

The Impact of Environmental Factors on Arizona Tiger Salamander Larval Growth

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Abstract:

Amphibians have been hit particularly hard by the recent era of biodiversity loss (Collins 2009). Understanding the factors that influence their growth and development can help us understand their global decline as well as the dynamics of populations in widely different habitats. Growth rates have been observed to vary dramatically between low elevation (6000 ft) and high elevation (12,000 ft) habitats (Whiteman and Wissinger 2005). Larval growth rates have important implications for life fitness and reproductive success, influencing the morphology of sexually mature salamanders and the size and age at first reproduction (Whiteman 1994; Semlitsch et al. 1988). Larval growth rates and physical characteristics are impacted by environmental factors like temperature, elevation, and pond hydroperiod (Wissinger et al. 2010; Semlitsch et al. 1988). This study will investigate the influence of environmental conditions on larval growth rates in Arizona tiger salamanders. Salamander larvae as well as sexually mature paedomorphic and metamorphic salamanders will be collected and measured from sites ranging in elevation. This data will be compared against multiple environmental variables such as elevation, water chemistry, solar input, and pond hydroperiod. Statistical analysis will reveal which variables or combination of variables have the greatest influence on larval growth rates and ultimately inform our understanding of salamander population dynamics between vastly different sampling sites.

Introduction:

Climate change, habitat fragmentation, and increasing human expansion have led to an era of alarming biodiversity loss. Changes in land use have resulted in modified habitats through disturbances such as logging, burning, and agriculture (Collins 2009). While these issues threaten almost all species on Earth, the recent and dramatic decline of amphibians has been particularly concerning. Many extinctions in the past few hundred years have been a result of changes in land use, and species that rely on both terrestrial and aquatic habitats such as amphibians are particularly vulnerable (Arntzen 2017). Amphibians are ecologically important for energy flow and nutrient cycling in aquatic and terrestrial systems, and have been seen to decline or even disappear in a variety of ecosystems throughout the last few decades (Whiteman and Wissinger 2005; Collins 2009). These declines have been observed by herpetologists since the late 1980s and have been occurring a rate exceeding that of birds and mammals (Collins 2009). While this downward trend in amphibian populations is widely recognized to be a result of habitat loss and land use, there is a surprising lack of research and understanding around the factors driving these patterns (Whiteman and Wissinger 2005; Arntzen 2017).

Studying the environmental conditions influencing the growth and development of amphibians provides us with a greater understanding of the threats they face and the conditions required for their ecological success. Most amphibians hatch as aquatic larvae and must survive for a period of time as they reach the minimum body size necessary to undergo metamorphosis into their sexually mature form. The larval stage is a period of rapid growth, competition for resources, and high mortality, so success during this period is critical for survival and long term reproductive success (Wilbur and Collins 1973). The plasticity of the larval form means that the time an individual remains as a larva varies greatly with environmental conditions such as temperature and resource availability (Wilbur and Collins 1973). Delayed metamorphosis as a

result of limited resources is common in amphibian populations (Whiteman 1994). Larval growth rates have been demonstrated to influence whether or not salamander larvae become paedomorphic or undergo metamorphosis into terrestrial adults, therefore having a large impact on the lifestyle and overall fitness of sexually mature salamanders (Whiteman 1994). The timing of metamorphosis as well as body size at metamorphosis also correlates with size and age of first reproduction as well as number of eggs laid, traits that impact the fitness of sexually mature morphs (Semlitsch et al. 1988). The composition of the salamander community in ponds where hatchlings are developing can greatly impact growth rates through a phenomenon known as the ecology of fear (Wissinger et al. 2010). Paedomorphic salamanders are more successful at cannibalizing smaller larvae than metamorphs. As a result, hatchlings in ponds with high numbers of paedomorphs must avoid being cannibalized, reducing their ability to feed freely and ultimately limiting their growth rates (Wissinger et al. 2010). Pond hydroperiod also plays a large role in these dynamics, as larvae in drying ponds are under a great deal of pressure to grow and reach metamorphosis before their pond disappears (Semlitsch et al. 1988).

Arizona tiger salamanders (*Ambystoma mavortium nebulosum*) at the Mexican Cut Nature Preserve in south central Colorado have been studied and monitored by Rocky Mountain Biological Laboratory (RMBL) for 40 years (Whiteman and Wissinger 2005). Long term studies on specific amphibian populations are rare but are critical for understanding global amphibian decline. This type of study deepens our understanding of the factors driving dramatic population fluctuations, the influence of human activity, and the general threats that salamander populations face (Whiteman and Wissinger 2005). Salamander populations in Colorado extend over a huge range in elevation, stretching from about 6000 ft to almost 12,000 ft at Mexican Cut. This elevational range results in a wide variation of environmental conditions between the warmer low elevation ponds to the icy high elevation systems. The last 40 years of research have revealed that salamanders at Mexican Cut tend to have dramatically slower growth rates than other populations ranging between 6000 and 11000 ft in elevation (Whiteman and Wissinger 2005). These sites also vary in other important factors such as exposure to cattle traffic and therefore nutrient inputs. Understanding the differing conditions that result in these vastly different growth rates can help us understand the population dynamics of lower elevation populations that lack the long term information gained from 40 years of study at Mexican Cut. I aim to contribute to the understanding of the salamander population at Mexican Cut Nature Preserve as well as other nearby populations in south central Colorado by analyzing a group of biotic and abiotic variables that potentially influence salamander development and ultimately their lifetime fitness.

I collected hatchlings from a variety of study sites across south central Colorado and measured their body size and weight. I also documented other physical traits such as coloration and gill length. Analysis of these traits against multiple biotic and abiotic factors reveals their relative impact on larval development. The variables I analyzed include slope aspect, dissolved oxygen, pH, temperature, elevation, pond hydroperiod, hatchling gill length, body size, and weight.

Methods:

Salamander hatchlings as well as paedomorphic and metamorphic adults were caught from a variety of study sites ranging from low elevation desert ponds to high elevation subalpine sites using long handled nets and a seine net before being stored in minnow buckets for analysis. Animal length and weight were recorded using a ruler and a portable scale. Snout-vent length (SVL) and total length (TL) were recorded. Hatchlings were photographed next to a reference ruler and gill length was measured using ImageJ in order to investigate their relationship to dissolved oxygen.

Dissolved oxygen percentage and concentration were measured using a DO probe at three separate locations within each sampling pond between 11:00 am and 2:00 pm to account for daily fluctuations due to photosynthesis. These sampling times were intended to capture data during peak photosynthetic productivity. The dissolved oxygen probe was also used to collect temperature and elevation data at each site. pH was also measured from three separate locations within each pond using a pH probe between 11:00 am and 2:00 pm to account for these daily fluctuations. Nitrogen samples were gathered using a syringe and filtered in the field with a glass fiber filter before being stored in opaque sample bottles and frozen quickly to prevent light contamination. Four 20mL duplicates were taken at each site. Samples will be analyzed for nitrogen content at Rocky Mountain Biological Laboratory. Chlorophyll samples were gathered by collecting and scrubbing algae off of submerged rocks in a known volume of water. Sample bottles were wrapped in tinfoil and frozen quickly to prevent light contamination. Algal samples from each study site will be filtered through a glass fiber filter and placed into a spectrophotometer. Rock size was determined using ImageJ to calculate chlorophyll by substrate area. Slope aspect was determined using the *r.slope.aspect* function in GRASS GIS (GRASS Development Team 2017) and adjusted to reflect the number of degrees between 1 and 180 away from North, since more southerly facing slopes generally receive more sun in this region. Pond hydroperiod was determined using data from previous years of study. Analysis was completed by determining correlations among independent variables and collapsing them into principal components. We analyzed our data using a principle components analysis as we expected high correlation among our environmental variables. All data analyzed has been standardized in order to compare variables measured on different numerical scales. We used the first two principle components for our analysis.

We gathered our data from a variety of sites in Gunnison County in south central Colorado. These sites ranged greatly in elevation, extending from 6000 ft to 11200 ft. They also ranged in environment, including hot and dry sagebrush communities to cooler high elevation subalpine areas. Notable downvalley sites include Lake City and Rainbow Lake. Other sites include the Kettle ponds just south of Gothic, and Mexican cut located north of Gothic.

Pond	DO (mg/L)	Temperature (C)	pH	Elevation (ft)	aspect	Hydroperiod
Rainbow1	1.27	21.00	7.70	9220	304	Temporary
K1	12.43	24.03	10.67	9404	112	Temporary
K6	10.32	20.40	9.80	9442	201	Temporary
Rainbow2	5.41	22.13	7.73	9466	303	Temporary
Lake City Cabin	6.90	20.87	7.67	8878	90	Temporary
Lake City	2.12	16.97	7.33	8963	305	Permanent
K2	5.85	21.90	7.03	9397	156	Temporary
Rainbow Marsh	7.54	26.28	7.80	10920	135	Temporary
Rainbow Road	5.09	19.93	8.10	10931	193	Permanent
Stampede	5.93	21.93	7.65	9709	116	Temporary
Rainbow forest	6.07	21.47	7.93	10481	174	Permanent
MC8	4.56	19.60	7.00	11197	16	Temporary
MC10	5.03	20.25	6.00	11201	8	Temporary

Results:

PC1 contained 50.25% of the variance while PC2 contained 23.7% of the variance (Figure 1). PC2 is mostly composed of variation in elevation while PC1 holds most of the remaining variation in the other variables. We tested relationships between hatchling traits and principle components using linear models, except for specific hypotheses such as the relationship between gill length and dissolved oxygen (Figure 2). Hydroperiod was also included as a categorical variable between temporary and permanent ponds. We calculated the ratio of snout vent length (SVL) to gill length in order to control for differences in body size between pond sites, however gill ratio was still strongly correlated to body size ($t=9.0$, $p<.001$). After controlling for body size, gill length was not correlated to dissolved oxygen levels (Figure 2). Gill length was also not related to principle components 1 or 2 ($p>0.1$). Hatchling body length was not found to be related to PC1 but is moderately positively related to PC2, which is associated with a decrease in elevation. Regression also revealed a positive correlation between dissolved oxygen and temperature ($t = 2.2$, $p = 0.052$).

Discussion:

Despite vastly different sampling sites with strong variation in environment and elevation, early analysis seems to indicate that abiotic factors during the hatchling growing season do not explain the strong variation in body size, characteristics, or growth rates between populations. Elevation does not explain variation in water quality variables between sites that ranged in elevation from as low as ~8900 ft to as high as 11200 ft (Figure 1). Even after controlling for variation in body size between ponds by calculating gill length to body size ratios, relative gill length was still found to be positively correlated to body size. Gill ratio was found to not be related to dissolved oxygen variance or either of our principle components. This result suggests that body size is the main driver of variation in gill length, and gill length is relatively unaffected by the measured environmental variables. Simply stated, larger salamander hatchlings tend to have larger gills. Hatchling body length was found to be unrelated to PC 1, but there was a slight positive correlation to PC 2. PC2 is associated with decreasing elevation, suggesting there is a slight negative relationship between elevation and hatchling body length (Figure 3). This fits with the observations made at Mexican Cut in the past decades of study, where salamanders at this high elevation site have significantly slower growth rates and lower body sizes in general (Whiteman and Wissinger 2005). These differences in body size and growth rates are likely related to a shortened growing season and late pond melting, as well as limited resources at higher elevations (Whiteman and Wissinger 2005). However, the relationship between body size and PC2 could also be a reflection of bias in sampling times, as the smallest body sizes tended to be from sites that had been visited earlier in the season. In addition to this, Rainbow Lake contains some of the largest and most numerous paedomorphs out of all the sites we had visited despite its high elevation. Future work will involve looking further into factors that affect snow such as solar irradiance (Scott Thomas, personal communication).

The insignificant impact of our measured environmental variables collected during the growing season suggests that these variables may have a larger impact on growth rates and differences in salamander populations during different times of the year. It is possible that these factors play a larger role through their influence on the phenology of the growing season, especially at the beginning of the summer. The date of pond melting and winter snowpack conditions determine the length of the growing season, so the impact of environmental factors such as elevation or temperature during this time could carry over for the entire growing season (Kirk et al. 2023). In addition to the date of melting and freezing at the tail ends of the season, the rate of warming and cooling could have an influence as well. Larger and deeper ponds may melt and cool at a slower rate, changing the dynamics of growth rates in salamander hatchlings.

The influence of cattle grazing is difficult to determine without direct data on cattle numbers and nutrient inputs such as nitrogen content. However, a comparison of similar sites that are both known to be impacted by cattle or horse grazing can indicate the relative impact of grazing animals. For example, a cursory look at the Stampede pond and the kettle ponds suggests that grazing animals do not have a strong impact on salamander growth rates or larval characteristics. These ponds have similar inputs from grazing animals yet differ in terms of larval body size. While a strong conclusion cannot be reached from this simple observation, it appears that other factors are driving the differences in these populations.

Dissolved oxygen in pond systems fluctuates significantly throughout the day due to changing levels of solar radiation and the resulting changes in photosynthetic productivity of the

pond's plants and algae (Antonopoulos 2004). Dissolved oxygen and pH measurements were taken during the hours of peak photosynthetic productivity between 11:00 am and 2:00 pm in order to account for these daily changes. There is a slight negative correlation between pH and dissolved oxygen levels and later sampling times, however the correlation is relatively insignificant. This means that the dissolved oxygen and pH readings were not significantly impacted by bias in sampling times and are therefore comparable between sites.

There are multiple correlations of varying strength between our environmental variables. Most of these are relationships that are understood to impact each other, such as temperature and elevation. It is generally understood that water at a lower temperature holds less dissolved oxygen than warmer water (Srikanth 2018). However, our data shows a positive correlation between temperature and dissolved oxygen. There are a few possibilities for this correlation. The first and most obvious possibility has to do with equipment error, where the probe used to measure dissolved oxygen and temperature was inaccurate. This result could also be related to sampling error or inconsistencies, where samples were taken at inconsistent depths or biased locations in the pond. It is also possible that a variable other than water temperature had a greater impact on dissolved oxygen levels than temperature on its own. For example, pond depth could have a stronger influence on dissolved oxygen than water temperature. Plant life in the ponds could also be strongly influencing dissolved oxygen levels (Antonopoulos 2004). In addition to this, many temporary pond sites were sampled late in the season while they were in the process of drying. This process could potentially bias the readings, as some ponds had partially dried and became partitioned by the shallow topography, creating sections that potentially varied in condition and chemistry.

Tables and Figures:

Figure 1:

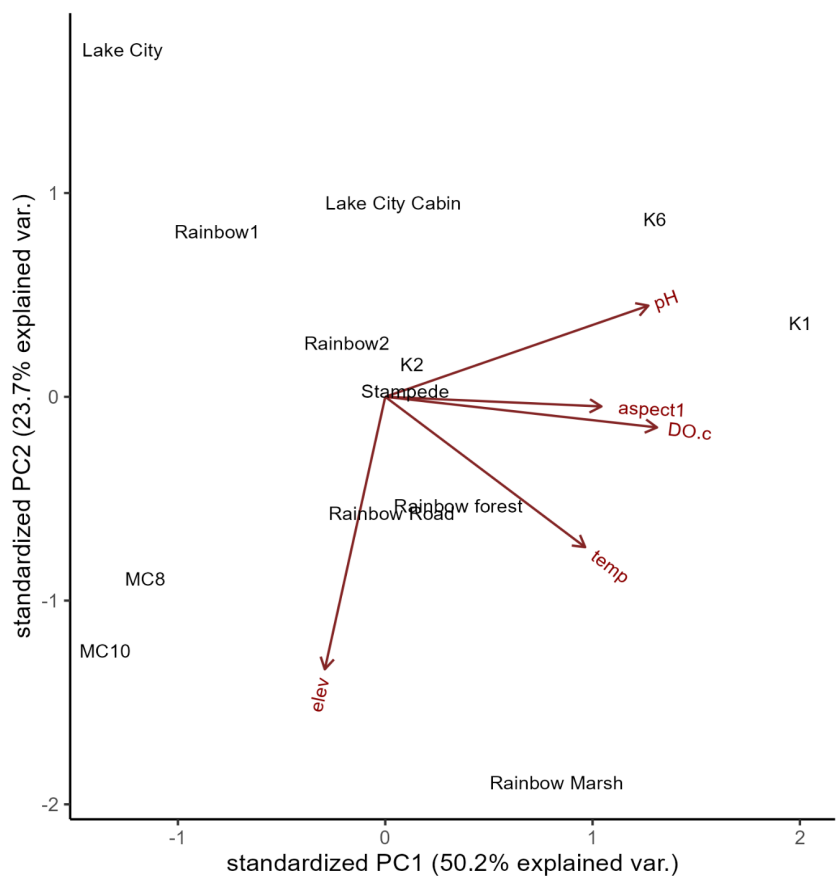


Figure 2:

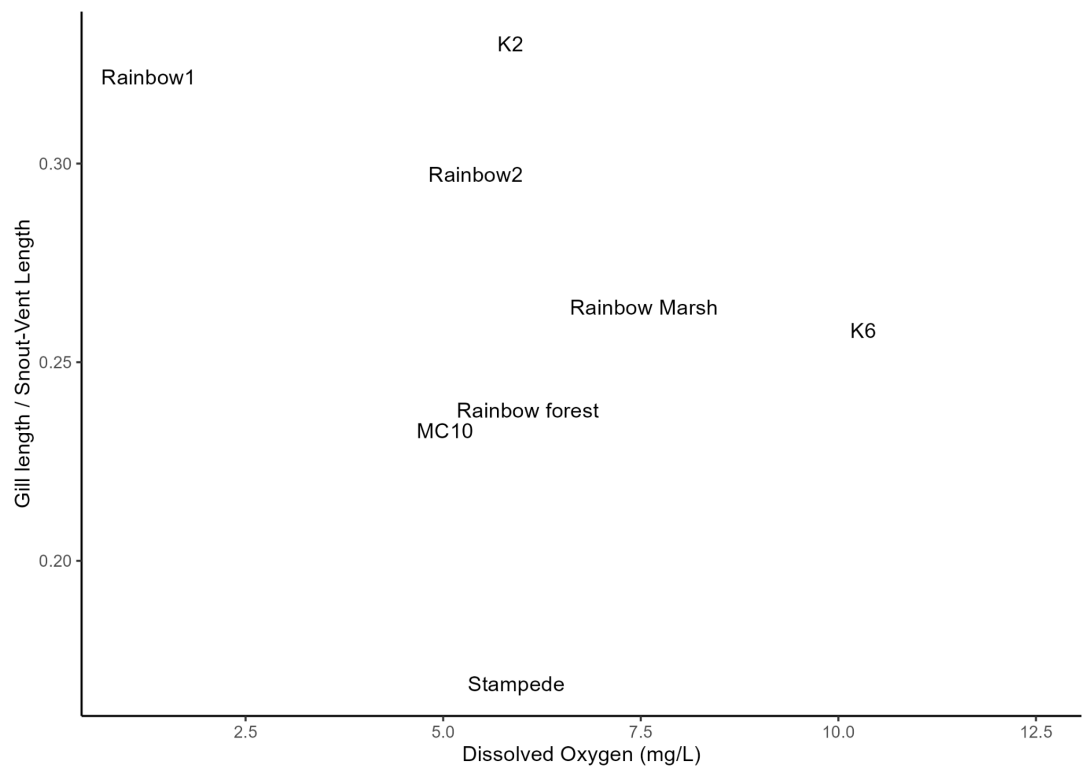
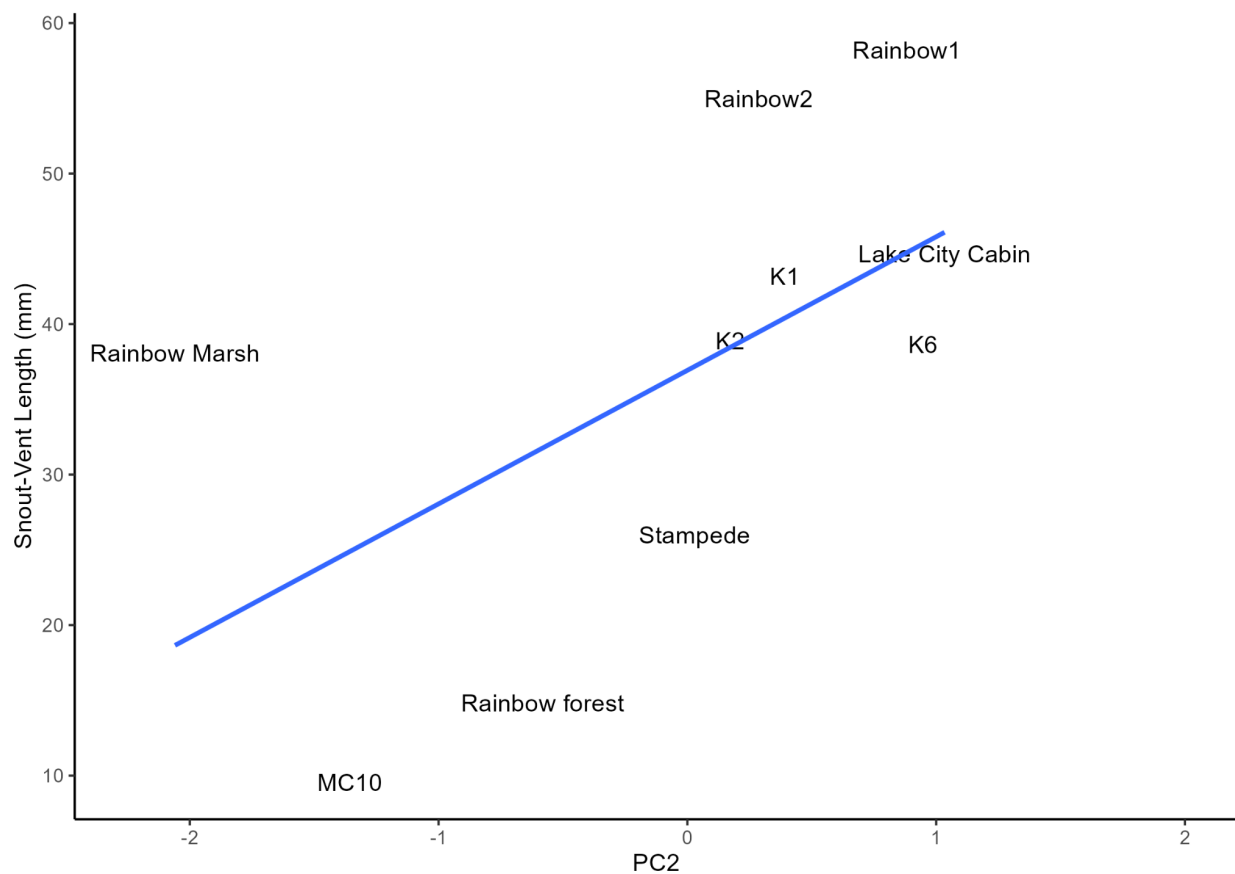


Figure 3:



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