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Effects of warming and drying on the survival and performance of giant salmonfly nymphs (Pteronarcys californica)

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Abstract

Rising temperatures and anthropogenic influences threaten to alter aquatic ecosystems as oxygen supply and demand is altered depending on temperature, flow, and water presence. In late November of 2021, a dam malfunction occurred on the Madison River in Montana that caused the river to dry rapidly downstream of the dam and reduced flow by 70% for 50 hours. My experiments were designed to determine the effects of this event by assessing how long giant salmonfly nymphs (*Pteronarcys californica*) can survive in still water and in air depending on temperature and relative humidity. In the laboratory, we exposed salmonfly nymphs to four temperatures (5, 12.5, 20, and 25 °C) in still water. We measured survival, feeding, and growth, as well as time spent performing 'push-ups' – a common respiratory movement that ventilates gills and increases oxygen supply rates. My study demonstrates that increases in temperature significantly increase the rate of pushups performed and decrease the survival of salmonfly nymphs. Next, we exposed nymphs to air – representing extreme dewatering – at five temperatures (5, 12.5, 20, 25, and 35 °C) and two relative humidities (75% and 100%) to measure their survival. Survival times declined at higher temperatures, particularly in lower humidity, with an average survival of less than a day. Notably, the most significant factor influencing salmonfly survival was the type of medium they were in, as most of the nymphs in still water were able to survive the duration of the two weeks, while all the nymphs in air died.

Introduction

Climate change and other anthropogenic influences are warming streams and increasing drought frequency and severity (Isaak et al. 2012). We currently know little about how these changes are affecting aquatic invertebrates. Because aquatic invertebrates are ectotherms, physiological rates are largely controlled by the temperature of the ambient environment (Isaak et al 2012). Indeed, warming temperatures increase metabolic demand (Jacobsen 2020, Frakes et al 2021), and, if severe, can cause rates of oxygen demand to exceed rates of supply, leading to lower performance and potentially, death (Verberk and Bilton 2011).

However, ratios of oxygen supply and demand also depend on flow. Flow increases the rate of oxygen delivered to insect respiratory surfaces and higher flows can help invertebrates tolerate higher temperatures and deeper hypoxia (Frakes et al. 2021). No-flow conditions brought on by warming temperatures, de-watering, and drought could restrict the transport of oxygen to aquatic nymphs and negatively affect their survival. Balancing oxygen supply and demand poses a key challenge to aquatic nymphs (Frakes et al. 2021). Oxygen concentrations and diffusion coefficients are much lower in water than in air, leading to low rates of environmental oxygen supply. For nymphs, this oxygen problem is often exacerbated at warm temperatures and low flows. While warming decreases the solubility of oxygen in water, it also increases the rates of oxygen supply by decreasing water viscosity and increasing diffusion coefficients (Woods 1999; Verberk et al. 2011). However, warm water also strongly increases the metabolic demand of aquatic nymphs, such that oxygen supply can no longer meet the demand (Frakes et al. 2021). Temperature-induced functional hypoxia can have profound effects on aquatic organisms and lead to reduced performance and, eventually, death (Portner & Knust 2007). Flow relates to heat and hypoxia by promoting gas exchange between atmosphere and water (increasing oxygen concentrations in water) and between water and the respiratory surfaces of nymphs. In warm water, higher flows can offset low oxygen supply and high demand by increasing oxygen transport across thinner boundary layers (Frakes et al 2021).

Anthropogenic activities such as water diversion and dam construction are substantially modifying the natural hydrology of streams and rivers and can impact rates of oxygen supply

(Walters and Post 2011). The dam malfunction that occurred on the Madison River, on November 30th, 2021, is an example of a human caused disturbance that may alter aquatic communities. There was a mechanical error in the dam that caused rapid drying downstream as well as a 70% instantaneous flow reduction that lasted 50 hours. The Albertson Lab at Montana State University is conducting a study on these effects by examining how de-watering affects the quantity and timing of nymphs emerging from the Madison over multiple years, and my research has been done collaboratively with them.

My work expands on the Albertson Lab study by testing how temperature affects the performance and survival duration of salmonfly nymphs during no-flow and dry conditions. My study will provide valuable information for conservation by determining how severe droughts and dewatering may affect salmonflies in the future depending on the temperatures when the events occur. We examined how long salmonflies can live in dry (i.e., air) conditions as well as no-flow conditions in the laboratory at different temperatures for two weeks. We measured performance as rates of feeding and growth before and after the experiment. We also measured how much time nymphs devoted to push-ups, which involve repeatedly moving their bodies up and down or side to side to ventilate their gills and to get more oxygen (Malison et al. 2022). Therefore, we expected the nymphs to perform more push-ups at higher temperatures so that they would receive more oxygen when their metabolic demand increased with temperature. Push-ups are an important aspect of salmonfly behavior since they provide short-term feedback on the organism's perception of its local oxygen availability.

Methods

For the no-flow experiment, 60 salmonfly nymphs of different sizes were collected from Rock Creek at mile marker 1, on October 4th, 2022, by lifting rocks and kicking the sediment to wash nymphs into a net. Only 48 nymphs were used for the experiment, but we collected extras in case of pre-experiment fatalities. The nymphs were acclimated for 24 hours at 12.5 ℃ – the average temperature of the river at the time of collection – in a 5-gallon bucket. Water was aerated with bubblers to provide flow and keep the water at 100% air saturation. After acclimation, nymphs were moved to 20-gallon plastic containers filled with water to experience one of four no-flow temperature treatments (5, 12.5, 20, and 25 ℃) with 12 nymphs per treatment (i.e., 48 nymphs total). Nymphs were separated from each other within treatments by placing each individual into a mesh bag closed by a labelled zip tie. Nymphs of different sizes were each weighed and distributed evenly across treatments. One conditioned leaf (i.e. soaked in stream water for two weeks) was also weighed and placed in each bag as a food source. Each treatment had one extra bag with a control leaf (no salmonfly) to account for effects of bacterial decomposition and temperature on changes in masses of leaves.

Methods for controlling temperature differed by treatment. One container was cooled to 5 ℃ in a refrigerator. Another was cooled to 12.5 ℃ using a recirculating water bath where cold water from the machine was pushed through tubing and coils placed through the container to cool the water. The 20 ℃ treatment was established at room temperature. The final treatment was warmed to 25 ℃ using a recirculation water bath. Water was held at ~100% of air saturation by pumping air through plastic tubing which wound through the bottoms of each container. Holes in the tubing let small streams of bubbles into the water but without creating significant amounts of flow or turbulence. Before the experiment was started, we tested whether the bubbles in the containers indeed produced minimal flow by adding food coloring to each one and monitoring how rapidly the dye spread over time. We observed negligible movement of the food dye, and therefore considered flow to be functionally zero.

Rocks were placed along the tube to hold it down. We also used an Onset HOBO temperature logger to record water temperatures every ten minutes in each treatment container. To mimic daylight, one lamp was set up in the refrigerator and another near the other three treatments on the lab bench and timed to turn on at sunrise and turn off at sunset.

For the dry experiment, we collected 120 salmonfly nymphs from the same area as above on February 14th, 2023. Nymphs were then acclimated for one week in 12 ℃ water, the acclimation temperature of the no-flow experiment. Ten 5-gallon buckets, which would eventually hold the nymphs, were filled with 2.4 L of water (1/8 of the total volume of each bucket). Five of the buckets had only water in them, holding air at 100% relative humidity. Water in the other five buckets were fully saturated with NaCl following the methods of Winston and Bates (1960), which held relative humidities at 75%. Hobo humidity loggers were placed in one bucket from each of the treatments to confirm the relative humidities to be near 100% and 75%, as intended. After acclimation, nymphs were divided into three groups by size: small, medium, and large and were evenly distributed among the two humidity treatments (75% and 100% relative humidity) at five different temperatures (5, 12.5, 20, 25, and 35 ℃) for a total of 10 treatments. Individuals were then placed in meshed bags marked by their temperature and humidity treatment numbers. To ensure nymphs did not fall into the water at the bottom of the buckets, mesh was taped inside the top of each of the buckets. Buckets were then placed into temperature-controlled incubators, (one of each humidity per incubator) and the incubators were set to a 12-hour diurnal light cycle. There was insufficient incubate space available, so the 20 ℃ buckets were placed in water in a 20-gallon plastic container that was kept at 20 ℃ using a recirculating water bath.

Data Collection

For the no-flow experiments, we observed nymphs multiple times per day between 8 AM and 8 PM for 2 weeks. When dead individuals were found, body masses and masses of the leaves were weighed. We checked temperatures (with a thermocouple; BarNant) and oxygen saturation levels (with an oxygen optode connected to a meter; FireSting Pyroscience) once a day to ensure they were at the appropriate levels. Each day, how many seconds per one minute four nymphs in each treatment performed push-ups was measured by observing a different subset of four individuals each day. At the termination of the experiment (two weeks), all surviving nymphs and leaves were weighed. Feeding and growth were measured by subtracting initial from final masses of both nymphs and leaves.

For the dry treatment, we observed how long 12 salmonfly nymphs survived in no water in each temperature (5, 12.5, 20, 25, and 35 ℃) and relative humidity (75% and 100%) treatment. As above, the duration of survival of nymphs in each treatment was determined by checking for movement multiple times a day between 8 AM and 11 PM. If they were not moving, we picked them up to make sure they were deceased. Dead individuals were dried in ovens at 60 ℃ for three days and then weighed.

Data Analysis

We analyzed the data using different models in R Studio version 2023.03.0 implementing R version 4.2.3. For the pushups, we used a linear mixed effects model implemented in package *nlme*. The fixed effects were the temperatures of each treatment, and the mixed effects were the individuals' responses, as we measured four different individuals from each treatment every day. Survival data from the no-flow experiment were analyzed using logistic regression and total hours survived were analyzed using censored regression (package *censReg*) since the experiments were ended after two weeks regardless of whether nymphs were still alive. To analyze the effects of temperature on growth and feeding, we used linear regression models. We also used linear regression for analyzing the effects of humidity and temperature on salmonfly nymph survival in air. To measure whether mass had significant effects on the total hours survived, we used a censored regression model for the nymphs in water and a linear regression model for the nymphs in air. Most data points in the no-flow experiment were censored since the experiment ended after 311 hours, and 34 nymphs survived the entire experiment. To account for this, we fit a censored regression model to the data. To validate these results, we also fit a linear regression model.

Results

No-Flow Experiments in Water

Survival: A total of 14 of 48 nymphs died, with at least one death in every treatment. Most of the deaths, however, occurred in the 25 °C treatment (Figure 1). Temperature had a significant effect on survival, with higher nymph mortality at higher temperatures ($P = 0.049$) (Figure 1). There was also a significant effect of temperature on total hours survived ($P = 0.007$). As temperature increased, survival duration decreased (Figure 2). The model only had information up to 311 hours so anything beyond that was predicted using the known data. Together, both models (linear regression and censored regression) suggest that survival times were lower at high temperatures. Mass was not a significant factor on salmonfly survival ($P = 0.80$) or duration of survival ($P = 0.68$**).**

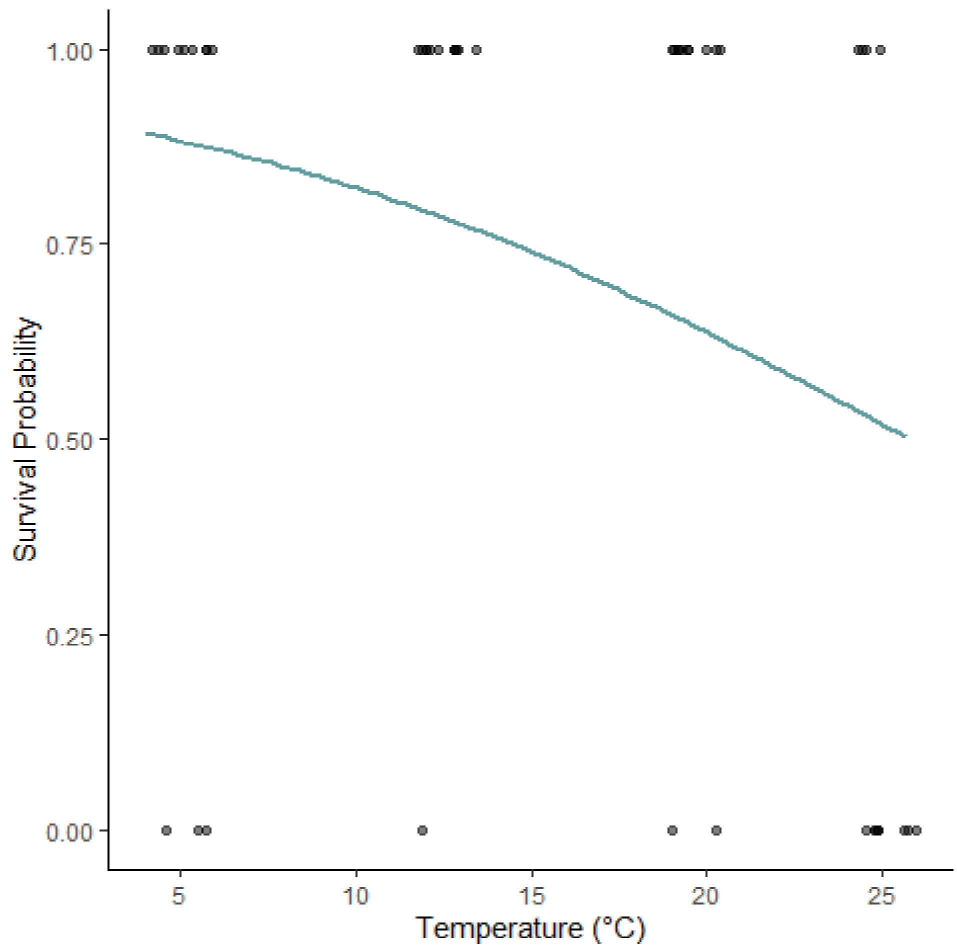

Figure 1: Survival of salmonfly nymphs across temperature treatments over the span of 2 weeks. Curve represents the fitted logistic regression model.

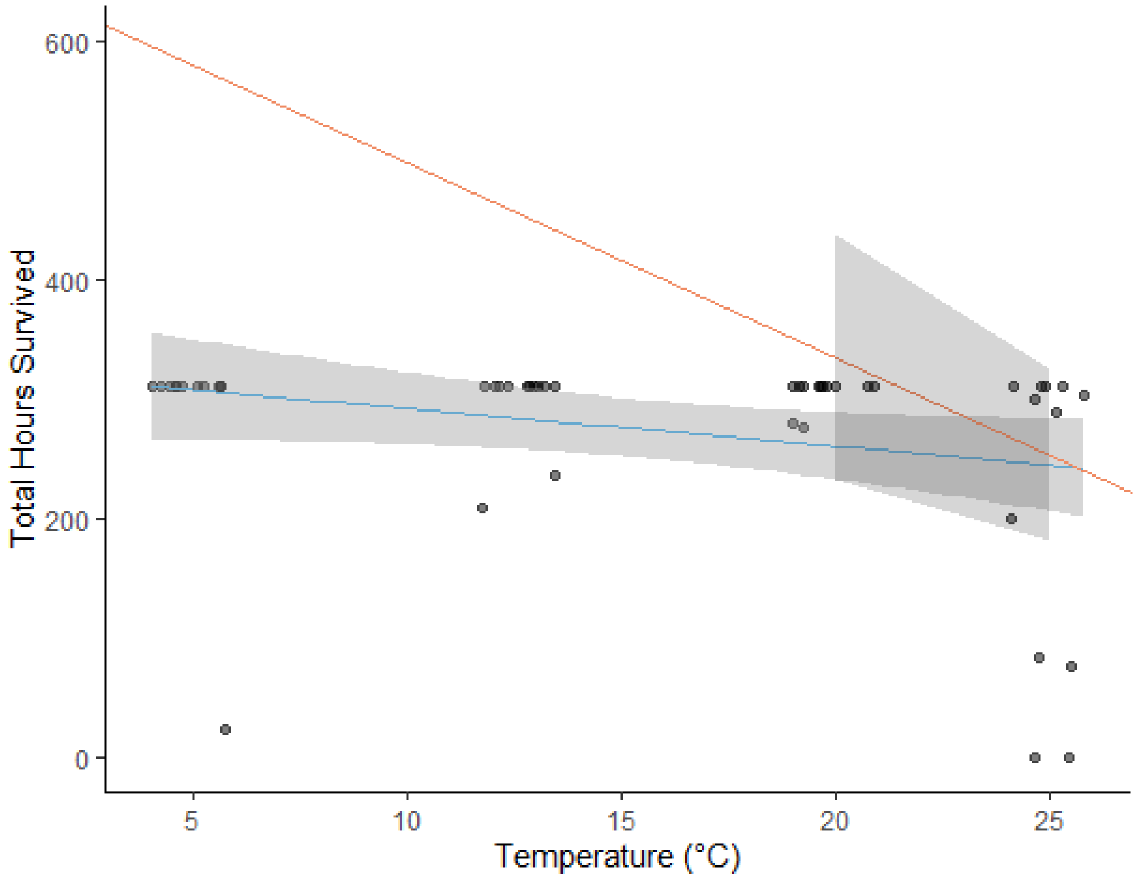

Figure 2: Duration of hours nymphs survived at five temperatures. Blue line is fitted linear regression model and red line is the fitted censored regression model. Shading is the standard errors of each model.

Pushups: Temperature significantly affected the rate of pushups performed ($P < 0.0001$), with nymphs performing more pushups at higher temperatures (Figure 3) with the average time spent doing pushups in the 5 ℃ being 1 second and in 25 ℃ the average being 14.2 seconds.

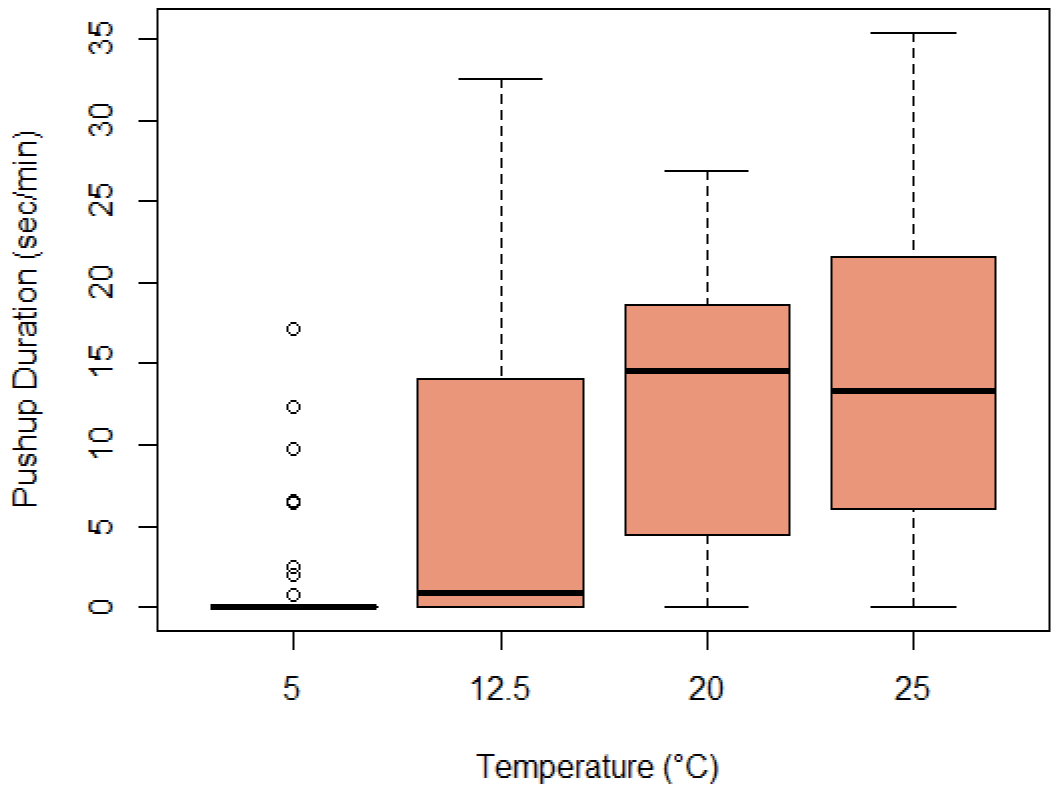

Figure 3: Duration of seconds at which salmonfly nymphs performed respiratory movements (pushups) in a minute.

Feeding and Growth: Analyzing data on growth (of only nymphs that survived) showed that there was no significant effect of temperature on the growth of nymphs ($P = 0.70$). Dead nymphs were excluded from analyses because they swell rapidly with water after death. Temperature also did not significantly affect leaf consumption of the living nymphs ($P = 0.41$). Although not significant, the data on feeding appeared to follow a thermal performance curve with feeding reaching its maximum around 18 °C (Figure 4). Mass of the alive nymphs at the start did significantly affect their growth ($P = .008$) but not their feeding ($P = 0.26$). The nymphs that were smaller grew more than the large nymphs and the large nymphs lost more body mass (Figure 5).

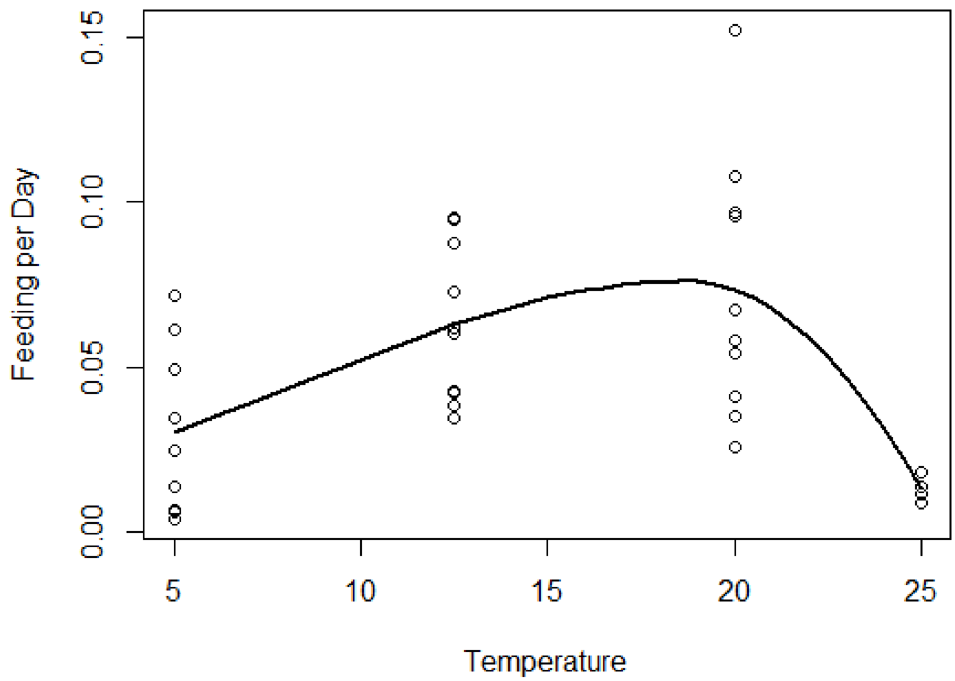

Figure 4: Thermal performance curve for the rate of feeding of the 34 nymphs that survived.

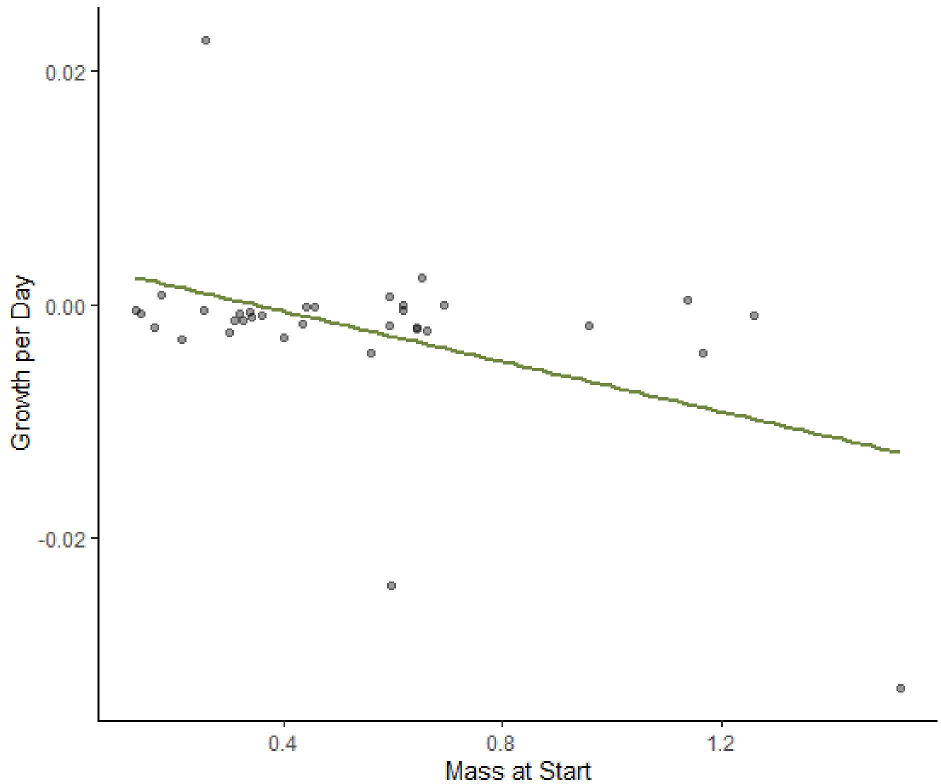

Figure 5: Growth of different sizes of nymphs.

Dry Conditions

Survival: Temperature and humidity both significantly affected survival duration, as did their interaction (Table 1). Nymphs survived longer in 100% relative humidity than in 75% relative humidity at the same temperature (Figure 6). Nymphs also survived longer as temperature increased (Figure 6). Nymphs survived the longest at the lowest temperature (5 °C) at 100% relative humidity (Figure 6). At 35 °C, they survived only a few hours, regardless of relative humidity (Figure 6). Temperature affected survival duration more profoundly at 75% than at 100% relative humidity (significant interaction term in Table 1). Starting body mass did not significantly affect their survival (P = 0.11).

	Degrees of Freedom	Sum of squares	F value	P value
Temperature	1	21921.8	131.749	< 2.2e-16

Humidity	1	9298.9	55.886	1.585e-11
Temp:Humidity	1	4024.8	24.189	2.906e-06

Table 1: ANOVA table for the linear regression model used to analyze the survival duration.

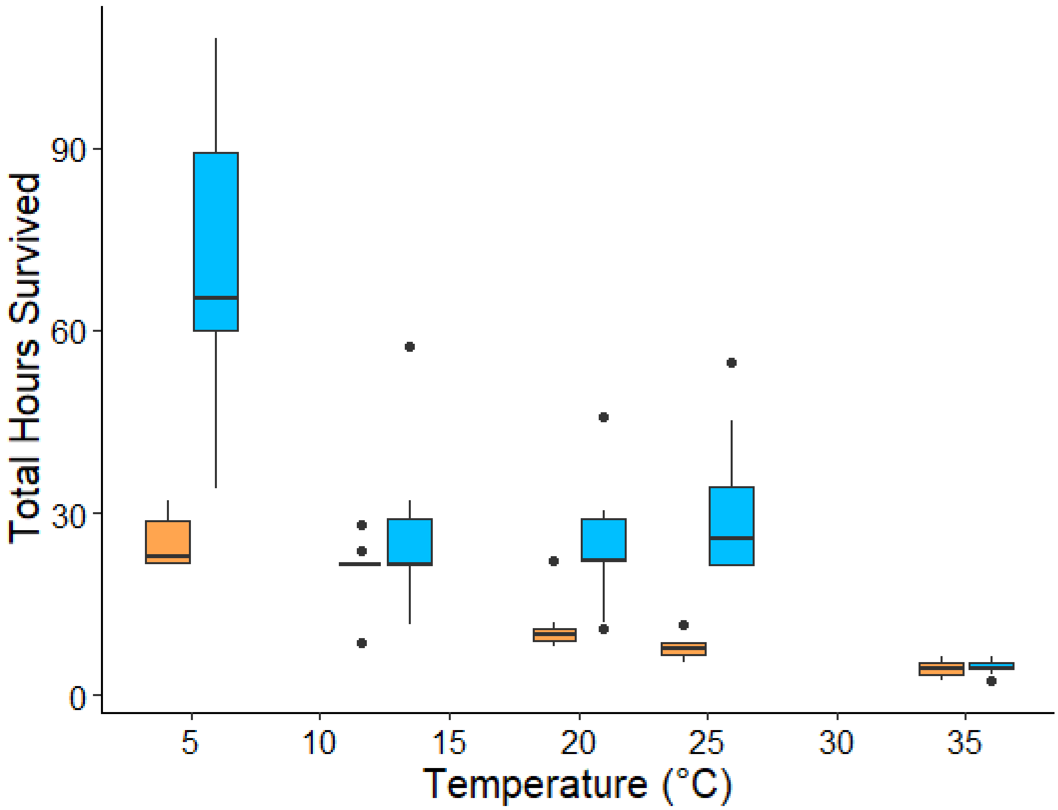

Figure 6: Boxplot showing survival duration of salmonfly nymphs exposed to either 75% RH (tan) or 100% RH (blue) at five temperatures. Boxes are box-and-whiskers, showing median, interquartile range (IQR), and 1.5x (IQR). Individual dots are possible outliers.

Survival in Air versus Water

Although the experiments were carried out at different times on different individuals, the temperature treatments between experiments (except 35 ℃) were the same. The 35 ℃ individuals were removed and the two relative humidities (75% and 100%) were combined for data analysis. We therefore were able to compare survival times of individuals in air versus water. Both temperature and environment type were significant and interacted to affect survival (Table 2). Environment type had a larger effect on survival than temperature did (Table 2). The nymphs survived longer in the water than in air at the same temperature (Figure 7). All the nymphs died in air within four days, whereas 70% of the nymphs survived for the two-week duration in water. Their survival time also decreased as temperature increased (Figure 7).

	Degrees of Freedom	Sum of squares	F value	P value
Temperature	1	84691	26.289	9.596e-07
Environment	1	1636423	507.973	< 2.2e-16
Temp:Humidity	1	47930	14.878	0.0001743

Table 2: ANOVA table for the linear regression model used to analyze the significance of temperature and environment on total hours survived.

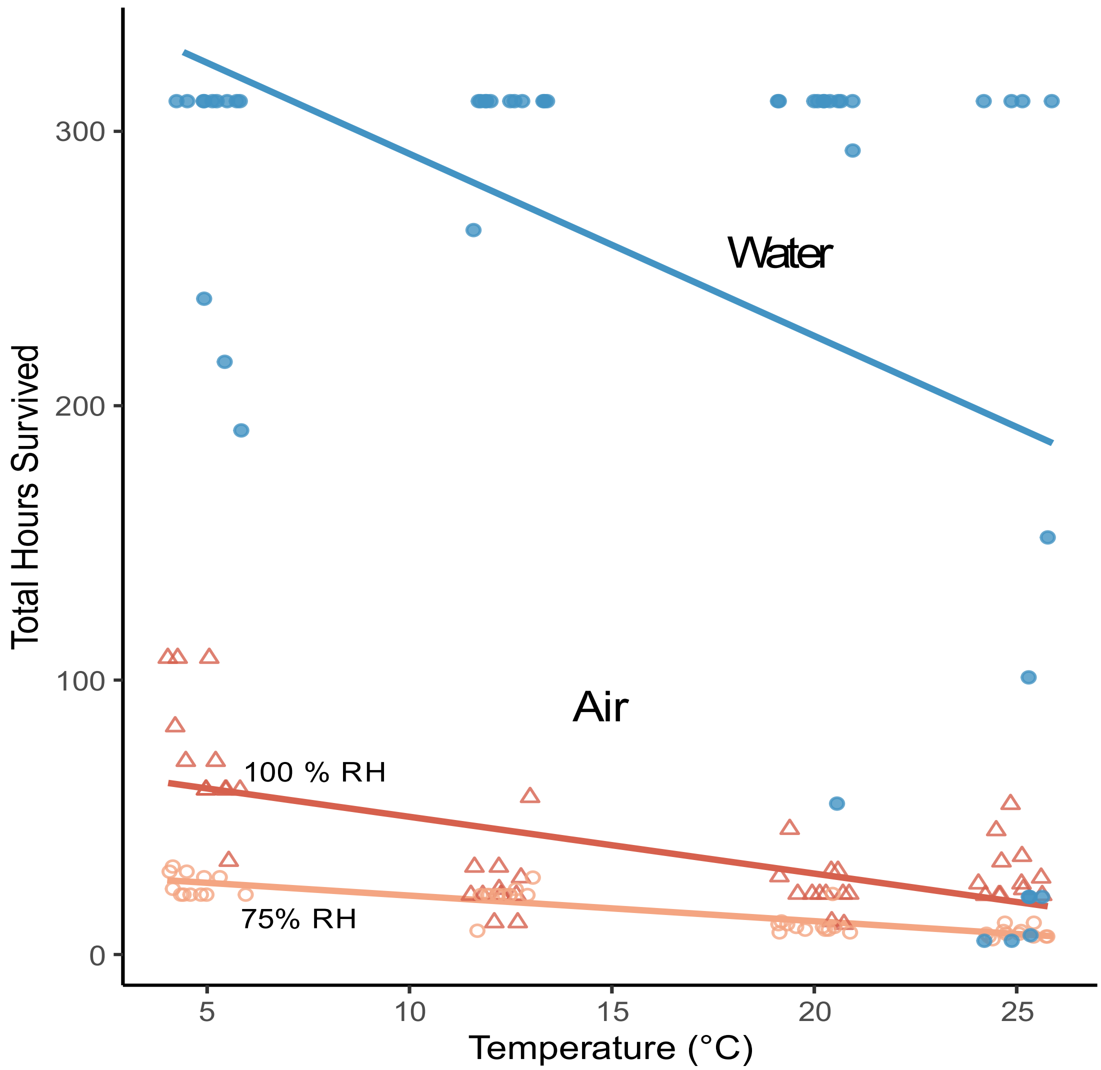

Figure 7: Survival duration (hours) of nymphs in water and in air.

Discussion

Aquatic invertebrates are crucial members of rivers and streams and understanding how they are impacted by de-watering, climate change, and anthropogenic disturbances will be crucial to forecasting species vulnerabilities and prioritizing conservation strategies (Birrell et al. 2020). Salmonfly nymphs, which are widespread across the western United States, are particularly important because they make up a large percentage of trout diets, increase nutrient cycling by shredding leaves, and move substantial amount of carbon into terrestrial ecosystems during their large, synchronous emergences (Walters et al. 2018; Albertson et al. 2022; Nehring et al. 2011). Their emergences are also followed closely by fly-fishers because they stimulate aggressive feeding behavior by trout (Nehring et al. 2011). Despite their importance, salmonflies are undergoing local declines and extirpations, and they may be disproportionately affected by climate change and de-watering (Anderson et al 2019, Birrell et al. 2019). Indeed, the temperature of air as well as rivers is increasing globally and is predicted to continue increasing (Liu et al. 2020). Rivers are also being altered from reduction in flows caused by increased aridity, water withdrawal, and river damming (Overpeck and Udall 2020). This is concerning since the results of my research established that drought, reduction in flow, and increasing temperatures reduce salmonfly performance and survival.

Data from this study support the idea that temperature and flow play a role in oxygen limitation in water which affects aquatic insects (Frakes et al. 2021). The results suggest that the dam malfunction in the Madison River may have caused declines in salmonfly survival and performance. Since the malfunction happened in the winter, the effects may not be as significant as they would have been if it happened when the temperature was warmer. In the cold, their metabolism is low which decreases demand for oxygen, meaning they may not have exceeded the oxygen supply in the non-flowing parts of the Madison River. As climate change progresses, warming and drought pose a serious threat to nymph survival and performance, which is supported by the significant decline in nymph survival as temperature increased.

Performance was also affected by increasing temperatures in the no-flow study. As expected, nymphs performed more pushups as the temperature of the treatments increased. This further supports the idea that they use pushups as a respiratory movement to ventilate their gills when transportation of oxygen is reduced from lack of flow. Additionally, more nymphs died in warmer treatments, and they also died faster. Growth and feeding were not affected by temperature, which was surprising since we expected them to lose weight and feed less as they became more stressed and spent more time doing pushups. Possibly, two weeks was not long enough to see change in growth and feeding. Also, the experimental population of nymphs covered a wide range of sizes and life stages, which may be the reason their growth was significantly affected by starting sizes of the nymphs.

In the dry experiment, humidity and temperature both affected nymph survival. Warmer temperatures caused nymphs to die more rapidly, as did the lower humidity. Humidity and temperature interacted significantly to affect survival. This interaction reflects that nymphs in 75% relative humidity died rapidly almost regardless of ambient temperature, whereas nymphs in 100% relative humidity survived much longer at lower temperatures (but still died relatively rapidly at high temperatures). In addition, salmonfly nymphs survived longer in no-flow conditions than in the air since, in dry conditions, all nymphs died within four days whereas, in water, most survived for two weeks. These data suggest that, in nature, nymphs survive best in flowing rivers, then in still water, then in air. So, the dam malfunction that occurred in November 2021 may not have killed the nymphs outright if they were able to remain in water since they can survive in cold still water for at least two weeks. If the malfunction had occurred in the summer, our data suggest that rates of mortality would have been much higher. Further research needs to be conducted to determine the long-term effects on performance and survival of nymphs. Also, altering oxygen levels in future studies is important to understand how fish and aquatic insects compete for oxygen in small pools of water that occur from dewatering events.

Such research will be conducted at the Albertson lab at Montana State University. The Albertson lab is expanding on my research and using it to inform their studies on salmonflies. They are investigating salmonfly survival and movement when they encounter dry conditions in the laboratory as well as studying their emergence in the field at the Madison River. They will be taking into account factors such as body size, refuge availability, extent of drying, and duration of drying. My work fills the knowledge gap for them regarding how long salmonflies can survive at different temperatures in both no-flow and dry conditions. To establish how general my results are, we need additional work on responses of other aquatic insects to warming and drying. Such work is pressing given the looming climate crisis and the ongoing scope of anthropogenic changes to the world's waterways.

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