimes smaller for very small individuals. I viewed marks with a UV light in a darkened area to enhance visibility. Tadpoles were marked on the tail in upper and lower (i.e., dorsal and ventral to the midline, respectively) and front and back positions (the front position being directly posterior to the vent, the back position being posterior to the mark closer to the vent; Figure 3). Marking methods followed Chelgren and Adams (2017) and 625 individual marking codes were generated using SalaMarker (MacNeil et al. 2011).

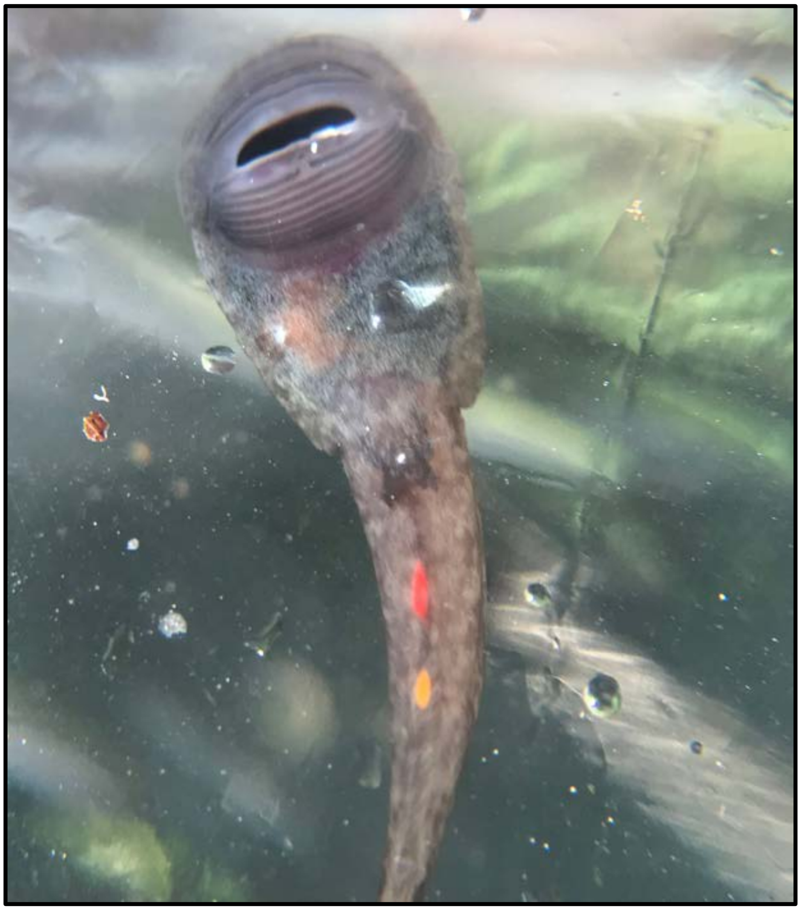


Figure 3. Coastal Tailed Frog tadpole with visual implant elastomer (VIE) marking.

Field collection

Because there are no museum collections of Coastal Tailed Frogs from any population over 2000m elevation, I collected 36 larvae that were either found dead, found inside snakes, or died incidentally from anesthesia/handling, and eight larvae and one sub-adult from newly described high elevation sites. Collections were vouchered at the HSU Vertebrate Museum (Appendix B).

Statistical methods

I performed all analyses in the statistical program R (Version 3.2.2, R Core Development Team 2017).

Growth rate

I estimated growth by subtracting the body size of the last capture of each individual animal by the body size of the first capture of each individual animal, this was compared to the number of days between captures. I compared the relationship between growth and number of days between captures across elevation categories using a linear mixed effects regression with elevation category as a fixed additive effect and each individual as a random effect. Growth rates were calculated by dividing the amount of growth by the number of days between captures and were compared across the survey months using non-parametric Kruskal-Wallis and Conover-Iman tests (Dinno 2017).

Number of larval cohorts and age at metamorphosis

I visually inspected density frequency histograms of total length vs. frequency of capture for each site. Since tail resorption during metamorphosis may confound size-class analysis, I defined metamorphosing tadpoles as having emerged forelimbs (developmental stage 41 and later) and excluded them so they would not create false peaks or confound existing peaks. Peaks within histograms were considered distinct age classes and the metamorphosing age class was considered a distinct separate age class. This has been the method used to estimate larval period in the literature to date (Brown 1990, Bury and Adams 1999, Metter 1968, Wallace and Diller 1999). I also compared the cohorts across the survey season at sites if I completed repeated surveys. This allowed me to see how the tadpole cohorts change across the season and determine timing of metamorphosis. I also made histograms of developmental stage following the same protocol as above. This aided in defining tadpole cohorts.

Bergmann's rule

I compared body size (SVL) of tadpoles across elevation categories, using the body size of tadpoles of development stage 37, and analyzed differences using a non-parametric Kruskal-Wallis and Conover-Iman tests (Dinno 2017).

Results

In total, I took 2,280 measurements of tadpoles across a total of 16 sites (Figure 3) over 70 days from May-August 2018. One thousand two hundred and thirty larvae were individually marked, 210 (17%) of which were recaptured at least once: 161 were caught twice, 37 caught three times, 11 caught four times, and one caught five times. The total number of tadpoles captured was similar across elevation categories: 408 individual tadpoles were caught in the low elevations, 484 in the mid elevations, and 338 in the high elevations. Tadpoles were found in all the ancillary sites, but only Upper Cedar Creek, East Stuart Fork, Kirkham Creek, and Little Bidden Creek had over 10 individuals captured.

Growth/developmental rates and body size of tadpoles

Growth rates were significantly higher at high elevations compared to low elevations (Std. Error=0.29, D. F.=159, P=<0.01) and mid elevations (Std. Error=0.26, D. F.=159, P=<0.04) (Figure 4 and Table 2). Even though there was an observable difference, there was no significant difference between the growth rates of low and mid elevations (Std. Error=0.26, D. F.=159, P=0.34). There was no significant increase of growth rates between the months of July and August in low elevations (***W***=0.99, D. F.=1, P=0.16), and sample sizes were not sufficient for growth rates to be compared between the months of May and June. In mid elevation sites growth rates increased significantly across the months of the season (***W***=15.44, D. F.=2, P=<0.01). Lastly, an opposite pattern was observed in the high elevations, in which growth rates decreased across the months of the growing season, however there was no significant difference between the months (***W***=4.31, D. F. =3, P=0.23) (Figure 5).

Table 2. Growth rates of *A. truei* tadpoles from May 2018 to August 2018 across an elevation gradient in northern California, USA.

Elevation Categories	Mean Body Growth (mm/day)	Standard Error
Low	0.06	0.01
Mid	0.08	0.02
High	0.11	0.02

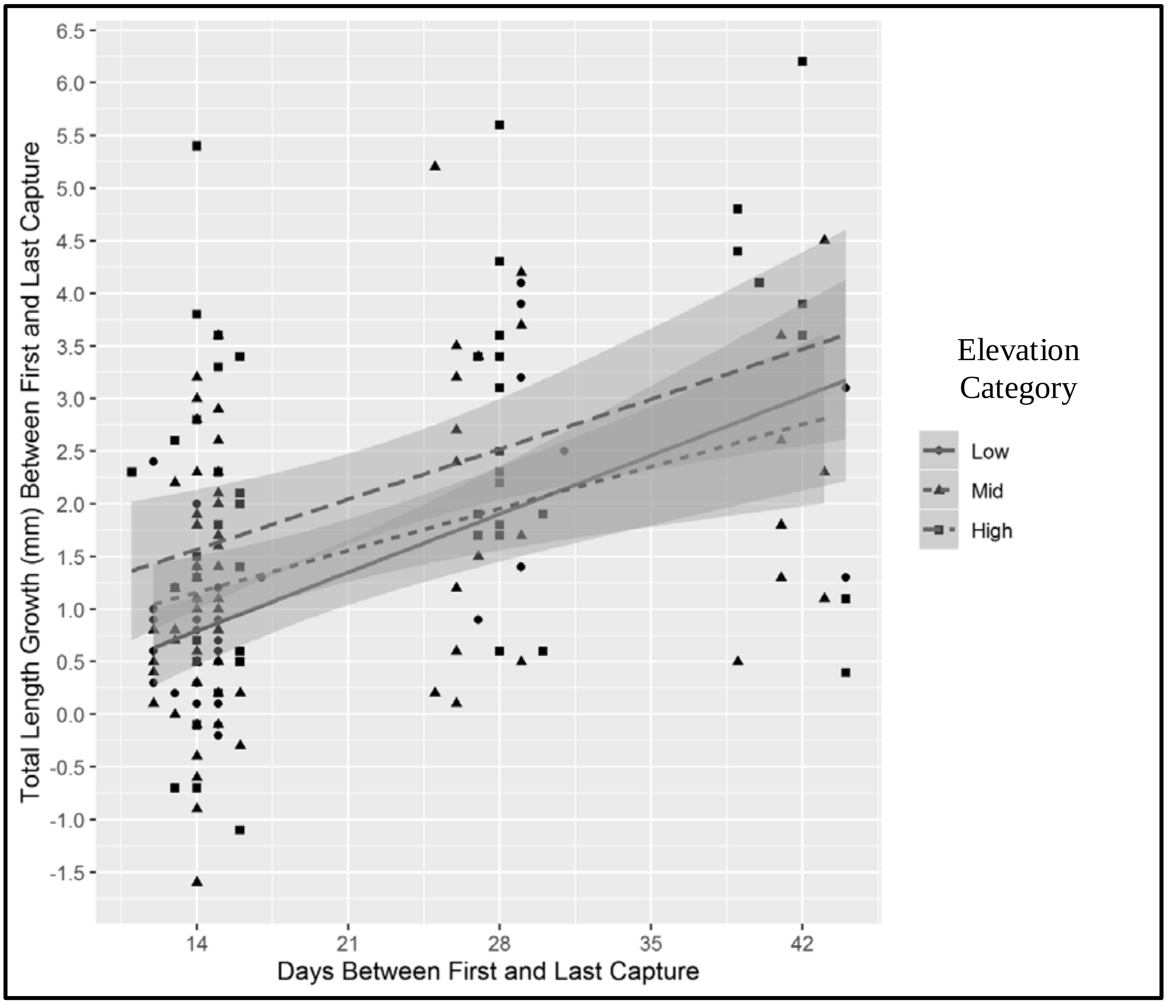


Figure 4. Growth rate of tadpoles across an elevational gradient in northern California, USA. Model variance is reported in standard error.

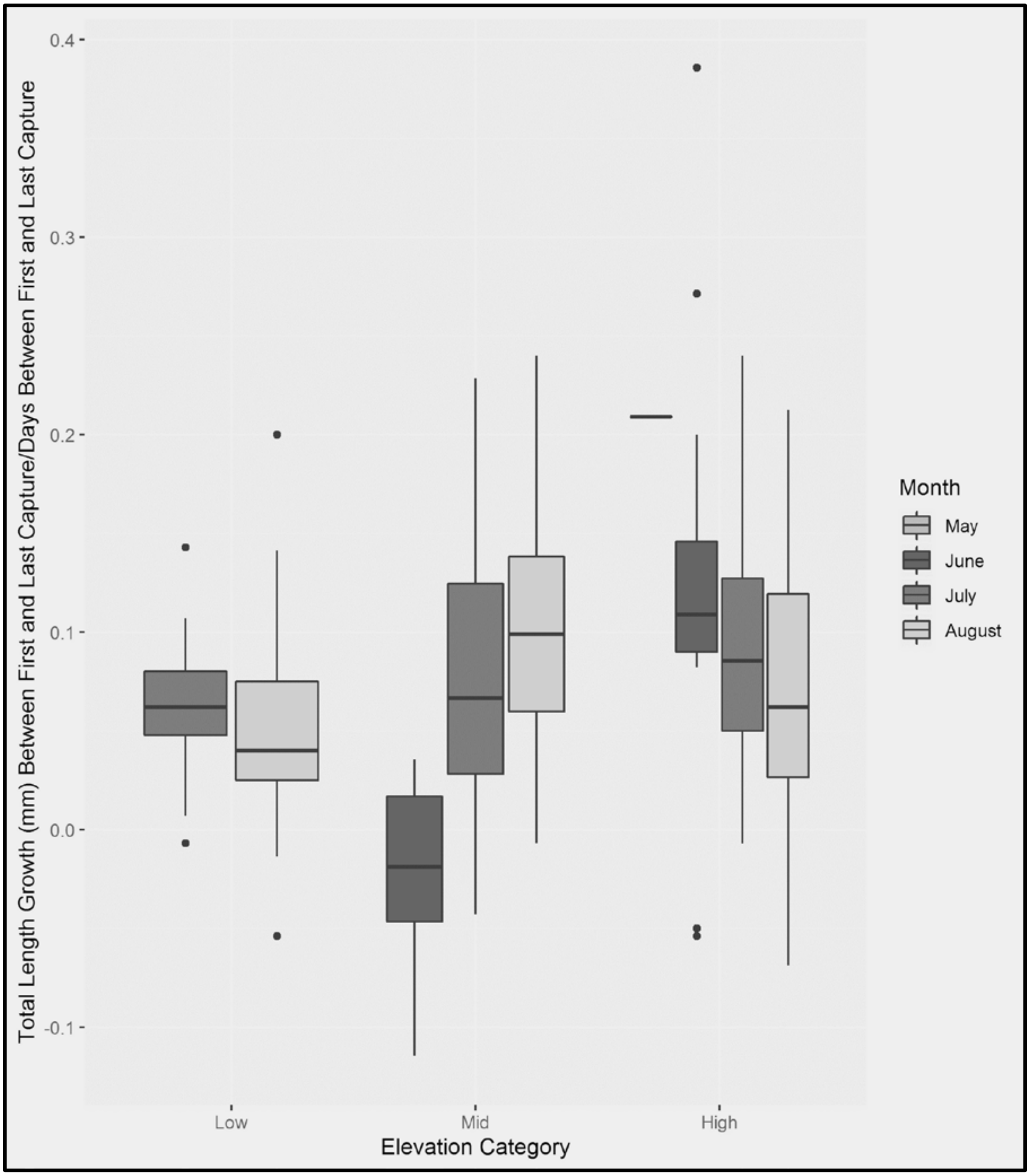


Figure 5. Growth rates of tadpoles across the months of June (left), July (middle) and August (right) of the survey season between elevation categories in northern California. The horizonal lines represent the medians and the lower and upper hinges correspond to

the first and third quartiles (the 25th and 75th percentiles). The whiskers extend from the hinge to the largest value (upper) and smallest value (lower) no further than 1.5 times the interquartile range.

Size cohorts and length of larval period

At the beginning of the summer, populations from the high elevations showed two main peaks of total length and developmental stage, followed by a third smaller peak (Figure 6). These peaks correspond to three larval cohorts. Samples from low elevations and mid elevations showed a single main size peak, followed by a second smaller peak (Figure 7 and 8). These peaks correspond to two larval cohorts. Later in the summer, the largest cohort begins to metamorphose and shrink. They were removed to avoid false peaks. Density histograms of development stage across the season as well as ancillary sites that were only sampled once gave further support for a larval period of two years in low and mid elevations and three years in high elevations (Appendix G through K).

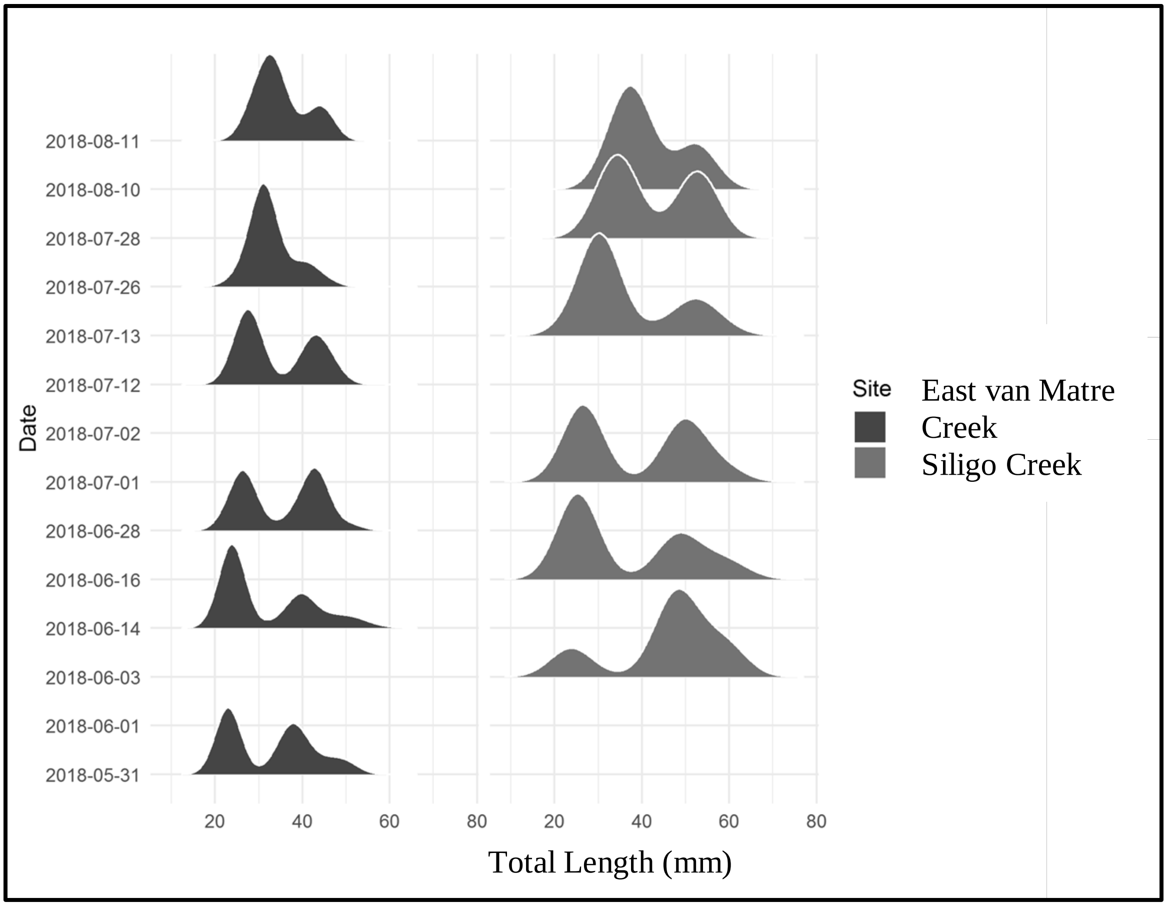


Figure 6. Density plots of total length histograms of Coastal Tailed Frog tadpoles from the high elevations of northern California. Once a tadpole began metamorphosis it was removed.

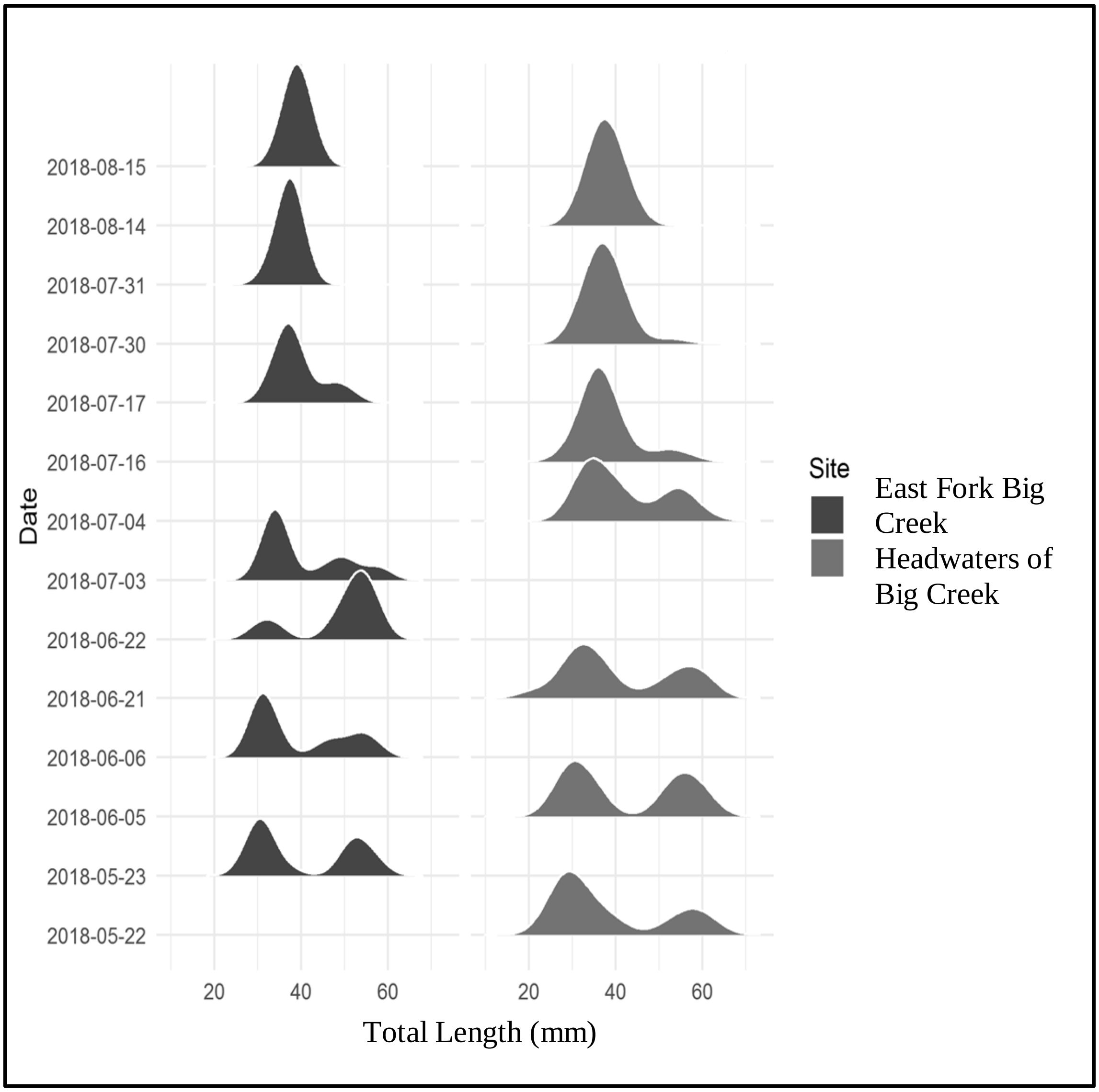


Figure 7. Density plots of total length histograms of Coastal Tailed Frog tadpoles from the mid elevations of northern California. Once a tadpole began metamorphosis it was removed.

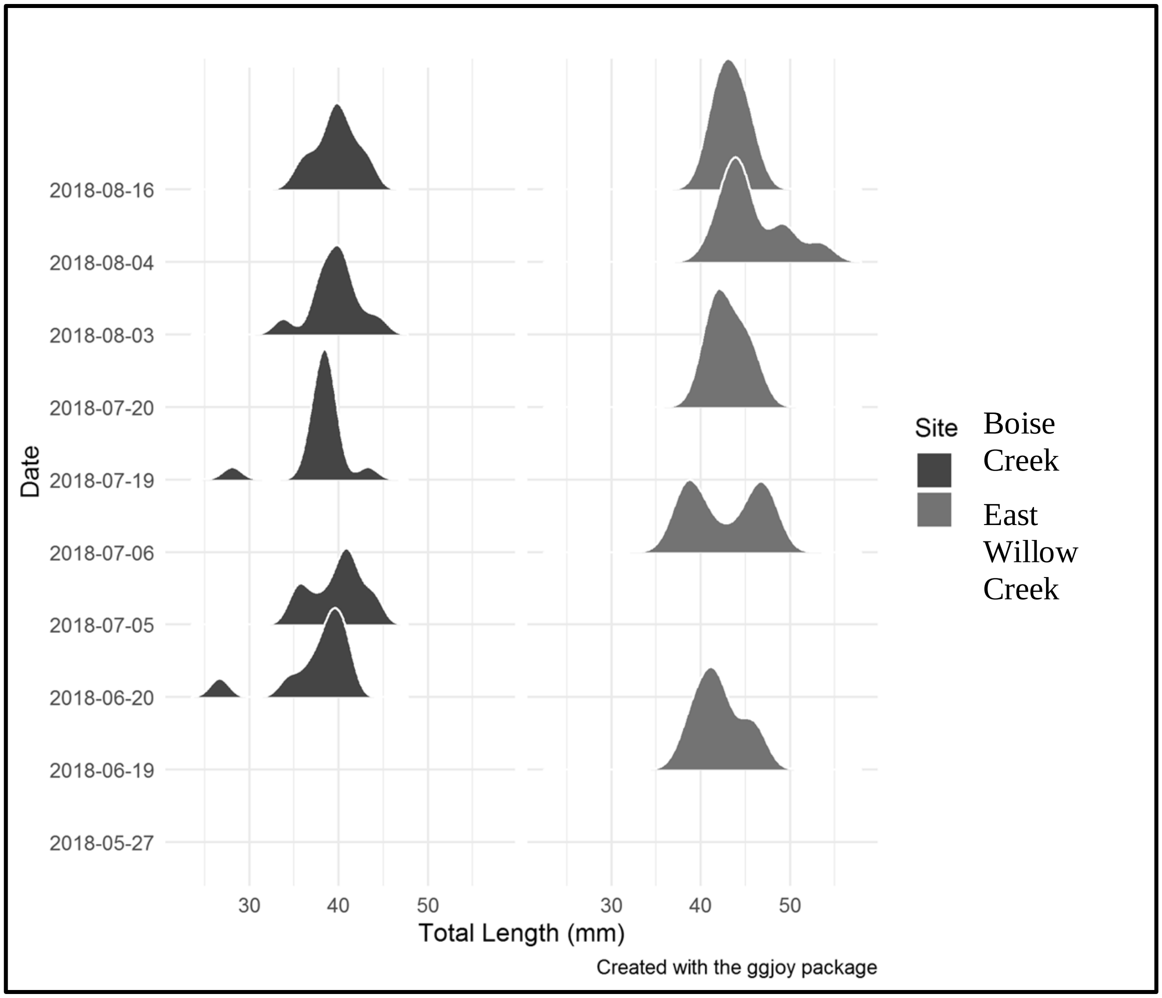


Figure 8. Density plots of total length histograms of Coastal Tailed Frog tadpoles from the low elevations of northern California. Once a tadpole began metamorphosis it was removed.

Bergmann's rule

Tadpoles at developmental stage 37 were larger in low elevation sites compared to mid (***t***=7.04, D. F=1, P=<0.01) and high elevation sites (**t**=6.75, D. F.=1, P=<0.01) (Figure 9). There was no significant difference in body size of tadpoles between mid and high elevation sites.

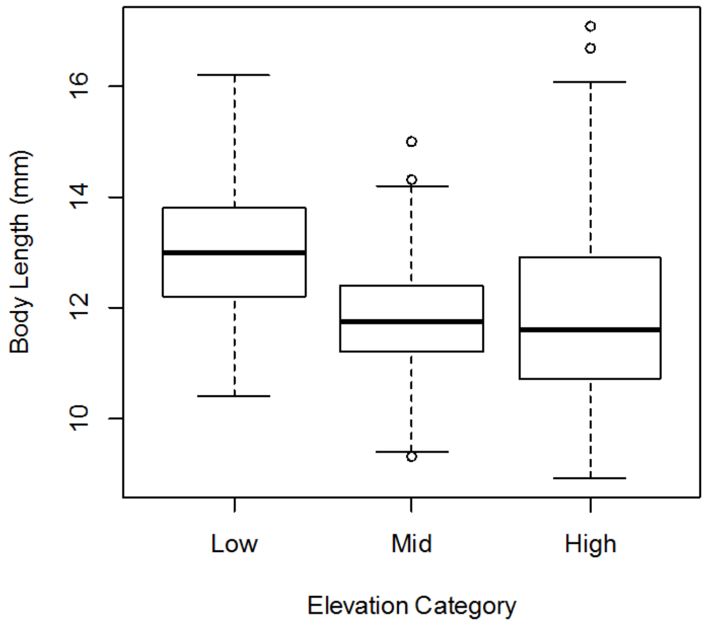


Figure 9. Body size of *A. truei* tadpoles at developmental stage 37 across elevation in inland northern California, USA. The horizontal lines represent the median, and the lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The whiskers extend from the hinge to the largest value (upper) and smallest value (lower) no further than 1.5 times the interquartile range.

Discussion

Growth

It is advantageous for species to have phenotypic plasticity in growth so that they may respond to differing environmental conditions (Berven 1979, Conover et al. 1995). This study describes increased rates of growth present in tadpole populations at higher elevations compared to lower elevations. This contrasts with the general trend seen in other amphibians in which growth and development rates are lower in high elevations and latitudes (Morrison and Hero 2003). This may be a case of countergradient variation, in which high elevation tadpoles grow faster to compensate for the differences in environmental conditions, in particular because of a reduced growing season. As described in the introduction, frogs at high latitudes can have higher growth rates due to higher growth efficiency (Lindgren and Laurila 2005). Increased growth rates in high elevation populations have also been described for Western Spotted Frogs (Licht 1975). Countergradient variation is often missed because genotypic traits such as faster intrinsic growth can be masked by environmental conditions. One would expect that cooler temperatures experienced in high elevations would mask the presence of increased growth rates. This may be a case when growth rates are able to overcompensate for temperature effects, such that we can observe faster growth in cooler high elevation environments.

My results also suggest that growth rates in the high elevations decrease across the months of the growing season, while the opposite was observed in the mid elevations. This may be because of the prolonged period of inactivity during the winter that tadpoles face in high elevations. It is possible that in high elevations tadpoles are putting less energy into body growth, but rather focusing on improving body condition prior to the winter. On the other hand, mid and low elevation tadpoles can potentially grow year-round, this has been described in post-metamorphic *A. truei* in coastal California (Burkholder and Diller 2007). In colder regions, this has been described in populations of post-metamorphic Asiatic Grass Frogs (*Rana chensinensis*) in which frogs put on more liver and fat mass than growth in colder climates (Chen et al. 2011).

Larval period

The amount of time it takes from hatching to transformation from a tadpole to a post-metamorphic frog (larval period) is an important aspect of an anuran's life history. A multi-year larval period among anurans is rare, and within the species that do have one, there is often intraspecific variation with respect to larval period (Bury and Adams 1999, Berven 1979, Morrison and Hero 2003). This intraspecific variation in larval period within anuran species is likely in response to environmental conditions. In the case of the larval period of *A. truei* in northern California, the present study shows there is clear variation in the larval period across an elevational gradient. This study extends the previously known maximum larval period in California from one-two years on the coast (Wallace and Diller 1998) to three years in the Trinity Alps. The population previously known with the longest larval period is from the mountains of northwestern Washington, where a four-year larval period has been reported (Brown 1990). If it is real, this difference between populations in Washington and California could likely be explained by the decreased activity period and increased snowpack generally associated with higher latitudes. Even though the populations studied in California were at a higher elevation (2100m in California compared to 1500m in Washington), the combination of high elevation and high latitude in Washington may even further limit activity and growth.

However, some caveats must be considered when estimating the length of the larval period of overwintering populations of tadpoles based on a single season's sample.

First, and importantly, combining samples from different streams can yield false evidence of distinct cohorts. This occurs in the early summer samples of the East van Matre and Siligo Creek tadpoles if they are combined into one histogram (Figure 10). This is due to the difference in size range within a cohort between the two sites; the third cohort in Siligo Creek is much larger than the third cohort of East van Matre. When the two sites are combined, the larger cohort from Siligo Creek appears to be a separate, fourth cohort (Figure 10).

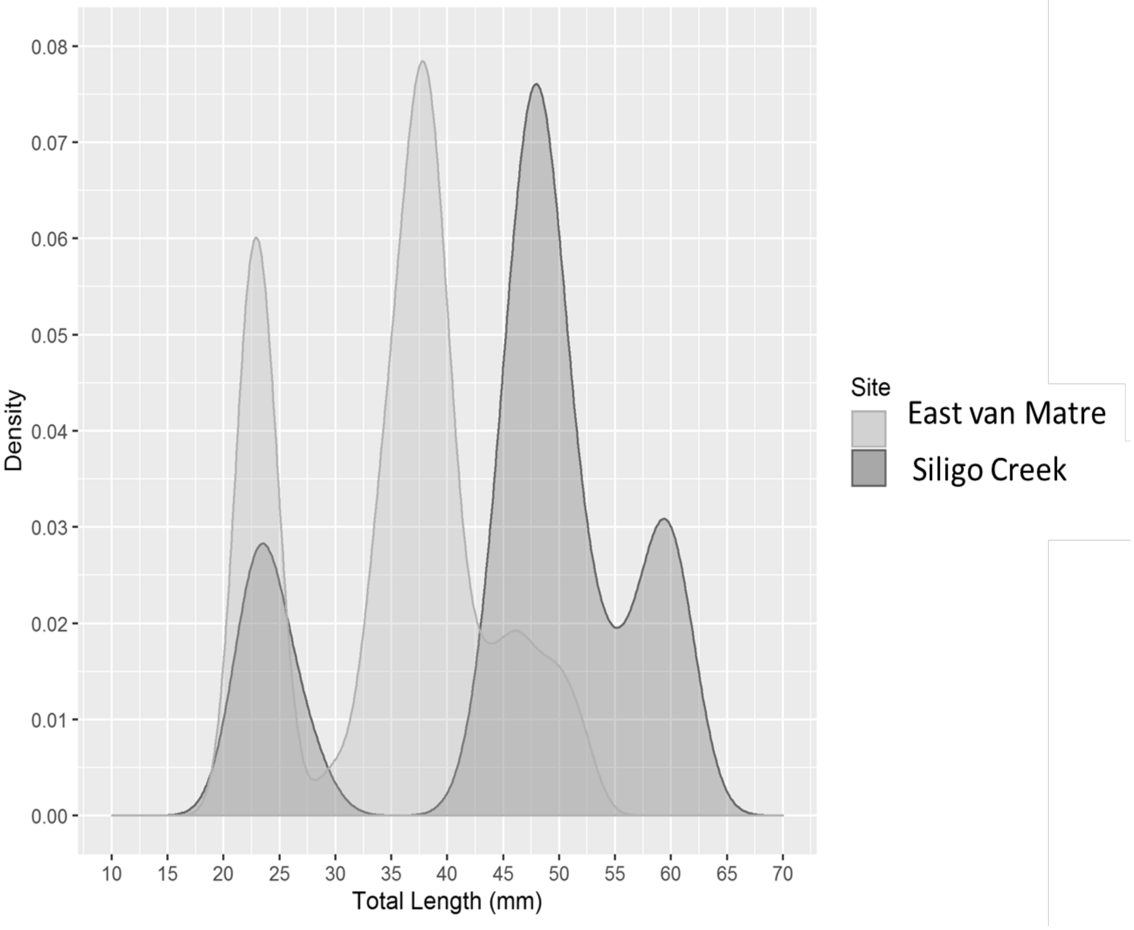


Figure 10. Density histogram of Coastal Tailed Frog tadpole total lengths between Siligo and East van Matre Creek from the Trinity Alps of northern California.

Second, the timing of sampling is important. In late summer in the mountains of Washington, it has been proposed that five larval age classes are present: hatchling tadpoles (cohort-0) within the gravels at the nest site, one-year, two-year, three-year, and metamorphosing tadpoles (cohort-4) (Brown 1990). By October, in the high country of Washington, cohort four has transformed into young of the year frogs, and three cohorts of tadpoles are left to overwinter (Brown 1990).

In the high Trinity Alps of northern California, during the early summer (May through early June) three cohorts of tadpoles can be observed (Figure 6). All three cohorts were observed throughout the rest of June and July, until August, when cohort-3 transformed into young of the year frogs (Figure 6). This leaves only two cohorts of tadpoles to overwinter in the stream, compared to what appear to be three cohorts in mountains of Washington (Brown 1990).

If managers are measuring abundances of tadpoles or attempting to define tadpole cohorts, the best time to do this is between May and August. If samples are taken during October, surveyors will only find two cohorts in the high elevation streams, and one cohort in the mid and low elevation streams.

Third, when looking at total length histograms to determine larval period it is important to treat metamorphosing tadpoles separately. Since tadpoles shrink as their tails are resorbed, false peaks can be made up entirely of metamorphosing individuals. Therefore, developmental stage in conjunction with total length is helpful when determining tadpole cohorts.

Bergmann’s rule

I found bigger larvae at the low elevation sites compared to high and mid elevation sites, after accounting for developmental stage. Even though the pattern appears opposite to Bergmann’s Rule, I considered a possible hypothesis: *A. truei* tadpoles have a general trend of being bigger at low elevations due to the expectation that the shortened growing season at higher elevations affect the ability of tadpoles to reach a larger size than tadpoles in low elevations. Although I found that populations in higher elevations experience higher growth rates and have an added year of growth compared to lower elevation populations, these factors do not appear to compensate for a shorter activity period and allow for larger body size in high elevations. Interestingly, larger larvae in lower elevations have also been described among populations of the Western Spotted Frog (Licht 1975).

Genetic differences between populations

Lastly, some preliminary data suggest that two different mitochondrial DNA based clades may exist across my study sites (personal communication: Bruce Bury, April 2018). The first clade encompasses my mid and low elevation sites, and the second encompasses my high elevation sites. Therefore, the life history and size differences between low and high elevation sites may be due to both genetic and environmental reasons, and the potential countergradient patterns of growth may be due to phenotypic or genetic plasticity among populations in my study area. Further studies are needed to distinguish phenotypic plasticity from genetic differences in these populations.

Management implications

This chapter describes populations of larval *A. truei* that exist in the highest known elevations for the species. Now that these new localities have been described, they can be considered in wilderness management. An extended larval period and delayed age at sexual maturity may make high elevation populations more susceptible to land use and climate changes. Land use in the high elevations in the Trinity Alps is generally limited to recreation, but climate change may have an impact in the long term. The climate changes in the high elevations is predicted to include more precipitation in the form of rain instead of snow (Snyder et al. 2004). This may affect recruitment because larvae stay in these steep, fast-moving streams for at least three years, it puts them more at risk of being crushed by rolling rocks or washed downstream in flood events or vulnerable to drying streams if summer snowpack is reduced. In general, mid and low elevations experience much more land use activity in terms of timber harvest, road building, etc.. There are also much higher stream and air temperatures in mid and low elevations than in high elevations. Increasing temperatures due to climate change can also raise the temperature of streams and reduce stream flow to potentially dangerous levels. Quantifying life history traits provides key demographic parameters for Coastal Tailed Frogs, which can have important implications regarding conservation and management of this species.

CHAPTER TWO: LIFE HISTORY OF POST-METAMORPHIC COASTAL TAILED FROGS (*ASCAPHUS TRUEI*) IN THE TRINITY ALPS WILDERNESS OF NORTHERN CALIFORNIA

Introduction

As noted in Chapter One, life history traits are known to vary in many taxa across environmental gradients (Begon et al. 1990). For example, amphibians tend to follow Bergmann’s rule, with larger adults at high elevations and latitudes (Ashton 2002). Intraspecifically, amphibian populations at high elevations and latitudes tend to have shorter breeding seasons, shorter activity periods, longer larval periods, reach sexual maturity later, and produce fewer and larger clutches per year relative to those at lower elevations (Baraquet et al., Bears et al. 2009, Lindgren and Laurila 2005, Miaud et al. 1999, Moore 1949, and Morrison and Hero 2003). The Green Frog (*Rana clamitans*) has a two-month breeding season in montane populations, compared to a five-month breeding season in lowland populations (Berven et al. 1979). Elevational variation in life history is also seen in the Argentinian anuran, the Córdoba Tree Frog (*Boana cordobae*). Across an elevational gradient ranging from 800-2,400m, males at higher elevations were larger and had greater longevity than those at lower elevations (Baraquet et al. 2018). In the Common Frog (*Rana temporaria*), mean adult body length, age at maturity, and longevity are increased at high elevations (Miaud et al. 1999).

Phenotypic plasticity in growth and development rates may occur in response to differing environmental conditions. Variation in growth and development at the proximate level can lead to differences in life history traits like longevity and maturity across latitudinal and elevational gradients (Lindgren and Laurila 2005, Licht 1975). The theoretical models developed by Reznick (1990) to understand the proximate origins for variation in age and length at maturity of guppies (*Poecilia reticulata*) were discussed in Chapter One, Reznick (1990), predicted that guppies in favorable conditions (more food) would grow faster and initiate the process of maturation earlier or at a larger size. In unfavorable conditions (less food), individuals grow slower and reach maturity later, or at a smaller size. These same models were applied to the Common Frog (*Rana temporaria*) with similar results – in environments with a shorter activity period, post-metamorphic frogs grew slower and reached sexual maturity later. However, adult Common Frogs at high elevations eventually attained a greater final length (Miaud et al. 1999). This was because they also had greater longevity than individuals in populations with a longer activity period. Since both guppies and amphibians are iteroparous and continue to grow after maturity, individuals in populations with greater longevity were able to grow larger after maturity (Miaud et al. 1999).

The Coastal Tailed Frog (*Ascaphus truei*) is an ideal species for the study of geographic and elevational variation in life history, because it ranges across most of the Pacific Northwest from northern California into British Columbia, and from near sea level to interior mountains.

Timing of oviposition and breeding are important components of life history, yet research on *Ascaphus* is difficult since egg masses are rarely found and copulation is rarely observed in the field (Adams 1993, Bury et al. 2001, Karraker et al. 2006, Metter 1967, Palmeri-Miles et al. 2010). Males develop darkened cornified nuptial pads prior to mating and these usually reach full development around mid-August to early September (Metter 1964). Copulation is thought to occur in early fall, about ten months prior to the laying of eggs in late June to late August (Karraker et al. 2006). However, Metter (1964) suggested that individuals in coastal *A. truei* populations might breed every year, whereas those in inland populations might lay eggs only every other year. This would be due to shorter activity periods in inland populations, which might decrease the time available for eggs to develop within the oviducts, and therefore delay oviposition to the following year. But this is just a hypothesis. To date, nothing is known about oviposition timing and breeding phenology across an elevational gradient in California, and any descriptions of variation in breeding season and oviposition timing will be valuable.

Little is known with regards to age at sexual maturity, growth rates, developmental rates, and longevity of Coastal Tailed Frogs across their range. In California, Burkholder and Diller (2007) used growth curves to predicted age at sexual maturity for six coastal populations in Humboldt County, CA and found that females reached sexual maturity at approximately two-and-a-half years of age and males at one-and-a-half years. They also found that growth rates of post-metamorphic frogs were maximized in summer and were consistent between sexes. No other studies have published post-metamorphic growth rates for the species.

Mark-recapture studies in Oregon, Montana, and British Columbia have examined movement and site fidelity (Landreth and Ferguson 1967, Daugherty and Sheldon 1982b, Wahbe et al. 2004). However only one study has described site fidelity of tailed frogs in coastal California (Burkholder and Diller 2007), and site fidelity has not been compared to inland or high elevation populations in California.

Longevity of Coastal Tailed Frogs is not known, but in its sister species, the Rocky Mountain Tailed Frog *(Ascaphus montanus)*, the oldest animal was estimated to be at least 14 years old, based on mark-recapture data (Daugherty and Sheldon 1982). Thus, there is ample opportunity to contribute to our knowledge of the life history of *A. truei* by documenting variation in post-metamorphic traits across an elevational gradient in California

Research objectives and predictions

My objective was to describe the following life history traits of post-metamorphic *A. truei* in the Trinity Alps Wilderness of northern California:

1. Growth rates of post-metamorphic frogs
2. Size range of adult and immature age classes
3. Age and size at sexual maturity
4. Timing of breeding
5. Site fidelity
6. Longevity

Importance and need

Focusing on data from larval populations limits our understanding of the ecology and life history of this species, especially regarding its sensitivity to land use and climate change. Vital demographic parameters of tailed frog populations in the high elevations of California are unknown. Examining populations of post-metamorphic frogs in the high elevations of California will improve our understanding on how populations may respond to environmental changes.

Methods

Study area

This study was conducted along an elevational gradient from the upper reaches of the Trinity River in Trinity County, CA down to the lower reaches of the Trinity River, Humboldt County, CA, with elevations ranging from ~150 m to ~2100 m. Details of the study sites used have been given in Chapter One, above.

Post-metamorphic frog capture

I primarily used night surveys to locate and capture post-metamorphic frogs. I started surveys at the bottom of the reach and walked in an upstream direction; I located frogs with a headlamp and captured them by hand. I placed captured frogs in plastic bags with cool stream water; the bags were numbered to correspond with a numbered flag placed at each site of capture. In each stream, I measured a linear transect as close to within the stream as possible. Each time a frog was captured I measured the distance from the start of the reach to the capture location to measure linear within-stream movement of individuals. After I completed the survey, all bags were collected, and I measured each frog and released it at its site of capture. I measured post-metamorphic frogs within the bag, then sanitized my hands with hand sanitizer, took the frog out of the bag and marked and toe clipped quickly without the use of anesthesia. I took toe samples from mid and low elevation frogs to estimate age and longevity.

Reproductive condition and development

I classified males as being in breeding condition while they had fully cornified and dark black nuptial tubercles on the palm, forearm, and chin.

I classified females as gravid when a mass of bright yellow eggs was visible through the transparent abdominal wall and their fingertips were black. Females develop black fingertips prior to oviposition to aid in digging of nest sites (Daugherty and Sheldon 1982a). I classified females as spent when no eggs were visible, but black fingertips were present and abdominal skin was loose. I assumed females were not laying that year when small eggs were present, and they had white fingertips. I assumed females with no eggs and white fingertips were immature.

Measurements

Snout-vent length: From the tip of the snout to the posterior end of the cloaca.

Mass: same protocol as for larvae (Chapter One).

I made all the measurements with dial calipers to the nearest 0.1mm and I measured mass with a 25g Pesola scale.

Marking

I marked frogs using visual implant elastomer (VIE) (Northwest Marine Technology Inc. Seattle, Washington). I marked each animal for individual recognition using a combination of four possible colors (yellow, orange, red, and blue). I made the marks just under the skin and they were about 3-5mm long and sometimes smaller for very small individuals. I viewed marks with a UV light in a darkened area to enhance visibility. Post-metamorphic frogs were marked in the hands and feet. This yielded four different body locations using four different colors (same as for tadpoles, as detailed in Chapter One). Marking methods followed Chelgren and Adams (2017) and 625 individual marking codes were generated using SalaMarker (MacNeil et al. 2011).

Field collection and skeletochronology

I collected a total of 96 bone tissue samples (all the phalanges of the longest toe on one hind foot) across the study area. Fifty samples had already been collected by California Department of Fish and Wildlife biologists in the high elevation sites prior to the study, and prepared by Matson's Laboratories, Inc. (MT, USA). I collected samples from mid and low elevations myself and prepared them in the laboratory at Humboldt State University (HSU). I decalcified phalangeal bone samples in RDO Rapid decalcifying solution (Apex Engineering Products Corporation, IL) for 2-3 hours, then embedded in ParaPlast. I made ten micrometer thick sections using a rotary microtome and stained them in either Ehrlich's hematoxylin for 16 minutes or Toluidine Blue (2g/100ml) for 2 minutes. I estimated longevity and age at sexual maturity using skeletochronology. Skeletochronological analysis involves counting lines of arrested growth (LAG)s in cross sections of bone tissue samples. Each LAG corresponds to each winter period of hibernation (Castanet and Smirina 1990). Counts of LAGs were made by at minimum two independent observers. When there were disagreements between observers, both viewed the sections again and agreed upon final estimates of post-metamorphic age. I added the skeletochronology estimate to the larval period estimate (see statistical methods) to get the complete age estimate. In some cases I could estimate age at sexual maturity as the point at which LAGs became closer together, due to a slowing of growth as more resources are dedicated to reproduction, this has been described in other skeletochronology work on amphibians (Guarino et al. 2003; Sinsch 2015). For my detailed skeletochronology protocol, see Appendix C.

Statistical methods

I performed all analyses in the statistical program R (Version 3.2.2, R Core Development Team 2017).

Growth rate

I estimated growth by subtracting the body size of the last capture of each individual animal by the body size of the first capture of each individual animal, this was compared to the number of days between captures. I compared the relationship between growth and number of days between captures using a linear mixed effects regression with each individual as random effects and sex and maturity as interactive additive effects.

Adult and immature age classes of frogs

I visually compared age classes of frogs by making snout-vent length frequency histograms in conjunction with reproductive male and female frogs (those exhibiting secondary sexual characteristics).

Size and age of mature frogs

First, I compared body size of mature frogs between males and females using a non-parametric Welsh's T-test. Then, I modeled the differences between females and males in size at reproductive maturity using a binomial general linear model with sex as a covariate. To compare body size for mature frogs across elevation categories, I used a non-parametric Kruskal-Wallis tests and Conover-Iman tests (Dinno 2017).

Site fidelity

I calculated movements by subtracting the location (linear distance from start of survey reach) at the last capture from the location (linear distance from the start of the survey reach) at the first capture. I visually assessed distribution of linear distance moved of *A. truei* in histograms, compared the absolute value of linear distance moved between frogs of different sex, maturity, and upstream vs downstream using non-parametric Welsh's T-tests, and compared across months of the survey season using a non-parametric Kruskal-Wallis and Conover-Iman tests (Dinno 2017).

Results

In total, I took 517 measurements of post-metamorphic frogs across a total of 16 sites (Chapter One: Figure 3) over 70 days from May-August 2018. One hundred and fifteen were individually marked: 66 were caught twice, 21 were caught three times, 14 were caught four times, five were caught five times, and one was caught six times. However, the majority of post-metamorphic frog captures were at high elevations. Fourteen were caught in low elevations, 32 in the mid elevations, and 110 in the high elevations. Sixty-three were recaptured in high elevations; only three individuals were ever recaptured in the mid elevations, and none were ever recaptured at low elevations. At the high elevation sites, 50 frogs were sampled for skeletochronology and 38 provided reliable age estimates; at mid elevation sites, 32 frogs were sampled and four provided reliable age estimates; and at low elevations 14 frogs were sampled and two produced reliable age estimates.

<u>Growth rates of post-metamorphic frogs</u>

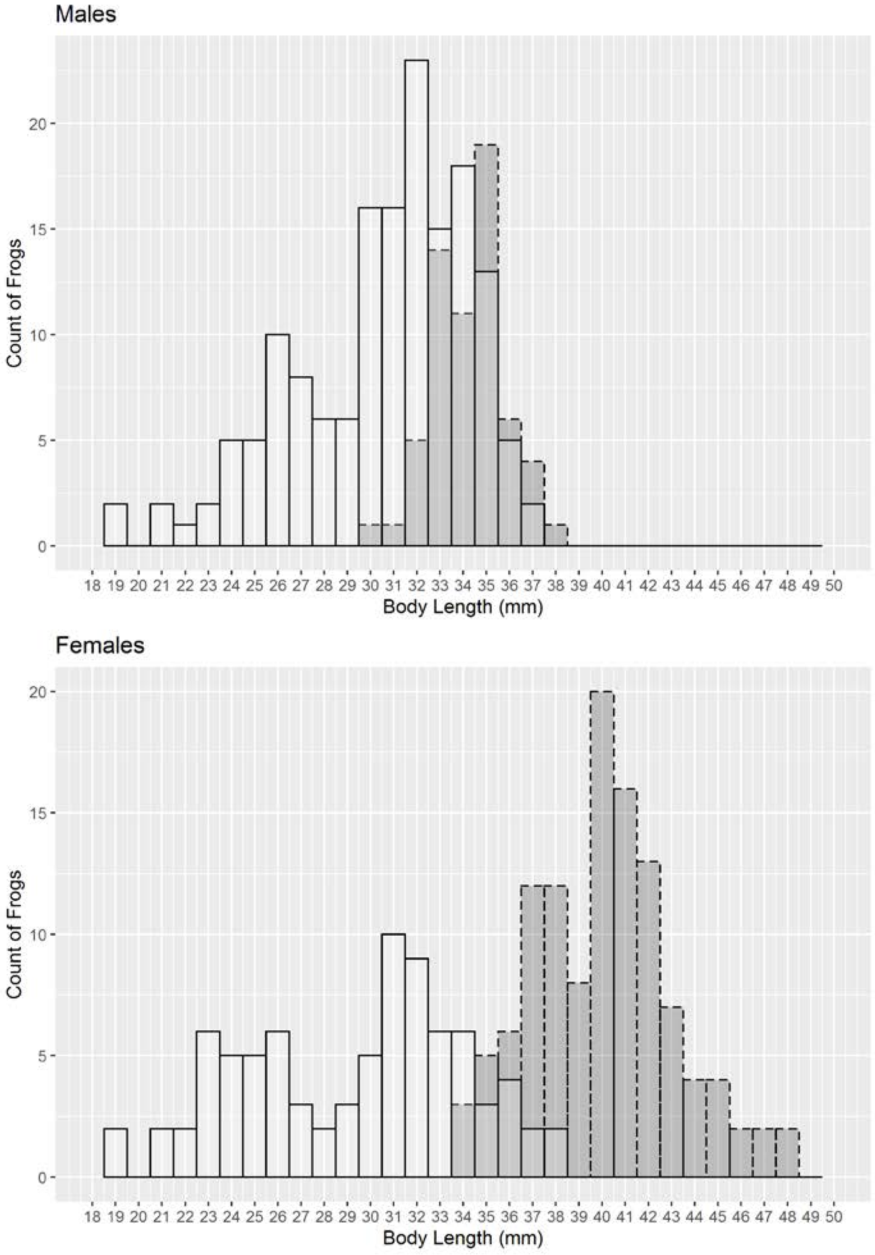
There was no significant difference in growth rates between males and females (χ^2 =0.01, D. F.=1.00, P= 0.94) nor between mature and immature frogs (χ^2 =0.04, D. F.=1, P=0.84). There was no significant difference between growth rates between the months of the season (χ^2 =2.39, D. F. =2, P=0.30). Overall, post-metamorphic frog growth rates were estimated to be 0.037mm per day (Std. Error= 0.12) in the Trinity Alps of northern California.

Adult and immature age classes

The SVL’s of the smallest reproductive frogs (based on secondary sexual characteristics) was about 30mm for males and 34mm for females. The size class distribution plots further supported these cut off values (Figure 11). I could not distinguish between subadult and juvenile frogs within the pre-reproductive category for males, however, there were distinct peaks for females, possibly corresponding to distinct cohorts. Juvenile females had a maximum size of about 28mm and subadult females had a maximum size of about 33mm (Figure 12).

Size at sexual maturity

Males and females reach sexual maturity at the same size – 50% are mature at about 33mm (Figure 12 and Table 4). Overall, they start out with immature males being significantly larger than immature females (*t*=2.16, D. F.=91.94, P=0.03) and females achieve significantly greater size as mature frogs (Figure 13 and Table 5) **(**_t_=9.67, D. F.=135.3 P<0.00).


Males

Females

Figure 11. Snout-to-vent length frequency histograms for mature (in dark gray and dashed borders) and immature (in white and solid borders) male and female Coastal Tailed Frogs from the Trinity Alps Wilderness of northern California, USA. Bins are 1mm wide.

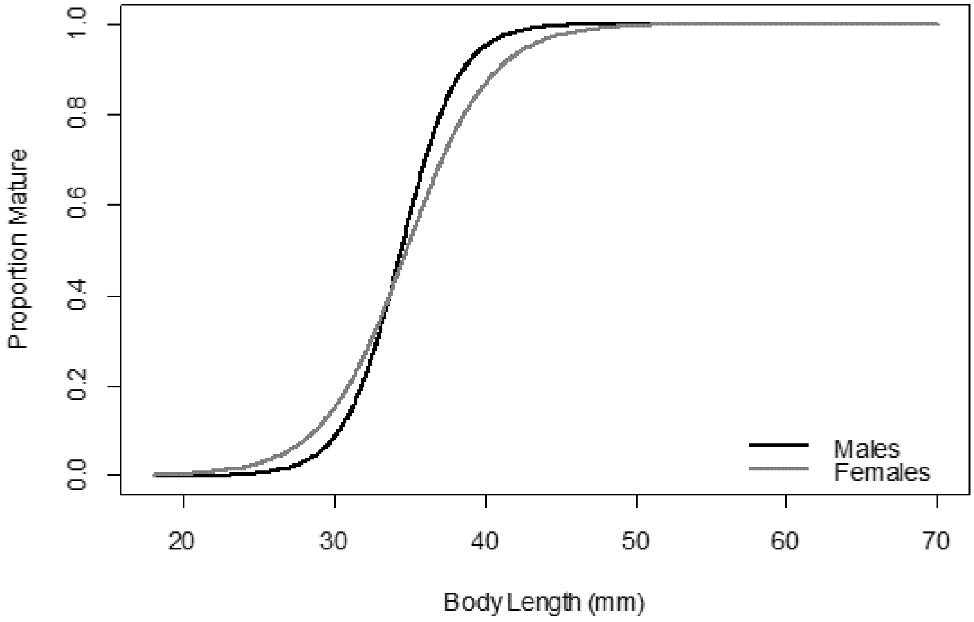


Figure 12. Binomial logistic regression of body size at sexual maturity of *A. truei* in northern California, USA. Plot of least squares regression on body length.

Table 3. GLM model of the influence of body length, sex, and their interaction on sexual maturity of *Ascaphus truei* in northern California, USA.

Parameters	P-value
Body Length	$2.0\text{x}10^{-16}$
Sex	0.09
Body Length:Sex	0.08

Table 4. Mean snout-vent length (SVL) of post-metamorphic frogs in the Trinity Alps of northern California,USA.

Immature	Mean SVL	Standard Error
Males	30.70	0.30
Females	29.45	0.52

Mature	Mean SVL	Standard Error
Males	34.32	0.42
Females	39.89	0.36

Body size of mature animals was significantly smaller in high elevations compared to mid elevations (t=2.74, D. F. =1, P=0.0034) (Figure 14). Body size of mature animals did not significantly differ between low and mid elevations (t=0.63, D. F.=1, P=0.26) and between low and high elevations (*t*=0.95, D. F.=1, P=0.17).

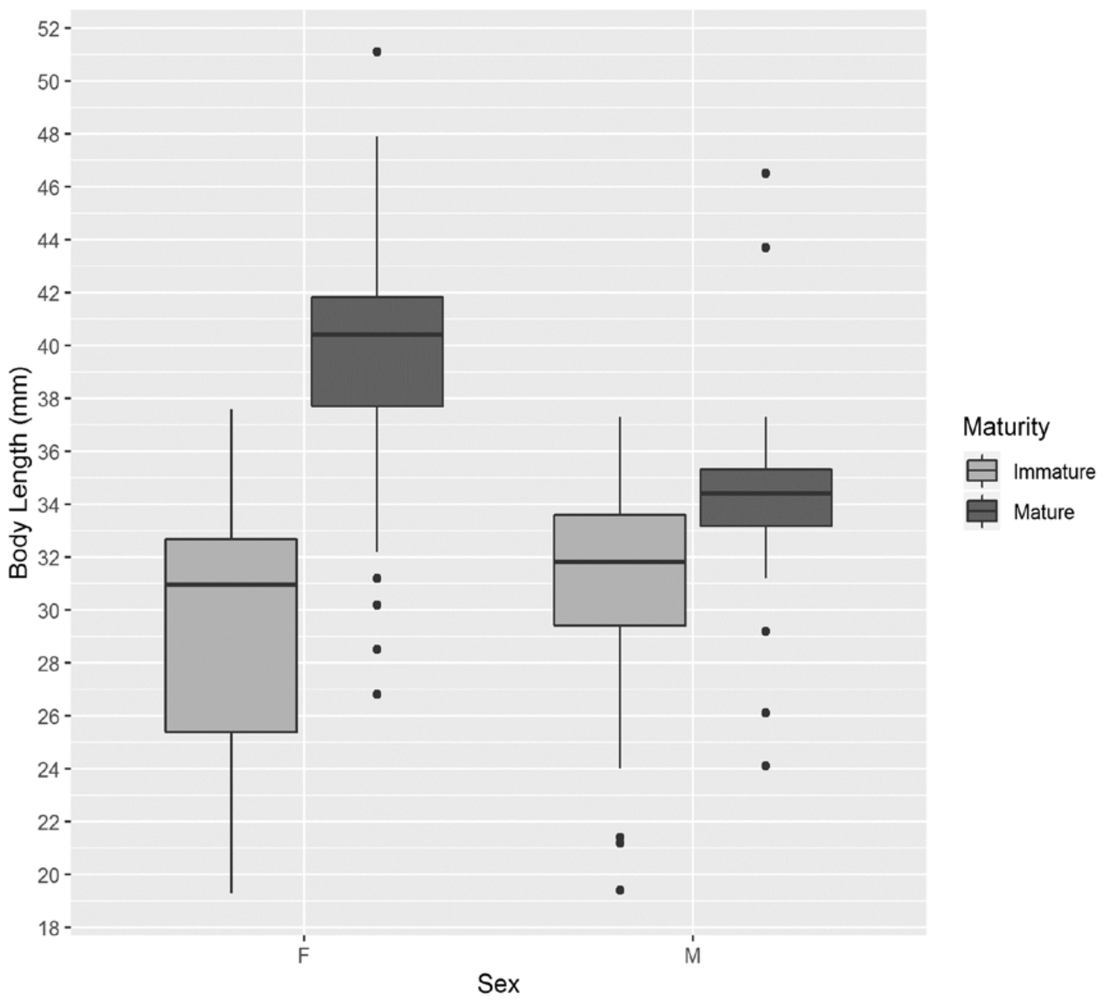


Figure 13. Body length of mature (right) vs immature (left) females (F) and males (M) Coastal Tailed Frogs in northern California, USA. The horizonal lines represent the medians and the lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The whiskers extend from the hinge to the largest value (upper) and smallest value (lower) no further than 1.5 times the interquartile range.

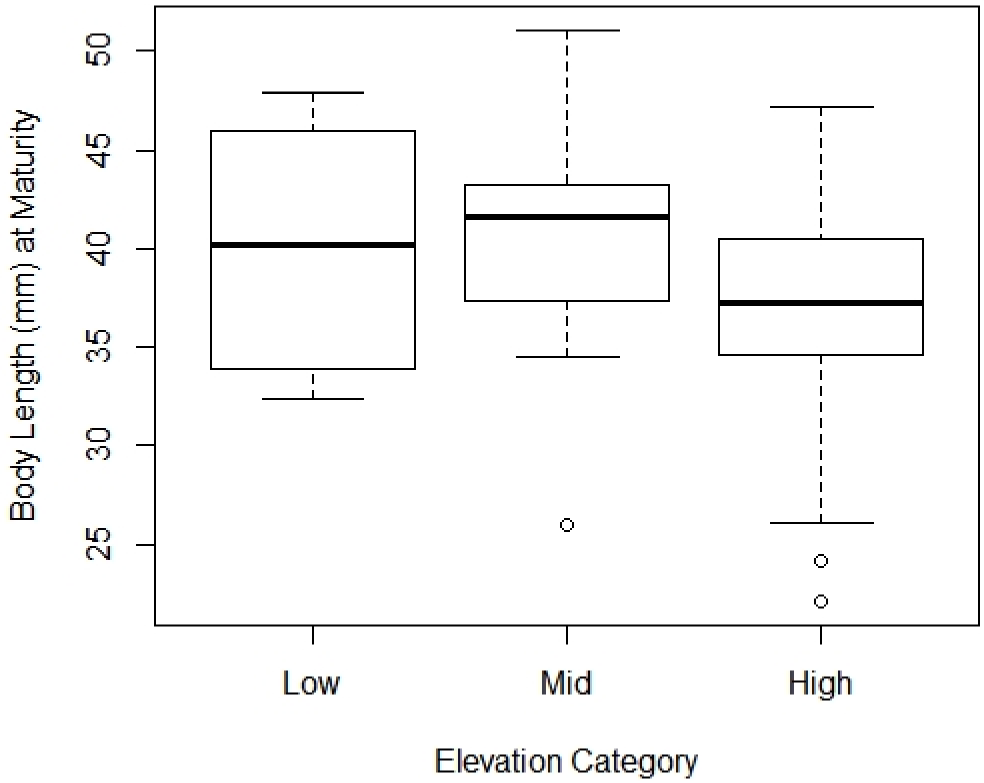


Figure 14. Body length of mature *Ascaphus truei* across elevation in northern California, USA. The horizonal lines represent the medians and the lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The whiskers extend from the hinge to the largest value (upper) and smallest value (lower) no further than 1.5 times the interquartile range.

Timing of breeding and oviposition

Males in the high elevation sites were first seen forming tubercles on their forearms on June 29th, and tubercles were fully formed in all mature males by July 27th. No egg masses were ever found in the stream beds during my study. However, my colleague and I found three *A. truei* eggs in the stomach of an Oregon Aquatic Gartersnake (*Thamnophis atratus hydrophilus*) during late July-early August 2016. I also have data on recaptures of a single female that lacked visible eggs, after having previously been observed as gravid. Based on this observation, oviposition must have happened sometime between July 28th and August 8th of 2018 in the high elevation. Interestingly, no females with large eggs or egg masses were found during the summer of 2017.

Longevity

Mean age of post-metamorphic animals was significantly higher in high elevation populations compared to mid-elevation populations (t = 3.74, p = 0.0003) and low elevations **(**t=2.711, p=0.0049**)** (Figure 15, 16, and 17). The oldest individual captured in mid elevation populations was three years post-metamorphosis, whereas the oldest individual in high elevations was eight years post-metamorphosis. However, the sample size for high elevations was much higher than for mid and low elevations.

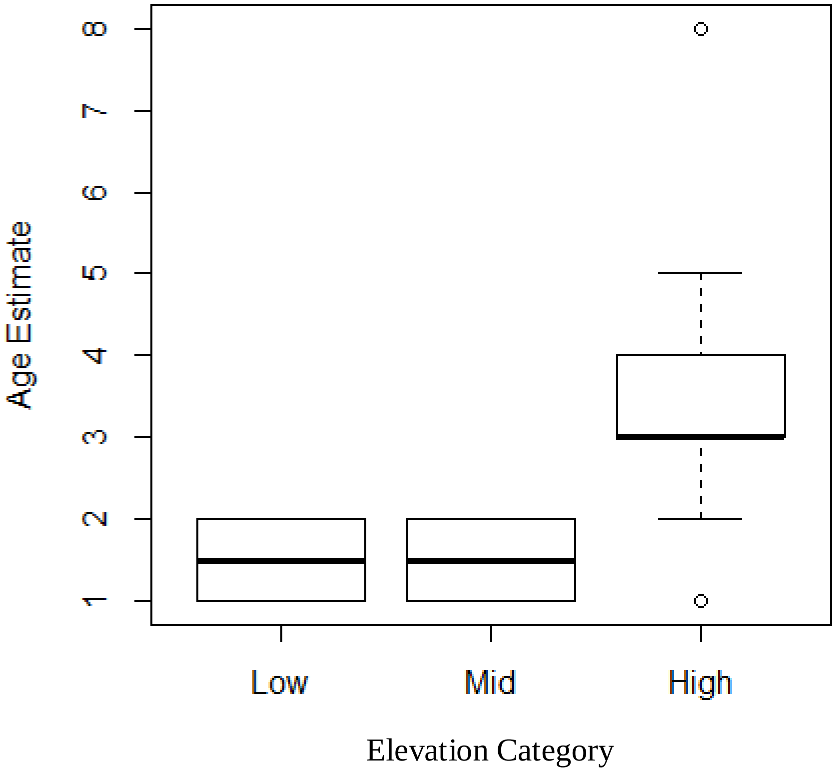


Figure 15. Median age (post-metamorphosis) of *A. truei* across elevation categories in northern California, USA. Sample size: high elevation: 38, mid elevation: four, low elevation: two. The horizontal lines represent the medians and the lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The whiskers extend from the hinge to the largest value (upper) and smallest value (lower) no further than 1.5 times the interquartile range.

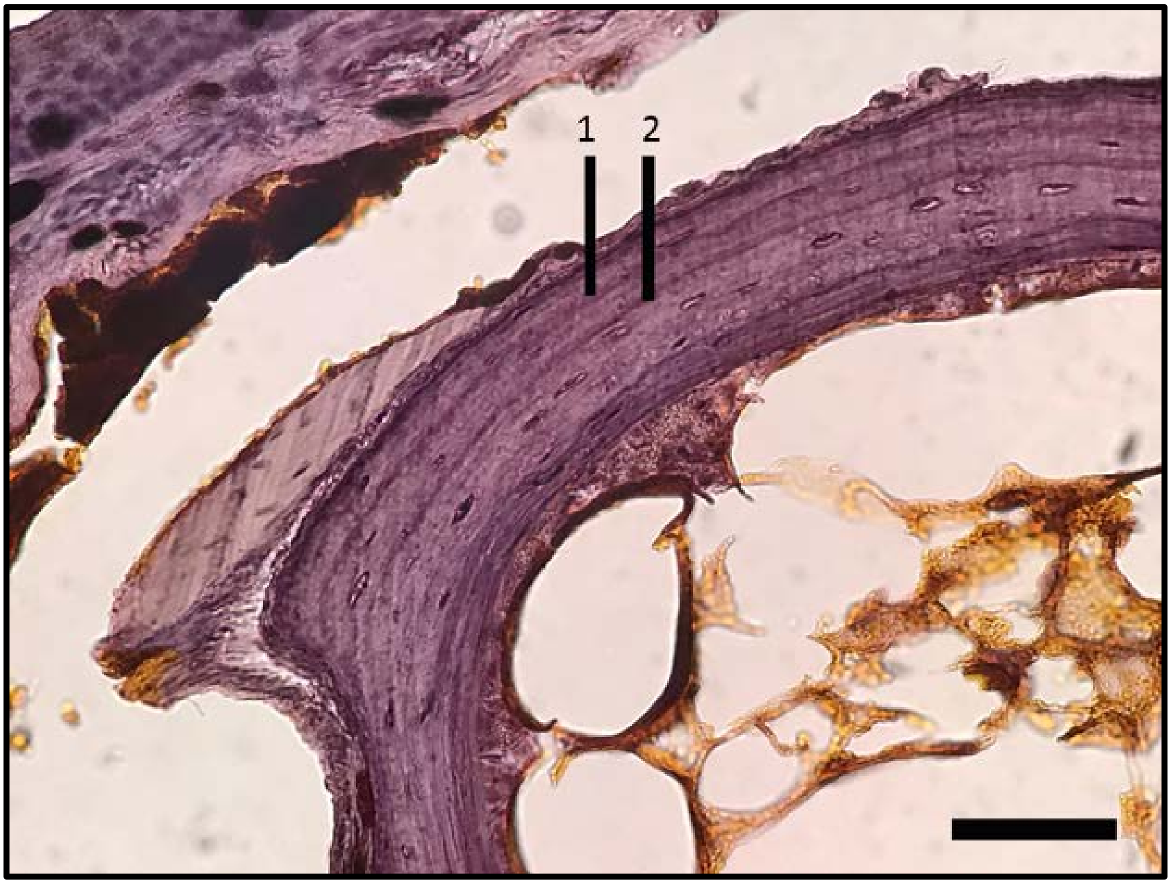


Figure 16. Phalanx section showing two LAGs from a post-metamorphic *A. truei* from a low elevation population site in northern California, USA. Scale bar = 200um.

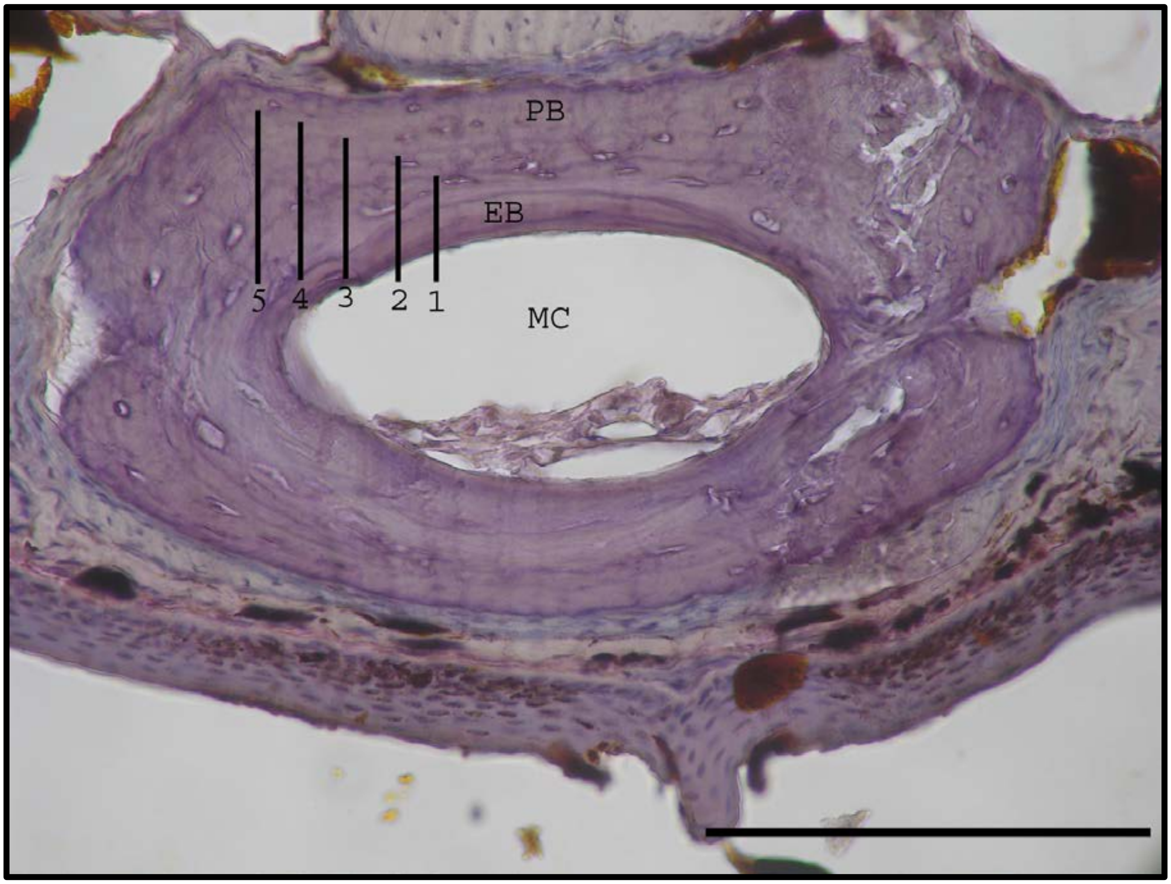


Figure 17. Phalanx section showing five LAGs from a post-metamorphic *A. truei* from a high elevation population site in northern California, USA. Scale bar = 100um.

Site fidelity and movement

Overall, Coastal Tailed Frogs from the Trinity Alps exhibit high site fidelity to a specific area in the stream channel (n=73, mean=12.4m, Std. Error=1.29). Both upstream and downstream movements were minimal (Table 6). Histograms of linear movements showed a range of 0-67.7m with most ranging from 0-20m (Figure 19). There were no significant differences between linear movement distances between males or females, nor between immature and mature frogs (Figure 18). There was, however, a significant drop of movement distances during the month of July between the months of the survey season ($\boldsymbol{W}^2$=41.9, D. F.=2, P=<0.01) (Figure 19). There was also significant upstream movement during the month of June compared to July (t=7.92, D. F.=1, P<0.01) and significant downstream movement during the month of August compared to July (t=4.60, D. F.=1, P<0.01) (Figure 20). There were no significant differences between movement of males and females nor mature and immature frogs between the survey months.

Table 5. Longitudinal (upstream/downstream) movement distances of the Coastal Tailed Frog populations of the Trinity Alps Wilderness of California, USA.

Adult Females	Mean Movement (m)	Standard Error	*n*
Upstream	13.9	3.32	19
Downstream	12.4	2.75	20

Immature Females	Mean Movement (m)	Standard Error	*n*
Upstream	6.23	1.58	10
Downstream	11.1	3.76	10

Adult Males	Mean Movement (m)	Standard Error	*n*
Upstream	11.1	2.9	17
Downstream	12.2	4.06	12

Immature Males	Mean Movement (m)	Standard Error	*n*
Upstream	14.9	4.58	16
Downstream	16.9	5.61	11

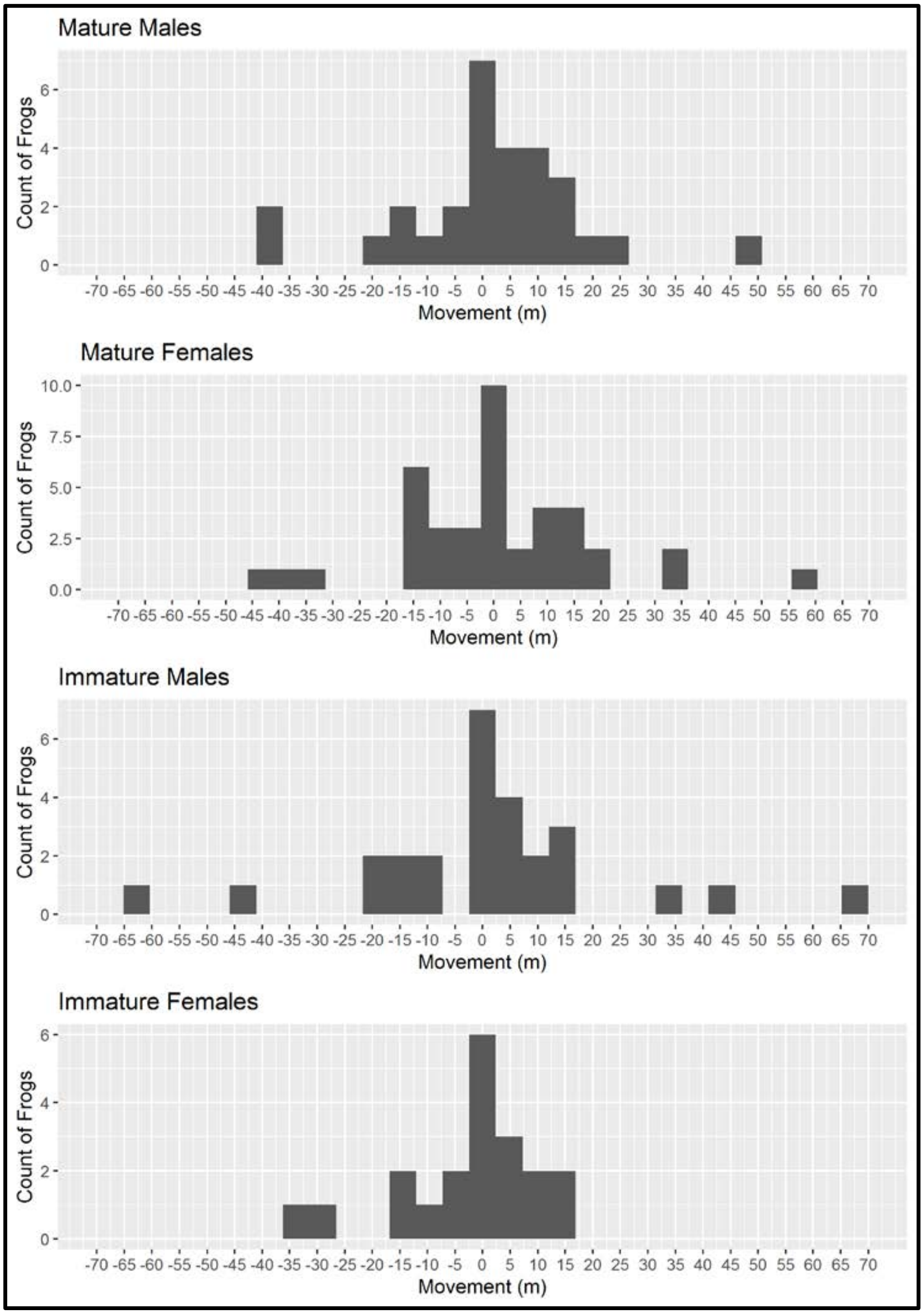


Figure 18. Distribution of longitudinal (upstream>0.0, downstream<0.0) movement distances relative to each capture of Coastal Tailed Frogs in the Trinity Alps Wilderness of northern California, USA.

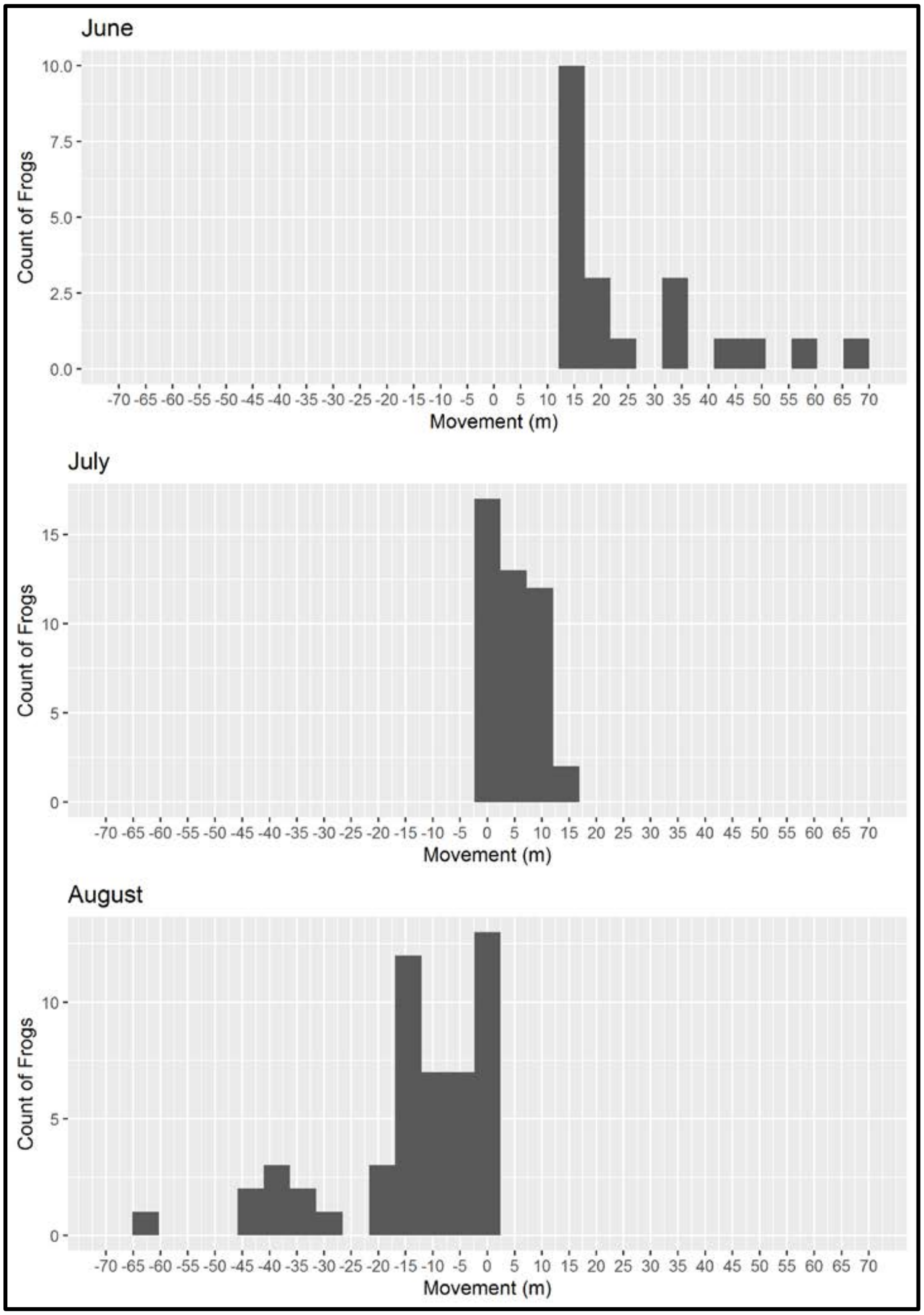


Figure 19. Linear movement distance (m) of Coastal Tailed Frogs across the months of the survey season in the Trinity Alps Wilderness of northern California, USA.

Discussion

Growth and development

It is advantageous for species to have phenotypic plasticity in growth and development rates so that they may respond to differing environmental conditions (Berven 1979, Conover et al. 1995). Variation in growth and development can lead to the differences between life history traits such as longevity and maturity across latitudinal and elevational gradients (Lindgren and Laurila 2005, Licht 1975). This study describes mean growth rates of 0.037mm per day (1.10mm per month) for both males and females in inland populations of *A. truei* in California. The only other published growth rates estimates for post-metamorphic frogs in California are from populations on the coast, which had summer growth rates of just slightly higher for males (1.42mm/month) and for females (1.5mm/month) greater than my estimates from the Trinity Alps (Burkholder and Diller 2007). This suggests during the summer growth rates are similar yet slightly slower among inland high elevation and low coastal populations of *A. truei* in California. However, one important consideration is that during the winter and much of spring, the high elevations are covered by snowpack, during which time the growth of high elevation frogs is likely arrested. However, frogs in coastal populations can continue to grow at approximately 0.89 mm/month through the winter (Burkholder and Diller 2007). This suggests that overall annual growth is greater in coastal populations compared to inland high elevation populations.

The relative consistency of observed growth rate does contrast with the general trend seen in other amphibians in which growth were slower in high elevations and latitudes (Morrison and Hero 2003).

Body size of sexually mature animals

The effects of shortened activity periods and lower temperatures on growth at high elevations can influence body size and age at maturity as well as sex individuals. This chapter describes growth rates to be similar to those in coastal populations, therefore I expected body sizes across elevations to also be similar (Burkholder and Diller 2007). In this species and most anurans, females tend to be larger than males and I expected the same for be true for populations in the Trinity Alps (Burkholder and Diller 2007). In general, studies have found that sexually mature frogs tend to be larger in high elevations (Morrison and Hero 2003), therefore I expected mature frogs in the Trinity Alps to also be larger than lowland populations.

My results show that size of sexually mature *A. truei* varies across elevations; mature frogs at high elevations were significantly smaller than those at mid-elevation. However, with only six mature frogs measured at low elevations, I could not determine whether these were generally larger than mid-elevation frogs. Comparing to coastal California, adult males had an average SVL of 36.7mm (Burkholder and Diller 2007), whereas adult males in the Trinity Alps averaged 34.3mm in SVL. On the coast of California adult females averaged 44.4mm SVL whereas adult females in the Trinity Alps averaged 40.0mm SVL. The same pattern occurs with immature frogs from the coast having a larger average SVL than immature frogs of both sexes from the Trinity Alps.

My findings contrast with Bergmann's rule, and with data from the European Common Frog (*Rana temporaria*; Miaud et al. 1999) and other amphibians (Morrison and Hero 2003). Coastal populations have been documented to grow year-round (Burkholder and Diller 2007). It appears frogs in coastal areas can reach larger sizes than inland Trinity Alps populations.

My results show that sexually mature males were on average 3.7 mm smaller than mature females, similar to the populations examined by Burkholder and Diller (2007) on the northern California coast. However, interestingly, in the Trinity Alps, immature males were on average 0.45 mm larger than immature females. This is opposite to what was described in populations on the coast of California and opposite to what I found for mature frogs in the Trinity Alps. When I modeled size at sexual maturity between the sexes a similar pattern emerged, with immature males being larger than immature females, but after about 50% of frogs are mature at a size of 33mm, females begin to be larger than males and this difference becomes greater as the proportion of mature frogs increases. This suggests that sexual maturity is strongly associated with size (even though adults are smaller at higher elevations), which may be useful to managers and researchers when secondary sexual characteristics may not be visible and they need to determine maturity.

Timing of copulation and oviposition

Timing of breeding and oviposition is important in studying the life history of a species, and little is known with respect to variation across the environmental gradients. My results suggest that copulation occurs in late July in the Trinity Alps, which is earlier than it was thought to occur by Metter (1967) and Karraker et al. (2006), who described copulation occurring in August and September in populations in Oregon and Washington. However, Sever and others (2001), reported mating during May in the North Fork of Mad River, Humboldt Co., California, and Noble and Putnam (1931) observed copulation from June 12 to July 6 in the Olympic Mountains, Washington. Therefore, there appears to be a high degree of variation in this regard. In this study, small sample sizes in mid and low elevation did not allow me to address the question of whether breeding season differed across an elevational gradient in California.

By contrast, my findings on oviposition timing in the high elevations of northern California were similar to what is described in the literature for other populations (July 24th) (Karraker et al. 2006). Determining if populations or individuals breed every other year in the high elevation was difficult, however the lack of females with large eggs captured in 2017, and the instances of egg predation in 2016, and oviposition in 2018 suggests this could be a possibility.

Site fidelity and movement

Post-metamorphic tailed frogs have been reported to exhibit site fidelity, with juveniles being the main dispersers (Daugherty and Sheldon 1982b, Wahbe et. al. 2004). In my study area tailed frogs also exhibited high site fidelity. However, there were no significant differences between the sexes nor between immature and adult frogs. Movement distances in populations in coastal California mostly ranged from 0-30m (Burkholder and Diller 2007) whereas in the Trinity Alps mostly ranged from 0-20m. The biggest difference was an average of 28.0m movement downstream of mature females on the coast, compared to an average of only 12.4m of movement in the Trinity Alps. It was interesting to find significant upstream movement in June and significant downstream movement in August, and a significant lack of movement in July. This increase of upstream movement in June could be individuals trying to feed or establish home ranges after emerging from 6-7 months of cold winter conditions. Others have also described strong upstream movements and hypothesized this is because headwaters provide more food (Hayes et al. 2004). I documented breeding to be potentially occurring in the sites in late July and August as well as oviposition occurring this time. Perhaps the increase in movement distances downstream in August is associated with breeding and/or oviposition. Further studies over broader timescales need to be conducted.

Longevity

Longevity is an important life history and demographic trait that is notoriously difficult to estimate. Skeletochronology allows for the estimating of post-metamorphic age from lines of arrested growth in bone tissues. This study describes the potential for the use of the this technique on the species and the potential for greater longevity in populations of high elevations. This may be a response to an overall slower life history pattern in high elevation Trinity Alps populations. The data however are limited due to low sample sizes. Further research needs to be done in perfecting and validating skeletochronology techniques for this species.

Management implications

Focusing on data from larval populations limits our understanding of the ecology and life history of this species, especially in regard to its sensitivity to land use and climate change. Vital demographic parameters of tailed frog populations in the high elevations of California described in this study, particularly information on site fidelity, movement, age at sexual maturity, breeding and oviposition timing, and longevity, are all important in managing populations. Examining populations of post-metamorphic frogs in the high elevations of California will improve our understanding on how populations may respond to environmental changes.

. This study describes populations of *A. truei* that exist in the highest known elevations for the species, and that now that these localities have been described, they can be considered in wilderness management. An extended larval period (described in Chapter one) and delayed age at sexual maturity may make high elevation populations more susceptible to land use and climate changes. Land use in the high elevations is limited to recreation, but climate change is predicted to include precipitation in the form of rain instead of snow. This study reports more robust adult populations in high elevations. The low captures of adults in mid and low elevations may signal significant declines in these areas, but also may a reflection of reduced capture probability, since larval numbers in the same streams are comparable (see Chapter One). In general, mid and low elevations experience much more land use activity in terms of timber harvest, road building, etc.. There are also much higher stream and air temperatures in mid and low elevations than in high elevations. More research is needed to assess potential population declines and reasons for declines in mid and low elevations and possible refugia in high elevations in northern California.

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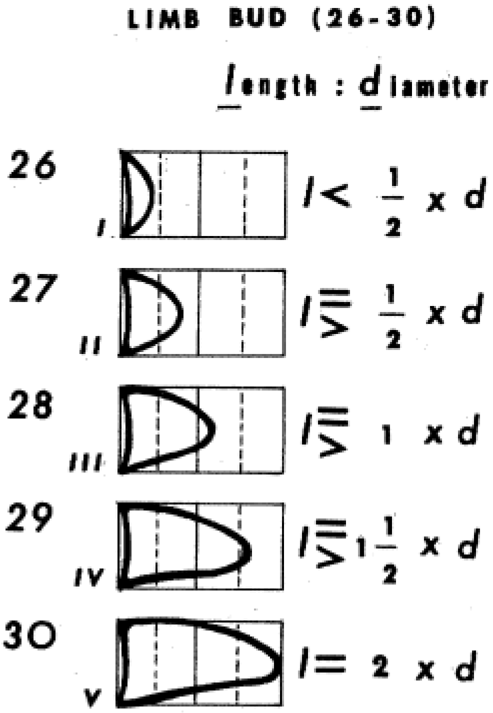
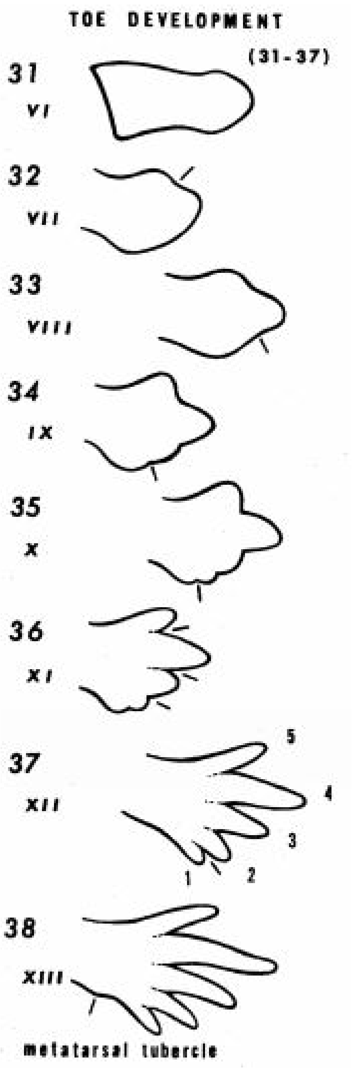
APPENDICES

Appendix A. Stages of development for Coastal Tailed Frog (*Ascaphus truei*) tadpoles, adapted from Gosner (1960), Brown (1990), Brown (1989), and John Reiss (unpublished). This table was design for use in the field on hatched tadpoles, therefore it does not include stages below 26.

Stage	**Initial criterion used here for *Ascaphus***	**Gosner (1960) initial criterion**	**# of days since hatching (and figures) from Brown (1990) and Brown (1989)**
26	Labial tooth formula is 1/3; the tooth row in the upper labium is continuous and in the lower labium, the first and second tooth rows are divided medially while the third is continuous. TL:18mm	View Hind limb diagram in **#28**	57 days
27	Dental ridges continue to develop: single ridge of the upper labium is continuous and consists of a closely set double row of teeth, and the second and third ridges are continuous and each remains as a single row	View Hind limb diagram in **#28**	66 days

Stage	Initial criterion used here for *Ascaphus*	Gosner (1960) initial criterion	# of days since hatching (and figures) from Brown (1990) and Brown (1989)
28-30	Tadpole with well-developed suctorial mouth, median opercular opening behind mouth, full dark coloration, and hind limb bud 0.5-0.7mm and completely covered by anal fold.		87 days

Stage	Initial criterion used here for *Ascaphus*	Gosner (1960) initial criterion	# of days since hatching (and figures) from Brown (1990) and Brown (1989)
31-33	Same as toe development diagram from Gosner (1960)		

Stage	Initial criterion used here for *Ascaphus*	Gosner (1960) initial criterion	# of days since hatching (and figures) from Brown (1990) and Brown (1989)
34	Tadpole with the appearance of ankle constriction and toes and ranging from 0.7-2.1mm long. Hind-limbs still hidden by the anal flap. Size of the oral disk increases but spiracle remains 0.3mm in width.		
35-36	Unidentifiable on live specimens in the field	View Gosner 1960 diagram in stage 31	
37	Tadpole with five toes distinct	Five toes distinct	
38	Tadpole with toes protruding beyond the anal flap. Oral disk enlarges in length and width, and the spiracle enlarges to width of 0.9mm	Metatarsal tubercle formed	38, 36 (Plate 1d, 1e)
39	Tadpole with ankle beyond anal flap	Pigment free areas indicate site of subarticular tubercle formation	
40	Tadpole with knee beyond anal flap. Spiracle enlarges to a width of 1.3mm and some may show fingertips at the spiracle opening	Subarticular tubercles formed; cloacal tail piece present	39, 40 (Plate Iib, If)

Stage	**Initial criterion used here for *Ascaphus***	**Gosner (1960) initial criterion**	**# of days since hatching (and figures) from Brown (1990) and Brown (1989)**
41	Tadpole with fingertips visible in spiracle	Skin over forelimbs thin & transparent, larval mouthparts begin to break down; cloacal tail piece lost	41 (Plate Ig, Ih)
42	Tadpole with forelimbs emerged	Forelimbs emerge; labial denticles and horny beak lost	42 (Plate Iic)
43A	Transforming tadpole with horny denticles and beak lost, oral disc retained	Angle of mouth between nostril and midpoint of eye	
43B	Transforming tadpole with oral disc reduced to upper and lower "lips"		43 (Plate Iid)
44	Transforming tadpole with upper and lower lips fused smoothly to head	Angle of mouth between midpoint and back of eye	
45A	Transforming tadpole with angle of mouth behind posterior border of eye	Angle of mouth behind posterior border of eye; tail reduced to stub	45 (Plate Iie)
45B	Metamorph frog with tail reduced to stub		
46	YOY frog with tail stub gone	Tail stub gone	46 (Plate Iif)

Appendix B. Larval Coastal Tailed Frog specimens collected from inland California and vouchered in the Humboldt State Vertebrate Museum (HSUMV).

HSU VM #	Site Name (refer to Chapter One for specific locations)	Developmental Stage (Appendix A)	Comments
3014	Headwaters of Big Creek	28	died during anesthesia
3013	Headwaters of Big Creek	40	found dead
3012	East van Matre Creek	40	died during anesthesia
3011	Siligo Creek	28	died during anesthesia
3015	East Fork Big Creek	34	died during anesthesia
3019	East Fork Big Creek	40	died during anesthesia
3020	Siligo Creek	37	died during anesthesia
3022	East Fork Big Creek	37	died during anesthesia
3023	Headwaters of Big Creek	37	died during anesthesia
3024	Headwaters of Big Creek	34	died during anesthesia
3025	Headwaters of Big Creek	34	died during anesthesia
3026	Headwaters of Big Creek	37	died during anesthesia
3027	Headwaters of Big Creek	43A	died during anesthesia
3028	Headwaters of Big Creek	34	died during anesthesia
3029	Headwaters of Big Creek	34	died during anesthesia
3030	Headwaters of Big Creek	34	died during anesthesia
3031	Headwaters of Big Creek	34	died during anesthesia
3032	Headwaters of Big Creek	37	died during anesthesia
3033	Headwaters of Big Creek	34	died during anesthesia
3034	Headwaters of Big Creek	34	died during anesthesia
3035	Headwaters of Big Creek	34	died during anesthesia
3036	Headwaters of Big Creek	34	died during anesthesia
3037	Headwaters of Big Creek	37	died during anesthesia
3038	Headwaters of Big Creek	34	died during anesthesia
3039	Headwaters of Big Creek	34	died during anesthesia
3040	Headwaters of Big Creek	37	died during anesthesia
3041	Headwaters of Big Creek	34	died during anesthesia
3051	East Fork Big Creek	37	died during anesthesia
3052	Headwaters of Big Creek	37	died during anesthesia
3053	Headwaters of Big Creek	38	died during anesthesia
3054	Siligo Creek	34	died during anesthesia

HSU VM #	Site Name (refer to Chapter One for specific locations)	Developmental Stage (Appendix A)	Comments
3055	East van Matre Creek	34	Eaten by a *Thamnophis atratus hydrophilus*
3057	East van Matre Creek	37	Eaten by a *Thamnophis atratus hydrophilus*
3058	East van Matre Creek	34	Eaten by a *Thamnophis atratus hydrophilus*
3059	East van Matre Creek	34	Eaten bya *Thamnophis atratus hydrophilus*
3060	East van Matre Creek	34	Eaten by a *Thamnophis atratus hydrophilus*
3016	Siligo Creek	34	Specifically collected for voucher
3017	Siligo Creek	38	Specifically collected for voucher
3018	Siligo Creek	34	Specifically collected for voucher
3061	Siligo Creek	34	Specifically collected for voucher
3062	Siligo Creek	37	Specifically collected for voucher
3063	Siligo Creek	38	Specifically collected for voucher
3064	Siligo Creek	37	Specifically collected for voucher
3065	Siligo Creek	34	Specifically collected for voucher

Appendix C. Post-metamorphic Coastal Tailed Frog Voucher Specimens at the Humboldt State Vertebrate Museum (HSUVM).

HSUVM #	Site Name	UTM Easting(WGS 84)	UTM Northing (WGS 84)	Comments
3021	Siligo Creek	508940	4531005	immature, sex unknown

Appendix D. Protocol developed to prepare slides of Coastal Tailed Frogs for estimating ages using skeletochronology.

Skeletochronology Protocol

Dissecting out Bone:

1. Get equipment: 70% Ethanol, flame, dissection tray, paper towels, scalpel, two forceps, glass screw top container, masking tape, and permanent marker.
2. **Between each sample,** sanitize tools (forceps and scalpel) by dipping in **70% Ethanol** and holding over flame until all Ethanol is burned off place them on paper towel (get a new paper towel between each sample)
3. Using sanitized forceps, remove the toe sample from the labeled centrifuge tube and place it on the paper towel. Set aside original centrifuge tube.
4. Using a pair of tweezers hold the toe bone, gently peel off the skin trying to get the largest pieces possible. Sometimes you may need to make an incision using a scalpel down the side of the toe, taking care to **not cut into the bone**.
5. Put tissue back in original centrifuge tube and copy the **exact label** from the toes previous container on the new glass screw top container using a fine tipped sharpie. Place the bone in the glass screw top container and fill with **70% Ethanol** to fully cover the bone.

Decalcification of Bone:

1. Tap water for **1 hour** (for pieces 1/2cm diameter or less)
2. Decalcify in RDO rapid decalcifier for **2-3 hours** (do not leave for too long!; Solution is **corrosive**)
3. Rinse with water for **2-3 hours**
4. **50% Ethanol** for **1 hour**
5. Store in **70% Ethanol**

Embedding (either in Autotechnicron or by hand):

1. **95% Ethanol** for **1 hour**
2. **100% Ethanol** for **1 hour**
3. **100% Ethanol** for **1 hour**
4. **Toluene** for **1 hour**
5. **Toluene** for **1 hour**
6. **50% Toluene/50% Paraffin** for **1 hour** inside **Paraffin Oven**
7. **Paraffin** for **1 hour** inside **Paraffin Oven**
8. **Paraffin** for **1 hour** inside **Paraffin Oven**

9. Label **and** Embed in **Molten Paraffin** in aluminum foil mold for **24 hours** in refrigerator

Sectioning:
1. Remove embedded specimen from mold, and trim off excess paraffin with a razor.
2. Fix specimen to wooden block
3. Adjust angle of razor to **0**
4. Make **10um** sections in microtome

Fixing Sections to Slides:
1. Take slide without cover slip and lightly smear with **Haupt's Solution**
2. Place sections onto slide
3. Float with **3% Formalin**
4. Place on warming plate for **24 hours**

Staining Slides:
1. Xylene for **2 minutes**
2. Xylene for **2 minutes**
3. 100% Ethanol for **2 minutes**
4. 95% Ethanol for **2 minutes**
5. 70% Ethanol for **2 minutes**
6. Water for **2 minutes**
7. Hematoxylin (Erlich's or Delafield's) for **16 minutes** for **Toluidine Blue** use **2 minutes**
8. Running **Water** for **4 minutes**
9. Ammonia for **2 minutes**
10. Water for **2 minutes**
12. 70% Ethanol for **2 minutes** for **Toluidine Blue just drip once**
13. 95% Ethanol for **2 minutes** for **Toluidine Blue just drip once**
14. 100% Ethanol for **2 minutes** for **Toluidine Blue just drip once**
15. 100% Ethanol for **2 minutes**
16. 50% Ethanol/50% Xylene for **2 minutes**
17. Xylene for **2 minutes**
18. Xylene for **2 minutes**

Mounting Slides
1. Take slide and add a few drops of **Permount**

2. Using **coverslide forceps**, place a **24x60 coverslip** over slide.
3. Adjust coverslip and remove bubbles
4. Set slides out to dry for **1 week.**

Viewing Slides:

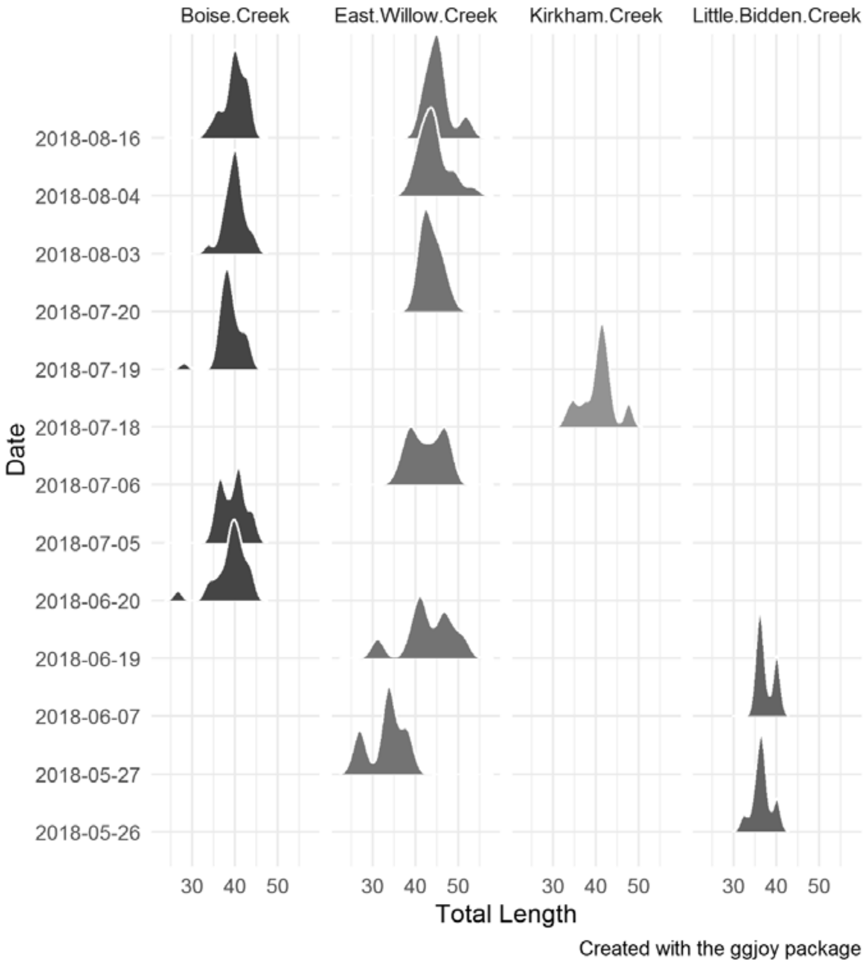
1. On skeletochronology data sheet, write down the Unique ID and Collection Dates prior to viewing slides.
2. On each slide there are **4** Unique IDs labeled on the **left**, and **4** phalange sections corresponding with each Unique ID (**Figure 1**).
3. View each slide using a microscope starting at **10x magnification**, and ending at **40x magnification.** Long bone sections can be reported at **10x magnification.**
4. Indicate which sections you will be reporting in the **Sections Used** column.
5. Indicate which area of the section you used by imagining the section as a clock (**Figure 2**)
6. Count each line of arrested growth (LAG) (**Figure 3 and 4**)
7. Two independent observers report their estimates, then agree on the final estimate of age.
8. Report endosteal resorption and other comments in the notes section.

Appendix E. Study site photos of low elevation habitat composed of a dense canopy of late seral Douglas-fir (*Pseudotsuga menziesii*), Big-leaf Maple (*Acer macrophylum*), Red Alder (*Alnus rubrus*), and Tan Oak(*Notholithocarpus densiflorus*) in northern California.

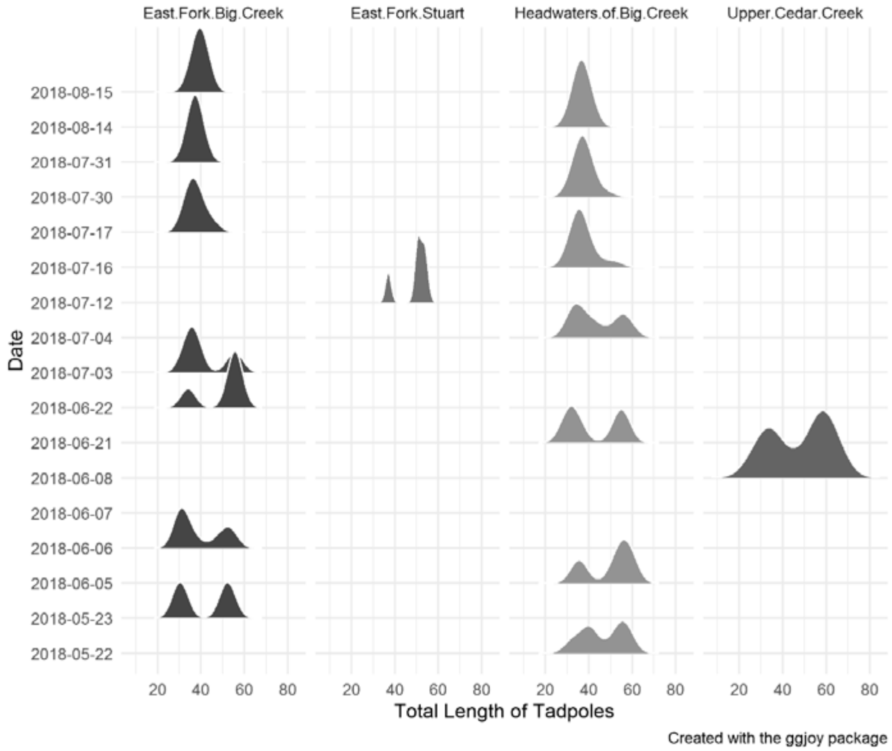
Appendix F. Study site photos of mid-elevation site habitat composed of a dense canopy of late seral Douglas-fir (*Pseudotsuga menziesii*), Big-leaf Maple Maple (*Acer macrophylum*), Red Alder (*Alnus rubrus*), and Tan Oak (*Notholithocarpus densiflorus*) in northern California, USA.

Appendix G. Site photos of high elevation habitat composed of very little canopy with a few scattered tree species, including Jeffrey Pine (*Pinus jefferi*), Western White Pine (*Pinus monticola*), and Foxtail Pine (*Pinus balfouriana*) in northern California, USA.

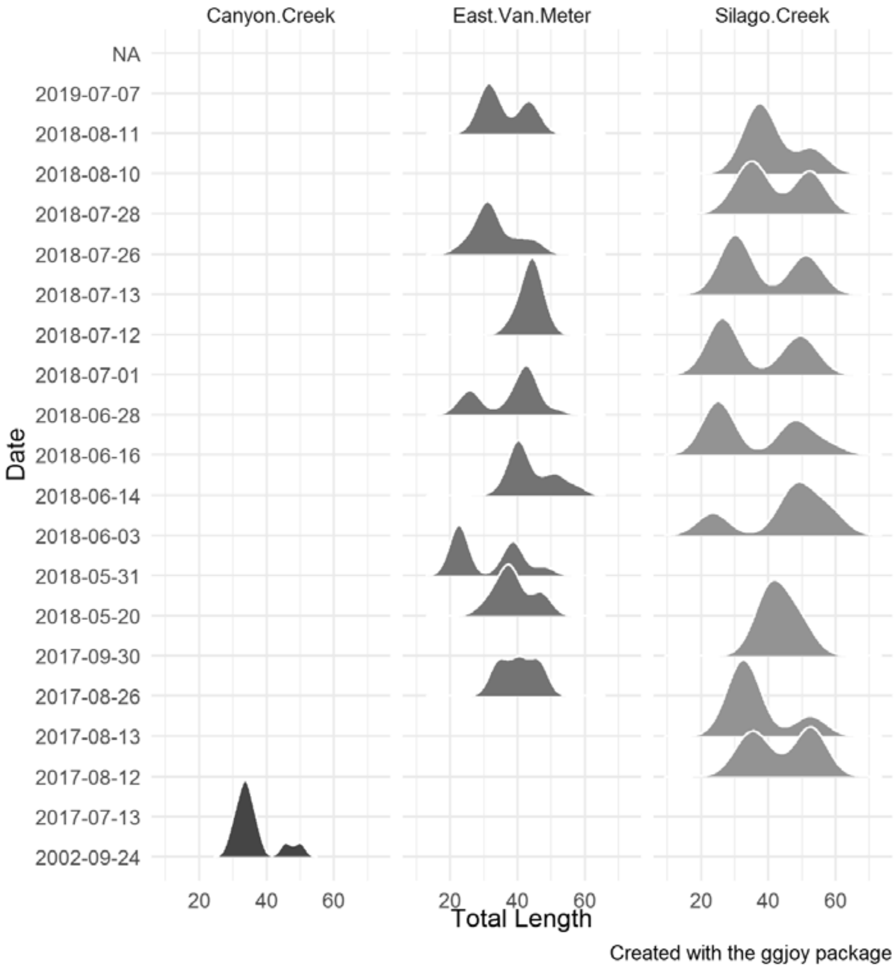
Appendix H. Total length frequency histograms of Coastal Tailed Frogs across low elevation study sites and dates they were surveyed.



Appendix I. Total length frequency histograms of Coastal Tailed Frogs across mid-elevation study sites and dates they were surveyed.



Appendix J. Total length frequency histograms of Coastal Tailed Frogs across high elevation study sites and dates they were surveyed.



Appendix K. Developmental Stage frequency histograms of Coastal Tailed Frogs across Elevation Category and dates they were surveyed.