

INFLUENCE OF THERMAL REGIME ON THE LIFE HISTORIES AND PRODUCTION OF
ROCKY MOUNTAIN AQUATIC INSECTS

by

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ABSTRACT

Life history traits of aquatic insect taxa such as metabolism, terminal body size, and fecundity vary along natural thermal gradients. Body size, in particular, is expected to respond to temperature and may have important consequences for fecundity and the production of insects. The Thermal Equilibrium Hypothesis (TEH) predicts that aquatic insect taxa are most abundant at an intermediate 'optimal' temperature where life history traits such as terminal body size and reproductive potential are maximized, i.e., the thermal 'optimum'. A competing hypothesis, the Temperature Size Rule (TSR), predicts that individuals developing at the coldest temperatures in their range will grow more slowly, but attain the largest body sizes and therefore exhibit greater fecundity than individuals growing at warmer temperatures. Implicit in both of these theories is that population-level production, a measure of population 'success', will be greatest where terminal body size and fecundity are maximized. Few studies have investigated the TEH in the field, and none have measured the relationship between production and other life history traits in the context of these theories.

Our study focused on three common Rocky Mountain aquatic insect taxa: *Drunella doddsii*, *Hydropsyche cockerelli*, and *Ephemerella infrequens*. We quantified the influence of thermal regime on growth rates, terminal body size, reproductive potential, and population-level biomass and production, all of which potentially limit the longitudinal distribution and success of these taxa. We found that growth varied strongly with season and site, leading to significant variation in the timing of growth and terminal body size. Reproductive potential was negatively associated with mean annual temperature as predicted by the TSR. Unexpectedly, reproductive potential was not always correlated with terminal body size. Population density, biomass, and secondary production were generally positively correlated with terminal body size for *D. doddsii* and *H. cockerelli*, as expected from both predictive models. In contrast, these relationships were not as consistent for *E. infrequens*. Our findings provide new insight as to how thermal variation influences the ecology of aquatic insects in the context of the TEH and TSR. Our results should be valuable for predicting population and community responses to ongoing changes in climate.

CHAPTER ONE

INTRODUCTION

Stream temperature is a fundamental environmental variable that influences the ecology and evolution of aquatic insects (Vannote and Sweeney 1980, Ward and Stanford 1982, Caissie 2006, Houghton and Shoup 2014, Sweeney et al. 2018). Stream temperature regimes vary widely across latitudinal and elevation gradients (Vannote 1973, Constantz 1998, Langan et al. 2001), and anthropogenic factors such as greenhouse gas emissions and land-use change are modifying thermal patterns globally (Cox and Rutherford 2000, Searchinger et al. 2008, IPCC 2013). These natural and novel thermal gradients have the potential to drive variation in species life history traits, population dynamics, community assembly and composition, and ecosystem-level productivity (Ward and Stanford 1982, Ward 1992, Angilletta 2009).

Climate change is influencing freshwater ecosystems globally, with major effects on water temperature (Meisner et al. 1987, Keleher and Rahel 1996, Woodward et al. 2010, IPCC 2013). Streams in the North American Rocky Mountains are warming faster than most and are expected to continue to warm by $\sim 1^{\circ}\text{C}$ per decade (IMAF 2012, Isaak et al. 2016, Whitlock et al. 2017). Even small changes in temperature ($\sim 1\text{-}3^{\circ}\text{C}$) are ecologically relevant, as these can cause significant changes in aquatic insect growth rates, metabolic rates, adult body size, emergence times, and gender ratios (Langford and Daffern 1975, Sweeney et al. 1986, Rempel and Carter 1987, Baker and Feltmate 1989, Hogg and Williams 1996), with the potential for large effects on fecundity (Sweeney 1978). Consequently, warming is likely to have a strong influence on composition and

productivity of stream macroinvertebrate communities (Bradshaw and Holzapfel 2006, Durance and Ormerod 2007, Hering et al. 2009, Kearney et al. 2009) by modifying some of these traits. Predicting climate-induced changes to freshwater communities will require additional research that links physiological traits to population-level 'success' in the context of warming. Understanding and quantifying life history plasticity can be useful for predicting how populations may respond to subtle changes in thermal regime, which is particularly important in light of global warming trends (Keleher and Rahel 1996, Hengeveld 1990, IPCC 2013).

Within heterogeneous thermal landscapes, physiological boundaries of insect taxa are defined by thermal performance curves, influencing the geographic range where species can survive and persist (Frederich and Pörtner 2000, Angilletta 2009, Verberk and Bilton 2011, Houghton and Shoup 2014). Although geographic ranges of many stream insects are relatively large, sometimes spanning whole continental regions (Yamahira and Conover 2002, Vinson & Hawkins 2003), significant thermal variation *within* these ranges can lead to plasticity in life history attributes that influence the relative success of populations. For instance, responses of growth, metabolism, and size at emergence to thermal variation can all lead to shifts in adult body size and, ultimately, fecundity (Sweeney and Vannote 1978, Vannote and Sweeney 1980, Sweeney et al. 1986, Li et al. 2011). Such differences in fecundity may, in turn, drive changes in abundance and population-level productivity (Vannote and Sweeney 1980), although these connections have rarely been assessed. Thus, thermal regime should have far-reaching potential consequences for populations and ecosystems.

The 'Thermal Equilibrium Hypothesis' (TEH) has been proposed for aquatic insects and predicts that species have 'optimum' thermal regimes, above and below which density may be reduced (Sweeney and Vannote 1978, Vannote and Sweeney 1980; Figure 1A). At super-optimal temperatures, where thermal energy (growing degree-days; Higley et al. 1986, Sweeney 1984) accumulates more rapidly in the environment, more metabolic energy is required for metabolism and therefore diverted away from growth (Sweeney and Vannote 1978, Sweeney et al. 2018). Under this scenario, individuals are predicted to reach maturity more quickly and emerge at a smaller size with lower potential fecundity (Stearns 1992, Honek 1993, Roff 2002). Survivorship, however, may be higher in response to shorter exposure to mortality factors (Reznick et al. 1990, Peckarsky et al. 2001, Angilletta et al. 2004). In contrast, at sub-optimal temperatures (slower accumulation of growing degree-days) less energy is allocated to metabolism, but growth rates may be reduced and development times protracted. Under these conditions, adults may achieve larger body size (Partridge and French 1996; Ashton 2004), but the extended time required for maturation may limit the number of generations possible during the growing period (Vannote and Sweeney 1980); in addition, the population may experience reduced survivorship (Stearns and Koella 1986). Therefore, natural selection should favor life history strategies between these two extremes – those with intermediate generation times, relatively large body sizes, and high fecundity to offset any losses to mortality. The temperature at which these life history gains and losses are optimized should vary among taxa depending on their evolutionary history and biogeography (Vannote and Sweeney 1980).

A competing hypothesis, the Temperature Size Rule (TSR; Bergmann 1847, Atkinson 1994, Atkinson and Sibly 1996; Figure 1B), predicts that maximum body size, and therefore other measures of success, such as fecundity (Stearns 1992, Honek 1993, Roff 2002) and population density and productivity (Chapman 1931, Buckley et al. 2008), should increase with declines in temperature. Similar to the TEH, the TSR utilizes evidence that decreases in temperature lead to increased energy allocation towards growth rather than metabolism, resulting in larger body size (Sweeney and Vannote 1978, Sweeney et al. 2018). In contrast to the TEH, however, the TSR does not incorporate environments that are “too cold” where metabolic processes cease. Indeed, aquatic insect activity and growth have been recorded at or below 0°C (Danks 1971, Ward 1976, Lillehammer 1978). Thus, according to the TSR, if constraints on survivorship are relatively similar across sites, species should be most successful at the coldest sites in their range, rather than at some intermediate optimum (Sweeney et al. 2018). However, some studies suggest there may not be a universal predictive model for how species’ life histories respond temperature, but rather that certain models apply to some species and not others (Angilletta and Dunham 2003; Angilletta et al. 2004). These same studies highlight the need for broad, multi-species investigations under natural environmental conditions.

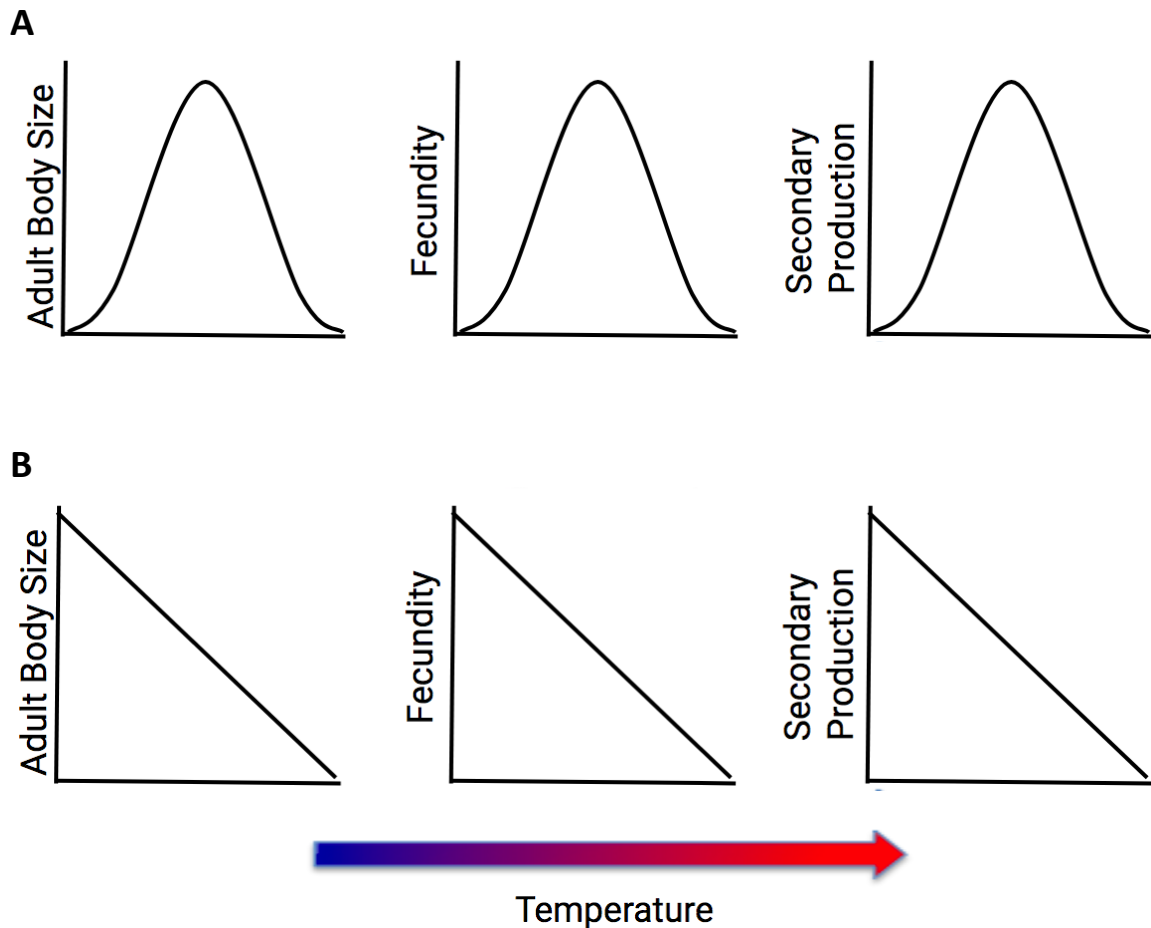


Figure 1. TEH (A) predicts an intermediate temperature optimum for aquatic insect life history traits, whereas the TSR (B) predicts optimization at coldest temperatures.

Most studies investigating these two hypotheses and other thermal growth models have been conducted under static temperature laboratory conditions (Atkinson 1994, Gillooley et al. 2001, Sweeney et al. 2018), and only a few have investigated them in the field (Sweeney 1976a, Stanley and Short 1988, Rader and Ward 1990). Though invaluable for understanding physiological responses to temperature gradients, these laboratory conditions do not adequately mimic the temperature regimes most aquatic insects experience in nature (Bergant and Trdan 2006, but see Sweeney 1976a and

Carrington et al. 2013). Most streams, for example, exhibit large diel, seasonal, and annual temperature fluctuations (Macan 1958, Edington 1965, Clifford 1978, Vannote and Sweeney 1980, Rader and Ward 1990), and this variation has been shown to significantly impact aquatic insect growth rates (Rader and Ward 1990), which may have cascading influence on other life history traits.

The magnitude and timing of accumulated growing degree-days have been shown to influence key aquatic insect life stages (Sweeney 1976a) such as diapause (Brittain 1982, Sweeney 1984), pupation (Chapman 1988), and emergence timing (Macan and Maudsley 1966, Brittain 1976, Sweeney 1976b, 1978), but few studies have examined their influence on growth, terminal body size (Ward 1976, Sweeney 1976a, Sweeney 1978, Rader and Ward 1990), and other life history traits *in situ*. Some studies have reported the fastest growth during the first instars (Brittain 1972, 1976) following egg hatching, while others have suggested it may occur during the penultimate and final instars before pupation and/or emergence (Heuvel 1963, Anderson and Cummins 1979, Hawkins 1990, Rader and Ward 1990, Davidowitz et al. 2004). In mountain streams with distinct seasonal temperature variation, these periods of fastest growth are often associated with warm temperatures in late summer-fall (Clifford 1977). Although cold winter temperatures are generally correlated with zero or extremely low growth rates (Brittain 1976, Clifford 1977, Rader and Ward 1990, Higley 2012), some studies have shown modest growth at near-freezing temperatures (Markarian and Lipovetskii 1976, Clarke 2003). This variation highlights the need for more focused study on periods and patterns of aquatic insect growth; how growth rates respond to thermal energy throughout

ontogeny may strongly influence terminal body size and population-level productivity. In addition, few studies have investigated how the magnitude and duration of growing degree-day accumulation within seasons falls within current life history hypotheses, such as the TEH and TSR. Revisiting these models to examine their value in predicting responses to thermal regime is increasingly important as streams and rivers experience unprecedented levels of thermal stress (Cox and Rutherford 2000, Searchinger et al. 2008, IPCC 2013).

In an effort to understand how natural variation in temperature regime influences aquatic insect life history and ecology, we studied patterns in growth rates, terminal body size, reproductive potential, and secondary production of common Rocky Mountain stream taxa. We focused on three species that coexist along a mountain river continuum to examine responses to changes in thermal regime over a relatively small geographic, but large elevation, gradient. We asked: (1) how does spatial and temporal variation in thermal regime influence life history characteristics (growth, final body size, fecundity) and distributions; and (2) does variation in these life history traits translate to differences in annual population-level biomass and secondary production? We expected that: (a) temperature regime will influence maximum body size and fecundity through its effects on seasonal growth rates; (b) the location (site) of the maximum terminal body size will differ among focal taxa; and (c) annual secondary production (the annual accumulation of biomass) will be positively correlated with body size and fecundity of mature females. Our results provide critical information (Resh and Rosenberg 2010) about how changes in stream temperature influence aquatic insect life histories and success. This knowledge

is important for predicting how aquatic insect populations and communities may respond to a changing climate.

CHAPTER TWO

METHODS

Study Area

This study was conducted over the course of one year from March 2017 through February 2018 along the west fork of the Gallatin River in southwest Montana. The Gallatin River Watershed has a catchment area of 3,480 km² and ranges in elevation from 1,225 m to 3,440 m (Gustafson 1990). The river originates in the mountainous northwest corner of Yellowstone National Park in Wyoming, flowing north to culminate at the Missouri River near Three Forks, Montana. This watershed experiences four distinct seasons. Maximum snowpack occurs during winter and melts through the spring, typically leading to peak flows during June. Following snowmelt, this watershed experiences a relatively dry summer and autumn (Farnes and Shafer 1972). Boulders, cobble, and gravel are the dominant substrate types within the Gallatin River. Bryophytes are common in the tributaries and upper Gallatin River, but not farther downstream where bed movement and scouring are more frequent (Gustafson 1990, English and Baker 2004).

Most of the landscape upstream of the Gallatin River valley contains montane coniferous forest. The upper Gallatin, near the western entrance of Yellowstone National Park, meanders through an open valley lined by exposed cliff on the east side, and hills to the west. The river then flows through a relatively narrow mountain pass where it is shaded by vegetation. Exposed rock and steep cliffs shade the river as it flows through

Gallatin Canyon. The river then emerges into an open, sun-exposed valley at Gallatin Gateway. Here, cottonwoods and other deciduous vegetation dominate the riparian zone. Suburban development, grasslands, and agricultural lands surround the river within the Gallatin valley (English and Baker 2004). The river is in a relatively “natural” state prior to the valley with no impoundments. However, significant agriculture and water withdrawals in the valley lead to reduced stream flows and warming in the summer (Poole and Berman 2001, English and Baker 2004, Cross et al. 2017).

Five sampling locations were established nearly equidistant from one another in river kilometers along the elevation gradient, beginning near the western boundary of Yellowstone National Park and terminating downstream near Manhattan, Montana (Figure 2; Table 1). The elevation difference between the highest to the lowest elevation site is 416 m.

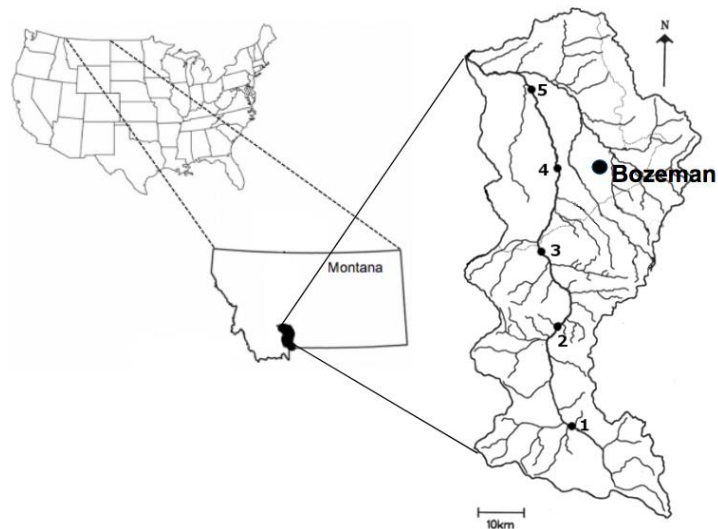


Figure 2. Map of project study sites along the mainstem of the Gallatin River, Montana. Site 1 is highest elevation near the west entrance of Yellowstone National Park. The river flows north, downstream to Site 5 near Manhattan, MT.

Table 1. Location, elevation and thermal characteristics of study sites. Tukey's HSD results lettered show differences among sites within each column. GDD = growing degree days; Avg = average.

Site	Latitude	Longitude	Elevation (m)	Total Annual		Summer-Autumn		Winter		Spring-Summer	
				GDD	Avg (°C)	GDD	Avg (°C)	GDD	Avg (°C)	GDD	Avg (°C)
1	45.06506	-111.18245	2,017	2,220 a	5.7 a	1,194 a	8.95 a	343 a	2.88 a	682 a,b	7.20 a,b
2	45.28152	-111.22528	1,816	2,507 b	6.5 b	1,386 b	10.48 b	391 b	3.24 b	726 b,c	7.78 b,c
3	45.47722	-111.26953	1,585	2,291 a	6.2 a	1,376 b	9.74 b	157 c	1.38 c	758 c	8.15 c
4	45.67308	-111.21047	1,430	2,590 b	6.6 b	1,560 c	11.97 c	151 c	1.38 c	879 d	9.39 d
5	45.86015	-111.29751	1,287	2,939 d	8.7 d	1,784 d	13.60 d	142 d	1.34 d	1,013 e	10.80 e

Measuring Stream Temperature

A pair of accuracy-checked ONSET HOBO 8k Pendant temperature loggers were deployed at each site to record stream temperatures continuously for the duration of the sampling period. These loggers were fixed to large boulders, bedrock, or submerged bridge pilings near the thalweg using climbing bolt hangers and protective casing made of PVC (Fogg et al., submitted). Thermal patterns recorded by the two loggers at each site were nearly identical and therefore averaged. To examine the potential for within-site thermal variation, four loggers were also deployed at site 4 at various locations within the stream water column: (1) 10 cm below the benthic surface near the thalweg, (2) at substrate level near the thalweg, (3) at substrate level near the bank, and (4) buried 10 cm below the substrate. Intra-site differences were greatest ($\pm 2.1^{\circ}\text{C}$) in late afternoon between the logger buried 10 cm below the substrate and one recording near-surface temperatures. Variation in temperature was minimal ($\leq 0.20^{\circ}\text{C}$) among all temperatures recorded at or above substrate-level. To examine seasonal patterns, annual thermographs were divided into winter, spring-summer, and summer-autumn periods. Seasons were chosen based on winter temperatures ($< 4^{\circ}\text{C}$) and collection dates (Rader and Ward 1990): (1) winter: November 1 – March 19 (temperatures below 4°C across sites and collection dates); (2) spring-summer: March 20 – July 20; and (3) summer-autumn: July 21 – October 31 (from the peak of the thermograph to November 1). For some insect response metrics, total and seasonal accumulated growing degree-days were used to compare influence of temperature across sites. Growing degree-days were calculated as both the seasonal and annual cumulative heat energy above the developmental threshold

of 0°C for all of the species in this study (Ulfstrand 1968, Ward and Stanford 1982, Hogue and Hawkins 1991; Ephemeroptera: Clifford et al. 1973; Trichoptera: Ross 1956, Ross 1967, Wiggins 1977).

Stream temperature can be influenced by both snowmelt influx and depletion of water. Therefore, discharge data from a USGS gauge at Gallatin Gateway (USGS Real Time Water Data 2019) were examined for abnormal patterns across the sampling period.

Study Taxa

Life history traits and population characteristics of three species (Ephemeroptera: *Ephemerella infrequens*, *Drunella doddsii*; Trichoptera: *Hydropsyche cockerelli*; Figure 3) were quantified at each of the five sites for the one-year study period. These species were chosen because they are relatively abundant across the thermal gradient (Gustafson 1990). In addition, these species are univoltine and exhibit relatively synchronous cohorts, enabling characterization of temporal life history patterns with field sampling over time (Gustafson 1990). Also, the species exhibit different feeding modes: *Ephemerella infrequens* has been characterized as a collector/gatherer, *D. doddsii* a scraper, and *H. cockerelli* a collector/filter feeder (Gustafson 1990, Merritt and Cummins 2008).

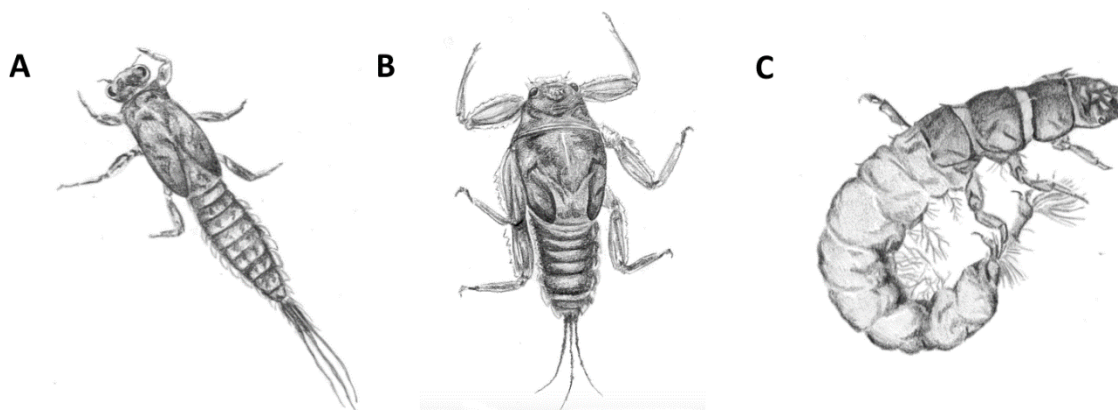


Figure 3. Study taxa: *Ephemerella infequens* (A), *Drunella doddsii* (B), and *Hydropsyche cockerelli* (C).

Immature Insect Sampling

Focal taxa were sampled once per month at each site. During each sampling event, five oversized surber samples (0.75m X 0.75m) were collected haphazardly from the same stretch of riffle at each site, representing a total of 2.81 m² of benthic habitat. The five collection points were selected to encompass a range of microhabitats such as near the thalweg to swift moving water near the bank, and “clean” substrate (little to no immediately visible masses of biofilm or algae accumulation) preferred by the mayfly species, as long as conditions allowed. These five surber samples were pooled and preserved in ethanol (80%) to provide a single representative sample on each date. This pooling precluded any estimate of spatial variability within a given sampling date and limited our ability to statistically compare population-level metrics (density, biomass, production) across sites, and thus, comparisons take place across time. On a few occasions, high stream flows or ice cover did not allow for safe sample collection at some sites.

In the laboratory, benthic samples were separated into fine ($<1.0\text{ mm}$ $>250\text{ }\mu\text{m}$) and coarse ($>1.0\text{ mm}$) fractions with a stacked sieve series. Focal taxa were removed from these samples in the laboratory with a dissecting microscope at 10X- 40X magnification. Smaller yield samples were picked completely, while others were typically subsampled to 1/8 with a Folsom Plankton Splitter until at least 100 individuals per sample were enumerated. Density (number/ m^2) and biomass (mg ash-free dry mass [AFDM]/ m^2) were quantified for each taxon (mass calculated using body length-mass conversions; Benke et al. 1999), though not all taxa were present at all sites during each collection month. Lengths of specimens were measured to the nearest 0.1 mm with an ocular micrometer ($N = 4,666$). Ephemerellids were measured from the tip of the head to end of the abdomen, and hydropsychids were measured from the tip of the head to the beginning of the anal proleg. Individual biomass was estimated using previously published taxon-specific length-mass relationships (Benke et al. 1999). The sample portion not used for quantifying density and biomass was further sorted to augment the number of each taxon included in size frequency distributions to reach at least 50 individuals per date.

Calculating Growth Rates and Annual Secondary Production:

Instantaneous growth rates (g) were calculated between each sampling period using differences in body size distributions between seasonal start and end dates as: $g = (\ln(M_f) - \ln(M_i))/d$, where M_f is average final body mass of the cohort, M_i is average initial body mass of the cohort, and d is the number of days in the season.

Population-level secondary production was estimated at each site using the instantaneous growth method (Benke 1984, Benke and Huryn 2017). This method calculates the product of average biomass and individual growth rates within each size class for each time interval and sums the production of these intervals over the year (13 sampling dates, 12 time intervals). Interval production ($\text{mg AFDm m}^{-2} \text{ interval}^{-1}$) was calculated as $P_{\text{int}} = \sum_i ([B_i(t+1) + B_i(t)]/2) \times g_i \times d$, where i = number of intervals collected for each species at each site, $B_i(t+1)$ = mean larval biomass at sampling interval $t + 1$, $B_i(t)$ = mean larval biomass at sampling interval t , g_i = size-specific instantaneous growth rate, and d = number of days in the interval (annual secondary production equals $\sum(P_{\text{int}})$). Annual secondary production for each population was estimated as the sum of all interval production values.

Mature Larval Sampling:

To calculate reproductive potential at this site, black-wing stage *E. infrequens* and *D. doddsii* nymphs about to emerge were separated from benthic samples or collected at each site close to the emergence periods (twice weekly: June – July 2017, Gustafson 1990), measured, and stored in 80% ethanol. Although *D. doddsii* were collected intermittently at site 4, no black-wing stage nymphs were found near or during emergence periods to calculate reproductive potential at this site. Most hydropsychids develop eggs after emergence (Dodson 1935, Unzicker 1968) and this eliminated our ability to describe the influences of stream temperature on reproductive potential of *H. cockerelli*. Eggs were collected from ephemerellid nymphs in the black-wing stage by dissecting gravid females and scraping/flushing all eggs onto a petri dish. Eggs were then

evenly dispersed across the petri dish, digitally photographed, and counted using ImageJ processing software (Version 2.0.0-rc-43/1.51w, Rasband 2016). ImageJ software final counts were checked for accuracy by hand-counting the eggs in 6 petri trays; minimal differences in count were detected. Female body lengths were converted to AFDM using methods described above (Benke et al. 1999). Reproductive potential during the emergence phase was estimated by multiplying half of June densities (assuming a 1:1 male to female ratio; Peterson et al. 2004, Sweeney et al. 2018) by the predicted number of eggs (based on regressions of length vs. number of eggs) of each species at each site. However, it should be noted that this approach may lead to biased estimates of actual reproductive potential because (a) June densities may not equal the actual number of adult females emerging, (b) temperature has been shown to alter aquatic insect sex ratios (Sweeney et al. 2018), and (c) post-emergence behavior can influence reproductive success (Ridley 1988, Taylor et al. 1997).

Measuring Food Availability and Quality:

Differences in food quality or quantity, along with temperature, have the potential to influence invertebrate life history traits and/or population-level metrics (Ward and Cummins 1979, Sweeney and Vannote 1984, Sweeney et al. 1986). Thus, seston and epilithon were measured quarterly during macroinvertebrate collections. Seston concentrations were measured with a seston net (63 μm) placed in the river at each site at the upstream boundary of the insect collection area for a recorded amount of time between X and Y minutes. Velocity into the center of the net was recorded for the duration of net deployment using a flow meter, and then multiplied by net volume to

calculate seston flux on a per liter basis (Hauer and Lamberti 2007). A subsample of seston was collected onto a glass microfiber filter (Whatman GF/F 0.7 μm), and chlorophyll *a* content was ethanol-extracted and measured using a Turner Trilogy Laboratory fluorometer (Hauer and Lamberti 2017). The remainder of the sample was separately dried, weighed, ashed, and reweighed in the laboratory to quantify total organic matter (as AFDM; Hauer and Lamberti 2007). These values were used to estimate the quantity (AFDM) and quality of food resources (using AFDM:chlorophyll *a* ratios as a proxy; Feio et al. 2010) for drift-feeding hydropsychids. High values of AFDM: chlorophyll *a* were interpreted as relatively low 'quality' seston.

To estimate food quantity and quality for the algal-grazing and omnivorous Ephemeroptera species, epilithic biofilm was scraped quarterly from benthic cobbles (10 cm^2 area; $n = 10$ per site) and pooled, totaling 100 cm^2 of rock surface area sampled at each site on each date. Samples were returned to the laboratory and analyzed in a similar fashion as above to examine inter-site variation in food quantity (AFDM) and quality (AFDM:chlorophyll *a*; Hauer and Lamberti 2007).

CHAPTER THREE

ANALYSIS

Temperature

All statistical analyses were performed in R 3.5.1 (R Core Team 2018). Among site variation in temperature was analyzed using a repeated-measures mixed effects ANOVA (rmANOVA), accounting for the fixed effect of season, and the random effect of time. If significant effects were detected ($p < 0.05$), then *post-hoc* Tukey's Honestly Significant Difference (HSD) multiple comparisons were used to determine differences in seasonal temperatures among sites as well as differences within sites among seasons (Gotelli and Ellison 2012).

Insect Life History Trait Variation

Shapiro-Wilks tests revealed that body sizes of *E. infrequens* and *D. Doddsi* were not normally distributed. Therefore, all body size data for these species were square root- (*E. infrequens*) and log- (*D. doddsii*) transformed for subsequent analyses (Ellison and Gotelli 2012). *H. cockerelli* body sizes were normally distributed and were not transformed.

Bootstrapping was used to quantify variance associated with seasonal growth rate estimates (Huryn 1996). Body size distributions were randomly resampled 1,000 times (Wilkinson and Engelman 1996) between sampling intervals for each season at each site and used to calculate 1,000 estimates of growth. These estimates were then used to

calculate median growth rates and 95% confidence intervals. A two-way ANCOVA was conducted to assess the effects of season and site on growth rates as well as growth rate per degree-day with body size as the covariate. Differences in terminal body size among sites were examined using one-way ANOVA and Tukey's HSD.

To quantify the relationship between female body size and egg count for comparison of reproductive potential, body sizes of nymphs in the black-wing stage were regressed against total number of eggs, providing replication for analysis. One-way ANOVA was used to determine differences in reproductive potential among sites.

Inter-site differences in density, biomass, annual secondary production, and annual production to biomass ratios (P:B) were examined qualitatively due to pooling of samples for each site-date combination.

Food Quality and Quantity

Among site variation in biofilm, seston, and autotrophic index were examined across quarterly collections using one-way ANOVA and post-hoc Tukey's HSD (Ellison and Gotelli 2012).

Reproductive Potential Versus Secondary Production

Pearson correlation coefficients were used to examine relationships between reproductive potential and production, body size, and average number of eggs per female.

CHAPTER FOUR

RESULTS

The temperature regime of the Gallatin River is exemplary of a Rocky Mountain stream. Temperatures were warmest in the summer and at or near freezing in the winter, with daily mean temperature generally decreasing in the autumn and increasing in the spring at all sites (Figure 4A). Mean annual temperature differed by 3°C between the highest and lowest elevation sites (sites 1 and 5, respectively; Figure 4A, Table 1) and increased progressively from site 1 to site 5, with the exception of site 3 in the Gallatin Canyon, which was slightly cooler than sites 2 and 4 (Table 1). Mean daily temperatures were significantly different among sites (repeated measures ANOVA, $F_{(1,4)} = 741.98$, $p < 0.0001$) on an annual scale, except for sites 1 and 3, and sites 2 and 4 (Tukey HSD; Table 1).

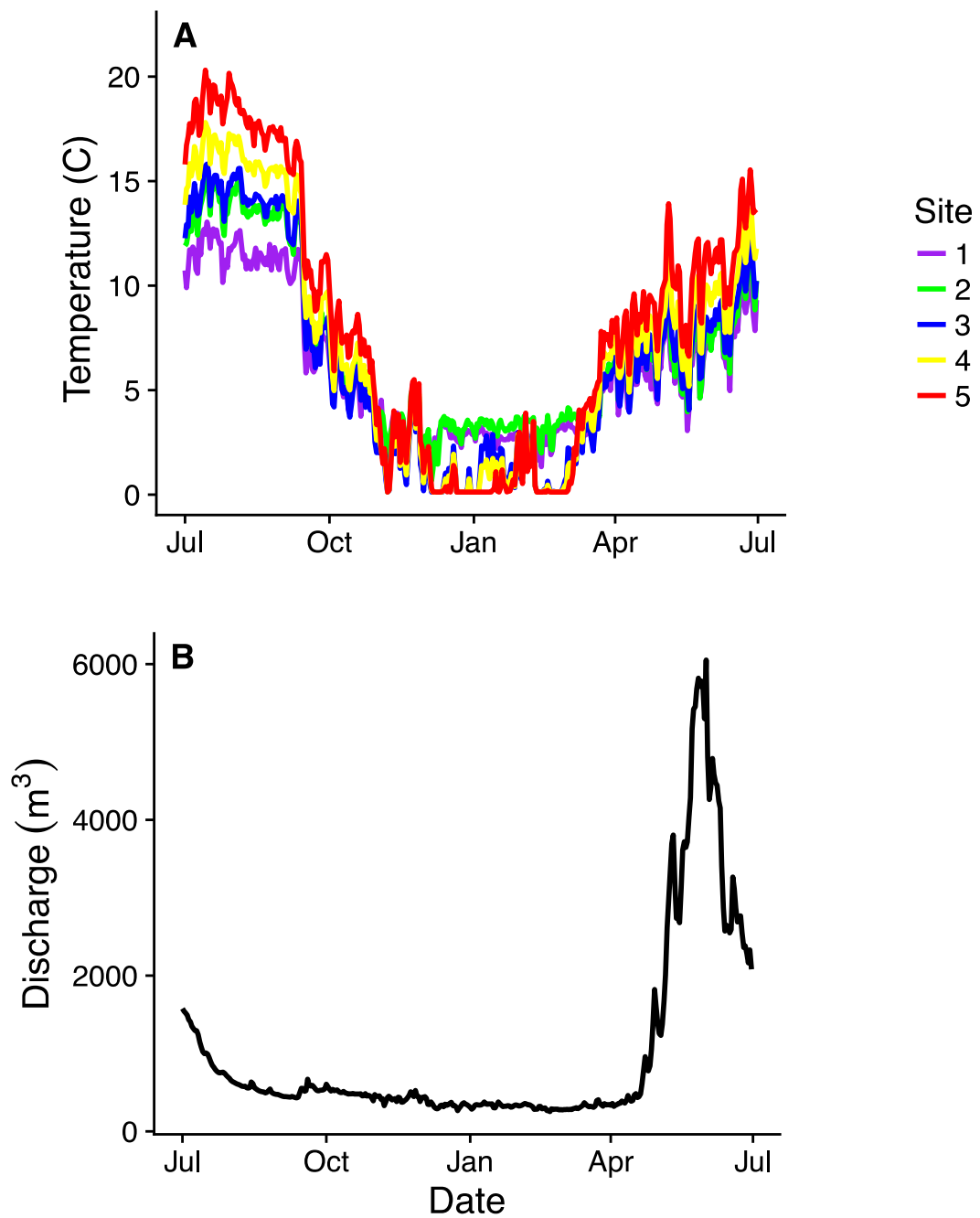


Figure 4. Mean daily temperature at five sites along the mainstem of the Gallatin River (A). Error between the two loggers at each site are omitted for visual appeal, simplification, and lack of differences. Discharge near site 4 (B; USGS Realtime Water Data) from March to December 2017, and January to March 2018.

Mean daily temperatures differed among sites (rmANOVA, $F_{(1,4)} = 118.61$, $p < 0.001$, Tukey HSD: $p < 0.05$) and seasons; they were highest at all sites in the summer-autumn and lowest in the winter (Figure 5; (rmANOVA, $F_{(1,4)} = 324.57$, $p < 0.001$, Tukey HSD: $p < 0.05$). Spring-summer temperatures were considerably warmer than winter, but cooler than summer-autumn (Figure 5; Tukey HSD: $p < 0.05$). Mean daily temperature generally decreased with increasing elevation in summer-autumn and spring-summer. The opposite was true in winter when mean daily temperatures were greatest at the highest elevation sites but near freezing at the lowest elevation sites. Variation in mean daily temperature at all sites was greatest in the summer-autumn (up to 4.65°C) and lowest in the winter (up to 1.90°C; Table 1).

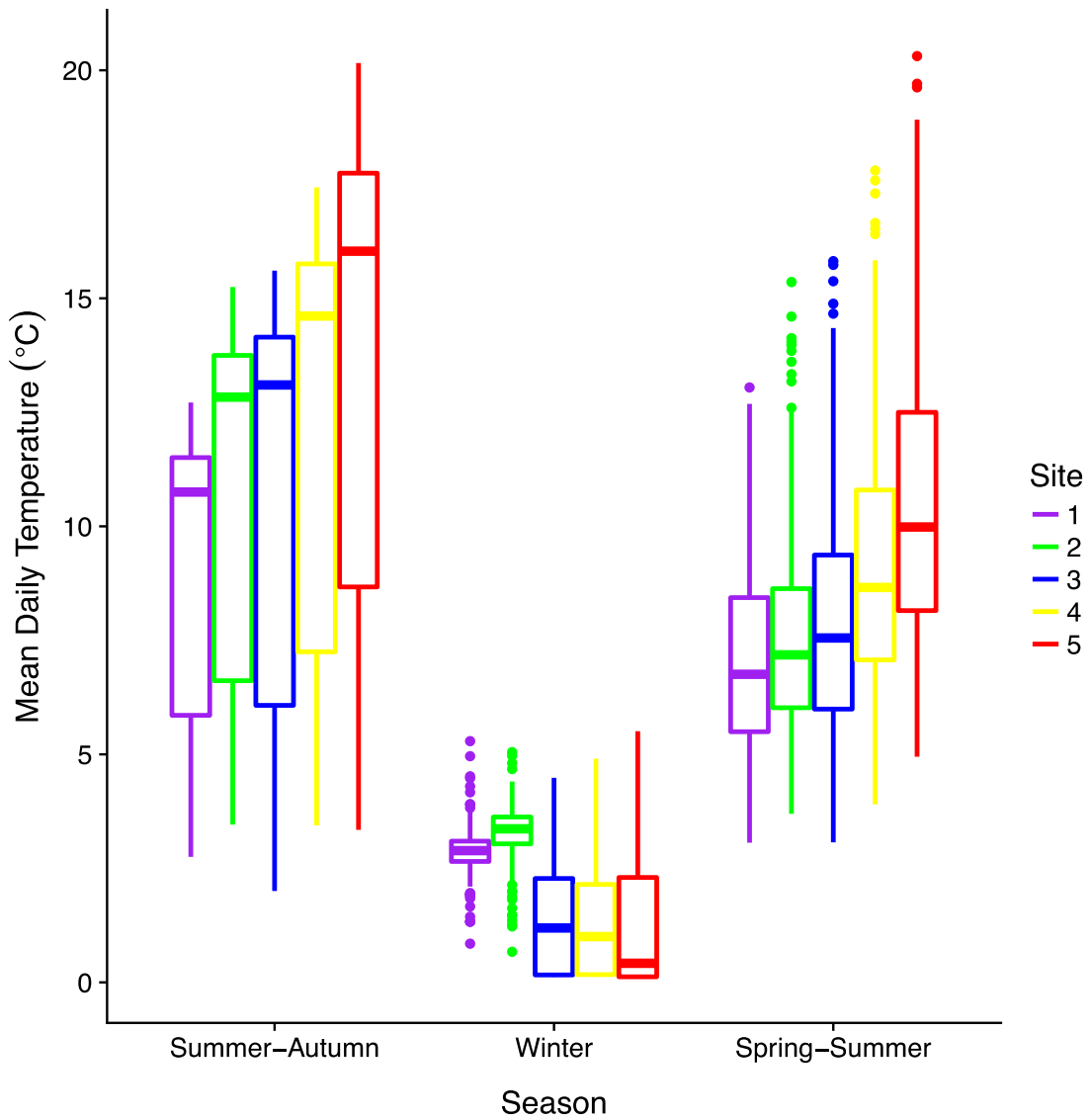


Figure 5. Mean daily temperatures across sites and seasons. Boxes show the median and the 25th-75th-percentile range. Whiskers extend to the 95th percentile.

Seasonal differences in temperature among sites and elevations were reflected in patterns of degree-day accumulation over time. Total growing degree-days across sites followed mean annual temperature trends (Figure 6A; repeated measures ANOVA, $F_{(1,4)} = 2,450.14$, $p < 0.0001$), with site 5 accumulating the greatest number of total growing degree-days (2,939), and site 1 the fewest (2,220; Table 1; Figure 6B; Tukey HSD).

Significant seasonal differences in growing degree-day accumulation were observed among sites (rmANOVA, $F_{(1,4)} = 613.14$, $p < 0.0001$). Total growing degree-day accumulation was greater in the summer-autumn than in other seasons, and was greater in spring-summer than in winter (Tukey HSD; $p < 0.05$; Table 1). Growing degree-days decreased with elevation in the spring through autumn, but increased with elevation in the winter (Figure 6A; Table 1; one-way ANOVA, $F_{(1,4)} = 101.6$, $p < 0.0001$; Tukey HSD; $p < 0.05$). In the winter, sites 3-5 accumulated growing degree-days at an average rate of ~1 per day; in contrast, sites 1 and 2 accumulated over twice as many winter growing degree-days (2.83 per day; Table 1). This disparity was evident in the slopes of cumulative distributions in winter; sites 3-5 had slopes near zero, whereas slopes at sites 1 and 2 remained positive (Figure 6B). These patterns underscore the large differences in winter heat accumulation between high and low elevation sites.

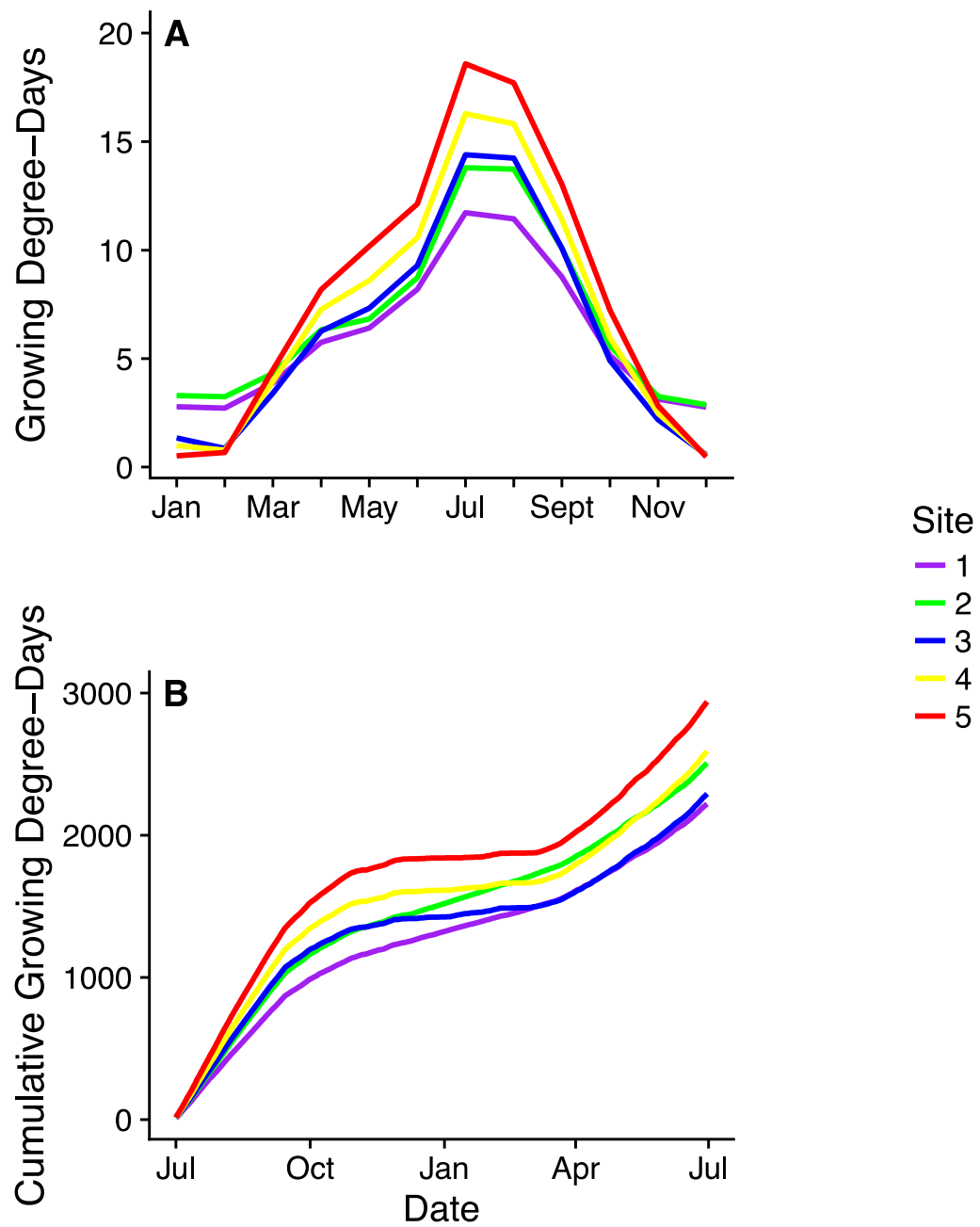


Figure 6. Growing degree-days across study sites, showing (A) mean daily growing degree-day trends throughout the year and (B) accumulation of degree-days over time.

River discharge data (USGS Real Time Data 2019) revealed no abnormal patterns and were consistent with temperature trends. Discharge was also variable across the year, timed with high snowmelt peaking in the spring-early summer and reduced discharge in the fall, winter and late-summer (Figure 4B).

Measures of food quantity and quality showed a relatively high degree of variation and no significant differences were detected among sites (Figure 7; Table 2) with the exception of biofilm chlorophyll *a* to AFDM ratios at site 4 (one-way ANOVA, $F_{(1,4)} = 101.6$, $p = 0.01$; Tukey HSD, $p < 0.05$; Figure 7F). Because our focal taxa did not show any notable anomalies in life history trends at site 4, these food resource data were not explored further.

Table 1. One-way ANOVA results comparing food quality and quantity among sites. A single test was run for each Food Type-Measurement combination. The autotrophic index is the ratio of AFDM to chlorophyll *a*. Bold type indicates significant relationship.

Food Type	Measurement	Degrees of Freedom	F value	<i>p</i>
Seston	chlorophyll <i>a</i>	1, 4	1.73	0.22
	AFDM	1, 4	1.62	0.25
	autotrophic index	1, 4	0.81	0.55
Biofilm	chlorophyll <i>a</i>	1, 4	2.59	0.10
	AFDM	1, 4	0.64	0.65
	autotrophic index	1, 4	5.91	< 0.01

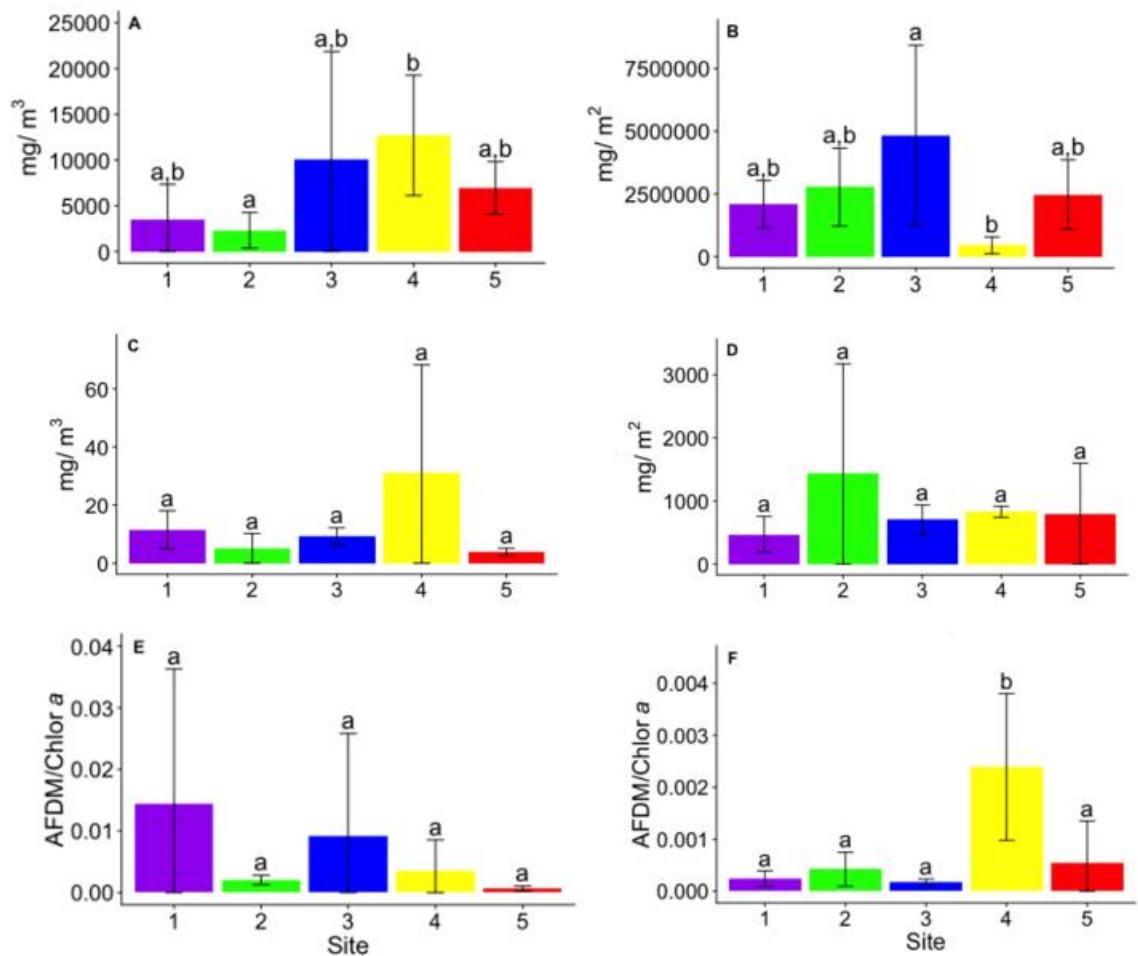


Figure 7. Estimates of food quality and quantity among study sites. Seston (A) and biofilm (B) chlorophyll *a* (Chlor *a*), seston (C) and biofilm (D) ash free dry mass (AFDM), and seston (E) and biofilm (F) autotrophic index (mean $\pm$ 1SE). Tukey HSD comparisons were made among sites and differences are shown with above-bar letters.

Temporal and spatial variation in body size was observed for each of the focal taxa. Body sizes of both ephemereid species (*E. infrequens* and *D. Doddsi*) increased slowly through summer-autumn and winter, and rapidly during spring-summer across all sites (Figures 8A and 8B). Body sizes of both ephemereid taxa were larger at site 1 than all other sites during summer-autumn and winter. Although *D. doddsii* was not collected

at site 4 in spring and early summer, body size trends at this site were roughly similar to other sites (Figure 8B). Body sizes of *H. cockerelli* increased rapidly at sites 2 and 4 in summer-autumn, while changes in body size were much smaller at the cooler sites 1 and 3 (Figure 8C). By the end of summer-autumn, average body size of *H. Cockerelli* was 42% larger at sites 2 and 4 (17.76 ± 1.49 mg) than at sites 1 (7.65 ± 0.99 mg) and 3 (7.13 ± 0.42 mg). During the winter, changes in body size were minimal at all sites for this species. *Hydropsyche cockerelli* body sizes increased slightly at sites 1 and 3 near the end of winter and early spring (Figure 8C). Although *H. cockerelli* were detected at site 5 in August, they were not collected again until spring; thus, body sizes at site 5 were excluded from the analysis.

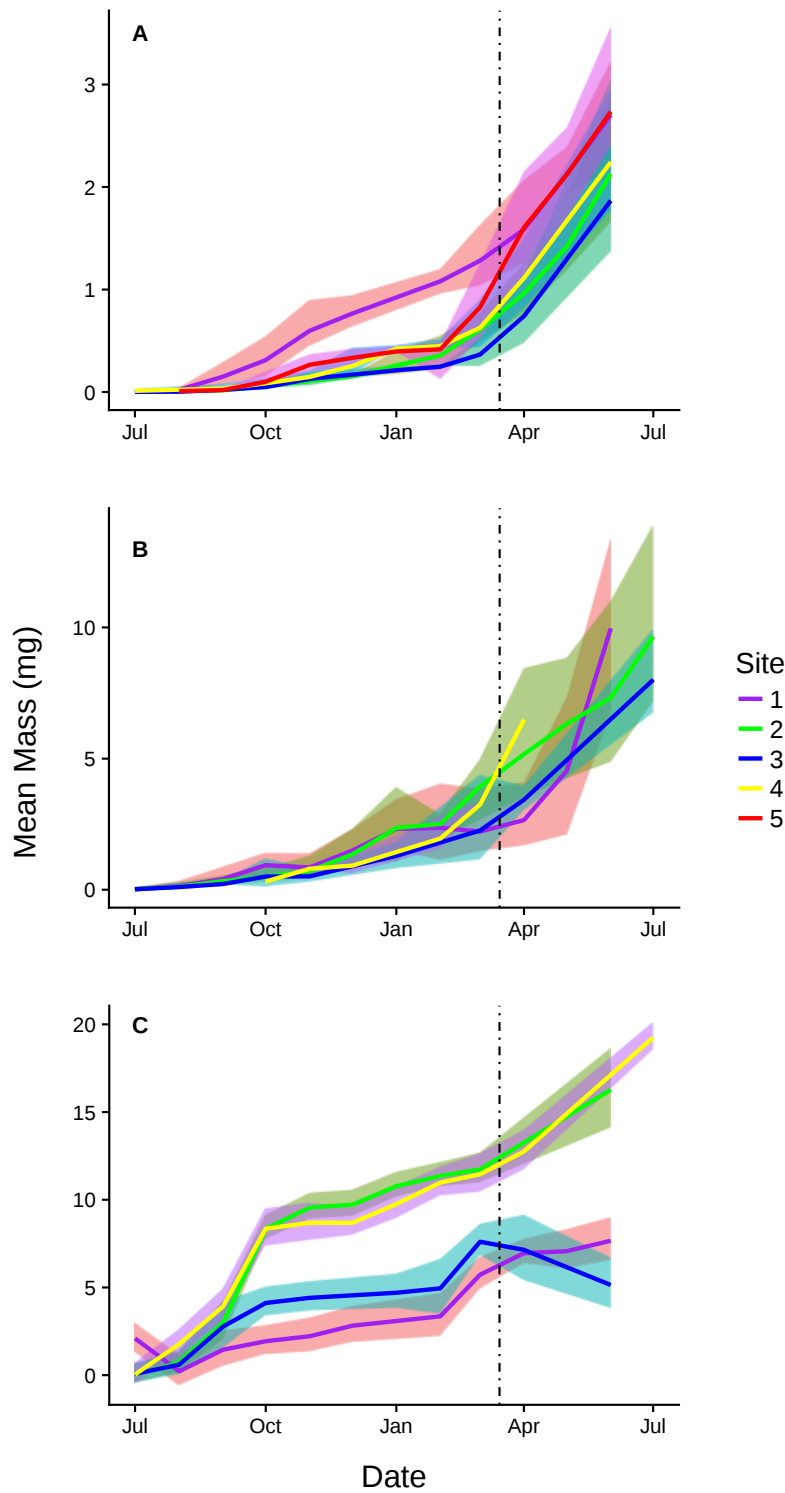


Figure 8. *Ephemera infrequens* (A), *Drunella doddsii* (B), and *Hydropsyche cockerelli* (C) per capita body sizes (mean $\pm$ 95% CI) over time. The vertical dashed lines indicate the break in cohort sampling, March to July 2017 and July 2017 to March 2018.

Although daily growth rates for all species generally decreased with body size (Figure 9; Table 3), there was still a significant effect of season and site on growth after controlling for differences in body size (Figure 10; Table 4). Growth rates were generally fastest during the summer-autumn for all sites and species (Figure 10); this trend corresponded with the warmest temperatures during the year and the presence of early-instar life stages. Growth of ephemereids slowed during winter and then increased in spring-summer as temperatures increased, though not to the magnitude observed in summer-autumn (Figure 10A and 10B). Ephemereid growth rates were not significantly different among sites (Figure 10A and 10B). Growth rates of *H. cockerelli* were consistently low through winter and spring at all sites (Figure 10C), but considerably faster at sites 2 and 4 in the summer-autumn than at other sites.

Table 3. Linear regression model describing relationships between body mass (mg) and natural log[daily growth rate (d^{-1})] for each species at each site. Bold type indicates significant relationship.

Species	Site	Equation	R ²	p
<i>E. infrequens</i>	1	$y = -3.81x - 1.15$	0.35	0.07
	2	$y = -4.04x - 0.30$	0.07	0.45
	3	$y = -3.56x - 1.67$	0.20	0.23
	4	$y = -4.17x - 0.59$	0.07	0.48
	5	$y = -4.09x - 0.58$	0.12	0.34
<i>D. doddsii</i>	1	$y = -4.57x - 0.09$	0.01	0.78
	2	$y = -3.81x - 0.21$	0.47	< 0.05
	3	$y = -4.05x - 0.13$	0.01	0.84
	4	$y = -5.00x - 0.39$	0.11	0.59
<i>H. cockerelli</i>	1	$y = -4.57x - 0.30$	0.27	0.13
	2	$y = -2.72x - 0.33$	0.80	< 0.01
	3	$y = -3.07x - 0.56$	0.74	< 0.01
	4	$y = -3.36x - 0.25$	0.44	0.05

Table 4. Two-way ANCOVA results comparing average seasonal growth rates among sites for each species. A single test was run for each species and measurement across either seasons or site, as denoted in the ‘Measurement’ column. Bold type indicates significant relationship.

Species	Measurement	Degrees of Freedom	F value	<i>p</i> <
<i>E. infrequens</i>	IGR ~ season	1,2	3,203.86	0.0001
	IGR ~ site	1,4	24.61	0.0001
	IGR/GDD ~ season	1,2	128.84	0.0001
	IGR/GDD ~ site	1,4	75.56	0.0001
<i>D. doddsii</i>	IGR ~ season	1,2	1,522.77	0.0001
	IGR ~ site	1,3	1,138.65	0.0001
	IGR/GDD ~ season	1,2	32.24	0.0001
	IGR/GDD ~ site	1,3	13.70	0.0001
<i>H. cockerelli</i>	IGR ~ season	1,2	982.21	0.0001
	IGR ~ site	1,3	544.85	0.0001
	IGR/GDD ~ season	1,2	617.48	0.0001
	IGR/GDD ~ site	1,3	328.13	0.0001

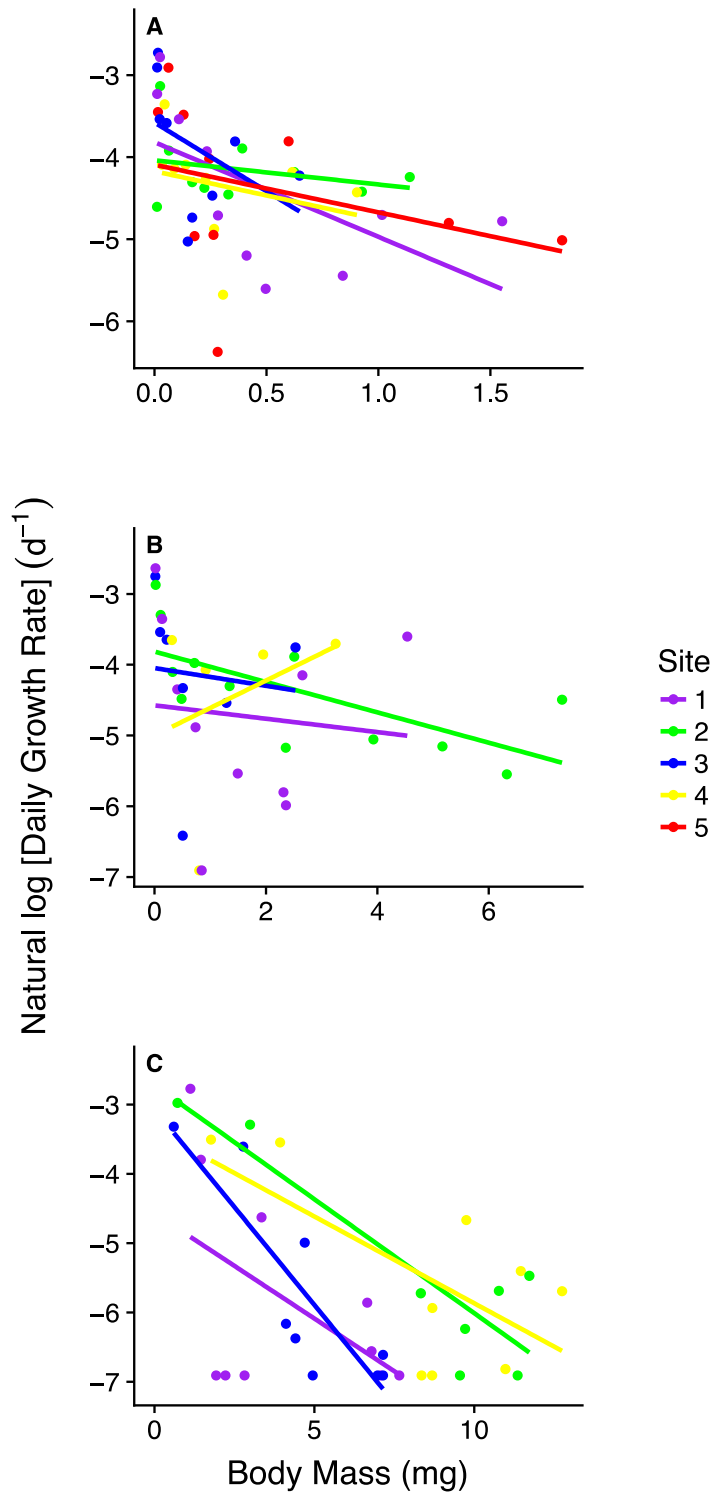


Figure 9. Relationships between body size (mg) and daily growth rate for *Ephemerella infrequens* (A), *Drunella doddsii* (B), and *Hydropsyche cockerelli* (C) across sites.

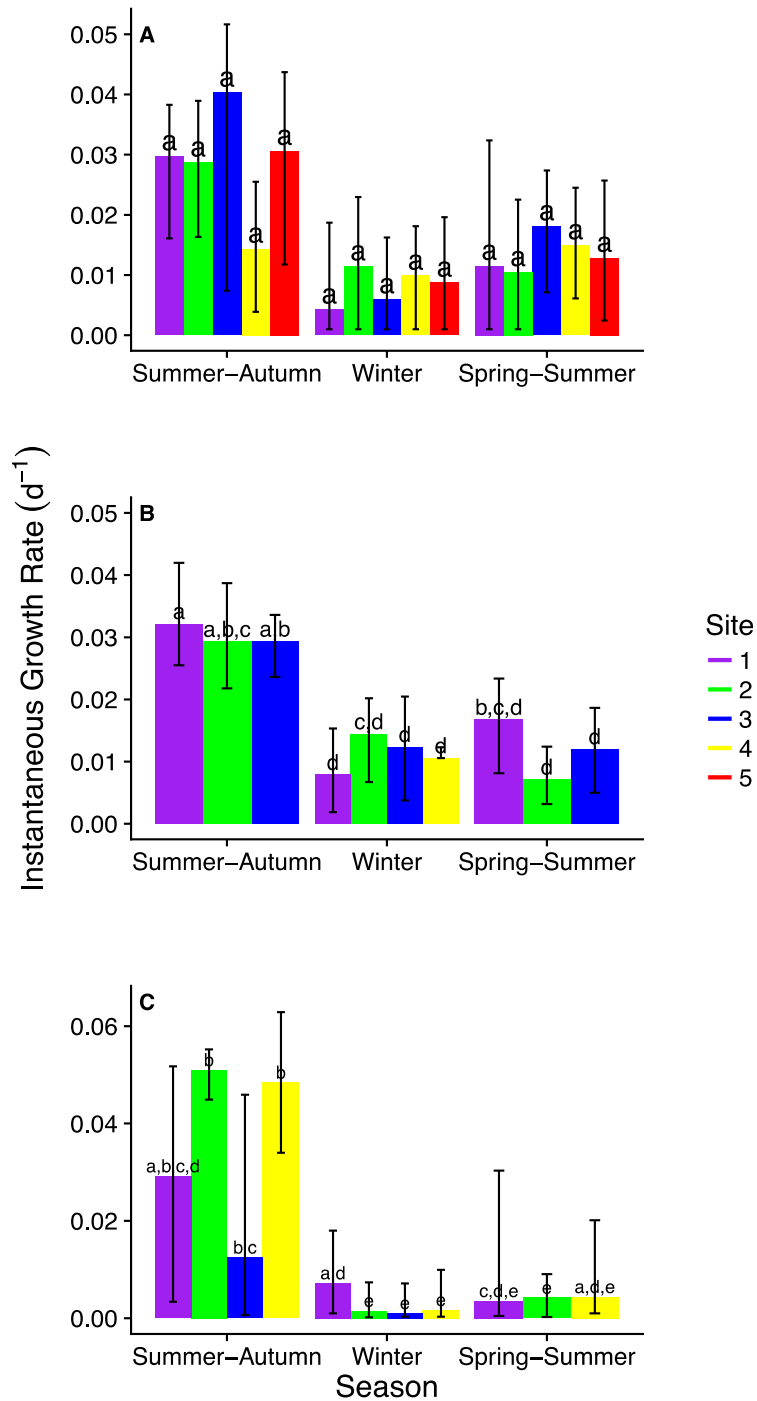


Figure 10. *Ephemerella infrequens* (A), *Drunella doddsii* (B), and *Hydropsyche cockerelli* (C) instantaneous growth rates (median $\pm$ 95%CI) across seasons. Tukey HSD comparisons are shown with letters above bars.

Growth rates were also examined on a per-degree-day basis to determine whether growth differed among sites given the same amount of thermal energy. There was a significant effect of season and site on growth per degree-day for all species after controlling for body size (Figure 11; Table 4). Growth per degree-day was highest in the winter for both ephemereleid species, and higher in summer-autumn and winter than spring-summer for *H. cockerelli* (Figure 11; Table 4). This metric of growth was also consistently highest for both ephemereleid species at the seasonally coldest sites (sites 1 and 3 in summer-autumn and spring-summer, and sites 3-5 in the winter; Figure 11A and 11B), but this pattern was not significant. *Hydropsyche cockerelli* growth per degree-day was fastest at intermediate temperature sites 2 and 4 in the summer-autumn (Figure 11C) and highly variable among sites in winter and spring-summer.

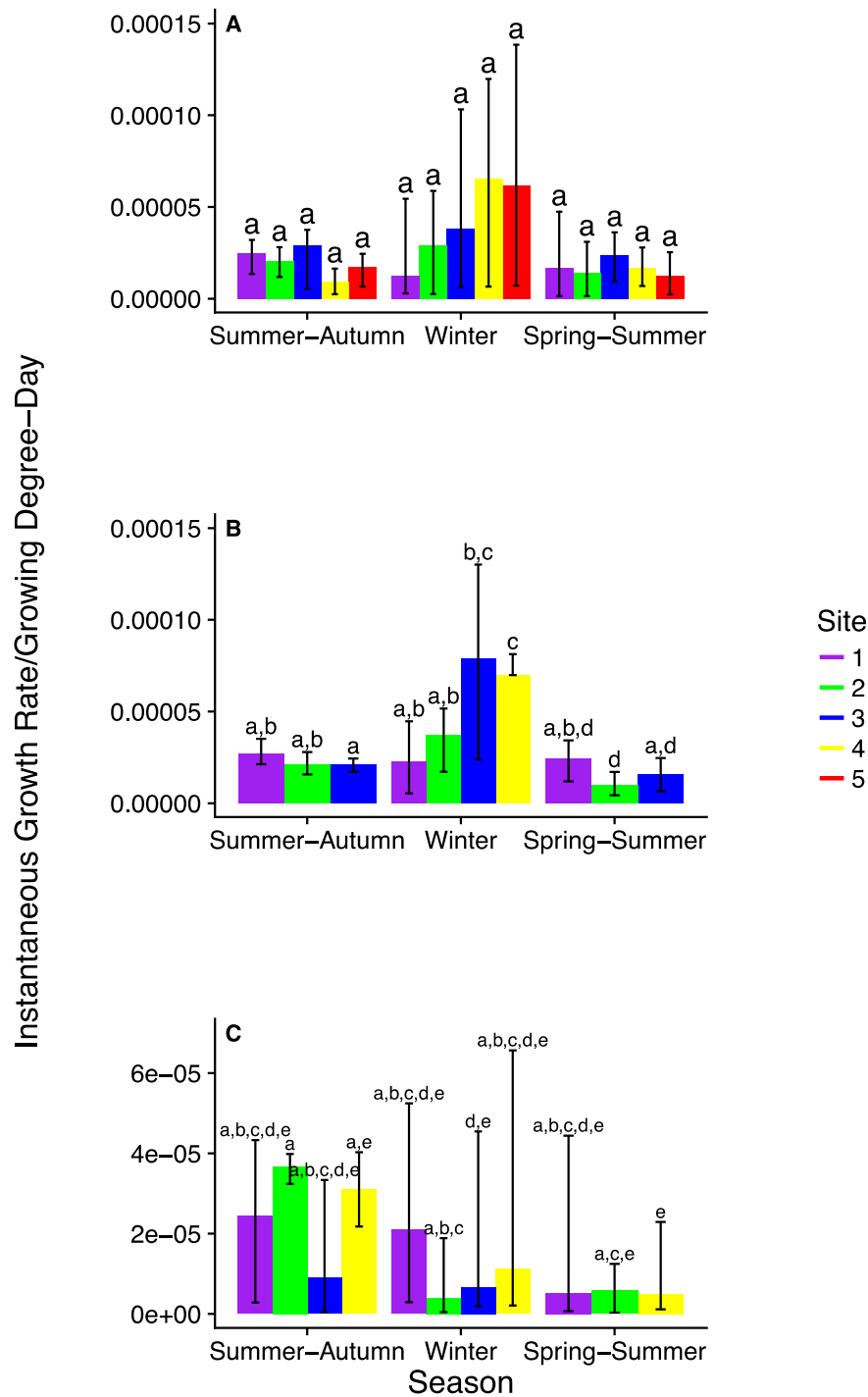


Figure 11. *Ephemerella infrequens* (A), *Drunella doddsii* (B), and *Hydropsyche cockerelli* (C) seasonal instantaneous growth rate per degree-day (median $\pm$ 95% CI). Tukey HSD comparisons were made across all sites and seasons; differences marked with letters above bars.

Terminal body size varied across sites and species (Figure 12; Table 5). Although not significantly different, average terminal body size of *E. infrequens* was greatest at the highest and two lowest elevation sites, and smallest at intermediate elevation sites (Figure 12A). Terminal body size of *D. doddsii* decreased significantly at lower elevations and increasing mean annual temperature (Figure 12B; one-way ANOVA, $F_{(1,3)} = 8.5$, $p < 0.05$; Tukey HSD, $p < 0.01$). *Hydropsyche cockerelli* achieved largest terminal body sizes at sites 2 and 4 where annual mean temperatures were intermediate (Figure 12C; one-way ANOVA, $F_{(1,3)} = 13.7$, $p < 0.01$; Tukey HSD, $p < 0.01$).

Table 5. One-way ANOVA results comparing terminal body size and reproductive potential among sites for each species. Bold type indicates significant relationship.

Species	Measurement	Degrees of Freedom	F value	$p <$
<i>E. infrequens</i>	Terminal body size	1,4	1.41	0.05
	Reproductive potential	1,4	14.33	0.0001
<i>D. doddsii</i>	Terminal body size	1,3	9.02	0.01
	Reproductive potential	1,3	993.40	0.0001
<i>H. cockerelli</i>	Terminal body size	1,3	19.25	0.01

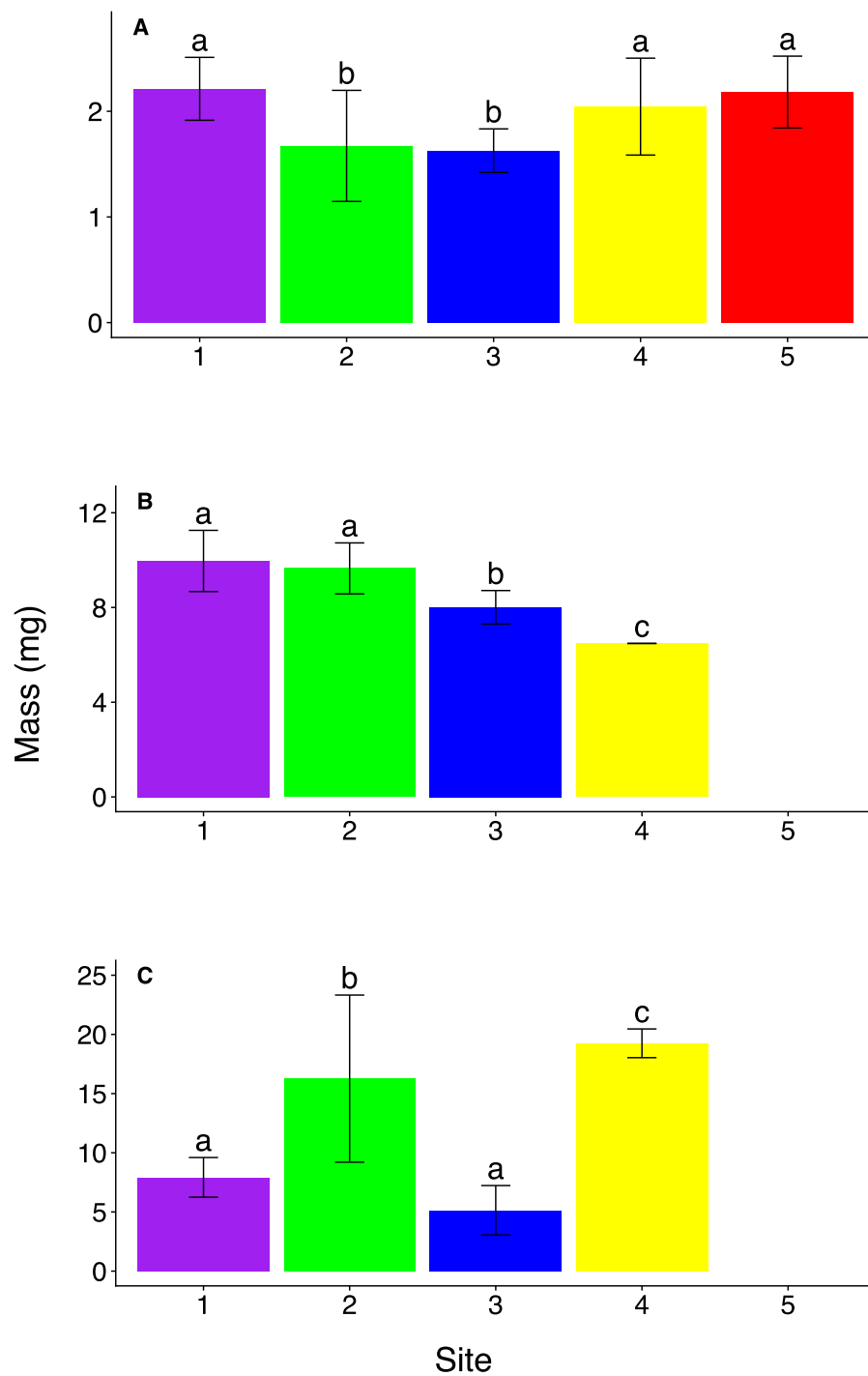


Figure 12. Terminal body size ([mg] mean $\pm$ 1SE) of *Ephemerella infrequens* (A), *Drunella doddsii* (B), and *Hydropsyche cockerelli* (C) among sites. Tukey HSD comparisons were made across all sites and differences marked with letters above bars.

Terminal body size explained relatively little variation in the number of eggs produced by black-wing stage ephemereid females when the species were considered separately (Figure 13; *D. doddsii*: $R^2 = 0.06$, $p = 0.10$; *E. infrequens*: $R^2 = 0.27$, $p < 0.001$). However, when both species were combined, body size was positively associated with the number of eggs, explaining 81% of the variation (Figure 13C; $R^2 = 0.81$, $p < 0.001$). Reproductive potential of both ephemereids decreased at lower elevations (Figure 14; one-way ANOVA, Tukey HSD, $p < 0.01$; Table 5). Reproductive potential was correlated with terminal body size for *E. infrequens* (Pearson's $r = 0.52$, $p < 0.001$) but not *D. doddsii* (Pearson's $r = 0.24$, $p = 0.10$).

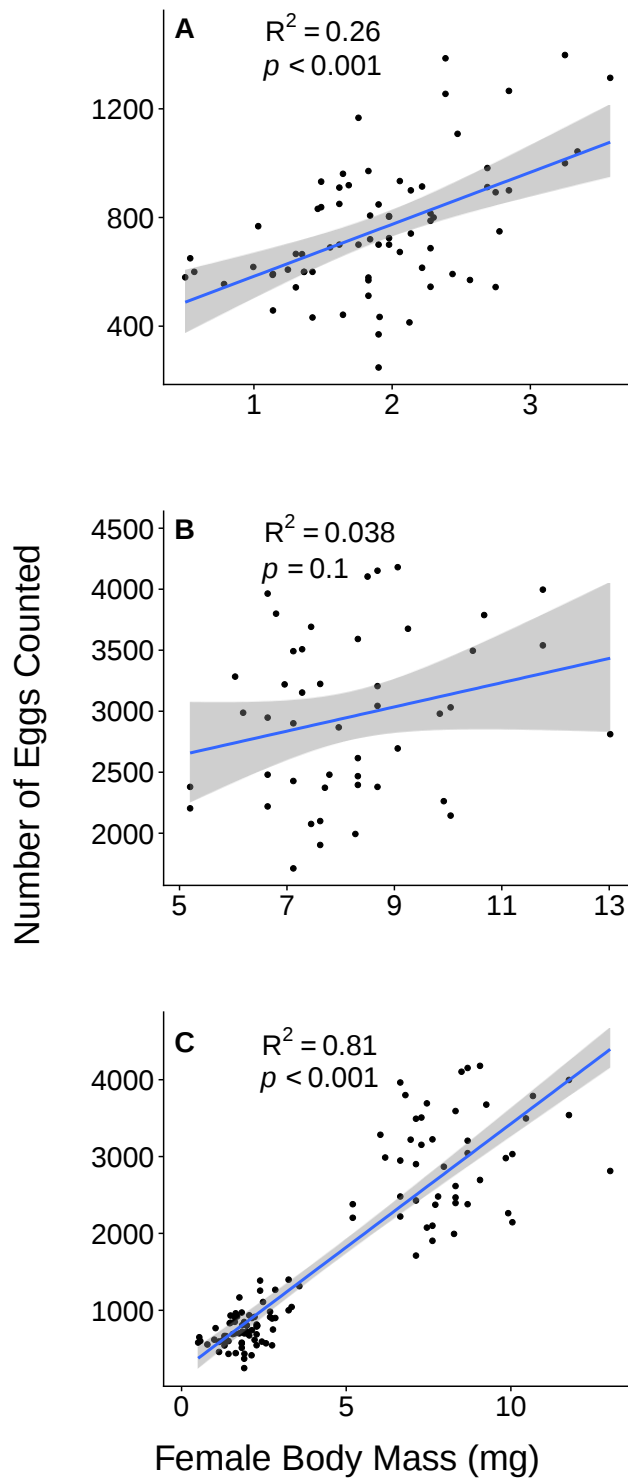


Figure 13. Linear regression model of *Ephemerella infrequens* (A; N = 72), *Drunella doddsii* (B; N=46) and combined (C; N=118) female body mass versus egg count with 95% confidence interval.

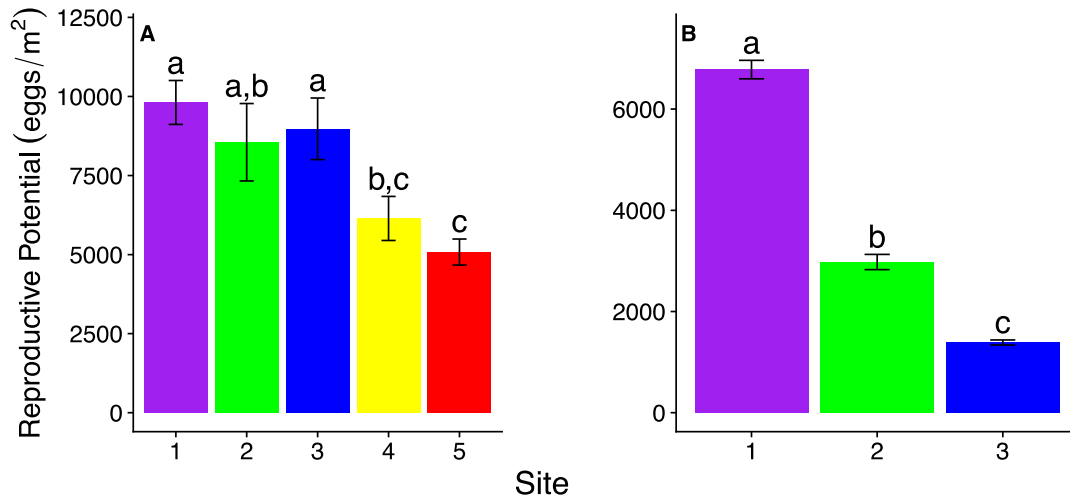


Figure 14. *Ephemerella infrequens* (A) and *Drunella doddsii* (B) reproductive potential (mean $\pm$ 1SE) estimated as number of eggs per area of benthic substrate across sites. Tukey HSD comparisons were made across all sites and differences marked with in-bar annotations.

Patterns in average population density and biomass varied by species and site (Table 6), but did not consistently follow predictions for either the TEH or TSR. For *E. infrequens*, there was no clear pattern in mean annual density among sites. Density of *D. doddsii* declined somewhat at lower elevation and increasing temperature. *Hydropsyche cockerelli* densities were clearly highest at sites with intermediate annual mean temperatures (2 and 4); *H. cockerelli* was the only species for which density was positively correlated with terminal body size (Pearson's $r = 0.77$, $p < 0.0001$). No specific pattern in biomass was observed for *E. infrequens*. *Drunella doddsii* biomass declined with increasing mean annual temperature. *Hydropsyche cockerelli* biomass was highest at sites with intermediate annual mean temperatures (2 and 4).

Annual secondary production also varied by species and site (Table 6) and was often correlated with other life history traits. Yet, there was no consistent support for the TEH or TSR. No spatial pattern in secondary production was observed for *E. infrequens*, but production was highest at sites 1 and 5 where the highest terminal body sizes were recorded. Secondary production of *D. doddsii* declined with increasing mean annual temperature and was positively correlated with final body size (Pearson's $r = 0.60$, $p < 0.0001$) and fecundity (Pearson's $r = 0.63$, $p < 0.001$). Secondary production of *H. cockerelli* was highest at sites 2 and 4 (Table 6), and positively correlated with final body size (Pearson's $r = 0.57$, $p < 0.0001$). No distinct pattern in P:B across sites was observed for *E. infrequens* (Table 6). P:B decreased with increasing temperatures for *D. doddsii* (reduced biomass turnover at lower elevations), but was positively related to temperature for *H. cockerelli*.

Table 6. Average annual density, biomass, and production at each site for each study species.

Species	Site	Density (individuals/m ²)	Biomass (g/m ²)	Production (mg/m ² /y)	P:B (yr ⁻¹)
<i>E. infrequens</i>	1	179.65	108.25	439.74	4.06
	2	67.36	28.01	101.46	3.62
	3	541.1	55.78	321.79	5.77
	4	79.67	16.99	77.24	4.55
	5	401.89	97.46	449.56	4.61
<i>D. doddsii</i>	1	31	50.93	292.71	5.75
	2	12.31	13.34	52.41	3.93
	3	14.28	9.30	36.05	3.87
	4	1.5	2.65	8.22	3.10
<i>H. cockerelli</i>	1	29.7	110.73	164.06	1.48
	2	60.9	561.92	1073.76	1.91
	3	36.2	106.58	329.03	3.09
	4	40.82	216.21	1211.10	5.60

CHAPTER FIVE

DISCUSSION

Our study investigated how life history traits of aquatic insect taxa relative to the TEH and TSR vary along a natural thermal gradient. Using measurements from a full annual cycle for three common Rocky Mountain taxa, we examined how the relationship between temperature and growth results in variation in body size, reproductive potential, and population-level metrics, all of which potentially limit the longitudinal distribution and success of these species. We found that growth varied with season and site, leading to significant variation in the timing of growth and terminal body size. We also found that reproductive potential was negatively associated with mean annual temperature as predicted by the TSR, but was not necessarily correlated with terminal body size. Population metrics such as density, biomass, and secondary production generally followed similar patterns as terminal body size for *D. doddssii* and *H. cockerelli*, as expected relative to predictive models, though were unusually high for *E. infrequens* at site 3. Our findings provide new insights into how thermal variation influences the ecology of aquatic insects in the context of the TEH and TSR, and should be valuable for predicting responses to changes in climate.

Differences in the seasonality of temperature among sites were reflected in the timing of species' growth rates and biomass accumulation. High elevation sites were warmer in the winter than lower elevation sites, likely resulting from high proportions of thermally stable groundwater inputs closer to the headwaters (Poole and Berman 2001); sites were cooler downstream in winter months. In contrast, high elevation sites were

coldest in the summer as a result of these same groundwater inputs and addition of late-spring snowmelt. Thus, low elevation sites were warmer in the summer because of increased travel time and insolation. The physiological adjustments of our focal taxa tracked these temperature trends because generation times spanned most of the year. For instance, although growth was generally greatest in summer-autumn and lowest in winter, differences among sites *within* seasons were more closely tied to temperature than elevation. In the winter, the highest elevation sites were warmest and this is also where seasonal growth rates of all species were highest. These growth-elevation patterns were reversed in the summer when the highest elevation sites were coldest. Ultimately, seasonal changes in thermal regime across the elevation gradient were reflected in the physiological responses of the study species.

Temperature, rather than food quantity or quality, was likely most important in driving life-history variation among the study taxa. Previous work has shown that differences in food resource quality and quantity can influence invertebrate life history traits and/or population-level metrics (Fuller and Mackay 1981, Ward and Cummins 1979, Sweeney and Vannote 1984, Sweeney et al. 1986). For instance, Fuller and Mackay (1981) showed that growth rates were highest among three hydropsychid species when food quantity and quality was greatest. Our results suggest there were no systematic or significant differences in food resources among the study sites, with the exception of higher biofilm chlorophyll *a* to AFDM ratio (higher food quality) at site 4 relative to other sites. Moreover, both of our focal mayfly taxa consume biofilm, and we did not observe elevated growth and/or terminal body size at site 4 where biofilm was greatest

that would indicate an effect of food resources. Based on these patterns, we suggest that the observed variation in life history traits may be principally attributed to changes in thermal regime and not variation in food resources.

Two general theoretical frameworks have been used to understand how temperature influences insect life histories, specifically patterns in terminal body size and fecundity: the 'Thermal Equilibrium Hypothesis' (TEH; Vannote and Sweeney 1980, Stanley and Short 1988, Rader and Ward 1990) and the 'Temperature Size Rule' (TSR; Atkinson 1994, Atkinson and Sibly 1996, Sweeney et al. 2018). The TEH predicts that each species exhibits a thermal optimum that maximizes body size and fecundity near the center of their geographic range, with declines at cooler and warmer temperatures. These expectations differ from those of the TSR, which predicts that individuals will be largest, and potentially most fecund and productive (Chapman 1931, Stearns 1992, Honek 1993, Roff 2002, Buckley et al. 2008) at the coldest temperatures within their range. Comparing field data to these theoretical predictions requires that study sites encompass the full thermal range experienced by these taxa; Our study spans a sufficient thermal gradient for investigating the TEH and TSR predictive life history frameworks *in situ*. For instance, in our study, high elevation sites ranged from near freezing to $\sim 12^{\circ}\text{C}$, while low elevation sites warmed to $\sim 21^{\circ}\text{C}$ in the summer. These temperatures are roughly similar to those observed in streams at the northern (Alaska and Canada) and southern (southern California and New Mexico) geographic boundaries of our study taxa (Heinold 2010, Mauger et al. 2017, NatureServe 2018, Zuellig et al. 2010, USGS Real Time Water Data; *E. infrequens*: Allen and Edmunds 1962, Hawkins 1984; *D. doddsii*: Hawkins 1984,

Kapur 2014, Tomlinson 2016; *H. cockerelli*: Short et al. 1980, Hauer and Stanford 1982, Lowe 1999, Voelz and Ward 1996), excluding the warmest streams in southern Colorado and northern New Mexico (up to 27 °C; Zuellig et al. 2010). The lack of extreme warm temperatures from our otherwise comprehensive thermal gradient does not restrict the interpretation of our results within the context of the TEH and TSR, however; both frameworks predict a decrease in body size, fecundity, and therefore abundance as temperatures near the warmest in a species' geographic range, patterns which are supported by our findings. Therefore, it can be assumed that capturing these extreme warm temperatures would simply yield more evidence of decreasing success rather than contribute novel data to the patterns observed.

While we were not able to quantify all parameters described by the TEH (weight-specific respiration [i.e. metabolism] and generation time), this theory predicts that terminal body size, female reproductive potential, and population metrics such as secondary production, would be maximized at an intermediate temperature optimum for each species. *Hydropsyche cockerelli* exhibited highest growth rates at intermediate temperatures, which translated to the largest terminal body sizes. This result is more consistent with the TEH than the TSR (Warren and Davis 1967; Brett 1979), suggesting these intermediate temperatures may be close to *H. cockerelli*'s thermal optimum. Lowe (1997) found similar evidence both *in situ* and in the laboratory while investigating *H. cockerelli*'s metabolic response to temperature. Lowe (1997) suggested that an unidentified complex mechanism drives *H. cockerelli*'s optimized growth at warmer (but not hot) temperatures. In Lowe's (1997) study, these larger body sizes led to higher

potential reproductive output which influenced spatial patterns of abundance and helped to reduce competition with other Hydropsychidae species. Such results suggest that species may evolve varying life history responses to temperature as a result of specific environmental pressures (such as competition) to optimize growth, terminal body size, and therefore higher potential reproductive output.

Our results, together with previously published examples, suggest that different life history models (TEH or TSR) may apply to the same taxon under different environmental conditions. For instance, we found that ephemereleid life history traits such as terminal body size, reproductive potential, density, and biomass were often maximized at the coldest average temperatures, most consistent with predictions from the TSR (Atkinson 1994, Atkinson and Sibly 1996, Sweeney et al. 2018). We also found that growth per degree-day was generally greatest for both mayfly species at the seasonally coldest sites (high elevations in summer-autumn and spring-summer, and lowest elevation sites in winter). In contrast, Rader and Ward (1990) found that *E. infrequens* grew faster and reached larger body sizes at intermediate temperatures with cool-summer and relatively warm-winter temperatures. In this case, cool summer temperatures allowed this cold-adapted species (Edmunds 1972) to grow quickly due to low metabolic costs, and the relatively warm winter temperatures (near 2 °C) were not cold enough to completely inhibit growth. However, Rader and Ward (1990) suggested that water released from the dam at this intermediate temperature site contained high amounts of diatoms, increasing the food quality and quantity. These results demonstrate there may be intraspecific variation in responses to temperature that can interact with differences in

other environmental variables, thus making general predictions for a given taxon somewhat difficult.

Vannote and Sweeney (1980) predicted that fecundity in ectotherms is positively correlated with female body size, and therefore should be greatest at temperatures that produce low weight-specific respiration and intermediate generation times (which were not measured in this study). Furthermore, they predicted that reproductive success, and the effect of one generation on the next, will depend on the abundance of emerging females. However, Vannote and Sweeney (1980) use the term ‘reproductive potential’ synonymously with individual fecundity. In our study, we estimated reproductive potential by multiplying the average number of eggs per female by female densities at each site prior to emergence. Similar to other studies, we found that ephemereleid female body size was positively correlated with the total number of eggs prior to emergence (Stearns 1992, Honek 1993, Roff 2002), especially when considered over a broad range of body sizes. Such patterns of female terminal body size represent the cumulative effect of differences in seasonal growth throughout the year. However, we found that our measure of reproductive potential did not directly correlate with terminal body size as expected. Instead, we found that reproductive potential decreased linearly with increasing cumulative degree-days. This pattern is consistent with the TSR, and has been observed for other aquatic insects (Sweeney et al. 2018). This decoupling of terminal body size and potential reproductive output at low elevation sites may be attributed to much lower densities of fecund females collected prior to emergence. The mechanism for reduced densities is unknown but could be driven by increased mortality with increasing spring-

summer temperatures. At low elevation sites, daytime temperatures reached $\sim 20^{\circ}\text{C}$, which may have strong negative effects on performance (Sweeney 1978, Sweeney et al. 2018, Ward and Stanford 1982). In addition, warm temperatures could accelerate predation by invertebrate or fish predators (Kishi et al. 2005). Although we were unable to determine *H. cockerelli* reproductive potential due to post-emergence oocyte maturation (Dodson 1935, Unzicker 1968), understanding how reproductive potential varies across taxonomic groups and temperature may provide a deeper understanding of temperature-life history relationships. The reproductive output of ectotherms is likely one of the most important factors determining success of subsequent generations (Chapman 1931, Buckley et al. 2008, but see Bovill et al. 2018).

Vannote and Sweeney (1980) predicted that largest terminal body size and fecundity at a species' thermal optimum would translate into highest local abundance and secondary production. We simultaneously measured average annual density, biomass, and production in an effort to explicitly test this prediction. Secondary production generally followed spatial patterns of terminal body size across our study species, consistent with TEH predictions. This pattern was also mirrored in the reproductive potential of *D. dodd sii*, suggesting that this species exhibits the greatest 'success' at temperatures that produce the most offspring. *Ephemerella infrequens* was the exception to this pattern. Terminal body size was greatest at sites 1 and 5, but secondary production was greatest at sites 1, 3, and 5. Results at sites 1 and 5 corroborate TEH predictions linking terminal body size and secondary production. At site 3, relatively high production was driven by high mean annual biomass, suggesting an important role of cohort

survivorship at this site. These results suggest that average terminal body size may not always predict secondary production and the relative success of populations (Rader and Ward 1990).

There are inherent tradeoffs associated with conducting life-history research *in situ* as opposed to controlled laboratory conditions. For instance, although our sites were chosen to examine the effects of temperature, dewatering for agriculture and potential siltation and chemical runoff at the lower elevation sites could have influenced the distributions and life history traits examined in this study. In addition, while we attempted to sample populations exhaustively (Li et al. 2001), we were at times limited by thick ice-over during winter or dangerously high flows during spring run-off. Thus, sample location, even at a microhabitat level, may have influenced our abundance and productivity results. It is also likely that drift and longitudinal movement of insects occurred during the study (Waters 1972), intermixing the range of temperatures experienced by individuals collected at a given site. However, the magnitude and distance of such movement was likely not large enough to influence our conclusions at a relatively large scale (Brittain and Eikeland 1988; but see Bovill et al. 2018). Finally, we were not able to separate individuals by sex, though life history protandry has been shown for some aquatic insects (Fischer and Fiedler 2002, Karl and Fischer 2008, Sweeney et al 2018). Future studies could benefit by examining species with distinct sexual dimorphism allowing for separation of individuals by sex, and therefore eliminating potential variability caused by protandry. Despite the aforementioned limitations, our study

effectively captures several of the critical components necessary for investigating the TEH *in situ* and provides a solid foundation for future research.

A comprehensive test of the TEH would require examination of all key life-history components simultaneously. In our study, weight-specific respiration and generation times were not investigated, and these factors are central to the TEH theory. For instance, terminal body size is best explained in the TEH through the relative balance of weight-specific respiration and growth over time. Thus, our results could be better informed by direct measurements of respiration across time and space. Finally, future investigations should focus on mechanisms driving variation in cold-specific seasonal growth, and the potential for amplified growth rates under cold conditions. Our study suggests the potential for cold adaptation among some taxa (Danks 2007, Lencioni 2004, Pritchard et al. 1996), as evidenced by faster growth per degree-day during the winter. This line of inquiry is ripe for additional research. All of these modifications may lead to greater insight into life history plasticity of aquatic insects in response to thermal regimes, helping us to better predict how aquatic insects will be impacted by a changing climate.

Stream temperatures are changing at unprecedented rates (Meisner et al. 1987, Keleher and Rahel 1996, Woodward et al. 2010, IPCC 2013). Rocky Mountain streams are particularly vulnerable because they are warming more quickly than other regions due to changes in climate, urbanization, and decreasing snowpack (IMAF 2012, Isaak et al. 2016, Whitlock et al. 2017). The Gallatin River is no exception, and the effects of nearby urbanization and dewatering for agriculture and livestock exacerbate broader climate

effects on stream temperatures (Gallatin River Taskforce 2017). These changes are worrisome, as mountain streams provide refugia for cold-adapted species (Ross 1956, Isaak et al. 2016). In addition to the indirect effects of climate change on aquatic insects such as loss of habitat and resources (Allan and Flecker 1993), increased predation (Lampert 1989, Kishi et al. 2005), and changes to water oxygen levels (Verberk and Bilton 2013), our results suggest that increasing stream temperatures will directly affect the life histories of cold-adapted species. In response to warming stream temperatures, cold-adapted species will likely experience increased metabolism at the cost of growth (Sweeney and Vannote 1978, Sweeney et al. 2018), leading to reduced growth rates, terminal body size, and reproductive potential. As the output of offspring decreases, so might population metrics such as biomass and secondary production, potentially leading to changes in population abundance and restructuring of whole communities (Gonzalez et al. 2003, Huryn and Wallace 2000). These predictions corroborate several studies suggesting that warming will lead to measureable shifts in stream biofauna (Bradshaw and Holzapfel 2006, Durance and Ormerod 2007, Hering et al. 2009, Kearney et al. 2009). Anecdotally, such changes might already be occurring in our study system. For instance, in the present study *D. doddsii* and *H. cockerelli* were not observed at the lowest elevation site 5, but were prolific between sites 4 and 5 just 30 years prior (Gustafson 1990). These changes in distribution and density are likely a result of anthropogenic alterations to habitat characteristics, especially stream temperature and sedimentation. These results underscore how the influence of stream thermal regime and its interaction

with other anthropogenic stressors may influence insect populations and community composition.

Understanding patterns of aquatic insect life histories along natural thermal gradients can help to better predict how aquatic insects may be influenced by a changing climate. In response to global warming trends, there is renewed focus on deciphering how insects respond to variation in temperature (Harper and Peckarsky 2006, Nelson et al. 2017, Sweeney et al. 2018). The TEH (Vannote and Sweeney 1980, Stanley and Short 1988, Rader and Ward 1990) and TSR (Atkinson 1994, Atkinson and Sibly 1996, Sweeney et al. 2018) provide useful conceptual frameworks for predicting how aquatic insect life history traits may vary across temperature gradients. However, our study suggests there may be no universal model that allows us to predict how all species – or even a single taxon - respond to changing temperatures (Angilletta and Dunham 2003, Angilletta et al. 2004). It is possible that species such as *E. infrequens* and *D. dodd sii* have evolved compensatory cold-temperature mechanisms which drive TSR patterns, whereas *H. cockerelli* and other species that trend towards TEH patterns have not evolved such compensatory mechanisms. Biochemical adaptations that maximize growth and development and become less efficient with increases in temperature (Sweeney et al. 2018), such as increased efficiency of food assimilation (Neat et al. 1995, Robinson and Partridge 2001) and a decrease in the thermal optimum for growth efficiency with increasing body size (Atkinson and Sibly 1997), may help to explain the success of some cold-adapted species. However, there is potential for other unidentified mechanisms to explain differences in life history patterns among taxonomic groups (Partridge and

French 1996). Perhaps once identified, the presence or absence of these mechanisms could be used to refine a predictive framework. Our results call for more widespread investigation of multiple species, taxonomic groups, and river systems as changes in temperature continue to lead to spatial range shifts. Predictive temperature-life history models may be valuable for foreseeing these shifts in aquatic species distributions and the consequences for the ecology of stream ecosystems.

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