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Title: The effects of cattle derived nutrients on growth rates of Arizona Tiger Salamander hatchlings in pastureland

Abstract

Nitrogen is a critical element in biological systems, with its cycling increasingly influenced by human activities, particularly in agriculture. In Colorado, where 50% of land is used for agriculture, cattle grazing may impact the ecosystems of montane and subalpine ponds inhabited by *Ambystoma mavortium nebulosum*. My study examined how cattle presence affects ammonium, phosphorus, and hatchling growth rates in these ponds. I collected and measured hatchlings from ten ponds, and qualitatively assessed cattle presence. Water samples were analyzed for ammonium and phosphorus concentrations. Results showed that low to medium cattle presence significantly increased hatchling growth rates but there was no significant correlation with ammonium or phosphorus concentrations. However, phosphorus was significantly correlated with reduced growth rates in a linear model, though I have uncertainty about this trend due to slight inconsistencies in data analysis. Additional trends suggested potential correlations between cattle presence and other water quality variables, such as dissolved oxygen and pH, though these were not statistically significant. The study highlights the need for further research with larger sample sizes and more comprehensive water quality measurements to better understand the impacts of cattle on high-elevation pond ecosystems and salamander populations.

Introduction

Along with carbon, hydrogen, and oxygen, nitrogen is one of the most important elements in biology. Nitrogen cycles naturally through the biosphere, but as human activity has increased this cycle has sped up (Burns, 2004). Agriculture is one such area where the cycling of nitrogen has been accelerated. In Colorado, 50% of the state's land is used for agriculture, and, of that half, 17.6 million acres is pastureland (USDA, 2022). Much of this pastureland is in the Rocky Mountains, where populations of *Ambystoma mavortium nebulosum* use and inhabit montane and subalpine ponds and lakes. Cattle that use this pastureland are likely to directly disturb these ponds through defecation or wading, increasing the nutrient content and turbidity of the ponds. In Argentina, high densities of cattle on pastureland have been shown to increase concentrations of nitrogen, as well as phosphorus, in wetlands (Agostini & Bilenca, 2024). This was also associated with decreased richness and abundance in various species of frogs.

Global amphibian populations have seen declining numbers since the mid-1900s (Houlahan et al., 2000). No single impact can account for this decline, rather different regions are susceptible to and affected by a variety of drivers (Grant et al., 2016, 2020). There is a need to develop a better understanding of amphibian population dynamics as they relate to these drivers. In the case of tiger salamanders in ranchland ponds, little is known about the impact of cattle on their population dynamics and thus how these populations might best be managed.

Tiger salamanders have a facultatively paedomorphic life cycle, with terrestrial metamorphic and aquatic paedomorphic adults (Whiteman, 1994). Whether a larva becomes a metamorph or paedomorph depends largely on environmental conditions such as whether the pond it's living in is permanent or temporary. Larvae in temporary ponds must metamorphose to leave the pond before it dries out while those in permanent ponds will often become paedomorphic as the pond will not dry up and thus it is advantageous not to leave. A larva's growth before and time of metamorphosis will affect its traits at maturity. Larger larvae tend to be larger adults at first reproduction and those that mature earlier in the season tend to be larger at the first two reproductions (Semlitsch et al., 1988). For female salamanders, earlier metamorphosis means they will be younger for their first reproduction than those that metamorphose later in the growing season (Semlitsch et al., 1988). Larger females can create a larger egg clutch (Wise & Jaeger, 2021). What can be inferred here is that individuals who are able to grow quickly and morph earlier will be able to reproduce sooner and, in the case of females, produce more eggs. This leads to population growth which is associated with a healthy population, so the more individuals that are able to do this, the healthier the population. Thus, it can be inferred that larval growth rates are a good proxy for the health of a population.

There is conflicting research about the effects of nutrients on amphibians. One study found significant increases in growth rates of two-lined salamanders (*Eurycea wilderae*), a species of salamander native to eastern North America, after enrichment of stream waters (Johnson et al., 2006). On the other hand, Earl and Whiteman (2009) looked at nutrient pulses and potential impacts on amphibian development. Pulses of nitrate were found to have the largest effect on organisms during the most sensitive stages of development such as metamorphosis.

The impacts of cattle on pond and wetland ecosystems have been previously researched. As noted above, cattle cause an increase in the nutrient concentrations of surrounding wetlands and as a result decrease the health of associated amphibian populations (Agostini & Bilenca, 2024). Additionally, cattle have been found to have adverse effects on the biodiversity and fitness of amphibians in nearby ponds and wetlands. If the cattle have open access to a pond, they can wade and defecate in the pond which directly adds nitrogen and decreases the overall water quality (Knutson et al., 2004). This was correlated with low reproductive success for the amphibians living in the affected ponds.

While there is previous research on the effects of cattle and nutrients on a variety of amphibian species, there is limited research looking at these impacts on Arizona tiger salamanders or at high elevations like in the Rocky Mountains. My study aims to determine how the presence of cattle around subalpine and montane ponds affects the growth rate of Arizona tiger salamander hatchlings. I tested four hypotheses to answer this question, 1) a higher presence of cattle will lead to higher concentrations of ammonium, 2) a higher cattle presence will lead to higher concentrations of phosphorus, 3) higher concentrations of ammonium will decrease growth rates, and 4) higher concentrations of phosphorus will decrease the growth rates.

Materials and Methods

Hatchling Measurements

I collected approximately 15-25 *A. m. nebulosum* hatchlings were caught from ten pond sites around Gunnison County. Sites include ponds along Rainbow Lake Road (CR724) and Colorado SH 149 as well as some around the Deer Creek Trail. Hatchlings were measured by hand with a ruler and a scale or using ImageJ. Those measured by hand had their snout-to-vent length (SVL), total length (TL), and weight recorded. They were released after being measured and weighed. Hatchlings measured using ImageJ were placed in a shallow tray with a 15 cm ruler. A picture was taken and the hatchlings were released. SVL and TL were recorded for individuals measured with ImageJ, but they were not weighed. Hatchling data was collected across a 41 day period. I revisited ponds roughly 14 days after the initial visit, although a few sites were revisited 23 and 36 days apart. The time between visits isn't as important, however, as growth rates were standardized to units of mm/day.

Determination of Cattle Presence

At each site, I walked a transect around the pond at approximately 1-3 m away from the bank. I took pictures of the bank every ten steps and then qualitatively analyzed and categorized each pond into four groups of cattle presence: high, medium, low, and none. High cattle presence is indicated by fresh cattle tracks and trampling of the pond's bank as well as fresh cow pies. Low presence is noted by few cow tracks, older cow pies, and the bank isn't disturbed.

Ammonium Analysis

Ammonium sampling and analysis followed the 2017 Taylor Lab Ammonium Analysis Protocol. In short, quadruplicate samples were collected at each pond site and stored in a cooler to prevent light contamination. Back at the RMBL, the quadruplicates had a working reagent added that reacts with ammonium. The result is a fluorescent compound, which was measured using a fluorometer. By comparing quadruplicates and correcting for background fluorescence, the concentration of ammonium in each pond was determined.

Phosphorus Analysis

The phosphorus sampling and analysis followed the 2017 Taylor Lab Phosphorus Analysis Protocol. A single water sample from each pond was needed for phosphorus analysis and samples were stored in a cooler after collection. Samples had a mixed/color reagent added so that a spectrophotometer could determine the concentration of phosphorus in the sample.

Other Measured Variables

In addition to nitrogen and phosphorus, I measured other water chemistry variables at each pond. These variables include temperature, elevation as barometric pressure, pH, and dissolved oxygen (DO). A pH probe will be used to measure pH and a dissolved oxygen probe will be used to measure DO, temperature, and barometric pressure. Dissolved oxygen was measured both as a percent saturation and as milligrams of oxygen per liter of water. Typically, milligrams per liter is the preferred unit of measurement for DO, so that is what was used in figures and statistics. These variables were measured at three spots around each pond between 11:00 pm and 2:00 pm during peak photosynthetic activity.

Statistical analysis

I used ANOVAs to do statistical analyses and comparisons of cattle presence to any of the quantitative variables. I then used Tukey's test to further analyze results from ANOVAs if significance was found. I compared quantitative variables with either Pearson correlation or Spearman's rank correlation tests depending on the distribution of the data. I also created a linear model to determine if there was any statistical significance between growth rates and both nutrient concentrations.

Results

Hatchling Data

Within the first sampling period, hatchling SVL ranged from 13.71-41.56 mm and the range for 28.65-76.38 mm. After the second set of sampling, SVL ranged from 30.36 mm to 57.08 mm and TL ranged from 57.51 mm to 109.54 mm. Growth rates by pond varied from 0.21 mm/day to 1.33 mm/day for SVL and 0.33 mm/day to 2.58 mm/day for TL (Figure 1). Growth rates were significantly correlated with both cattle presence and barometric pressure. When looking at growth rates and cattle presence, Tukey's test revealed that hatchlings in ponds with low and medium presence had significantly higher growth rates than those in ponds with high presence (Figure 2). Growth rate was negatively correlated with barometric pressure (Figure 3). Spearman's rank correlation test revealed that this trend was significant ($p < 0.01$).

Growth rates were also compared to the average size of hatchlings at the start of the season (Figure 4). There is a clear downward trend in the data showing that larger individuals grow significantly slower than smaller hatchlings ($p < 0.01$).

Cattle Presence

During the first set of visits, two ponds had high presence, two with medium, four with low, and two with no cattle presence. Then during the second set of visits, one pond, LDC1, increased in cattle presence from medium to high. Additionally, another pond, RBF, increased from low to medium cattle presence.

Ammonium and Phosphorus

During the first sampling period, phosphorus concentrations ranged between 0 µg P/L to 422 µg P/L. The mean was 111.5 µg P/L. The range increased during the second sampling period to between 0 and 960 µg P/L. The mean concentration also increased to 166.6 µg P/L. Phosphorus concentrations were insignificantly correlated with cattle presence ($p = 0.64$). Hatchling growth rates were also not significantly correlated with phosphorus concentrations. The Pearson correlation test yielded an insignificant p-value ($p = 0.74$). Ammonium concentrations ranged from 0.091 µg/L to 1.530 µg/L during the first samplings, with a mean of 0.439 µg/L. During the second sampling period, the range shifted up to be between 0.225 µg/L and 3.054 µg/L. The mean also increased to 1.576 µg/L. Ammonium was also not statistically correlated with cattle presence or growth rates. Ammonium against cattle presence yielded a p-value of 0.45 while growth rates against ammonium gave a p-value of 0.78. Additionally, phosphorus and ammonium concentrations were not significantly correlated ($p = 0.83$).

The linear model comparing growth rates to both ammonium and phosphorus yielded a significant p-value ($p < 0.01$). However, the only significant correlation in this model was between growth rates and phosphorus concentrations ($p < 0.01$), where growth rate was decreasing as phosphorus increased. Ammonium, again, showed no significant correlation with growth rates.

Other Water Chemistry

Average temperatures measured in all ten ponds ranged from a low of around 15 ˚C to a high of about 21 ˚C across both sampling periods. Temperature fluctuations varied between ponds. LC and RB3 saw a decrease of 6.2 ˚C and 3.8 ˚C, respectively and LCC increased by 4.0 ˚C. The other seven ponds saw less than a degree of change between samplings. For barometric pressure, all ponds saw a slight increase in pressure of no more than 2.0 mmHg. Dissolved oxygen widely varied between ponds and changes to DO concentrations also widely varied between ponds. One pond saw concentrations above 11 mg/L while others were severely desaturated at less than 1 mg/L. pH ranged between 7.2 and 9.3 for the first set of sampling and then between 7.2 and 9.0 for the second set. The average between the first and second sets of sampling decreased from 8.3 to 7.7, although decreasing pH was not seen in all ponds. Both DO and pH were compared against cattle presence to see if a correlation exists between them (Figure 5). DO showed a slight negative, nonsignificant trend with cattle presence ($p = 0.1$) and pH yielded a similar trend, but the ANOVA gave a p-value that was closer to significance ($p = 0.08$).

Discussion

Some significant trends were found in the data collected during this study, however the majority were insignificant. The first two null hypotheses cannot be rejected as both phosphorus and ammonium were insignificantly correlated with cattle presence. The third null hypothesis can also not be rejected as ammonium was not correlated with growth rates. On the other hand, initial statistical tests made it appear like the fourth null hypothesis couldn't be rejected either, however, the linear model I used to look at how both phosphorus and ammonium affect growth rates yielded a significant p-value for the overall model as well as a significant p-value when looking at the impact of phosphorus on the model. So in this case, the null hypothesis that phosphorus does not affect hatchling growth rates can be rejected. However my certainty in this outcome is low as some of the ammonium concentrations were too high to be determined using the Taylor Lab Ammonium Analysis Protocol, so some data points were left out of the model. I believe this is why comparing growth rates to just phosphorus yielded an insignificant p-value while comparing growth rates to both nutrient concentrations showed significance. There was a large outlier in the phosphorus data that likely skewed the results and overall trend towards insignificance when looking at the two-dimensional comparison.

Outside of testing the hypotheses, I found additional significance, namely when directly comparing growth rates to cattle presence. These particular results show that cattle presence does have an impact on growth rates, but the scope of my study could not parse out what the link might be. My sample size was quite small which could have made it impossible for me to see some of the trends I was trying to uncover. I think this is especially true when looking at the data I used to test my hypotheses, as I do not believe that 20 total data points is enough to fully uncover trends between cattle, nutrients, and hatchling growth rates. Other studies have found similar trends between growth rates and cattle presence. One paper I cited in the introduction could offer a link between cattle presence and growth rates (Knutson et al., 2004). Their study offered a much more in depth analysis of the quality of a pond as they looked at the morphometry of ponds, vegetation, and many other water quality variables that I did not measure. This allowed them to look at many other potential links between cattle presence and its effects on salamanders than I was able to. Although their study did not specifically look at growth rates, they were able to correlate cattle's access to ponds with decreased species richness and reproductive success. They attribute this to decreased water quality, which is something that I did not look into thoroughly. Along with the study above, another similar study found that cattle grazing affects various amphibian species differently (Burton et al., 2009). While this study found variable effects of cattle on amphibians, they seemed to have found a consistent trend in the pond vegetation. The researchers saw all three vegetation variables that they measured increase in wetlands that cattle couldn't access. Knutson et al. also found this trend in their study.

Looking at the other variables I measured, growth rates were significantly correlated with barometric pressure, although I don't know what the mechanism driving this trend could be as neither pH or DO were correlated with growth rates. Other notable areas in my data include a potential correlation between cattle presence and pH and DO. The data was insignificant, but a noticeable trend is present where ponds with no cattle presence had the highest DO and pH while any amount of cattle presence decreased those values.

If I were to continue or repeat this project, my main goal would be to increase the amount of data I am collecting. I see this being done in a few ways. Firstly, starting data collection earlier in the season would allow me to revisit sites more than twice, allowing me to gain more accurate growth rate data. Additionally, increasing the number of ponds in my study would increase the amount of data I have as well as the diversity of my samples. I would also like to measure more variables (i.e. vegetation, algae, invertebrate populations, etc.) to gain a more comprehensive understanding of each pond, how the cattle are affecting them, and how salamander hatchlings may be impacted by these factors. I see mesocosms as a particularly effective way of looking at how nutrients impact growth rates. I would be able to control for various nutrient ratios in each mesocosm and generate a much more precise data set that would allow me to see any trends that I couldn't with my small sample size. Finally, I believe that moving to remote sensing for collecting water chemistry data. In an ideal world, I could place a buoy in each pond that has all the sensors and probes I need. I could then let it collect data for the entire season while I focus on collecting hatchling data. The logistics of this idea are much more complicated than any of my other ideas as I would need to engineer and build this system from the ground up as nothing like this exists right now.

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References

Agostini, M. G., & Bilenca, D. (2024). Grazing for biodiversity: Assessing the effects of cattle management practices on wetlands and amphibian communities in central Argentina. *Agriculture, Ecosystems & Environment*, *361*, 108801. https://doi.org/10.1016/j.agee.2023.108801

Burns, D. A. (2004). The effects of atmospheric nitrogen deposition in the Rocky Mountains of Colorado and southern Wyoming, USA—a critical review. *Environmental Pollution*, *127*(2), 257–269. https://doi.org/10.1016/S0269-7491(03)00264-1

Burton, E. C., Gray, M. J., Schmutzer, A. C., & Miller, D. L. (2009). Differential Responses of

Postmetamorphic Amphibians to Cattle Grazing in Wetlands. *The Journal of Wildlife Management*, *73*(2), 269–277. https://doi.org/10.2193/2007-562

Grant, E. H. C., Miller, D. A. W., & Muths, E. (2020). A Synthesis of Evidence of Drivers of Amphibian Declines. *Herpetologica*, *76*(2), 101–107. https://doi.org/10.1655/0018-0831-76.2.101

Grant, E. H. C., Miller, D. A. W., Schmidt, B. R., Adams, M. J., Amburgey, S. M., Chambert, T., Cruickshank, S. S., Fisher, R. N., Green, D. M., Hossack, B. R., Johnson, P. T. J., Joseph, M. B., Rittenhouse, T. A. G., Ryan, M. E., Waddle, J. H., Walls, S. C., Bailey, L. L., Fellers, G. M., Gorman, T. A., … Muths, E. (2016). Quantitative evidence for the effects of multiple drivers on continental-scale amphibian declines. *Scientific Reports*, *6*(1), 25625. https://doi.org/10.1038/srep25625

Houlahan, J. E., Findlay, C. S., Schmidt, B. R., Meyer, A. H., & Kuzmin, S. L. (2000). Quantitative evidence for global amphibian population declines. *Nature*, *404*(6779), 752–755. https://doi.org/10.1038/35008052

Johnson, B. R., Wallace, J. B., Rosemond, A. D., & Cross, W. F. (2006). Larval salamander growth responds to enrichment of a nutrient poor headwater stream. *Hydrobiologia*, *573*(1), 227–232. https://doi.org/10.1007/s10750-006-0272-3

Knutson, M. G., Richardson, W. B., Reineke, D. M., Gray, B. R., Parmelee, J. R., & Weick, S. E. (2004). Agricultural Ponds Support Amphibian Populations. *Ecological Applications*, *14*(3), 669–684. https://doi.org/10.1890/02-5305

Semlitsch, R. D., Scott, D. E., & Pechmann, J. H. K. (1988). Time and Size at Metamorphosis Related to Adult Fitness in Ambystoma Talpoideum. *Ecology*, *69*(1), 184–192. https://doi.org/10.2307/1943173

Whiteman, H. H. (1994). Evolution of Facultative Paedomorphosis in Salamanders. *The Quarterly Review of Biology*, *69*(2), 205–221. https://doi.org/10.1086/418540

Wise, S. E., & Jaeger, R. G. (2021). Maternal Body Size and Condition Predict Measures of Reproductive Success and Future Reproductive Allocation in Territorial Eastern Red-Backed Salamanders. *Ichthyology & Herpetology*, *109*(1), 55–63. https://doi.org/10.1643/h2019323

Figures

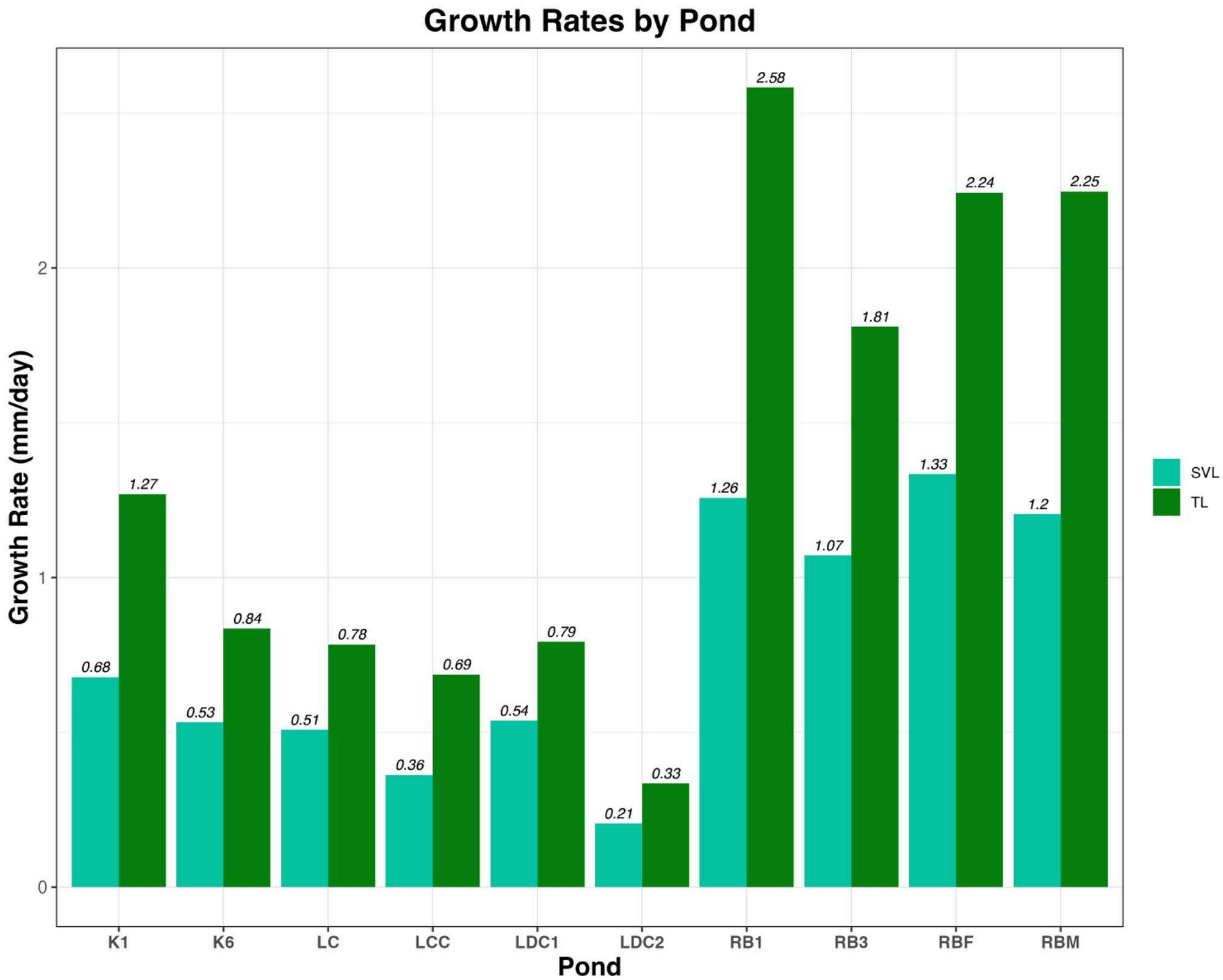

Figure 1: Growth rates by pond for both snout-to-vent length (SVL) and total length (TL) in hatchlings.

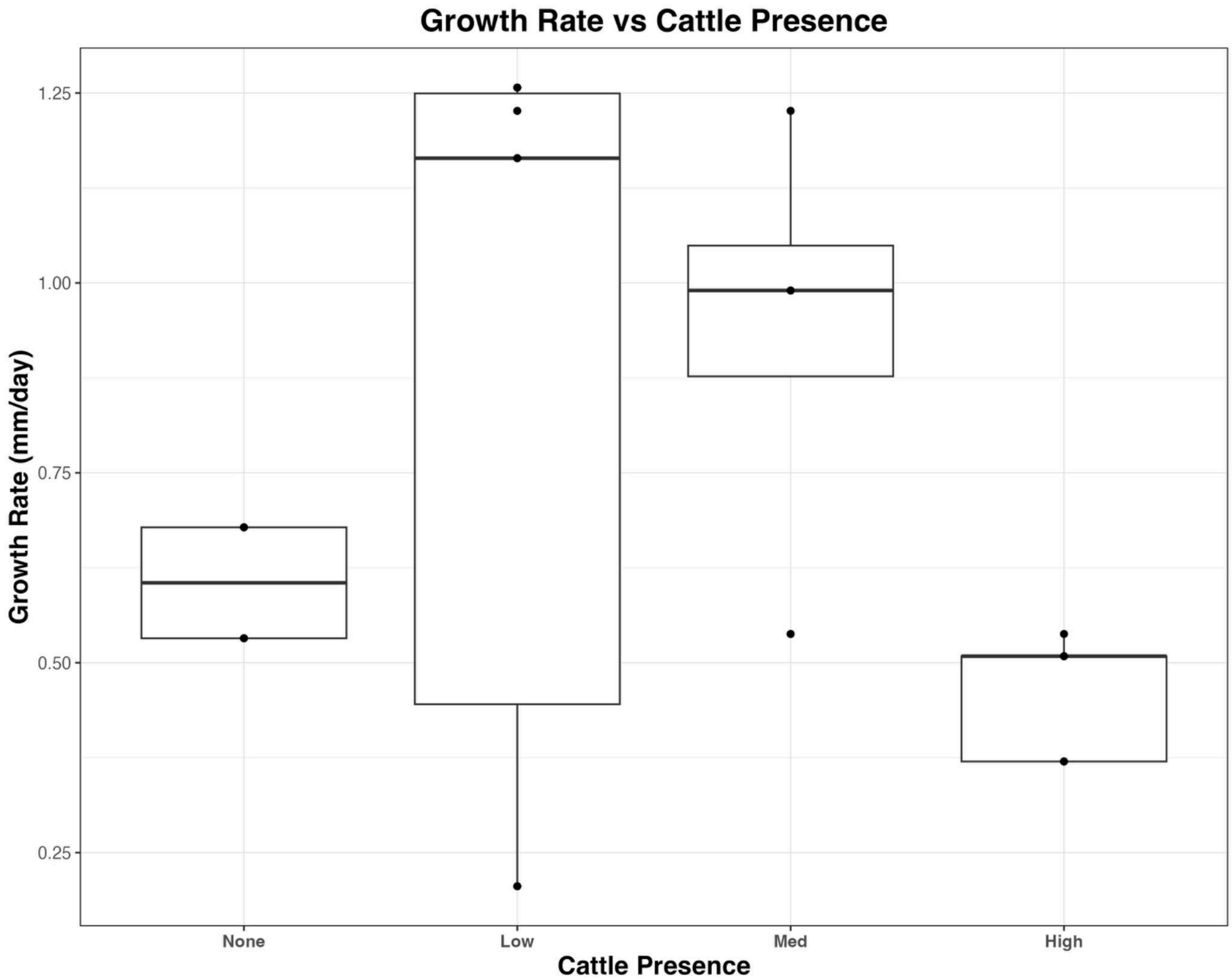

Figure 2: Growth rate of snout-to-vent length by cattle presence. Growth rates in low and medium presence ponds were significantly higher than those in high presence ponds ($p < 0.01$).

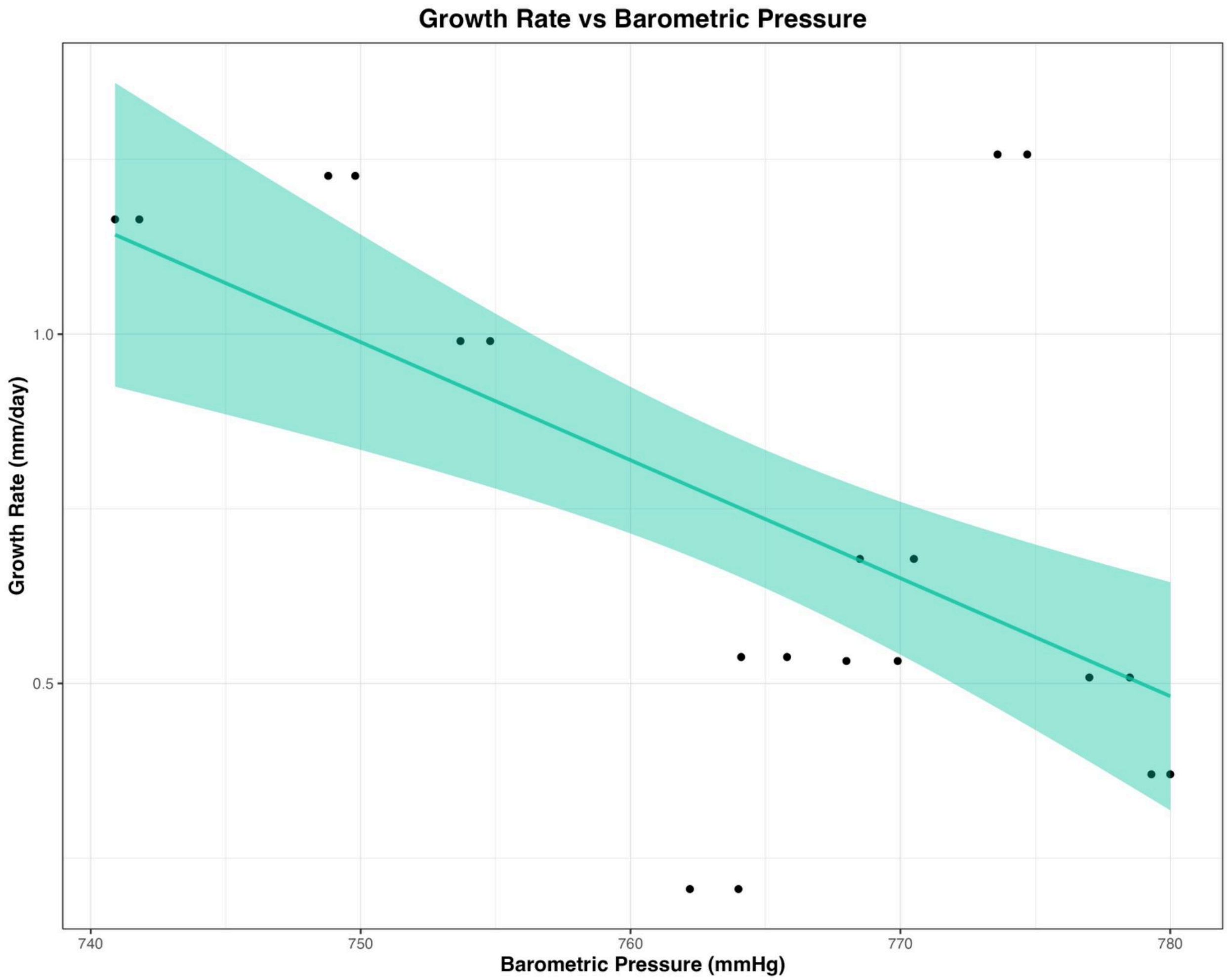

Figure 3: Growth rates are correlated with barometric pressure. The barometric pressure at each pond is analogous to the elevation of each pond. There are two barometric pressure measurements for each pond, but only one growth rate measurement. Each pair of points corresponds to a different pond.

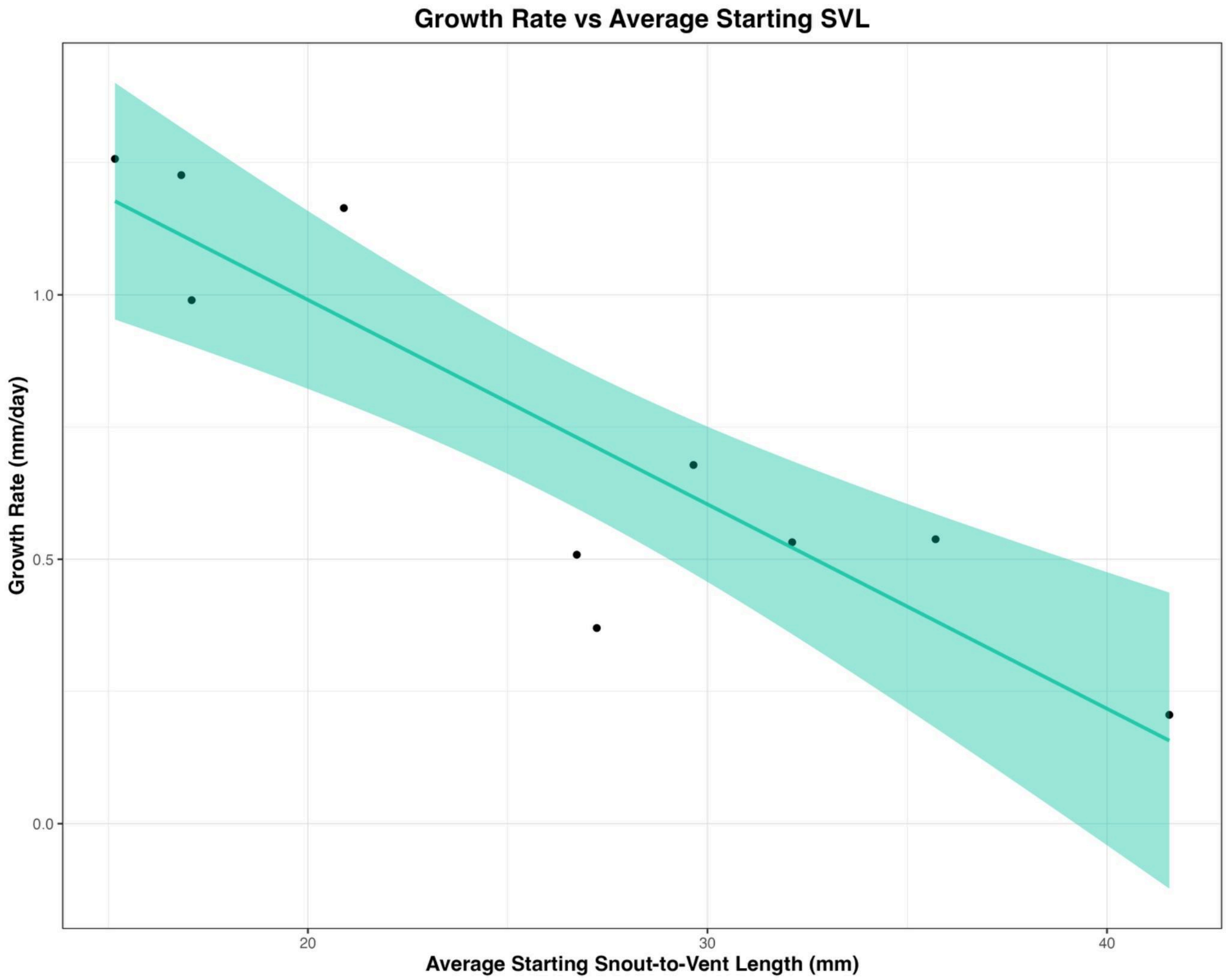

Figure 4: Growth rates are significantly correlated with the SVL of individuals at the start of the season.

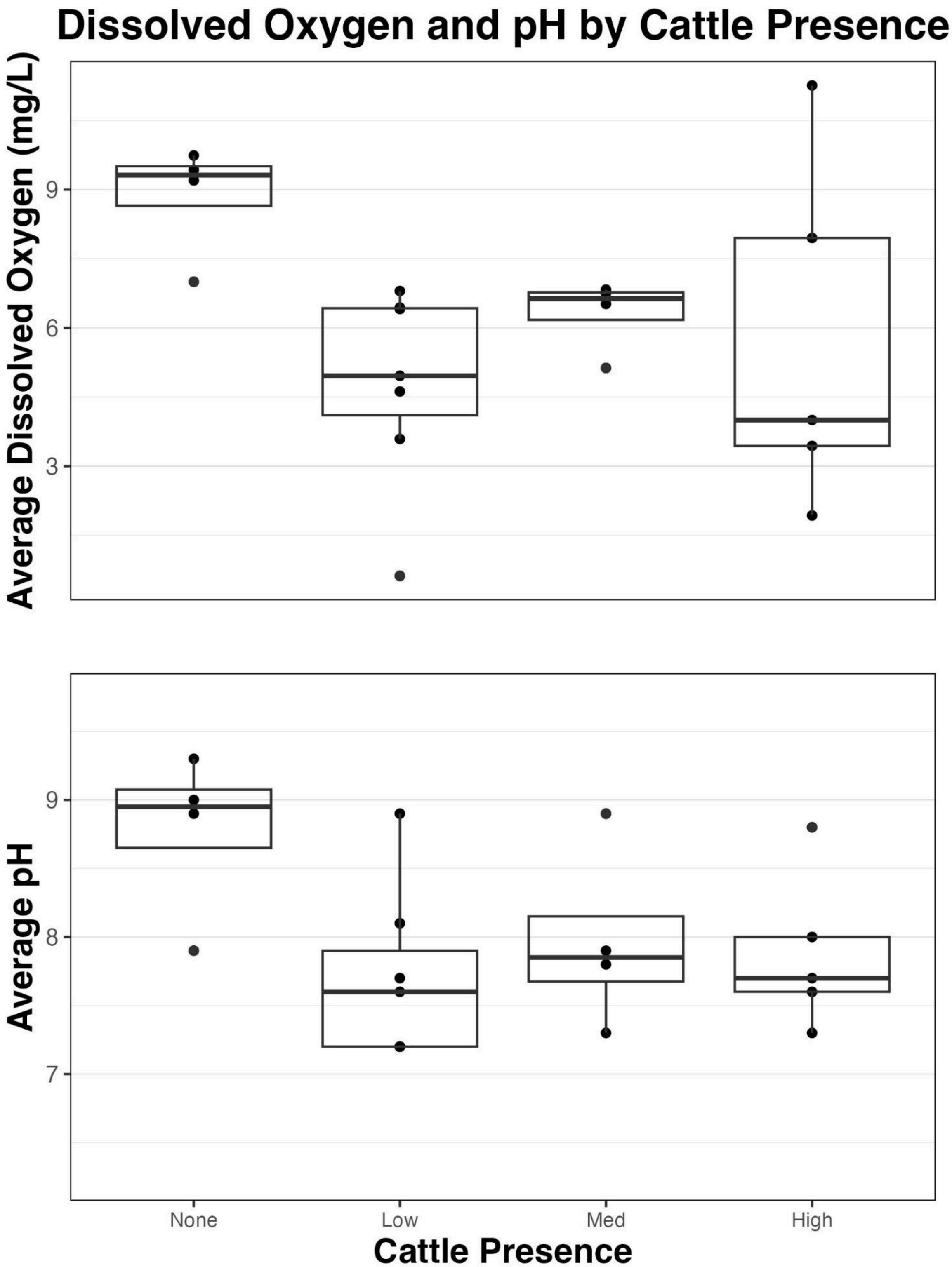

Figure 5: Average pH and average dissolved oxygen from all ponds and sampling periods compared against cattle presence. Both DO and pH show negative trends as cattle presence increases, but neither is a significant correlation ($p > 0.05$).