CLIMATE CHANGE AT THE AIR-WATER INTERFACE AFFECTS GIANT SALMONFLY (*PTERONARCYS CALIFORNICA*) EMERGENCE TIMING AND ADULT LIFESPAN

by

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DEDICATION

For the rivers and the lives they sustain— the salmonflies' and ours.

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ABSTRACT

Aquatic invertebrates experience complex temperature regimes throughout their life history, especially during the vulnerable life stage transition from aquatic to terrestrial habitats. When climate warming interacts with snowmelt in high elevation systems, it creates a novel set of conditions in which spring water temperatures remain within a narrow range from year-to-year while summer water and air temperatures rise. Giant salmonflies (Pteronarcys californica) depend on spring water temperature cues to time their large, synchronous emergence in early summer, but it is unknown how variable temperatures after this springtime cue affect life-history traits. We experimentally tested how changes in temperature in the 6 weeks before and after emergence affect emergence timing, emergence success, and adult lifespans. We found that the timing of emergence was 2.8 days earlier with each degree of warming during the weeks preceding emergence. However, there was no evidence that emergence success was affected by higher water temperature within our test temperature range (13-23°C). In the terrestrial adult stage, adult lifespans were shortened by increased air temperatures, especially when water temperatures during the aquatic juvenile stage had also been increased. The predicted lifespan was almost five times longer at the coldest air and water temperature combination than at the warmest (28 vs. 6 days). The shortest lifespans observed (3 days) are not likely to prevent successful reproduction, given that salmonflies can mate and oviposit within days of emergence. Still, because salmonflies can oviposit repeatedly for up to 80% of their lifespan, shortened lifespans may reduce total egg production and thus fitness. Our results indicate that rising water and air temperatures will impact not only the life history of the insects, but also the organisms in the riparian zone that rely on salmonfly emergence by altering the timing, magnitude, and duration of the nutrients provided by these large-bodied aquatic insects.

CHAPTER ONE

INTRODUCTION

Organisms rely on environmental cues to initiate and complete critical physiological transitions (Jacobs and Wingfield 2000). Many of these cues, such as temperature and hydrology, are related to climate, and global climate change has caused shifts in phenology across phyla (Parmesan and Yohe 2003). Because climate change can produce variable results across seasons and habitats, organisms with complex life histories can face novel combinations of environmental conditions as they transition from one life stage and habitat to the next (Both et al. 2010), yet little is known about how such changes in conditions across seasons and habitats can affect life history, survival, and reproduction.

Many life history transitions must begin in well in advance of a critical life history event, in anticipation of future conditions that are favorable for survival or reproduction. These two critical points in time and space, described as the "environment of decision" —where life cycle changes are initiated— and the "environment of selection"—where environment and timing affect organismal fitness— have evolved over many generations (Visser et al. 1998). However, as climate change unfolds unevenly across seasons and habitats, the adaptive links between the cues in the environment of decision and the environment of selection can become unreliable. For instance, the pied flycatcher (Ficedula hypoleuca) relies on daylength cues in North Africa that have remained stable, leading it to arrive late at breeding grounds in Europe and diminishing its ability to reproduce during the optimal window, which has shifted earlier due to climate change (Both and Visser 2001). Thus, changes in either the environment of decision or the environment of selection can alter outcomes for organismal fitness.

Aquatic insects may be particularly susceptible to climate change due to the strong influence of temperature on a number of life-history traits, including emergence timing and body size, as well as their geographic distribution (Bhowmik and Schafer 2015). Many cold-adapted aquatic species are already experiencing range contractions, and these trends are expected to intensify in the future (Giersch et al. 2015; Anderson et al. 2019a). Even species that do not face local extirpation are likely to encounter sublethal effects, such as alterations in growth, metabolism, phenology, and reproductive success (Dallas and Ross-Gillespie 2015). Temperature is widely studied as a driver of the timing of life history events in freshwater macroinvertebrates (Woods et al. 2021), yet few studies have investigated the influence of temperature across life stages and habitats. Since many studies of thermal tolerance focus on a single life stage, the range of temperatures that each species requires to successfully complete its life history may be different than we currently understand (Kingsolver et al. 2011).

In temperate ecosystems, where emergence is seasonally constrained, many aquatic insects rely on water temperature to time the transition to their terrestrial adult stage (Nash et al. 2023). Because seasonal temperature trends in water and air are not changing at the same rate, climate change is creating a novel set of conditions before and after emergence that could alter phenology, reproduction, and survival outcomes. For example, snowmelt driven streams in Western North America show dramatic patterns of warming in summer and fall, whereas spring and winter water temperatures remain stable or rise much more slowly (Jones et al. 2017, Isaak et al. 2012). By contrast, air temperature in the same region is rising more uniformly across seasons (Pederson et al. 2010). Accurately assessing species' response to these conflicting cues requires consideration of their whole life cycle, as aquatic insects experience distinct temperature regimes and changes in thermal tolerance as they progress from one life stage and habitat to the next (Levy et al. 2015, Radchuk et al. 2013, Kingsolver et al. 2011).

The emergence of giant salmonflies (*Pteronarcys californica*) plays a pivotal role in providing food subsidies to riparian ecosystems across the temperate mountain west. Because of their abundance and large size, salmonflies account for the majority of the annual nutrient flux from aquatic to terrestrial environments during their short, synchronous emergence period (Walters et al. 2018). However, salmonfly populations are declining in many parts of their range (Stagliano 2010; Birrell et al. 2019; Heinold and Pomeranz 2011), and previous research demonstrates that juvenile stages in the aquatic habitat are sensitive to temperature increases (Anderson et al. 2019a and 2019b; Gregory et al. 2000). Phenological mismatches can be especially impactful when the resource has a narrow temporal distribution (Miller-Rushing et al. 2010), so even small changes in salmonfly emergence timing could have large ecological consequences.

Previous studies have demonstrated that salmonfly emergence in early summer is cued by springtime water temperatures, which are predicted to be relatively stable in the future, whereas summer water and air temperatures will continue to rise due to climate change (Anderson et al 2019a, Hostetler et al. 2021, Isaak et al. 2012, Jones et al. 2017). However, salmonfly nymphs spend over a month in the water after receiving the springtime temperature cue, and the role of rising temperatures during this early summer period remains unclear (Fig. 1). Understanding how salmonflies respond to these elevated temperature cues will allow us to make more accurate predictions about their vulnerability. Thus, we investigated how changes to temperature in spring and summer affect multiple life stages of salmonflies as they cross the aquatic-terrestrial boundary during emergence.

We experimentally manipulated water temperature before salmonfly emergence and air temperature after emergence to test the impact of rising temperatures on emergence timing, emergence success, and adult lifespan. We predicted that elevated water temperature in the early summer period would lead to higher juvenile mortality, earlier emergence timing, and decreased emergence success, and that elevated air temperatures would shorten adult lifespans.

Study System

The Madison River in Southwest Montana is a large, regulated river with healthy populations of salmonflies in its upstream reaches. We collected salmonflies from two sites to simulate different springtime water temperature cues (Fig. 2). Both sites support high abundances of salmonflies (>84 nymphs/m^2; Anderson et al. 2019a). The downstream location, Varney Bridge (45.229977, -111.752063; hereafter, the warmer site), has warmer temperatures in the spring, whereas the upstream site, just below Hebgen Dam (44.856486, -111.349704; hereafter, the cooler site) has cooler temperatures in the spring (see Anderson et. al 2019a supplemental information for sites “3”, Hebgen Dam, and “7”, Varney Bridge). Because of this difference in temperature, the annual salmonfly emergence occurs several weeks later at the cooler site than at the warmer one (Anderson et al. 2019a). Other differences between the two sites include elevation and proximity to a dam; however, previous research demonstrates that spring water temperature is the primary driver of emergence timing (Anderson et al. 2019a) and thus temperature was the focus of this experiment.

CHAPTER TWO

METHODOLOGY

Sixty male and sixty female salmonflies from each site were collected for the experiment in the late spring, approximately one month (40 days) before their typical emergence. We chose this timing so that the nymphs at each site would receive the springtime water temperature cue in their natural habitat, but would still spend approximately the same amount of time in the laboratory at experimental water temperatures before emerging. We aimed to minimize time spent in the laboratory environment in order to reduce stress on the insects. Wild emergence was predicted by regressing May mean temperature on 'day of first emergence' using published records and our own emergence records from 2021 (Anderson et al. 2019a; Fig. S1).

Final instar salmonfly nymphs were collected using a kick net and sorted by sex at the time of collection. In the lab, each individual was photographed in a shallow pan of cooled water and its length was determined using ImageJ software (Schneider et al. 2012). A two-way ANOVA showed a significant difference in body length between the two sites and between males and females. A Tukey's Honest Significant Difference Test for multiple comparisons revealed an 8.08mm difference between the mean length of males and females (females are from 8.83 to 7.32mm longer, p-value adjusted <0.0001), and a 1.07 mm difference between the mean lengths of individuals from the warmer and cooler sites (nymphs from the cooler site are from 0.31 to 1.83 longer, p-value adjusted= 0.006). We accounted for the size difference between sites by including body length as a regression parameter in the first iteration of all statistical models.

Individuals were randomly assigned to a combination of air and water temperature treatments and housed in uniquely identified steel mesh tubes (8cm diameter x 40cm tall) within a temperature-controlled aquarium. We used circulation pumps and aerators to maximize flow and oxygenation, which are important for salmonfly nymphs (Birrell et al. 2020, Frakes et al. 2021). The tubes contained rocks from the corresponding site on the Madison River and conditioned leaves collected from a tributary of Bozeman Creek (45.671148, -111.03431) for habitat and food, and protruded from the surface of the water to allow the salmonflies to crawl up the side when they were ready to emerge (Nebecker 1971). Salmonflies were acclimated to assigned water temperature treatments gradually (2°C per 12 hours) before being transferred to the experimental setup.

There were four water temperature treatments established using tap water treated with an aquarium dechlorinating conditioner (Mars Fishcare North America, Inc., Chalfont, PA). These treatments were based on (i) the estimated mean temperature at the warmer site in the month prior to emergence (14 ºC); (ii) the estimated mean temperature at the cooler site during that same month (12 °C); (iii) the projected temperature in 2100 (17°C; based on +0.33°C/decade, Isaak et al. 2012); and (iv) a more extreme temperature that exceeds that determined to be suitable for salmonflies (19°C; Anderson et al. 2019a). A constant water temperature was maintained using four chillers (MC-1/4HP, AquaEuroUSA/Hamilton Technology, Gardena, CA) (Table S1, Fig. S2). Streams in the mountain west rarely fluctuate more than 3-4 degrees from April through July (Ferencz and Cardenas 2017), so stable temperature treatments are ecologically relevant. Light was controlled using an overhead LED shop light (Greenlite USA, Anaheim, CA) and a timer set to 16 hours light, 8 hours dark. To minimize the establishment of divergent bacterial communities or nutrient concentrations in the different temperature treatments over the course of the experiment, water from the four treatments was mixed in a common sump and redistributed to each experimental unit through chillers that maintained experimental temperatures.

Salmonfly individuals were visually inspected daily until all individuals had either died or emerged (93 days). After nymphs crawled out of the water and split their exuvia, we used two categories to describe the outcome. Individuals either succeeded in metamorphosing into adults (emergence success) or were unable to complete metamorphosis (emergence failure). Successful emergence typically happened overnight or, if during the day, took less than an hour to complete. Failed emergence typically involved several days of attempted metamorphosis before an individual died. We compensated for the time discrepancy by recording the date of a failed emergence as the date that the individual left the water rather than the date that it died if the dates were not the same. Because of the highly synchronous nature of salmonfly emergence, we counted all nymphal mortalities that occurred after the emergence period began (quantified as the first successful emergence within that treatment) as emergence failures even if their exuvia had not split.

Successfully emerged adults were transferred to 178ml (5 x 5.4 x 7cm) food-grade plastic containers with ventilation holes placed within temperature-controlled incubators built using established protocol (environmental chamber with dynamic temperature controller; Garcia-Robledo et al. 2020) at one of four fluctuating air temperatures. These treatments were based on (i) the mean air temperature experienced by the warmer population during the week of emergence (14 ºC); (ii) the mean temperature experienced by the cooler population during the week of emergence (12 °C); (iii) the projected air temperature during emergence accounting for a rise of about 2.88°C by 2100 under Realistic Concentration Pathway 4.5 (21°C, Hostetler et al.

2021); and (iv) a more extreme temperature that might help us determine curvature in the relationship between temperature and adult mortality (23°C). The range assigned to these temperature treatments (±11 degrees for day/night) is based on the mean diel fluctuation in riparian air temperature from data collected in 2016 (Fig. S3).

The incubators cycled between the maximum and minimum temperatures on a 24-hour cycle. We used HOBO TidbiT MX temperature loggers (Onset Computer Corporation, Borne, MA) to verify the temperature profile of each incubator and aquarium. In most of the treatments, the incubators kept the daily mean temperature within ±0.4°C of the nominal temperature (Table S2). The 21°C incubator treatment had a mean temperature closer to 19.7°C, which we accounted for by using the true mean temperature for each treatment in our statistical modeling. Additionally, because of a programming error, the 17°C incubator treatment had a 6-hour difference in the timing of maximum temperature as compared to the other treatments (Fig. S4). Peak temperature still occurred during daylight hours, and the outcomes for adult lifespan were within the expected range. Therefore, we determined that any differences caused by this discrepancy were minor.

Adults were checked daily for mortality and fed a 5% sucrose solution on a cotton ball, which was replenished every other day (Collier and Smith 2000). We note that salmonflies of both sexes did appear to consume the nectar, but determining the feeding habits of adult salmonflies was beyond the scope of this study. Adult lifespan was estimated as the number of days between an individual’s emergence and when it died or became completely unresponsive.

Data Analysis

We modeled mean emergence date using parameters for site, length, water temperature, sex, and an interaction between water temperature and site. The most parsimonious model from a backwards stepwise selection yielded a reduced linear model that included all of the aforementioned parameters except length.

We used a binomial logistic regression to model emergence success. The full model included water temperature, sex, length, site, and ‘emergence order’, which we defined as the number of days between an individual’s emergence and the first attempted emergence in its treatment group. This allowed us to preserve the information about finer scale emergence timing without being confounded with temperature. The final model included water temperature, site, and emergence order.

We modeled adult lifespans using a Poisson distributed generalized linear model using backwards stepwise selection from a full model that included sex, length, site, air temperature, water temperature, and an interaction between air and water temperature. The final model included site and an interaction between air temperature and water temperature. All statistical analysis was completed using R Statistical Software (v4.3.0; R Core Team 2021).

CHAPTER THREE

RESULTS

Emergence Timing

Emergence timing was strongly influenced both by temperature treatment and by site, (Fig.3; Table S3). Mean emergence date was 2-3 days earlier for each degree of warming after accounting for other parameters (se=0.086, $P<0.001$). Mean emergence date for individuals from the warmer site was approximately 26 days earlier than for those from the cooler site (se= 1.834, $P < 0.001$). Additionally, there was an interaction between site and water temperature. The mean emergence date for individuals from the warmer site was about 0.5 days later per degree of water temperature than for those from the cooler site (se=0.118, $P < 0.001$), which means that individuals from the cooler site reacted even more strongly to elevated water temperatures than did those from the warmer site. This pattern suggests a hierarchical, nested effect of water temperature across seasons. Springtime water temperature determines emergence timing most strongly, but early summer water temperature provides a second, proximate emergence cue that also modifies emergence timing.

As has been observed in the wild, emergence was highly synchronous within each population and water temperature (Fig. 4). Ninety-five percent of emergence occurred over 9.3 days on average (sd=1.29 days) in each event. Mean and median emergence dates for each treatment were similar (average difference = 0.23 days, sd = 0.47 days). Males emerged 1.7 days earlier than females, on average, within each temperature and site (-1.658, se=0.328, $P < 0.001$).

We exposed nymphs from the two sites to approximately the same number of days of experimental warming in the laboratory to minimize confounding interactions between time in the lab and temperature treatments. Nymphs from both sites displayed a similar pattern of emergence timing within each temperature treatment after they were brought into the lab, suggesting that our estimates of each site's emergence timing were accurate (Fig. 5).

Emergence Success

Water temperature had no effect on emergence success after accounting for site and emergence timing (binomial GLM: P= 0.21, Fig. 6). Instead, emergence success was dependent on the timing of emergence within the cohort and site (Fig. 7, Table S4). In general, salmonflies that emerged earlier relative to other insects in their treatment had a higher probability of successful emergence. The decrease in the log-probability of emergence success was estimated at 0.14 per day (se=0.04, P<0.001). Additionally, salmonflies from the warmer site had a higher log-probability of emerging successfully than those from the cooler population (increase=0.67, se=0.274, P= 0.015). Emergence success was low across both sites during the entire emergence period. Individuals from the warmer site had ~ 69% (95% CI from 56-79%) probability of emergence at the beginning of the emergence period, which was the highest probability of emergence success among all treatments. However, this probability of success dropped to 45% after the first week of the emergence period (95% CI from 36-54%). Individuals from the cooler population had an initial 53% probability of emergence success, which was reduced to only 30% by the end of the first week (95% CIs from 38-67% and 22-39%, respectively).

No salmonfly nymphs died at any water temperature between the end of the acclimation period and the beginning of emergence in each treatment.

Adult Lifespan

Increased temperatures in both air and water led to shortened adult lifespans (Figs 8A and S5, Table S5). In addition to the main effects of air and water temperature, the model estimated a 0.6% reduction in lifespan for each degree of warming in either water or air (Poisson GLM: se=0.002, P=0.034). Origin also influenced adult lifespan (Fig. 8B). The mean lifespan for individuals from the warmer site was 12% shorter than for individuals from the cooler site (se=0.052, P=0.013).

The mean lifespan for both populations combined, 15.8 days, closely matched what we expected based on a prior published adult lifespan and field observations (15±1.8 days at simulated field conditions; DeWalt and Stewart 1995); however, the longest model-predicted mean lifespan, 29 days (for the cooler site at the coolest combination of air and water temperatures), was roughly double what we expected. About 10% of individuals lived thirty days or longer, and the longest-lived individual survived for 58 days. The shortest model-predicted mean lifespan, six days, was for the warmer site at the warmest combination of air and water temperatures. The 10% of adult salmonflies with the shortest lifespans lived between three and six days.

CHAPTER FOUR

DISCUSSION

Climate change is altering conditions in rivers and riparian habitat across the globe and may have particularly pronounced effects in mountainous environments (Poff et al. 2002). Organisms with complex life histories rely on environmental cues to initiate key life-cycle transitions, and may suffer decreased fitness when conditions across multiple environments are misaligned due to climate change (Both and Visser 2001). Many studies have detailed the numerous ways that aquatic or terrestrial biota will be impacted by climate change in either environment (Hader and Barnes 2019, Prowse et al. 2009), but few have investigated the combined effect of stressors across multiple environments.

Changes in emergence phenology have been widely documented in aquatic insects (Parmesan and Yohe 2003), but there is mixed evidence that salmonfly emergence timing is advancing. In at least some parts of their range, emergence timing has remained relatively stable, likely due to the moderating influence of snowmelt on springtime water temperatures (Anderson et al. 2019a). In our study, the experimentally simulated warmer summer temperatures caused salmonflies to emerge earlier but did not have a significant impact on their emergence success, which was low across all treatments. Adult lifespans were shortened through the combined effects of air temperature and water temperature. Our findings confirm the importance of the springtime water temperature cue as a primary driver of salmonfly emergence timing and add a second, modifying cue from early summer water temperature. We also show that salmonflies are influenced by temperature both before and after emergence in ways that can influence their life history and interactions with the food web.

Multiple hierarchical cues for phenological outcomes may be an underappreciated yet important component of aquatic insect ecology. Aquatic insects rely on temperature cues to initiate and complete critical life history events such as egg development, diapause, and metamorphosis. Even if rising temperatures do not approach lethal limits, they can interrupt the necessary signals and cause local extirpation (Lehmkuhl 1974). Similarly, aquatic insect behavior depends on a sequence of conditions being met in the environment. Oviposition site selection in some species is influenced by a series of cues, beginning with selecting fast moving water at the reach scale, and continuing at the more granular level of choosing among individual rocks (Miller et al. 2020). We suggest that emergence timing depends on several sequential cues from water temperature, starting with the primary springtime cue which can then be modified by early summer water temperatures. If early summer water temperatures rise sufficiently, this secondary cue allows for emergence timing to advance by as much as several weeks, perhaps allowing salmonflies to better match favorable air temperatures for reproduction. This could be beneficial if it helps salmonflies avoid extreme daytime heat, thereby extending their adult lifespan and opportunity to oviposit.

The synchronicity of emergence is another trait that can potentially be influenced by temperature. In experimental manipulations of dragonfly larvae, warming decreased the synchronicity of emergence (McCauley et al. 2018), whereas in an observational study of salmonflies, sites with warmer water had shorter, more synchronous emergence periods (Anderson et al. 2019b). An accumulation of degree days has been suggested as the mechanism behind emergence timing and synchronicity in several studies (Adams et al. 2023, Gregory et al. 2000) but has not yet been shown to be the best predictor in observational studies (Anderson et al. 2019a). We saw a similar pattern of shortened emergence duration under warmer conditions as has been described in wild populations (Anderson et al. 2019b).

Metamorphosis is a physiologically stressful period for aquatic insects (Campero et al. 2008, Lowe et al. 2021). Background rates of emergence failure in the wild have not been extensively documented, but one estimate is about 7% for salmonflies (Wesner et al. 2019). We observed rates six to eleven times higher, emphasizing the sensitivity of this life stage to stress. Laboratory conditions and excessive handling (moving the cages daily to check for mortality) probably contributed to high rates of emergence failure across all temperature treatments in our experiment. Additionally, because of the effect of temperature on emergence timing, nymphs in cooler temperature treatments endured laboratory conditions and handling stress up to 25 days longer than their warmer treatment counterparts. Studies of other cold-adapted stoneflies (*Pteronarcys dorsata*, Nebecker 1971; *Lednia tumana*, Shah et al. 2023) have demonstrated temperature-dependent rates of emergence failure under similar conditions. We did not detect a statistically significant effect of temperature on emergence success in our study, but because of the high level of overall mortality, we cannot conclusively determine the role of temperature in emergence success more broadly. Future studies might document body condition and feeding rate (Nebecker 1971) as a function of laboratory and handling stress.

The temporal pattern within each treatment of a period of emergence success followed by a period of failure was an unexpected finding and may be worthy of further study. We are unaware of any other examples of this phenomenon in the literature. Healthier salmonflies may be able to complete their metamorphosis earlier because of better body condition or another unmeasured trait. If this pattern were consistent in the wild, it could indicate a mechanism for selection for earlier emergence under stressful conditions.

Our observations conform to the pattern of stage dependent mortality seen in other groups of aquatic insects under environmental stress prior to emergence. Heavy metals (Wesner et al. 2020), food and pesticide stress (Campero et al. 2008), and thermal exposure (Shah et al. 2023) have been shown to cause emergence failure, even when larvae were apparently unaffected. We saw no juvenile mortality across any water temperature treatment after the acclimation period, even at temperatures that reach the upper limit of salmonfly thermal range in the wild (19°C maximum summer maximum, Anderson et al. 2019a). This illustrates the resilience of the juvenile stage, and the relative sensitivity of metamorphosis. These types of stage- dependent thermal effects are increasingly recognized as key to our prediction of species' response to climate change (Levy et al. 2015, Radchuk et al. 2013), although it is still unclear whether heat resistance is genetically related across life stages. For example, if selection favors heat resistance in metamorphosis, the same genes may or may not lead to more heat resistant larvae and adults (Kingsolver et al. 2011).

The characteristics of adult aquatic insects are less well studied, although this life stage is important for reproduction, dispersal, and connecting food webs across aquatic and terrestrial boundaries. We found that adult lifespans were shortened by the interacting influences of warmer air and water temperatures, which demonstrates a carryover effect from their larval thermal exposure. Adult insect lifespans have been shown to decrease with higher air temperatures (Jackson 1988, Collier and Smith 2000), but carryover effects from larval stressors are not ubiquitous. Some physiological indicators of larval stress, such as fluctuating asymmetry (random deviations from bilateral symmetry), are not reflected in adulthood (Campero et al. 2008). In a study of *Pteronarcys dorsata*, Nebecker (1971) found similar carryover effects in adult lifespan, which he attributed to juvenile feeding rates and metabolic inefficiencies at high temperatures. Larval thermal stress has also been shown to affect adult dragonfly morphology (McCauley et al. 2018), which in turn can influence performance (McCauley and Mabry 2011).

Even though lifespans were reduced by over 75% from the warmest temperature combination in our experiment to the coolest, the reduction would not likely be sufficient to prevent reproduction. In a separate small (n=15 pairs) study of wild-caught mating pairs, adults were observed mating and ovipositing for the first time within the first three days of emergence at a range of air temperatures (author's unpublished data). While there were adults that died within three to four days, the shortest modeled mean lifespan was six days. However, it remains unclear whether shortened lifespans could affect population dynamics by reducing the number of fertilized eggs produced. Salmonflies from the Rio Conejos in Colorado lived about 15 days and produced between five and seven batches of eggs during the first 12 days of their lifespan, which suggests that shortened lifespans could reduce oviposition rates significantly in a laboratory setting (DeWalt and Stewart 1995). On the other hand, in wild populations predation pressure may be a stronger determinant of adult lifespan, and without estimates of lifespans under natural conditions, the importance of reduced lifespan is hard to assess.

The conservation of insects in the face of climate change is important to prevent the destabilization of food chains and loss of ecological services, in particular the connectivity of river and riparian food webs (Harvey et al. 2022, Isaak and Rieman 2013). Aquatic insects provide a significant flux of nutrients to terrestrial consumers as they emerge from rivers and become food sources for spiders, ants, birds, bats, and reptiles (Baxter et al. 2005, Collier et al. 2002, Fukui et al. 2006, Nakano and Murakami 2001, Sabo and Power 2002). Avian consumers depend heavily on emergent insects as a unique source of omega 3 fatty acids, which they require for successful reproduction (Twining et al. 2018, Parmar et al. 2022).

Although both predators and prey may advance their phenology in response to environmental cues, such advances may not occur at the same rate, causing phenological and nutritional mismatches that directly affect consumer fitness (Thackeray et al. 2010, Shipley et al. 2022). In some cases, however, species that have synchronous dynamics have responded to changing climate more congruently than expected, particularly in taxonomic groups linked by feeding relationships (Ovaskainen et al. 2013). Thus, how other organisms that rely on salmonfly emergence will respond to warming and other changing environmental conditions remains unclear. Habitat heterogeneity can act to increase consumer access to invertebrate prey by desynchronizing emergence at the basin scale (Adams et al. 2023). This is especially important for arriving migratory birds, for whom even small changes in salmonfly emergence timing could lead to complete exclusion from this subsidy, and who may themselves be receiving conflicting environmental cues from the various habitats they traverse (Adams et al. 2023, Both and Visser 2001).

Shortened adult lifespans or phenological mismatches could also alter the proportion of salmonflies that enter detrital pools rather than being eaten by predators, as they are available as flying adults for less time. At sites with high salmonfly abundance, modeled nitrogen deposition rates from 25% of salmonflies entering detrital pools rivaled rates of deposition due to salmon carcasses in Alaska (Wesner et al. 2019). If the percentage of salmonflies were to increase due to environmental stressors, the balance of nutrient subsidies could shift from predators to detritivores (Yang 2006).

Phenotypic plasticity and evolution may be important factors in the climate change story that are difficult to test in a laboratory environment and may help salmonflies and their predators adjust to novel climates (Kellermann and van Heerwaarden 2019). The demonstrated plasticity in emergence timing could prevent salmonflies from encountering both warmer air and water by emerging earlier, while temperatures are still favorable. Salmonflies can be vulnerable to late frosts when warmer water temperatures cause early emergence (Rockwell and Newell 2009); however, if water temperatures continue to favor later emergence, exposure to heat stress in the adult stage is more likely. In that case, microclimates provided by riparian forest cover can provide thermal refugia as well as structural habitat for mating adults (Collier and Smith 2000).

Individuals from the two sites showed similar trends in emergence timing but differed in their responses to warming during emergence and adulthood. Individuals from the warmer site had a higher probability of emergence success but shorter adult lifespans. Whether this is the result of a plastic response to their long-term thermal history or of genetic differentiation between the sites remains unclear.

CHAPTER FIVE

CONCLUSION

Our findings suggest that emergence timing and adult lifespan are strongly influenced by early summer water and air temperature, which is expected to rise in the coming decades (Hostetler et al. 2021, Isaak et al. 2012). The changes brought about by rising temperatures to emergence timing, emergence success, and adult lifespan could have cascading effects on the biological communities that depend on salmonfly emergence. Managers who wish to arrest the decline of salmonflies and promote predator access to emergent insects should focus on enhancing thermal heterogeneity in both air and water temperatures through tributary inputs (Adams et al. 2023), increased riparian vegetation (Collier and Smith 2000), and potentially new tools such as managed aquifer recharge (Van Kirk et al. 2020). Water quality, flow and groundwater contributions are also important across life stages (Harvey et al. 2022, Birrell et al. 2020, Frakes et al. 2021).

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Competing Interests

No competing interests are declared.

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Data Availability

All relevant data can be found within the article and its supplementary information.

CHAPTER SIX

FIGURES AND TABLES

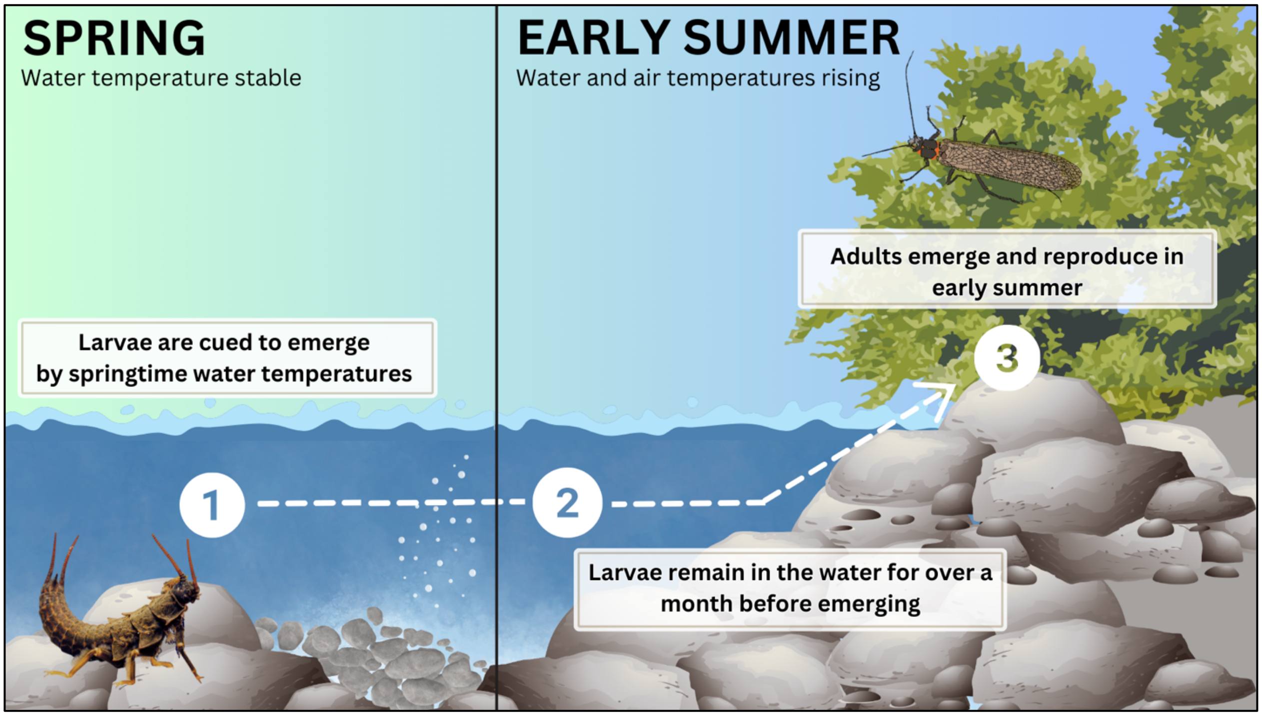

Figure 1. Graphic depiction of salmonfly partial life history. Giant salmonflies traverse the aquatic-terrestrial boundary during emergence to complete their life cycle as terrestrial adults. Warming trends are not evident in springtime water (1), which influences emergence timing later in the season. Early summer water temperature (2) and air temperature (3) are rising, but their effects on emergence timing and adult lifespan are unknown. Artwork by the author using original images of salmonflies from Jason Neuswanger at Troutnut.com.

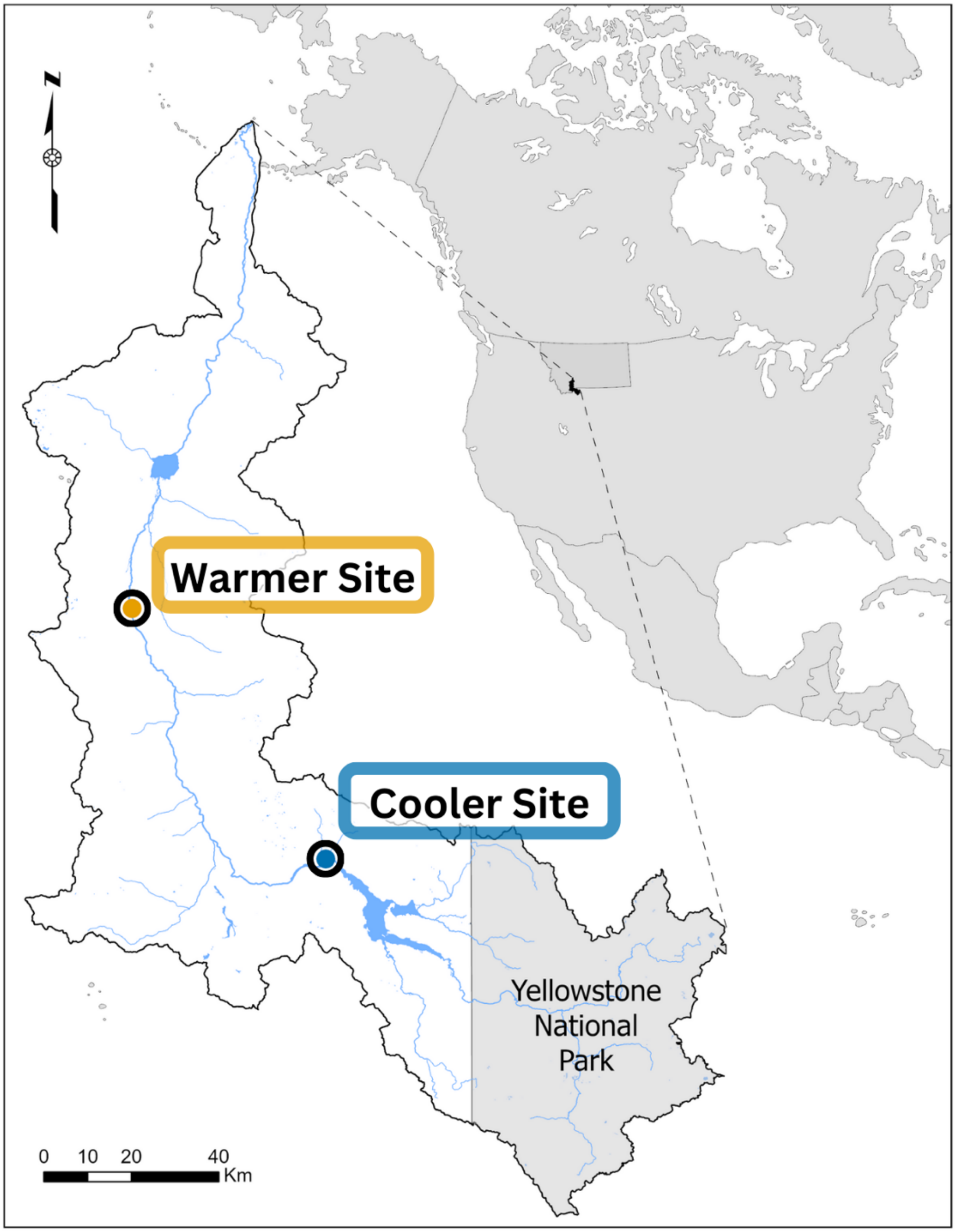

Figure 2. Map of the Madison River Watershed. The two study sites are located on the mainstem of the Madison River, which flows north and forms the headwaters of the Missouri River.

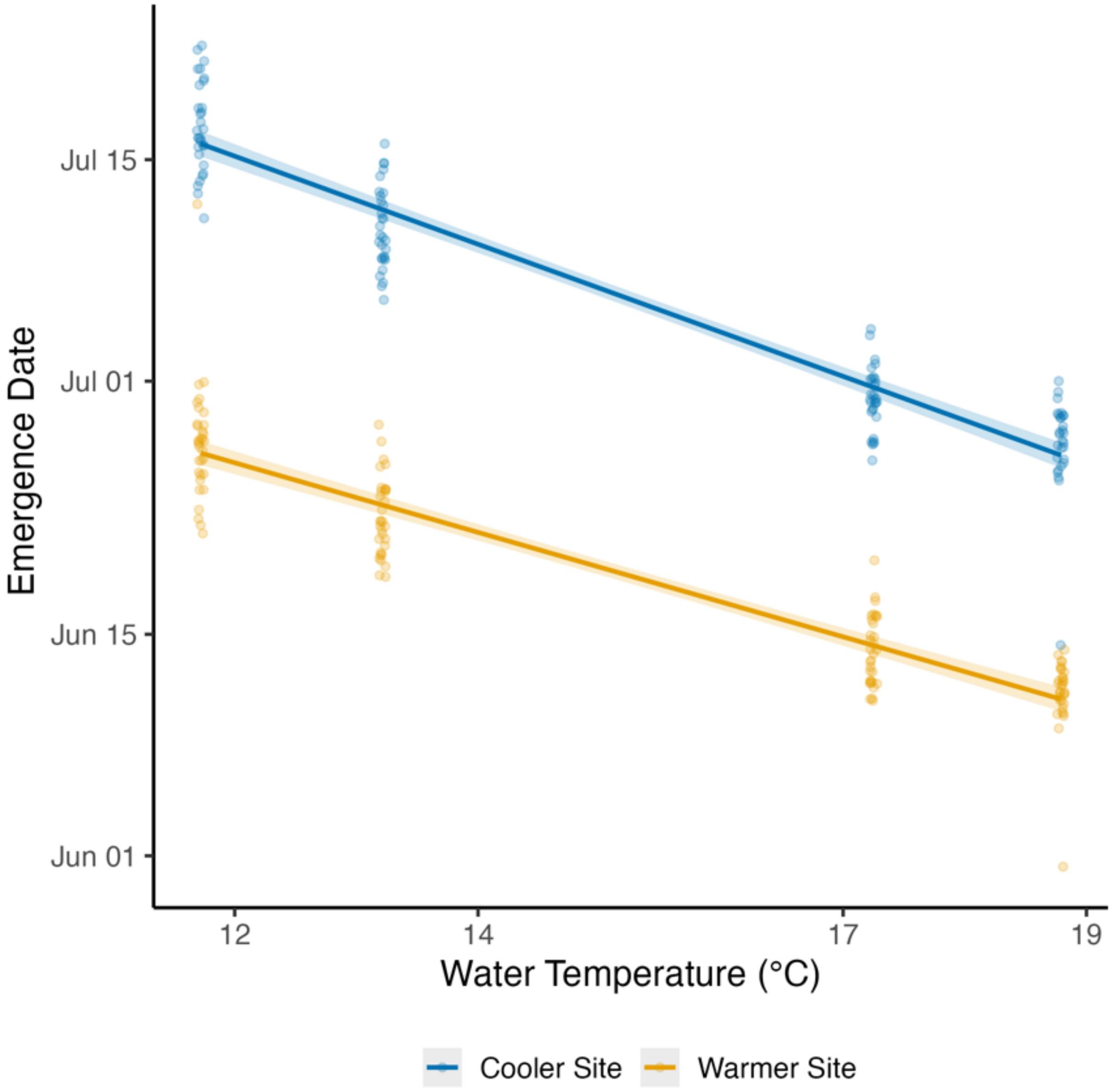

Figure 3. Emergence timing of individuals from the two populations at experimentally manipulated water temperature treatments. Shaded bands represent 95% confidence intervals on a linear model that includes sex and an interaction between water temperature and site.

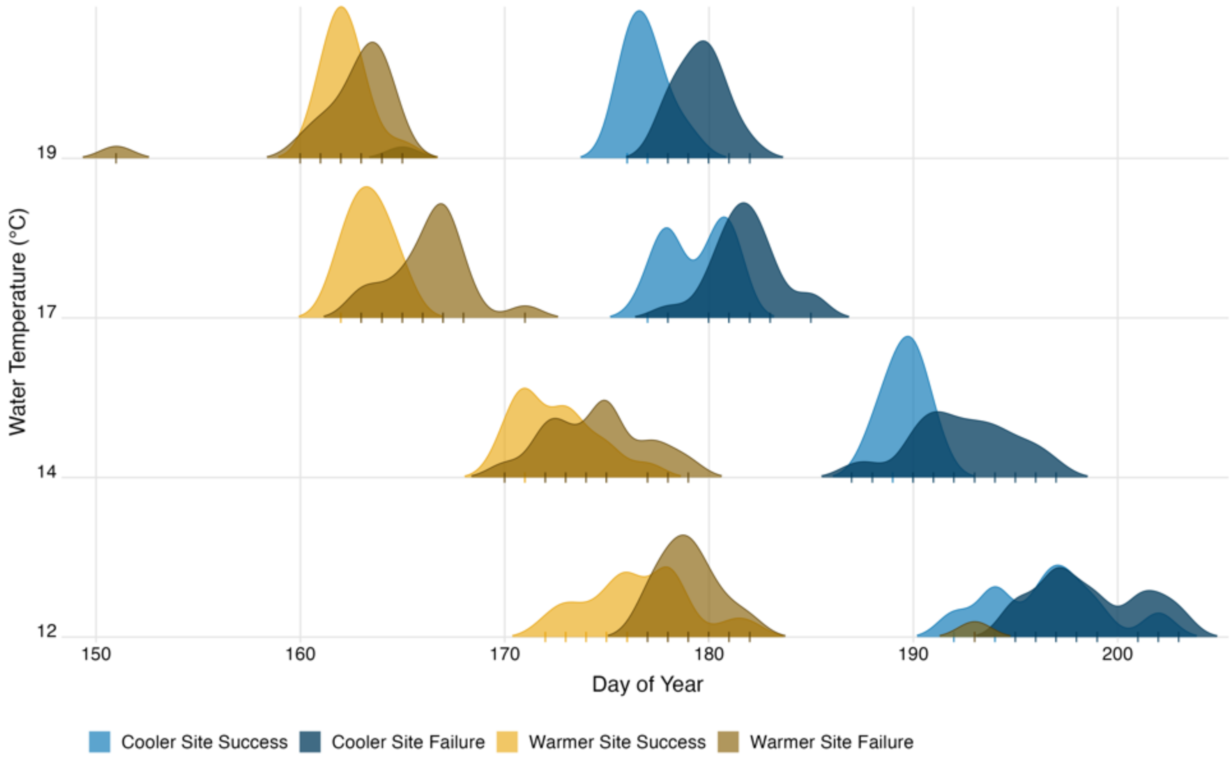

Figure 4. Timeline of emergence success and failure over the course of the experiment. Ridge height indicates the relative proportion of individuals that emerged each day. Individuals from the warmer site emerged sooner at all water temperatures (gold), followed by those from the cooler site (blue). In each combination of site and temperature, a period of emergence success (represented by lighter shading) preceded a period of emergence failure (darker shading). This is the phenomenon described by the "emergence order" variable.

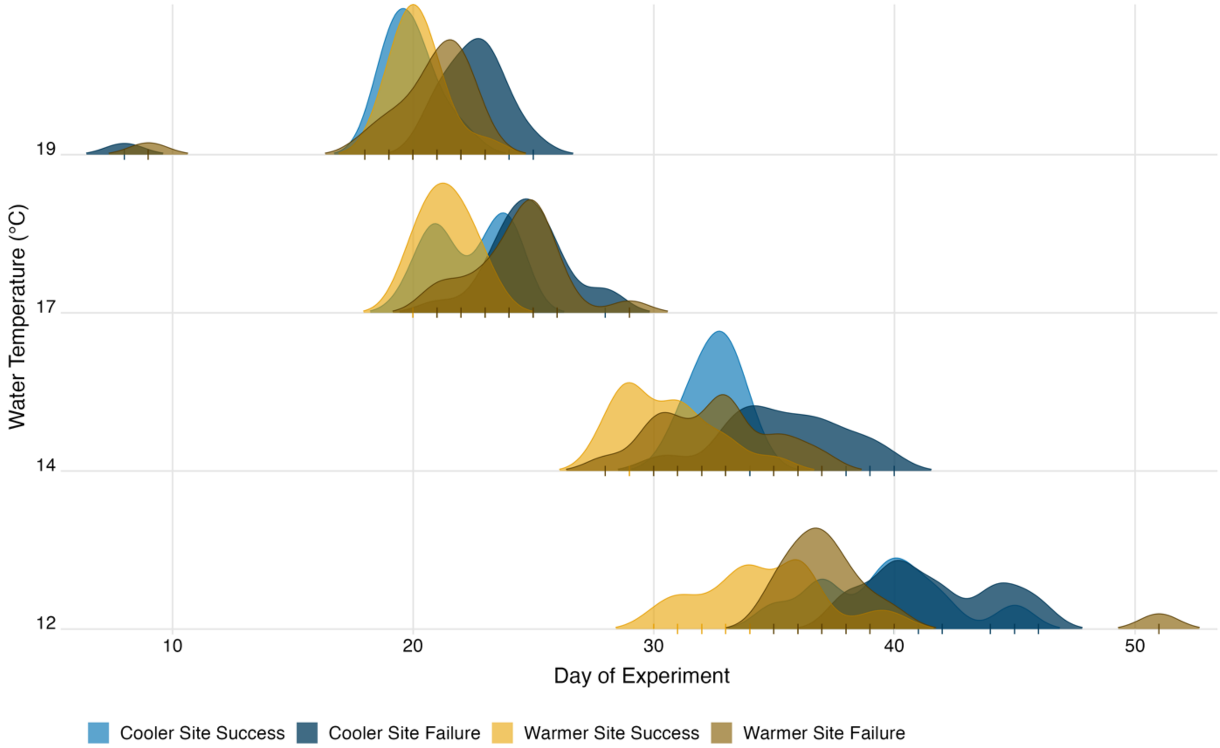

Figure 5. Timeline of emergence success and failure accounting for the staggered start of the experiment for salmonfly nymphs from the two different sites. The height of each ridge shows the relative proportion of individuals emerged each day, and the shading denotes emergence success (lighter shading), or failure (darker shading).

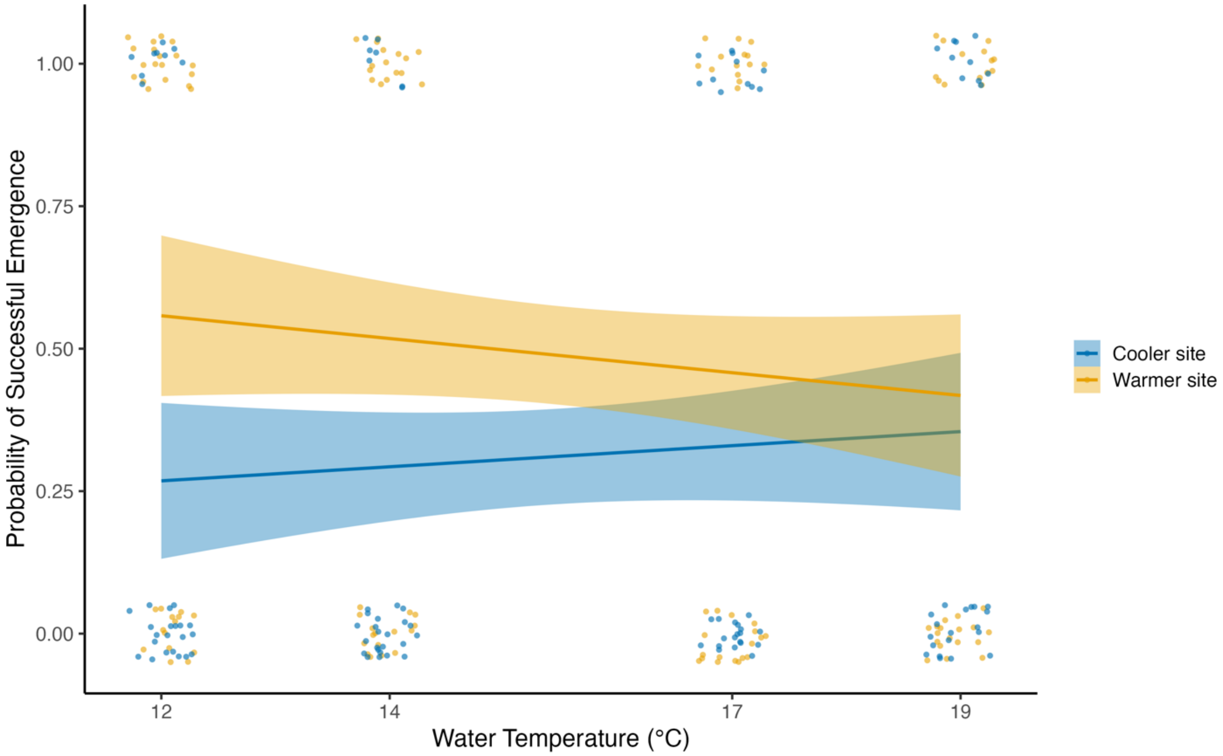

Figure 6. Predicted probability of successful emergence for individuals from the two sites across water temperatures, with 95% confidence intervals based on a binomial generalized linear model. We found little evidence for an effect of water temperature on emergence success, even after accounting for population (p= 0.19).

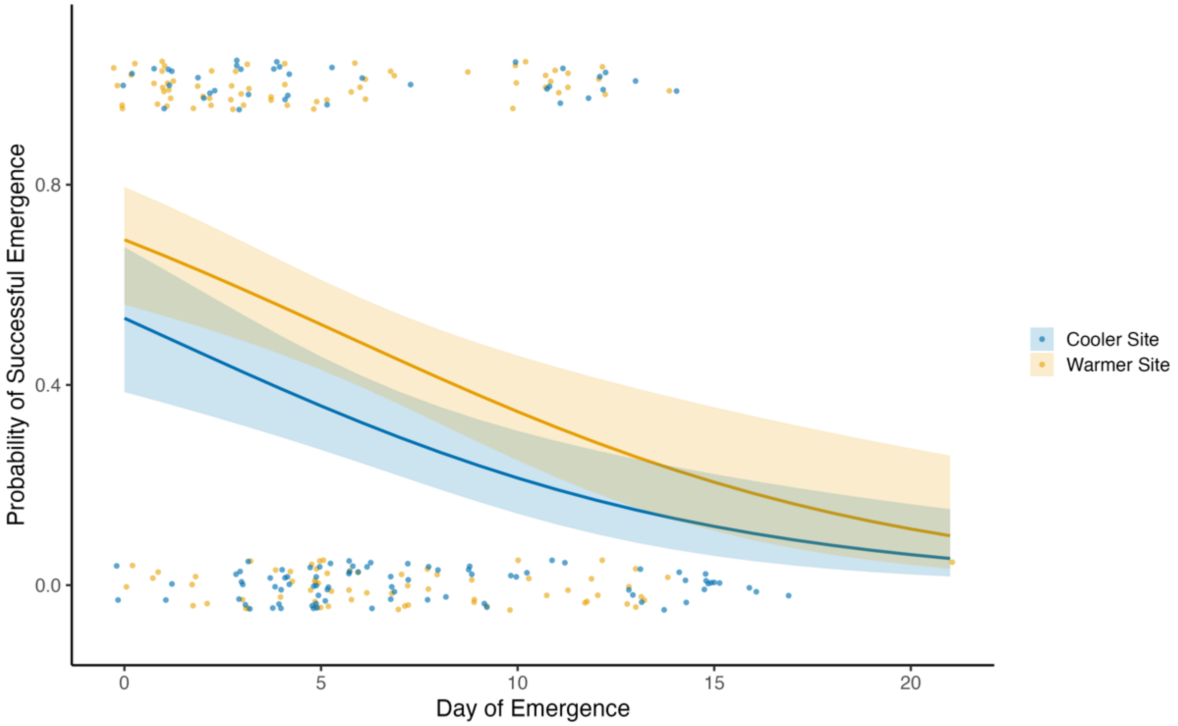

Figure 7. Predicted probability of successful emergence for individuals from the cooler (blue) and warmer (gold) populations over the days of the emergence period. Shaded bands indicate 95% confidence intervals on a generalized linear model with a binomial distribution.

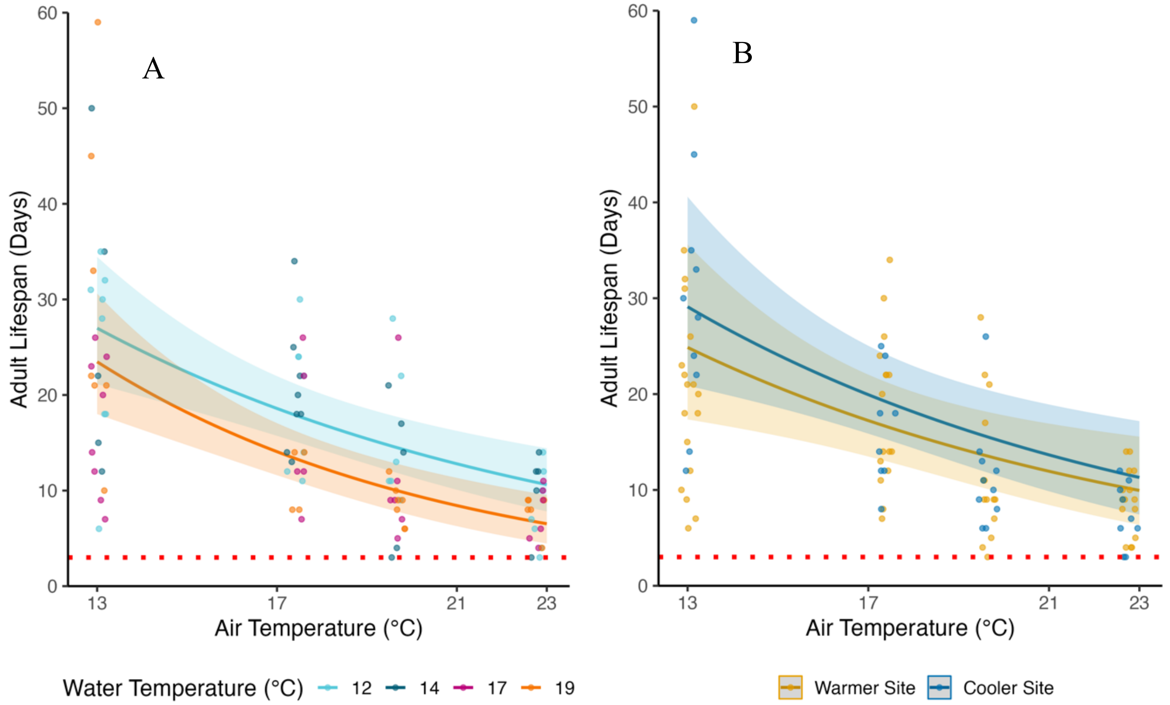

Figure 8. Predicted lifespan of adults from the two populations across four air temperature treatments. For better visualization, panel A shows median lifespan at just the warmest and coolest water temperatures and combines data from both sites. See Fig. S5 for the complete graph. Panel B illustrates the difference in predicted lifespan for individuals from the warmer and cooler sites exposed to 12°C water. Shaded bands show 95% confidence intervals for a Poisson distributed generalized linear model. The red dashed line shows the minimum lifespan required for reproduction observed in a separate, small study of 15 mating pairs.

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APPENDICES

APPENDIX A

SUPPLEMENTARY TABLES

Table S1. Summary of nominal and actual aquarium water temperatures.

Nominal temperature, °C	Mean daily mean temperature, °C	Mean daily max temperature, °C	Mean daily min temperature, °C	Range, °C
12	11.72	11.87	11.46	0.42
14	13.212	13.53	12.95	0.58
17	17.25	17.71	16.66	1.05
19	18.79	19.27	18.26	1.01

Table S2. Summary of nominal and actual incubator air temperatures.

Nominal temperature, °C	Mean daily mean temperature, °C	Mean daily max temperature, °C	Mean daily min temperature, °C	Range, °C
13	13.04	23.85	1.72	22.14
17	17.41	27.12	8.91	18.21
21	19.65	30.21	8.95	21.27
23	22.76	33.85	11.78	22.07

Table S3. Model summary for a linear regression that models emergence date using site, water temperature, sex, and an interaction between site and water temperature.

Variable	Regression Coefficient	95% Confidence Interval	Pr(>F)
Intercept	230.35	227.72, 232.99	---
Site (0=Cooler site, 1=Warmer site)	-26.44	-30.05, -22.83	<2e-16
Water Temperature	-2.79	-2.95, -2.62	<2e-16
Sex (0=Female, 1=Male)	-1.40	-2.07, -0.73	5.63e-05
Site*Water Temperature	0.59	0.35, 0.82	1.40e-06

Table S4. Model summary for a binomial logistic regression that models emergence success using water temperature, day of emergence period, and site.

Variable	Regression Coefficient	95% Confidence Interval	Pr(>Chisq)
Intercept	-0.88	0.09, 1.82	--
Water Temperature	0.07	0.96, 1.18	0.21
Day of emergence period	-0.14	0.81, 0.93	5.66e-05
Site (0=Cooler site, 1=Warmer site)	0.67	1.15, 3.36	0.014

Table S5. Model summary for a Poisson distributed generalized linear model that models adult lifespan using site, air temperature, water temperature, and an interaction between air and water temperature.

Variable	Regression Coefficient	95% Confidence Interval	Pr(>Chisq)
Intercept	3.99	2.75, 5.23	--
Site (0=Cooler site, 1=Warmer site)	-0.13	-0.23, -0.03	0.014
Air Temperature	-0.03	-0.10, 0.04	<2.2e-16
Water Temperature	0.05	-0.03, 0.13	2.417e-05
Air Temperature *Water Temperature	-0.01	-0.01, -0.0004	0.034

APPENDIX B

SUPPLEMENTARY FIGURES

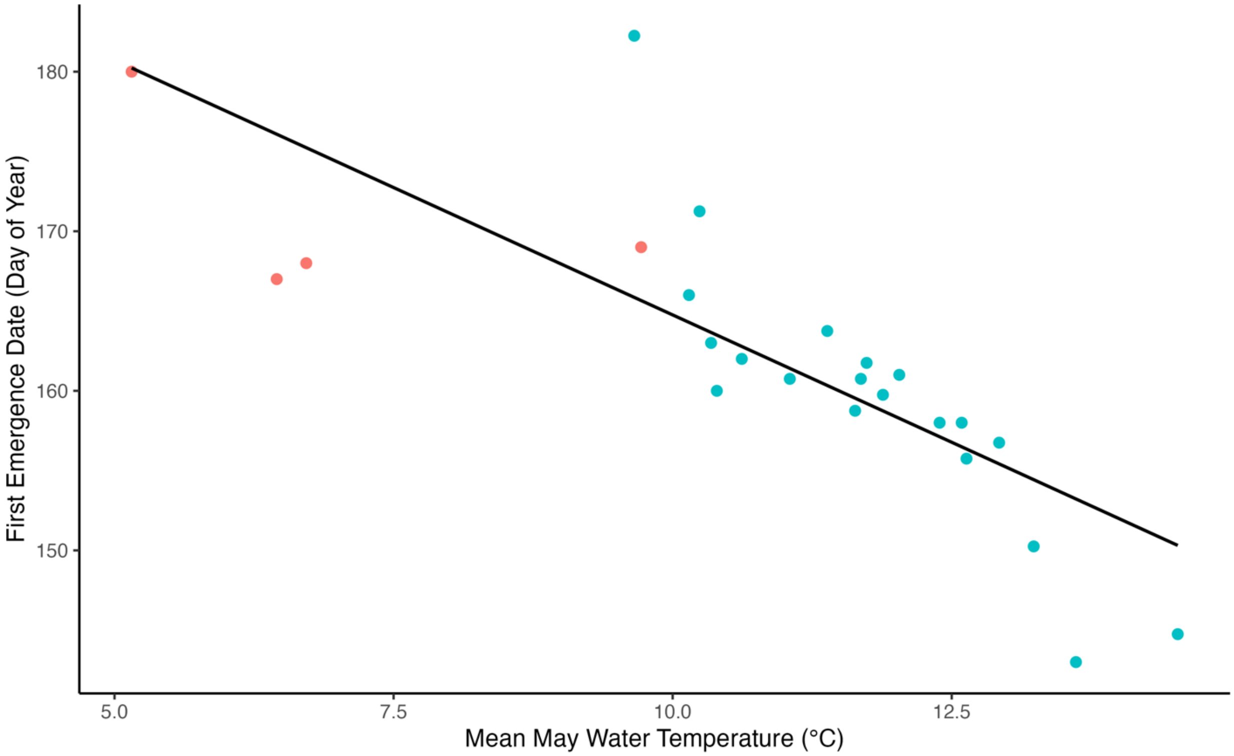

Figure S1. First emergence date (day of year) at any site was estimated by regressing day of year against mean May temperature of that site in degrees Celsius. We used historical records published in Anderson et al. 2019a Figure 3C (blue dots) and our own records from 2021 (red dots). The resulting formula was Emergence date = -3.1925*(May Temp) + 196.68

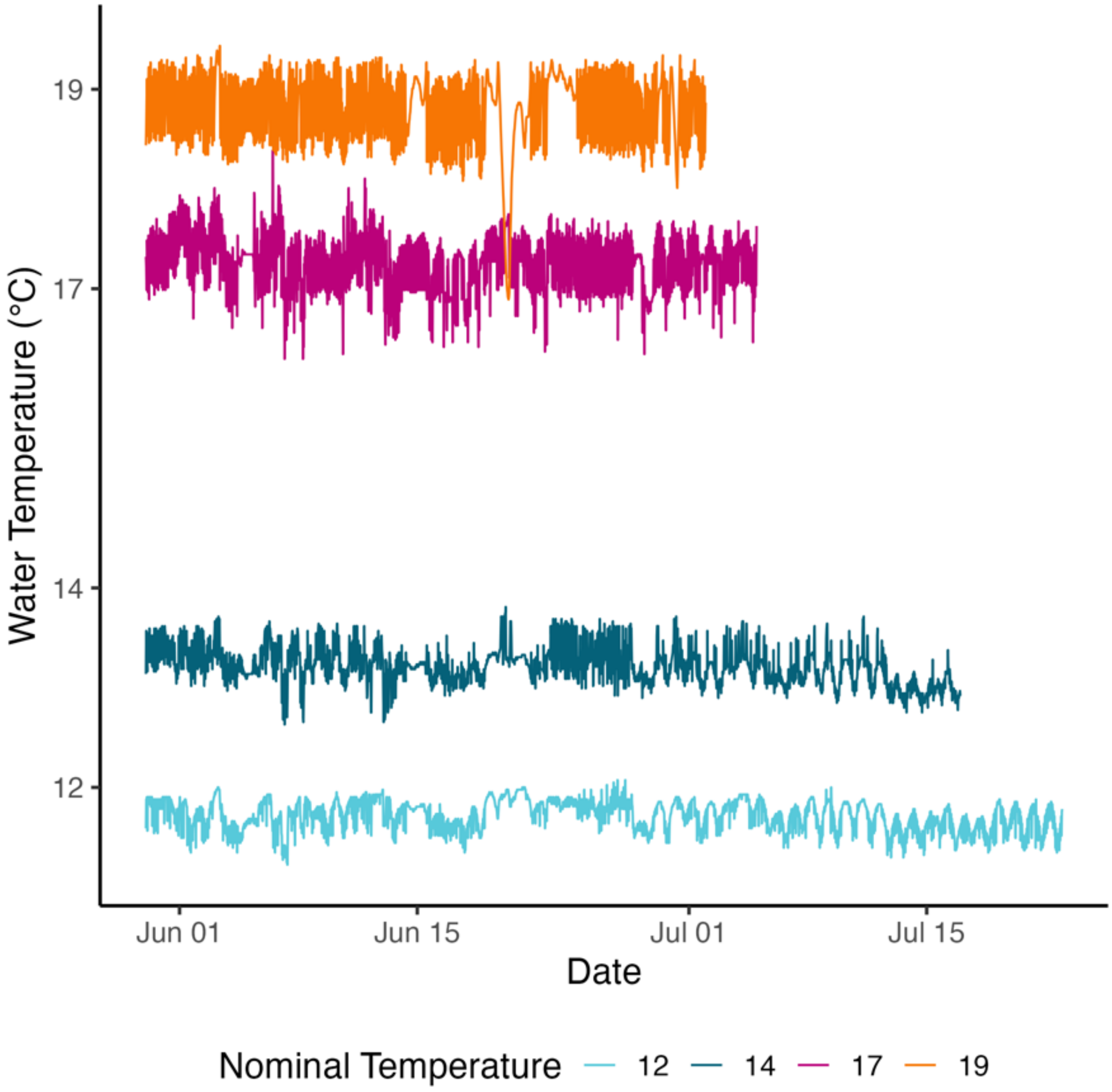

Figure S2. Graph of aquarium temperatures throughout the experiment. Aquarium temperatures over the course of the experiment remained within an average of +/- 0.38°C of the nominal temperatures. Fluctuations around the mean are the combined result of diel changes in lab temperature and chiller inefficiencies. Temperature data are shown and summarized only during the time periods when the aquariums contained salmonfly larvae.

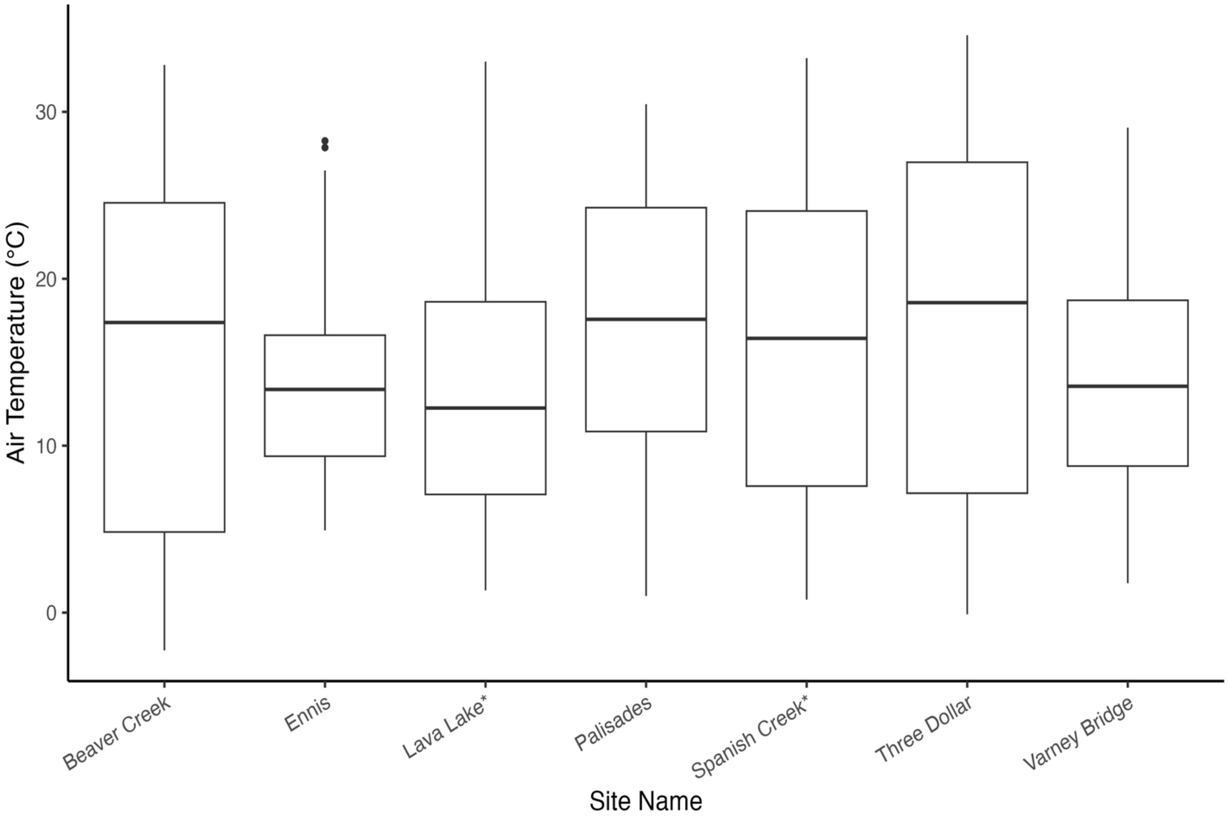

Figure S3. Graph of riparian air temperature ranges during salmonfly emergence in 2016. All sites are on the Upper Madison River in Southwest Montana, except for Lava Lake and Spanish Creek, which are on the Gallatin River. The mean daily temperature range using a one sample t-test is 22.67 °C (CI from 22.48-22.85, t_{4703}=240.36, p<.0001).

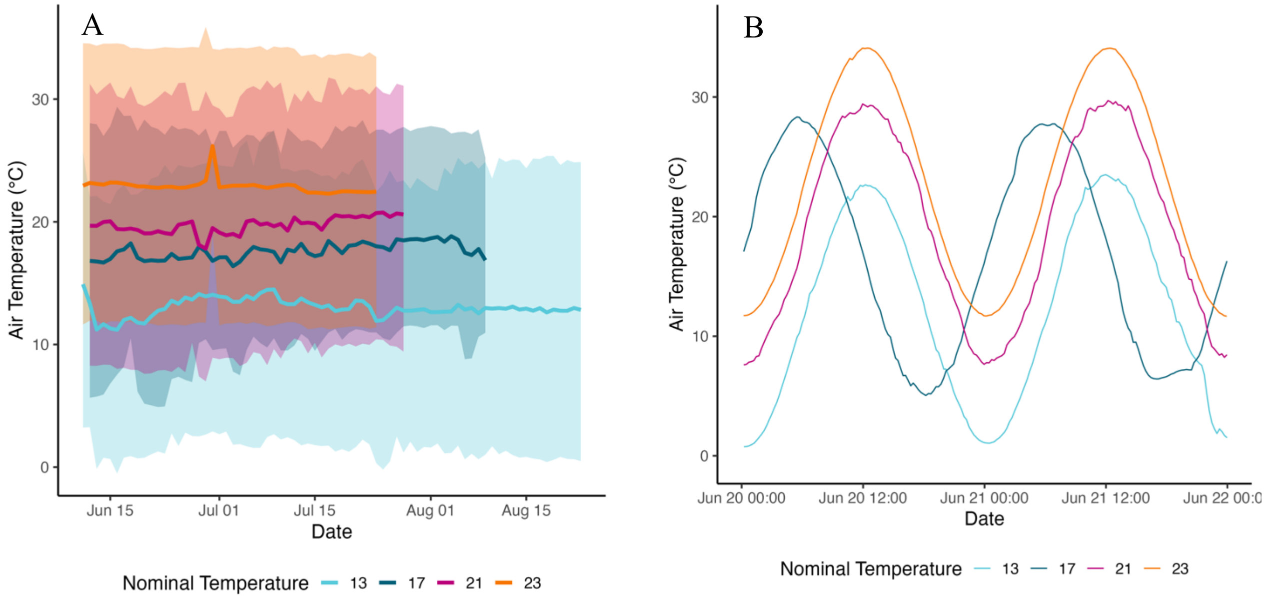

Figure S4. Graph of incubator temperatures throughout the experiment. The incubators fluctuated between maximum and minimum temperatures on a 24H cycle. The 17 °C treatment followed the curve and was very close to the nominal temperature, but the peaks occurred six hours earlier than those in the rest of the treatments. This caused a slight difference in daylight cues from the automated lights, but because the peak still happened during daylight hours and the data were within the expected range, we believe it is unlikely to have affected the outcome. Temperature data are shown and summarized only during the time periods when the incubators contained adult salmonflies.

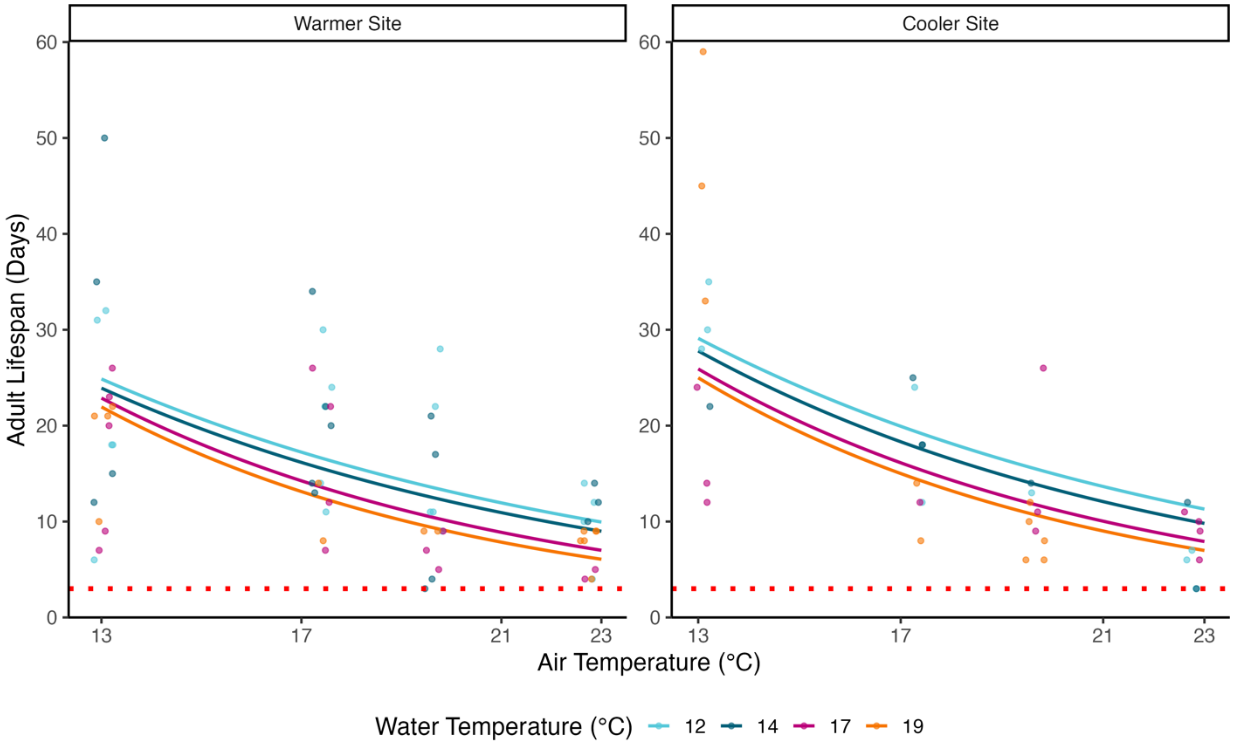

Figure S5. Lifespan of adults from the two populations across the four air temperature treatments. Colored lines show median lifespans at different water temperatures prior to emergence based on a Poisson distributed generalized linear model. The red dashed line shows the minimum lifespan required for reproduction, three days.