Metabolic rates of Arizona tiger salamanders in a multipond subalpine basin

Elle Beehler
Mentor: Kelsey Reider
Rocky Mountain Biological Laboratory 2026 Education Program

Abstract

Ectotherms are especially sensitive to climate change because their body temperature, and thus many fundamental body processes, are determined by the temperature of the surrounding environment. Understanding how metabolic rates vary ontogenetically is critical to predicting how climate change will affect amphibian populations. The population of Arizona tiger salamanders (*Ambystoma mavortium nebulosum*) at the Mexican Cut Nature Preserve allows for unique research due to their extended larval stage and the variety of environments they inhabit. We measured the standard metabolic rate of salamanders at multiple stages of development and in ponds that differ in environmental characteristics such as density, hydroperiod, and thermal quality. This study sought to inform how metabolic rate varies with mass and age class or origin pond. Age and pond were not significant predictors of metabolic rate. Mass did predict metabolic rate with the allometric equation $MR = 0.1398 \times M^{0.4432}$ for this population at 10–20 °C. These data can be used to guide future research on metabolism in this population, and predict future fitness consequences of elevated metabolic rates under projected climate change.

Introduction

Ectotherms rely on external conditions to regulate their body temperature, leaving essential core biological processes, including metabolic rates, physiological function, and behavior intrinsically linked to the temperature of their surrounding environment. (Navas et al., 2008). Consequently, ectotherms like amphibians, estimated to be the most threatened vertebrate class (Luedtke et al., 2023), are particularly vulnerable to the shifts in global temperatures caused by climate change (Huey & Berrigan, 2001).

Although they cannot thermoregulate internally, ectotherms achieve thermoregulation behaviorally via selection of microhabitats or timing of activities (Drakulić et al., 2026). For example, an aquatic salamander may achieve its preferred body temperature through its position in the temperature gradient of the water column. However, this may come with trade-offs such as reduced access to prey which live in deeper waters, or exposure to predators at the water surface (Piasečná et al., 2015).

Because metabolism requires continuous energy and sustains fundamental life processes, ectotherms' thermoregulatory behavior is heavily influenced by the need to optimize metabolic rates (Huey & Stevenson, 1979). Therefore, the impact of climate change on organismal metabolic rate has the potential to affect how they balance thermoregulation with other ecological demands such as hunting and avoiding predators (Piasečná et al., 2015). At certain ambient temperatures, this balance may be more difficult to achieve, creating new pressures on ectotherm populations. Hence, understanding an ectothermic population's metabolic rates becomes critical in order to predict its ability to cope with climate change.

Salamanders are an established model clade for studying ectotherm response to climate change and developmental ecology. Previous work with red-backed salamanders (*Plethodon cinereus)* revealed that metabolism increases with both body mass and temperature, and that

certain populations possess some plasticity in standard metabolic rate (Homyack et al., 2010; Muñoz et al., 2022). However, the species used in these studies is completely terrestrial, whereas most amphibians have an aquatic larval stage and some remain entirely aquatic throughout their lives. A study on six-week-old larval spotted salamanders (*Ambystoma maculatum*) found that metabolic rate varied based on population density of their pond, showing evidence of microgeographic evolutionary variation in metabolic physiology (Giery et al., 2021). Although the individuals in this study were in the aquatic larval stage, this species metamorphoses into terrestrial salamanders within the year they hatch. Thus, a critical research gap exists regarding the metabolism of aquatic salamanders and how their metabolic rate changes throughout larval development. This gap is significant because the stability of a population relies on larvae making it to maturity and reproducing, and they face additional pressures which adults do not such as cannibalism, increased sensitivity to temperature changes, and potentially decreased ability to thermoregulate (Dupré & Petranka, 1985; Wissinger et al., 2010).

The Mexican Cut Nature Preserve (MCNP; 39°02'N, 107°06'W, 3640 m a.s.l.) is a subalpine basin in south-central Colorado which is managed for ecological research by the Rocky Mountain Biological Laboratory. The MCNP supports a population of Arizona tiger salamanders (*Ambystoma tigrinum nebulosum*) which have an extended aquatic larval period due to the high-elevation climate (Wissinger & Whiteman, 1992), allowing individuals to be sampled at finer gradations of development relative to lower elevation populations. The MCNP system consists of over 60 ponds with varying sizes and levels of hydrologic permanence, leading to different growth rates and densities despite their geographic proximity (Wissinger et al., 2003). Therefore, two animals could be the same age but significantly different masses, creating a unique opportunity to examine the effects of age and mass as separate factors. The differing pond conditions also present potential for microgeographic evolution of metabolic physiology.

The aim of this study was to understand how mass and age affect metabolic rate, and whether pond of origin influences metabolic rate. We hypothesized that mass would be the main predictor for metabolic rate, and that the relationship between mass and metabolic rate would vary based on age class or pond.

Methods

Study species/population

The Arizona tiger salamander (*Ambystoma mavortium nebulosum*) population at the MCNP is the subject of an ongoing long term mark-recapture study beginning in 1988 which evaluates larval and adult salamanders from June to August every year (Kirk et al., 2026). Salamanders which are large enough are individually marked with unique passive integrated transponders (PIT) tags, which we used to determine each individual's age. To prevent repeated measurements of the same individuals resulting in pseudoreplication, we indicated in the mark-recapture database which PIT tagged animals had been used in our study; any animals too small to receive PIT tags were marked with tail clips. Each animal was captured using a net and careful techniques to avoid disturbing the benthos of the pond or harming the animal. Animals

were held for the minimum amount of time required to complete the metabolism testing and returned to the pond where they were captured.

Ponds in the MCNP system vary in density, hydroperiod, thermal quality, and shade from tree cover. Larger ponds typically have warmer temperatures, at least around their edges, and longer growth seasons than smaller ponds (Kirk et al., 2026), causing a difference in growth rates between ponds. We sampled from ponds L1, L3, L5, L6, L8, L9, and L18 in order to address a variety of pond types (Table 2). We sampled hatchlings (1st year larvae, hatched in 2026), 2nd year larvae, 3rd year larvae, and small paedomorphic salamanders (mature aquatic salamanders). However, because not all ponds contained replicates of each life history stage at the time of sampling (Table 1), sampling decisions were primarily based on obtaining individuals spanning the widest possible range of available masses. We also took pond oxygen measurements using an oxygen probe (Oxygen Dipping Probe DP-PSt3; PreSens, Regensburg, Germany) at multiple points along the edges of ponds L1, L3, L6, L8, and L9.

Measuring metabolic rate

Oxygen consumption rate was used as a proxy for metabolic rate and measured using a respirometry chamber constructed of a 585 mL clear sealing container equipped with an oxygen sensor spot (Oxygen Sensor Spot SP-PSt3-NAU; PreSens, Regensburg, Germany) and connected to an external oxygen meter (Fibox 4; PreSens, Regensburg, Germany) with a polymer optical fiber. The respirometry chamber was filled with water from the trial individual's pond of origin which we filtered to 0.2µm to prevent microbial respiration. We also ran a reference chamber alongside each trial with the filtered pond water in order to account for any remaining microbial oxygen consumption. The filtered water reservoir was aerated for 15 minutes prior to use in either chamber. Both the trial and reference respirometry chamber were equipped with a magnetic stir bar (and a mesh divider to protect the animal from the stir bar) to ensure consistent oxygen concentration across the entire respirometry chamber.

Measurements took place in a WeatherPort tent stationed at the MCNP, which led to varying light levels and temperatures during testing. We placed the respirometry chamber in a large open-top box during data collection, which reduced the amount of movement and light changes noticeable to the salamander in the chamber. We attempted to buffer temperature changes by placing the chamber inside a 2L water bath, however the chamber could only be partially submerged in order to protect the equipment. We determined the temperature mean ± SD of each measurement interval used in the analysis. Salamanders were fasted for 18–24 hours before testing to avoid recording oxygen consumption associated with the energy used in digestion. Each individual was held in the chamber while recording oxygen concentration for 30-75 minutes. We anticipated some levels of stress, which could interfere with our measurements as it would cause the individual to consume more oxygen than they would in a relaxed state. To assess the animal's oxygen consumption at rest only, we recorded any movement by the individual and used data from a ≥10 minute measurement interval in which oxygen consumption was constant and there was no movement. We also never used

measurements from the first 10 minutes of a trial to allow this time to serve as an acclimation period. These measures reduced the chances of recording oxygen consumption due to increased energy usage under stress or during movement. We did not allow the oxygen concentration of the chamber to fall below a predetermined minimum of 50% atmospheric concentration, in accordance with a metabolism study done on closely related spotted salamanders (*Ambystoma maculatum*) (Giery et al., 2021).

Statistical analysis

The linear regression comparing log-transformed wet body mass and log-transformed metabolic rate can be used to determine the allometric equation relating body mass to metabolic rate. This equation is represented as:

$$MR = aM^b$$

MR represents the metabolic rate and M represents the individual's mass, in our study the units for each are mg O_2 per hour and grams, respectively. The y-intercept of the linear regression (after reversing the log transformation) is the mass scaling coefficient, *a*, and the slope of the linear regression is the mass scaling exponent, *b* (Lighton, 2018). We used an Analysis of Covariance (ANCOVA) to compare the slopes and intercepts of the linear models for mass and each age class or pond group, a linear regression to compare oxygen consumption and mass, and a multiple regression to compare oxygen consumption with mass and temperature.

Results

Using an ANCOVA, we found no significant difference in the mass scaling exponent ($F = 1.1402$, $df = 3$, $p = 0.3426$) or the mass scaling coefficient ($F = 1.1402$, $df = 3$, $p = 0.3426$) of the allometric equation for any age classes (Figure 2). We also found no significant difference in the mass scaling exponent ($F = 1.3693$, $df = 1$, $p = 0.2473$) or mass scaling coefficient ($F = 0.8746$, $df = 1$, $p = 0.3539$) of the allometric equation for any ponds (Figure 3).

A linear regression predicting oxygen consumption from mass was statistically significant ($F[1, 54] = 325.1$, $p < 0.001$, $R^2 = 0.8575$). The linear equation was $y = 0.62235x – 0.8538$ (Figure 4). By using the slope as the mass scaling exponent, and reverse log-transforming the intercept for the mass scaling coefficient, this model gives the allometric equation:

$$MR = 0.14 \times M^{0.62235}$$

Although we attempted to buffer temperature changes, the temperature during the trials ranged from 10.6 to 20.6 °C, with an average of 15.9 °C (Figure 5). The maximum standard deviation of the temperature during the interval which resting oxygen consumption data was taken from was 0.511°C, with the mean standard deviation being 0.08°C. We ran a multiple regression with mass and temperature, and found that temperature had no significant effect on the mass scaling exponent ($F = 1.4318$, $df = 1$, $p = 0.23690$), but it did have a significant effect on the mass scaling coefficient ($F = 5.5808$, $df = 1$, $p < 0.05$), with higher temperatures having a higher intercept (Figure 6).

Oxygen measurements of ponds L1, L3, L6, L8, and L9 ranged from 17.91–23.76% oxygen saturation, with an average of 21.50% oxygen saturation and a standard deviation of 1.25% oxygen saturation. The oxygen saturation for L1 was 21.89 +/– 0.47%, 20.66 +/– 0.29% for L3, 19.275 +/– 0.72% for L6, 22.59 +/– 0.80% for L8, and 21.83 +/– 0.56% for L9 (Figure 7). The average starting oxygen saturation for our trials was 17.98%, and the lowest final oxygen concentration out of all trials was 54% the concentration of atmospheric conditions.

Discussion

We found that the metabolic rate of Arizona tiger salamanders at the MCNP at 10 – 20 °C can be estimated as a function of mass using the allometric equation $MR = 0.14 \times M^{0.62235}$, which explains about 86% of the variation in metabolic rate. Age class and pond do not seem to have an effect on metabolic rate, despite significant differences in oxygen concentration between certain ponds. Different oxygen concentrations between ponds could be due to variations in temperature, sunlight, and biological activity.

In a study on microgeographic evolution of metabolic rate, the mass scaling exponent remained constant between metapopulations, but the mass scaling coefficient varied (Giery et al., 2021). Our results similarly found an invariant mass scaling exponent, but contrasted in that they did not find variation in the mass scaling exponent. This could suggest that the ponds in this study were not different enough to induce microgeographic evolution, or that other factors in this environment prevent this population from experiencing microgeographic evolution as strongly. Additionally, Giery et al. found a mass scaling exponent of 0.87 for the spotted salamanders in their study (2021), while the Arizona tiger salamanders in this study had a mass scaling exponent of 0.62235. This decreased mass scaling exponent aligns with the slower growth rates of salamanders at the MCNP.

We also found that an increase in temperature correlates with an increase in the mass scaling coefficient, and thus an increase in metabolic rate. The relationship between temperature and metabolic rate could explain the approximately 14% variation in metabolic rate not explained by mass, but experiments more precisely controlling temperature or manipulating temperature would be required to fully understand this. Although there was a wide variation in temperature across trails, within the constant interval we took measurements from the temperature changed very little.

These findings inform future research on the metabolic rates of this population by showing that age class and pond do not affect metabolic rate, suggesting that mass should be the main covariate. However, this only applies to aquatic salamanders; further research would be required to understand potential variation in the metabolic rate trends of metamorphic or cannibal morph salamanders. In addition, Arizona tiger salamanders have very large geographic and elevational range (Shaffer & McKnight, 1996), so studies sampling across populations are necessary to fully understand this species.

These baseline data synthesized with future research on how metabolic rates in this population respond to temperature changes — or potentially even existing research on how other

salamander species' metabolic rates are affected by temperature changes — could be used to predict how this population's metabolic rates and thus population viability might respond to climate change. Those projected rates could have further implications regarding thermoregulatory behavior and trade offs, as well as habitat expansion or constriction.

Acknowledgements

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Figures/Tables

Table 1. Number of individuals from each pond and age class in this study. H: Hatchlings/first year larvae, hatched this season. L2: second year larvae, hatched one year ago. L3: third year larvae, hatched two years ago. P: Paedomorphic adults, mature aquatic salamanders. Pond descriptions in Table 2. No entry means zero individuals.

	Pond L1	**Pond L3**	**Pond L5**	**Pond L6**	**Pond L8**	**Pond L9**	**Pond L18**	**Total**
H	-	3	-	6	12	6	-	27
L2	5	4	3	-	-	-	-	12
L3	6	3	1	-	-	-	1	10
P	-	6	-	-	-	-	1	7
Total	11	16	4	6	12	6	2	56

Table 2. Descriptions of all ponds sampled in this study. Pond identification numbers are from the long term studies at the MCNP. Permanent ponds have been aquatic for at least the past 50 years, while semipermanent ponds may or may not dry out in a given year. Paedomorphic salamanders are fully aquatic, while metamorphic salamanders are mostly terrestrial and return to the ponds to breed. See Figure 1 for a map of ponds.

Pond	**Relative size**	**Hydroperiod**	**Demographic majority**
L1	Large	Permanent	Paedomorphic
L3	Small	Permanent	Paedomorphic
L5	Medium	Permanent	Paedomorphic

L6	Small	Semipermanent	Metamorphic
L8	Medium	Semipermanent	Metamorphic
L9	Small	Permanent	Metamorphic
L18	Small	Permanent	Paedomorphic

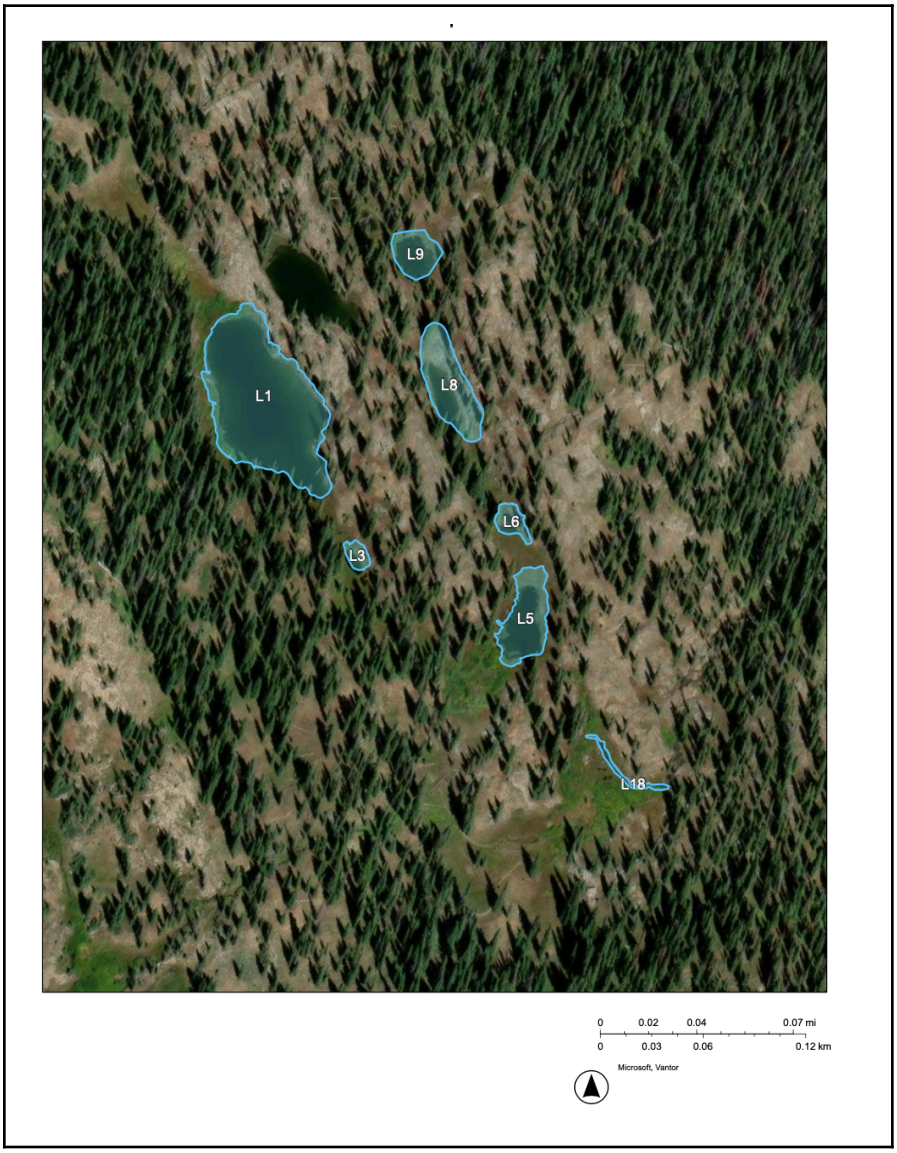

Figure 1. All ponds sampled in this study. See Table 2 for descriptions of each pond.

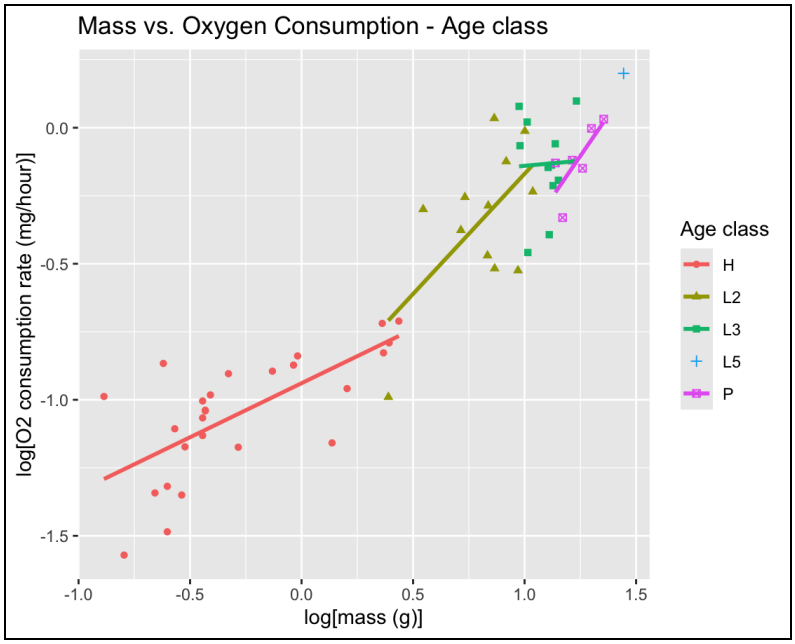

Figure 2. Full data, color/shape coded by age class. No significant difference in slope (ANCOVA: F = 1.1402, df = 3, p = 0.3426) or intercept (ANCOVA: F = 1.5447, df = 4, p = 0.2037) between any age classes.

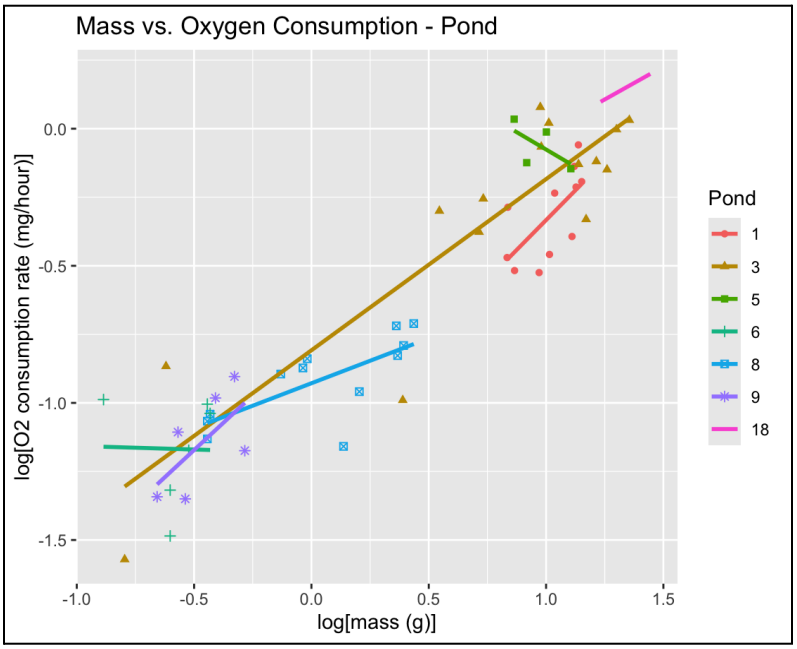

Figure 3. Full data, color/shape coded by pond. No significant difference in slope (F = 1.3693, df = 1, p = 0.2473) or intercept (ANCOVA: F = 0.8746, df = 1, p = 0.3539) between any ponds.

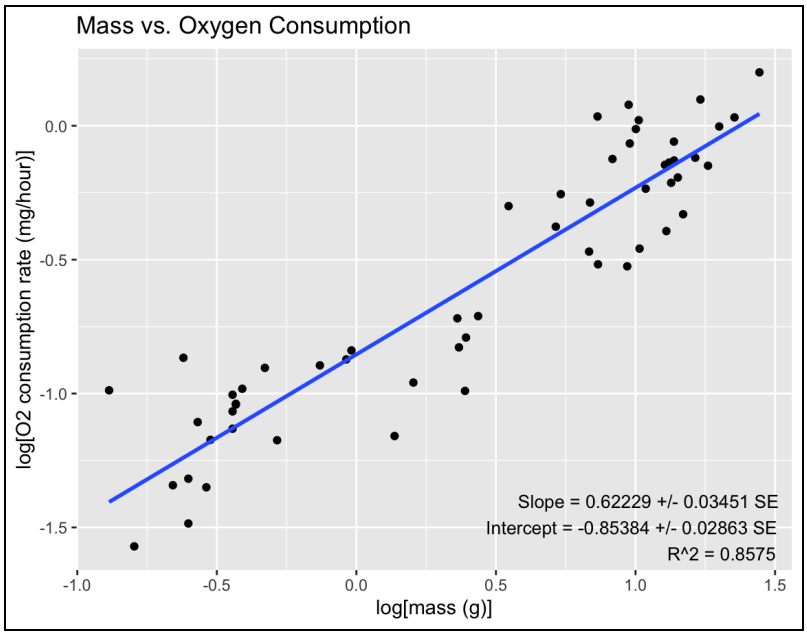

Figure 4. Full data comparing mass and oxygen consumption rate. Mass and oxygen consumption rate are significantly correlated ($F[1, 54] = 325.1$, $p < 0.001$, $R^2 = 0.8575$).

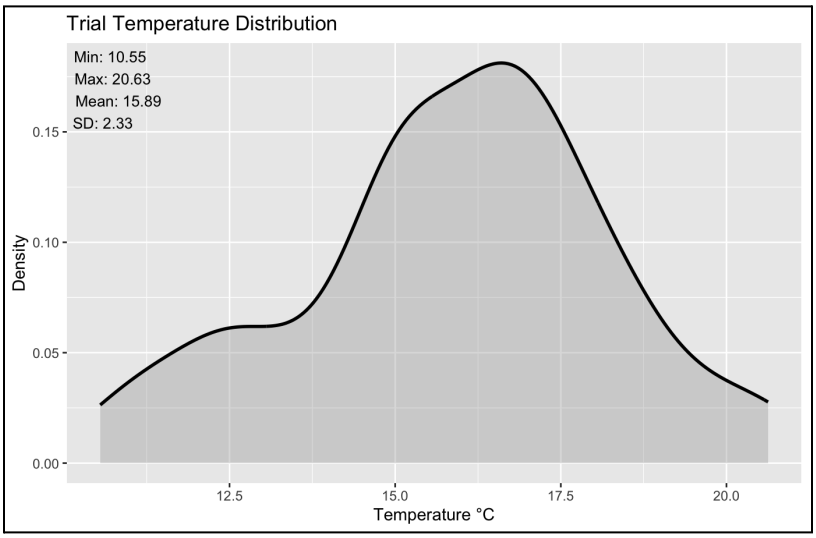

Figure 5. Distribution of average temperature during interval of constant oxygen consumption within trials.

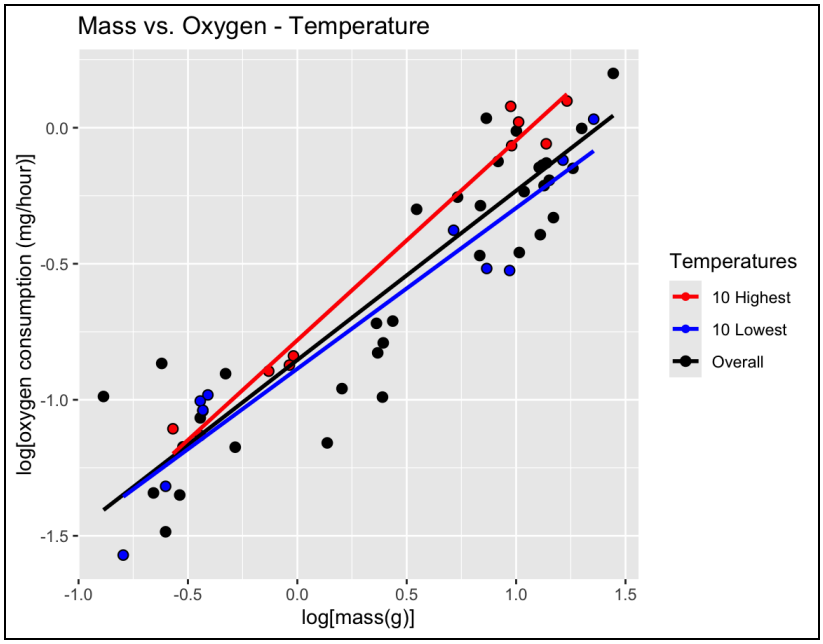

Figure 6. Overall data color coded by temperature groups: 10 highest trial temperatures, 10 lowest trial temperatures, and all temperatures. Temperatures had a significant effect on the intercept (multiple regression: $F = 5.5808$, $df = 1$, $p < 0.05$).

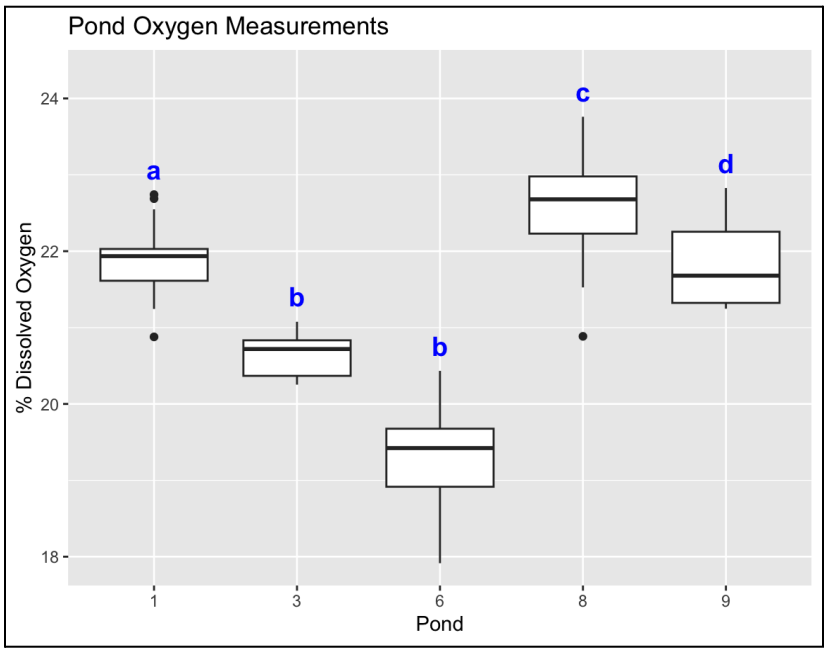

Figure 7. Pond oxygen concentration measurements.

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