CLIMATE EFFECTS ON FRESHWATER ECOLOGICAL COMMUNITIES: FROM LOCAL SPECIES INTERACTIONS TO CONTINENTAL BIODIVERSITY PATTERNS

By

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ABSTRACT

CLIMATE EFFECTS ON FRESHWATER ECOLOGICAL COMMUNITIES: FROM LOCAL SPECIES INTERACTIONS TO CONTINENTAL BIODIVERSITY PATTERNS

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Climate change is altering freshwater habitats in the United States by increasing water temperatures and changing stream flows. These habitat changes can directly impact survival and geographic ranges of ectothermic freshwater organisms through thermal stress and habitat loss, and indirectly through changes to life-history traits such as generation time. Thus, it is likely that many freshwater organisms will be vulnerable to extinction under climate change, and many more will undergo distributional shifts to track their climatic niche, driving large-scale alterations to freshwater biodiversity in the United States. I investigated the effects of climate change on freshwater biodiversity at multiple levels of ecological organization and spatial scales.

In Chapter 1, I examined how climate change could alter the survival of predators with different foraging traits. I used experiments to quantify the effects of temperature on predator-prey interactions of freshwater insects and zooplankton living in Southwest Michigan ponds. I found that freshwater insects increased their feeding and growth rates at higher temperatures. Further, predators with sit-and-wait foraging strategies may outperform insects with active strategies in a warming world, because active predators may be more likely than sit-and-wait predators to starve if their prey populations decline as a result of climate warming.

In Chapter 2, I investigated how increases in predator feeding and growth rates with climate warming could alter populations of freshwater insects with complex life cycles. Using data from experiments in Chapter 1 and field surveys, I parameterized a consumer-resource population model for damselflies (consumer) and (zooplankton) resource. I used this model to

simulate changes in populations with climate warming and found that damselflies shifted their life-history from one to two generations per year and increased their population size.

In Chapters 3 and 4, I scaled up my research by investigating the effects of climate change on freshwater insect biodiversity in the contiguous United States. I addressed the limitations of existing biodiversity databases for freshwater insects in Chapter 3 by assembling a database, *Freshwater insect occurrences and traits for the contiguous United States* (*Freshwater insects CONUS)*. This database contains 2.05 million genus occurrence records for 932 genera in the major freshwater insect orders, at 51,044 stream locations, and life history, dispersal, morphology, and ecology traits for 1,007 insect genera.

In Chapter 4, I paired this database with spatial environmental data on climate, hydrology, land cover, and topography of watersheds to assess the climate sensitivity of insect genera across the contiguous United States. Of the 488 insect genera that I examined, insects with erosional flow preference, cold-cool eurythermal preference, and univoltine life-histories, and insects in the orders Plecoptera and Trichoptera are likely the most sensitive to climate change. I also found that watersheds with the highest proportions of sensitive taxa are in the mountain West and Appalachian regions of the United States. These insect genera and regions should be prioritized for further research and protection against losing freshwater biodiversity.

I addressed two knowledge gaps that have hindered efforts to conserve freshwater biodiversity in a changing climate, including understanding physiological and demographic consequences of increasing temperatures and relationships between climate and geographic distributions of freshwater insects. Conservation practitioners can use the results of my research to prioritize freshwater organisms and watersheds for protection in a changing climate.

My dissertation is dedicated to my husband who braved the icy winters of Michigan with me for four years. Thank you for supporting me.

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Abstract

Climate warming and species traits interact to influence predator performance, including individual feeding and growth rates. However, the effects of an important trait—predator foraging strategy—are largely unknown. We investigated the interactions between predator foraging strategy and temperature on two ectotherm predators: an active predator, the backswimmer *Notonecta undulata*, and a sit-and-wait predator, the damselfly *Enallagma annexum*. In a series of predator-prey experiments across a temperature gradient, we measured predator feeding rates on an active prey species, zooplankton *Daphnia pulex*, predator growth rates, and mechanisms that influence predator feeding: body speed of predators and prey (here measured as swimming speed), prey encounter rates, capture success, and attack rates. Overall, warming led to increased feeding rates for both predators through changes to each component of the predator's functional response. We found that prey swimming speed strongly increased with temperature. The active predator's swimming speed also increased with temperature, and together, the increase in predator and prey swimming speed resulted in two-fold higher prey encounter rates for the active predator at warmer temperatures. By contrast, prey encounter rates of the sit-and-wait predator increased four-fold with rising temperatures as a result of increased prey swimming speed. Concurrently, increased prey swimming speed caused a decline in the active predator’s capture success at high temperatures but did not affect the sit-and-wait predator’s capture success, which increased with temperature. We provide some of the first evidence that foraging traits mediate the indirect effects of warming on predator performance. Understanding how traits influence species’ responses to warming could clarify how climate change will affect entire functional groups of species.

Introduction

Ecologists are challenged with predicting the consequences of climate change on ecological communities. This effort requires understanding how warming affects predators, because changes to predator populations can lead to larger consequences across communities through trophic interactions (Zarnetske et al. 2012, Urban et al. 2017). In particular, we require knowledge of how temperature affects individual predator feeding and growth rates (hereafter, *predator performance*) that in turn, influence individual predator survival and populations (Vasseur and McCann 2005, Lang et al. 2017). Species traits, such as predator and prey body speed and predator foraging strategy, that modify the effects of temperature on predator performance (Barton and Schmitz 2009, Vucic-Pestic et al. 2011, Öhlund et al. 2014) can help us predict the performance of different predators under climate warming.

Despite recent research into the effects of climate warming on predator foraging (Vucic-Pestic et al. 2011, Öhlund et al. 2014, Culler et al. 2014, Frances and McCauley 2018), the relationship between predator foraging strategy and temperature remains poorly understood (Dell et al. 2014). Predator foraging strategies include active pursuit whereby predators patrol for prey and sit-and-pursue or sit-and-wait, where predators remain in a fixed location and attack prey that move within their pursuit distance (Preisser et al. 2007). Predators can be especially sensitive to climate change and can amplify its effects on ecological communities (Zarnetske et al. 2012, Urban et al. 2017) by influencing predator-prey population dynamics (Vasseur and McCann 2005), community stability (Paine 1980), and trophic cascades (Shurin et al. 2002). Thus, understanding how predator foraging strategy interacts with temperature to alter predator performance will improve our ability to predict the direct and indirect effects of climate change on ecological communities.

Ectotherms experience especially strong effects of climate warming because rising temperatures alter their metabolism, which in turn influences other biological rates critical to survival, including body speed, feeding, and growth (Gillooly et al. 2001, Brown et al. 2004, Dell et al. 2011, Rall et al. 2012). Over sufficiently large temperature ranges, metabolism and feeding show unimodal responses to temperature, rising with temperature up to a thermal optimum, and declining at very high temperatures (Englund et al. 2011). Metabolic theory suggests that within the rising portion of the unimodal response, ectotherm rates of feeding and growth should scale exponentially with temperature as a result of increasing metabolism, with activation energies (strength of increase with temperature) between 0.60 to 0.70 eV (Brown et al. 2004). However, there is substantial deviation from this expectation in observed rates of predator feeding (Englund et al. 2011, Rall et al. 2012), which suggests that other components of predator-prey interactions, in addition to metabolism, are mediating the effects of temperature.

By increasing metabolism, rising temperatures also increase predator and prey body speed, for example, the swimming speed of aquatic animals (Dell et al. 2011, Grady et al. 2019). Foraging theory indicates that predator and prey body speed mediate a predator’s feeding rate by influencing each component of its functional response (Holling 1959), including its encounter rate, capture success (the number of successful attacks per encounter), and the attack rate parameter (Holling 1966, McGill and Mittelbach 2006). Specifically, encounter rates increase with predator or prey body speed (Holling 1966, McGill and Mittelbach 2006, Dell et al. 2014). In turn, a predator's attack rate increases with rates of prey encounter and capture success (Holling 1966). All else being equal, the higher the attack rate, the higher the predator's functional response (Holling 1959). Thus, by increasing body speed, warming results in higher encounter rates between predators and prey (Vucic-Pestic et al. 2011, Öhlund et al. 2014), which leads to higher attack rates (given constant capture success), and higher feeding rates (Rall et al. 2012). Little is known from contemporary theory about how warming should influence capture success. However, capture success should increase if warming increases relative predator to prey body speed such that predator attack speed exceeds prey escape speed, and capture success should decrease with higher relative prey escape speed (Grady et al. 2019).

The effects of temperature on predator metabolism and feeding can either increase or decrease predator individual growth rates. Higher feeding rates can result in faster individual growth rates if prey ingestion exceeds metabolism. However, predator growth rates can decrease at very high temperatures if metabolism exceeds ingestion (Culler et al. 2014, Lang et al. 2017). This process is referred to as a decline in energetic efficiency, whereby the biomass losses from metabolism exceed the biomass gains from feeding, resulting in predator starvation (Lang et al. 2017). Therefore, metabolism and predator and prey body speed mediate the effects of temperature on predator feeding and growth rates.

Ecological theory predicts that foraging strategy is another species trait that should interact with temperature to mediate the effects of climate warming on ectotherm predator feeding, and its components of body speed, prey encounter, and attack rates (Dell et al. 2014). This is because actively foraging predators have higher body speeds, rates of prey capture, consumption, and metabolism relative to sit-and-wait predators (Huey and Pianka 1981, Taigen and Pough 1983). Theory predicts that the temperature scaling of active predator encounter and attack rates will depend on the relative temperature scaling of predator and prey body speed (Dell et al. 2014). Theory also predicts that because the body speed of sit-and-wait predators will not vary with temperature, the temperature scaling of sit-and-wait predator encounter and attack rates will depend on the temperature scaling of prey body speed. Therefore, if body speed of active predators and prey increase under warming (Dell et al. 2011), prey encounter rates will also increase, resulting in higher feeding rates for active predators (Dell et al. 2014). Further, warming will increase feeding rates for sit-and-wait predators only if prey body speed (and thus encounter rate) increases with warming (Dell et al. 2014).

We expect that predator foraging strategy mediates the effects of temperature on ectotherm predator feeding rates through its effects on predator body speed and encounter rate (Dell et al. 2014). All else being equal, we predict that climate warming could be more advantageous for performance of active predators due to their increased body speed and feeding rates. In addition, we propose that foraging strategy mediates the effects of temperature on predator growth rates through its effects on predator metabolism, whereby active predators have higher metabolic rates than sit-and-wait predators due to the metabolic demands of movement during feeding (Huey and Pianka 1981, Taigen and Pough 1983). Therefore, we suggest that metabolism will increase more with temperature for active compared to sit-and-wait predators. If metabolic rates of active predators increase more with warming than feeding rates, this would result in in lower energetic efficiencies that reduce individual growth rates and could result in starvation (Lang et al. 2017). Thus, even if feeding rates of active predators are higher relative to sit-and-wait predators, their individual growth rates may be lower due to higher metabolic rates

(Huey and Pianka 1981, Taigen and Pough 1983), potentially leading to smaller population sizes or local extinction in warmed conditions (Vasseur and McCann 2005).

Here, we examine the relationships between foraging strategy and temperature on ectotherms using a three-species aquatic community module (sensu Paine 1980), consisting of an active prey species, the water flea *Daphnia pulex*, an active predator, the backswimmer *Notonecta undulata*, and a sit-and-wait predator, the damselfly *Enallagma annexum*. Our work provides one of the first tests of recent theory (Dell et al. 2014) predicting how foraging strategy mediates the effects of temperature to shape predator feeding. We test the following hypotheses with a series of predator-prey experiments across a temperature gradient where we measure each predator's individual growth rate and functional response to prey. As temperature increases, we expect:

H1: Swimming speed of the active predator and prey will increase as a result of increasing metabolism.

H2: Prey encounter rates and capture success will increase more strongly for the active predator as a result of its faster swimming speed.

H3: Prey attack rates will increase more for the active predator as result of its higher prey encounter rates and capture success.

H4: Individual growth rates of the active predator will increase less than growth rates of the sit-and-wait predator as a result of the active predator's higher metabolism.

Methods

Aquatic community module

Notonecta (Hemiptera: Notonectidae) are piercing-sucking predators that actively hunt by swimming toward and grasping prey (Fox 1975). *Enallagma* (Odonata: Coenagrionidae) are engulfing predators that hunt from aquatic plants by waiting for prey to move within striking distance of their labium, a prehensile mouthpart for grasping (Merritt and Cummins 1996). *Daphnia* (Cladocera: Daphniidae) are actively swimming prey that compose a large proportion of the diet of nymphal *Notonecta* and *Enallagma* (Lawton 1970, Scott and Murdoch 1983). Our community module is well suited to address the interactions between foraging strategy and temperature, because all three species coexist in shallow ponds (Hanly 2017, Twardochleb unpublished data) where predators share prey resources, and are of similar body sizes (McPeek and Crowley 1987, Gergs and Ratte 2009). Therefore, this community module can provide insight into how temperature influences measures of predator performance among species that differ in foraging strategy, but otherwise share similar ecological requirements and local adaptation to temperature.

Predator functional response experiments

We conducted functional response experiments to quantify relationships between predator feeding rates and temperature (*H3*), and we analyzed video-recordings of these experiments to assess how temperature affects predator and prey swimming speed (*H1*), and encounter rates and capture success (*H2*). Experiments were conducted from July to October 2017 at Kellogg Biological Station in southwestern Michigan, USA. We collected predators and prey from fishless ponds in Lux Arbor Reserve, Barry Co., MI and acclimated them to laboratory conditions at 20 °C until their first molt (several days to two weeks), then placed them inside an environmental growth chamber (I36LLVL, Percival Scientific, Perry, IA, USA) set to 10, 15, 20, 25, 30, or 35 °C, which encompassed the mean (24 °C) and range (10 – 31 °C) of ambient pond temperatures during spring to fall, and one temperature (35 °C) above ambient. We monitored chamber temperature using HOBO pendant temperature loggers (UA-001-64, Onset Corporation, Bourne, MA, USA). We fed predators pond zooplankton and allowed them to acclimate to a given temperature for 24 hours (Thompson 1978), and we then deprived them of food to standardize hunger levels. We acclimated *Daphnia* to a given temperature for two hours prior to trials (Thompson 1978).

For *Notonecta* trials, we used densities of 10, 20, 50, 100 *Daphnia* L^{-1}. For *Enallagma* trials, we used densities of 5, 10, 20, 50, 100 *Daphnia* 100 mL^{-1} for 10 – 20 °C, and 5, 10, 20, 50, 100, 150 *Daphnia* 100 mL^{-1} for 25 – 35 °C. Prey densities were determined from preliminary trials in 2016 and 2017 in order to capture the shape of the functional response curve. For each trial, we placed an individual predator into an experimental arena with *Daphnia* for one hour inside an environmental chamber. We quantified the number consumed as the difference between initial and final prey densities, including a correction for errors in enumerating *Daphnia*. We replicated each prey density at every temperature at least four times for a total of 100 trials for *Notonecta* and 158 trials for *Enallagma.* Methods followed those commonly used in functional response experiments (e.g., Thompson 1978, Vucic-Pestic et al. 2011).

Testing H1: Video analysis of predator and prey swimming speed

We video recorded functional response experiments at each temperature for *Notonecta* (29 total) and *Enallagma* (30 total) to test *H1 and H2*. Speed (cm s^{-1}) for *Notonecta* (28 total) and *Daphnia* (24 total) was quantified using Tracker Video Analysis software (https://physlets.org/tracker/). We calculated *Daphnia* speed over 20 second intervals three times during each trial by tracking the *Daphnia* closest to the center of the experimental arena. We averaged these three speeds by trial. *Notonecta* speed was calculated by accounting for two distinct swimming patterns; short bursts lasting 1-2 seconds each that were associated with attacks, and longer "patrolling" movements lasting 3-5 seconds each that occurred between bouts of attacking and consuming prey. We analyzed the relationship between *Notonecta* speed and temperature individually for "burst" and "patrolling" movements but found no effect of temperature on "burst" swimming speed (see Tables 2 and 3 in Appendix B).

Testing H2: Video analysis of encounter rates and capture success

We quantified encounters as the number of reactive plus non-reactive encounters. A reactive encounter was any instance when a predator visibly reacted to prey, and a non-reactive encounter was when prey moved within the predator's reactive distance, but the predator did not react visibly. We used a reactive distance of a 4x1 cm rectangle around *Notonecta* (Giller and McNeill 1981), and a 0.5 cm radius circle around *Enallagma* (Johansson 1993). We quantified capture success as the number of successful attacks per encounter (reactive and non-reactive) (Holling 1966).

Testing H3: Prey attack rates from functional response experiments

We fit separate, Type 2 functional response curves (Holling 1959) to feeding data for each predator species and each temperature using Rogers Random Predator Equation to account for prey depletion (Rogers 1972):

Algorithm 1. Rogers Random Predator Equation

$$N_c = N\left(1 - e^{-a(t - hN_c)}\right).$$

Here, N_c is the number of prey consumed (hr^{-1}), N is prey abundance, a is the attack, or search rate (L hr^{-1}), and h is handling time (hr.). We used the 'frair' R package version 0.5.10 (Pritchard et al. 2017) to fit the lambert *w* version of Algorithm 1 using maximum likelihood estimation (Bolker 2008). See Table 1 in Appendix B for fits of Algorithm 1 to feeding data.

We used estimates of a (Algorithm 1) to test *H3*. We fit three models to describe the temperature-dependence of a and h for each predator: an intercept (Algorithm 2), an exponential (Algorithm 3), and a quadratic model (Algorithm 4). The intercept model describes no effect of temperature on the biological rate of interest *Y* (in this case, a or h):

Algorithm 2. Intercept model

$$Y = c.$$

Here, *c* is the model intercept.

The Arrhenius equation (Gillooly et al. 2001) describes the temperature-dependence of biological reaction rates for temperatures below thermal optima (Englund et al., 2011):

Algorithm 3. Arrhenius model

$$Y = ce^{E_a(-\frac{1}{kT})},$$

where *c* is a fitted constant, E_a is the activation energy (eV) describing the strength of the temperature response, *k* is Boltzmann's constant (8.617×10^{-5} eV), and *T* is temperature (Kelvin). We also fit the Arrhenius-quadratic model describing the temperature-dependence of biological rates for temperatures below and above thermal optima (Englund et al. 2011):

Algorithm 4. Arrhenius-quadratic model

$$Y = ce^{b\left(-\frac{1}{kT}\right)+q\left(\frac{-1}{kT}\right)^2}.$$

Here, *c*, *b* (eV), and q (eV^2) are fitted parameters. Models were fit using linear regression in R, and regression fits were weighted by the inverse of the standard error around each estimate of *a* and *h* (n = 6 estimates of *a* and *h* per predator, n = 1 estimate per temperature, per predator).

Testing H4: Predator growth experiments

We ran experiments to quantify relationships between temperature and predator individual growth rates, from July to September 2018. Our temperature gradient included 10, 15, 20, 25, 30 or 35 °C. Predators were collected and acclimated the same way as above (see *Testing H1: Predator functional response experiments*). We measured initial wet mass by gently blotting predators dry on a paper towel and weighing them (± 0.01 mg) on a microbalance (Sartorius XM1000P, Goettingen, Germany). We then fed each predator *Daphnia* for three (*Notonecta*) or four (*Enallagma*) days. Each predator was fed ad libidum by ensuring that we fed enough *Daphnia* to recover at least two individual *Daphnia* the following day (5 to 50 *Daphnia* day^{-1}) (Culler et al. 2014). After three or four days of feeding, we deprived predators of food to allow for gut evacuation, blotted them dry, and then weighed them for final wet mass. We then dried predators for 24 hours at 60 °C and weighed them again for final dry mass. We calculated the relationship between wet and dry mass using linear regression (*Notonecta*: final dry mass = 0.0033 + 0.94*final wet mass, $R^2 = 0.98$; *Enallagma*: final dry mass = -0.61 + 0.22*final wet mass, $R^2 = 0.75$), and used this relationship to calculate initial and final dry mass for the trials (McPeek and Anholt 2004). We then calculated daily relative growth rate (RGR) as:

Algorithm 5. Relative growth rate

$$RGR = \frac{[(final\ dry\ mass) - (initial\ dry\ mass)]}{[(trial\ length\ (days)) * (W_e^m)]},$$

where W_e is the exponential mean dry mass and m is an allometric scaling exponent that accounts for the effect of body size on growth rate (Gordon 1968). The exponential dry mass accounts for the fact that insect growth rates are exponential rather than linear. Rather than dividing the mass change by the mean dry mass of the organism, we divide by the exponential dry mass to obtain mass-specific growth rates. We set m equal to -0.34 (Niven and Scharlemann 2005). We replicated each temperature treatment six times for *Notonecta* (36 trials) and seven times for *Enallagma* (42 trials). We used estimates of *RGR* (Algorithm 5) to test *H4*.

Statistical analyses

We used Analysis of Variance (ANOVA) to test whether biological rates varied with temperature and between predator species. We tested whether *Notonecta* or *Daphnia* speed varied with temperature using one-way ANOVA with temperature as the predictor. We used two-way ANOVA with temperature and predator species as predictors, and encounter rate, capture success, or *RGR* as responses. Data were log-transformed as needed to meet assumptions of normality and homoscedasticity. For significant ANOVA models, we ran Tukey's HSD to test pairwise differences between temperature and predator species. We also assessed temperature effects by fitting intercept (Algorithm 2), Arrhenius (Algorithm 3), and Arrhenius-quadratic (Algorithm 4) models for encounters, capture success, attack rates, and growth rates. We also fit intercept and Arrhenius models for *Notonecta* and *Daphnia* swimming speed, and a segmented Arrhenius model for *Daphnia* swimming speed. We fit the segmented model using maximum likelihood estimation (MLE) with the 'Segmented' R package version 1.1-0 (Muggeo 2008). We used AIC corrected for small sample sizes to select the best-fitting model (See Table 2 in Appendix B), except in the case of the segmented Arrhenius model. To compare models fit using ordinary least squares (intercept and Arrhenius) and MLE (segmented), we selected the model with the lowest residual variation and AIC value, and the highest R^2 (Table 4 in Appendix B). When the Arrhenius equation was selected as the best model, we based inference on whether 95% confidence intervals for E_a (Algorithm 3) overlapped zero or overlapped between predators. All analyses were implemented in R Version 3.5.1 (R Core Team 2018). Significance levels for all models were set to 0.05.

For more details on the experimental methods described above, see Appendix B.

Results

Testing H1: Video analysis of predator and prey swimming speed

Prey *Daphnia* swimming speed (cm s^{-1}) increased with temperature (ANOVA, $F_{5,18} = 16.73$, $p < 0.01$; Tukey's HSD, Table 5 in Appendix B). A segmented Arrhenius model with a breakpoint of 28.36 ± 1.46 (°C ± 1 SE) provided the best fit to the relationship between *Daphnia* speed and temperature (Fig. 1; Table 3, 4 in Appendices). We found an activation energy $Ea = 0.11 \pm 0.06$ (eV ± 1 SE) at temperatures below the breakpoint, and $Ea = 1.04 \pm 0.23$ (eV ± 1 SE) above the breakpoint. The Arrhenius equation was the best fit to the relationship between temperature and "patrolling" speed of the active predator *Notonecta* (Fig. 1; Table 2, 3 in Appendices), but ANOVA indicated no strong differences in speed among temperatures ($F_{5,22} = 0.83$, $p = 0.55$).

Testing H2: Video analysis of encounter rates and capture success

Prey encounters, quantified as an hourly rate at a density of 20 *Daphnia* per liter (hr^{-1} 20 *Daphnia* L^{-1}), differed across the temperature range ($F_{5,44} = 12.19$, $p < 0.01$; Table 6 in Appendix B), between the active predator *Notonecta* and sit-and-wait predator *Enallagma* ($F_{1,44} = 971.52$, $p < 0.01$), and there was an interaction between temperature and predator species on encounter rates ($F_{5,44} = 2.92$, $p = 0.02$). *Notonecta* had more prey encounters than *Enallagma* at all temperatures, and *Enallagma* had more encounters at higher than lower temperatures (Fig. 2a; Table 7 in Appendices). *Enallagma* had a four-fold increase in prey encounters between 10 and 35 °C, compared to a two-fold increase for *Notonecta* (Fig. 2, Appendix A). The fit of the Arrhenius equation revealed that E_a for encounters was greater for *Enallagma* than *Notonecta*

(Fig. 2a; Table 3 in Appendices). There were nonoverlapping 95% confidence intervals for E_a (*Notonecta*: 0.06-0.23, *Enallagma*: 0.30-0.66), indicating a stronger increase in *Enallagma's* encounter rate with warming.

Capture success (number of successful attacks per encounter) varied between predators ($F_{1,44} = 49.64$, $p < 0.01$) and was higher overall for *Enallagma* than *Notonecta* (Fig. 2b in Appendix A). Although there was not an overall effect of temperature on capture success ($F_{5,44} = 0.60$, $p = 0.70$), the Arrhenius-quadratic model (Algorithm 3) provided the best fit to the relationship between capture success and temperature for *Notonecta*, and the Arrhenius equation provided the best fit for *Enallagma* (Fig. 2b; Table 2, 3 in Appendices). This indicates that *Enallagma* capture success increased slightly with temperature, but *Notonecta* capture success increased up to a thermal optimum of 22.64 ± 0.01 (°C ± 1 SE), and then declined. There was no strong interactive effect of temperature and predator species on capture success ($F_{5,44} = 0.99$, $p = 0.43$).

Testing H3: Prey attack rates from functional response experiments

Predator functional responses (Algorithm 1) increased with temperature for both predators (Fig. 3 in Appendix A). Attack rates were higher for *Notonecta* than *Enallagma*, but their attack rates increased by a similar magnitude with warming (Fig. 2c; Table 3 in Appendices). The 95% confidence intervals for E_a (Algorithm 2) overlapped between predator species (*Notonecta*: 0.33-1.01, *Enallagma*: 0.42-0.73). Attack rates from video observations supported these results (Tables 2, 3 in Appendix B).

Testing H4: Predator growth experiments

Predator *RGR* (Algorithm 4, mg $mg^{0.34}$ day^{-1}) increased with temperature (Fig. 2d; $F_{5,67} = 15.88$, $p < 0.01$; Table 7 in Appendices) and differed between predator species ($F_{1,67} = 475.60$, p

< 0.01), but there was no interaction between temperature and predator species ($F_{5,67} = 1.02$, $p = 0.4$). *Enallagma* had an overall higher *RGR* than *Notonecta* (Fig. 2d in Appendix A). The Arrhenius equation provided the best fit to the relationship between *RGR* and temperature for *Notonecta* and *Enallagma* (Fig. 2d; Tables 2, 3 in Appendices). Overlapping 95% confidence intervals for E_a (*Notonecta*: 0.04-0.07, *Enallagma*: 0.02-0.06) indicate that *Notonecta* and *Enallagma* experienced a similar magnitude of increase in *RGR*.

Discussion

We found that both predators—*Notonecta* and *Enallagma*—increased their feeding rates with temperature (Fig. 3, Appendix A) as a result of changes to each component of the functional response, including predator and prey swimming speed, encounter rates, and capture success. Prey swimming speed increased with warming according to a segmented Arrhenius function (Fig. 1, Appendix A), which is consistent with other studies demonstrating that body speed can show biphasic responses to increasing temperature (Gibert et al. 2016). This overall increase in prey swimming speed increased encounter rates with both predators (Fig. 2, Appendix A). The active predator also increased its swimming speed (Fig. 1, Appendix A), which further contributed to increases in its prey encounter rate (Fig. 2, Appendix A). However, the increase in prey swimming speed more strongly influenced the encounter rate with the sit-and-wait predator; whereas the encounter rate with the active predator increased two-fold with warming, the encounter rate with the sit-and-wait predator increased four-fold (Fig. 2, Appendix A). In addition, increasing prey speed may have contributed to a decline in capture success for the active predator at high temperatures, whereas the sit-and-wait predator experienced a slight increase in capture success. The predators experienced similar increases in individual growth rates with increasing temperatures (Fig. 2, Appendix A).

Foraging strategy and prey body speed mediate the effects of warming

Feeding rates of sit-and-wait predators have been found to increase with warming when they forage for active prey (Culler et al. 2014, Frances and McCauley 2018) but not inactive prey (Novich et al. 2014). Thus, if prey speed remains constant or declines in warm climates, sit-and-wait strategies could be less energetically efficient because their encounter and feeding rates may not increase enough to offset their higher metabolism. By contrast, active predators are affected by their own body speed as well as prey speed. Therefore, when temperatures rise, active predators may be able to increase their encounter and feeding rates whether they are foraging for mobile or immobile prey (e.g., Vucic-Pestic et al. 2011, Öhlund et al. 2014) by increasing their own body speed. If prey speed does not increase with temperature, active predators could gain a relative performance advantage over sit-and-wait predators at high temperatures.

Foraging strategy and prey body speed also mediated capture success. Whereas the active predator's capture success declined at high temperatures, the sit-and-wait predator was successful at all temperatures, and its success increased somewhat with warming. This increase may have been due to unmeasured changes in predator movement that could influence capture success, such as faster protrusion of the labium, the raptorial appendage that damselflies use to capture prey. Overall, our observations suggest that the sit-and-wait predator may have had higher capture success because it was less detectable than the active predator. We observed that *Daphnia* moved away from the active predator as it approached but moved away from the sit-and-wait predator only after an unsuccessful attack, as prey were apparently unaware of the predator's location prior to the attack. Studies in aquatic environments have shown that prey rely on visual cues to assess predator location and risk. Prey respond to these visual cues through reduced activity and escape responses (Hall and Clark 2016, Fischer et al. 2017).

Given that prey detect predator movement as a predation risk, capture success may have decreased with warming for the active predator due to two mechanisms: 1) active predators are more likely to be detected by prey because they are moving, and 2) prey's escape ability was enhanced due to their faster body speed. This is supported by the fact that the active predator's "burst" swimming speed (associated with prey attacks) remained constant while the prey's swimming speed increased with warming (Tables 2, 3 in Appendix B). Our results are consistent with previous research showing that warming increases swimming speed of *Daphnia* (Ziarek et al. 2011) but contrast with other findings of predator body speed increasing more with temperature than prey speed (Dell et al. 2011). Our results are also consistent with studies showing that capture success decreases when prey relative speed increases with warming (Grigaltchik et al. 2012, Grady et al. 2019). Knowledge of the thermal responses of prey body speed is thus necessary to predict the outcomes of warming on predator-prey interactions.

We found that attack rates of the active predator increased with temperature with an activation energy of 0.67 eV (Fig. 3, Appendix A). However, encounter rate showed a weaker temperature scaling of 0.14 eV, and capture success decreased at high temperatures. These results suggest that unmeasured components of foraging, such as hunger, could have contributed to the increase in attack rates with warming. In addition, we did not test whether prey body speed responded differently to temperature between the two predators, which could have contributed to differences in the temperature scaling of their encounter rates. Other components of predator and prey biology may also influence how climate changes alters predator performance (Grady et al. 2019).

Our experiments used a single species of prey, and a single species of sit-and-wait and active predator. Therefore, our results may have been due to species-specific differences in temperature responses rather than differences in foraging strategy. We believe that additional research will reveal that foraging strategy affects species' responses to climate warming in predictable ways (e.g., Barton and Schmitz 2009, Archer et al. 2019). At present, more research using multiple species of active and sit-and-wait predator is needed to validate our results and predictions of theory (Dell et al. 2014). We outline below some additional hypotheses that could be tested to better reveal the influences of predator foraging strategy and prey body speed on predator performance at different temperatures and prey densities.

Conclusions

Foraging differences between active and sit-and-wait predators could scale up to influence their relative performance in environments that differ in prey density and temperature. We found that attack rates were higher overall for the active predator at ten-fold lower prey densities relative to the sit-and-wait predator (Figs. 2 and 3). In addition, encounter rates of the sit-and-wait predator were strongly affected by prey body speed, whereas encounter rates of the active predator were affected by predator and prey body speed. Thus, our results support previous research and theory predicting that active predators will gain a relative performance advantage when prey speed and density are low, and that sit-and-wait strategies will be advantageous when prey speed and density are high (Huey and Pianka 1981, Werner and Anholt 1993, Ross and Winterhalder 2015). In addition, we found that the sit-and-wait predator was more energetically efficient, because it had higher growth rates despite lower feeding rates (Fig. 2, Appendix A). This supports previous research showing that actively foraging species have higher metabolic rates than related sit-and-wait species (Huey and Pianka 1981, Taigen and

Pough 1983). Taken together, this evidence suggests that sit-and-wait predators may be more efficient in high prey density environments, where their lower metabolism results in higher growth rates. Thus, we hypothesize that when prey speed increases with warming, active strategies should be relatively advantageous at low temperatures when prey density is low, while sit-and-wait strategies should be advantageous at high temperatures when prey density is high (Fig. 4a, Appendix A). If prey speed decreases with temperature, active strategies should be advantageous at high temperatures when prey density is low, while sit-and-wait strategies should be advantageous at low temperatures when prey density is high (Fig. 4b, Appendix A).

Tests of these hypotheses could improve trait-based assessments of predator performance under climate change. Indirect effects of warming on predator performance could influence predator persistence and population dynamics (Vasseur and McCann 2005), and food web structure and stability (Gilbert et al. 2014). Knowledge of trait-mediated effects of temperature on predators could also help predict ecosystem responses to climate change, because sit-and-wait and actively foraging predators have different effects on ecosystem functions that may strengthen with warming (Barton et al. 2009). Ultimately, knowledge of how foraging traits mediate the effects of temperature on predator performance can help clarify how climate change will affect entire functional groups of species.

APPENDICES

APPENDIX A: Figures

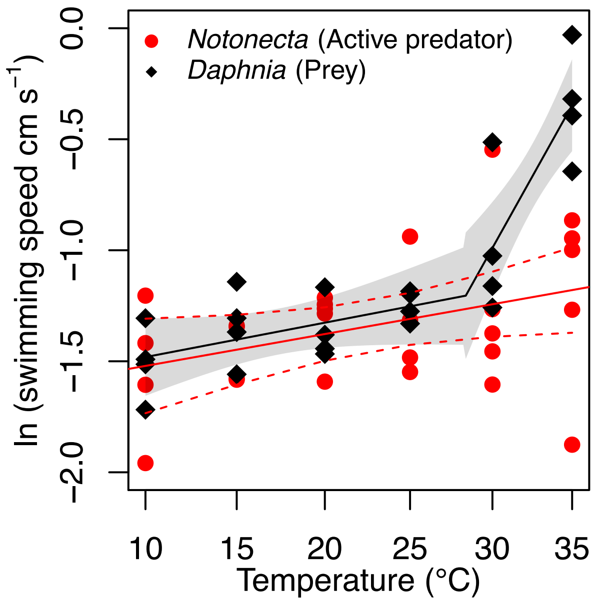

Figure 1. Relationships between temperature and swimming speed. The Arrhenius equation provided the best fit for the relationship between temperature and swimming speed (cm s^{-1}) of *Notonecta*, with slope *Ea* (activation energy, eV ± 1 SE) = 0.10 (± 0.05). A segmented model provided the best fit for *Daphnia*, with a breakpoint near 28 °C, *Ea* = 0.11 (± 0.06) at temperatures below the breakpoint, and *Ea* = 1.04 (± 0.23) above the breakpoint. The 95% confidence intervals around the best fit line are represented by gray bands for *Daphnia* and broken red lines for *Notonecta*.

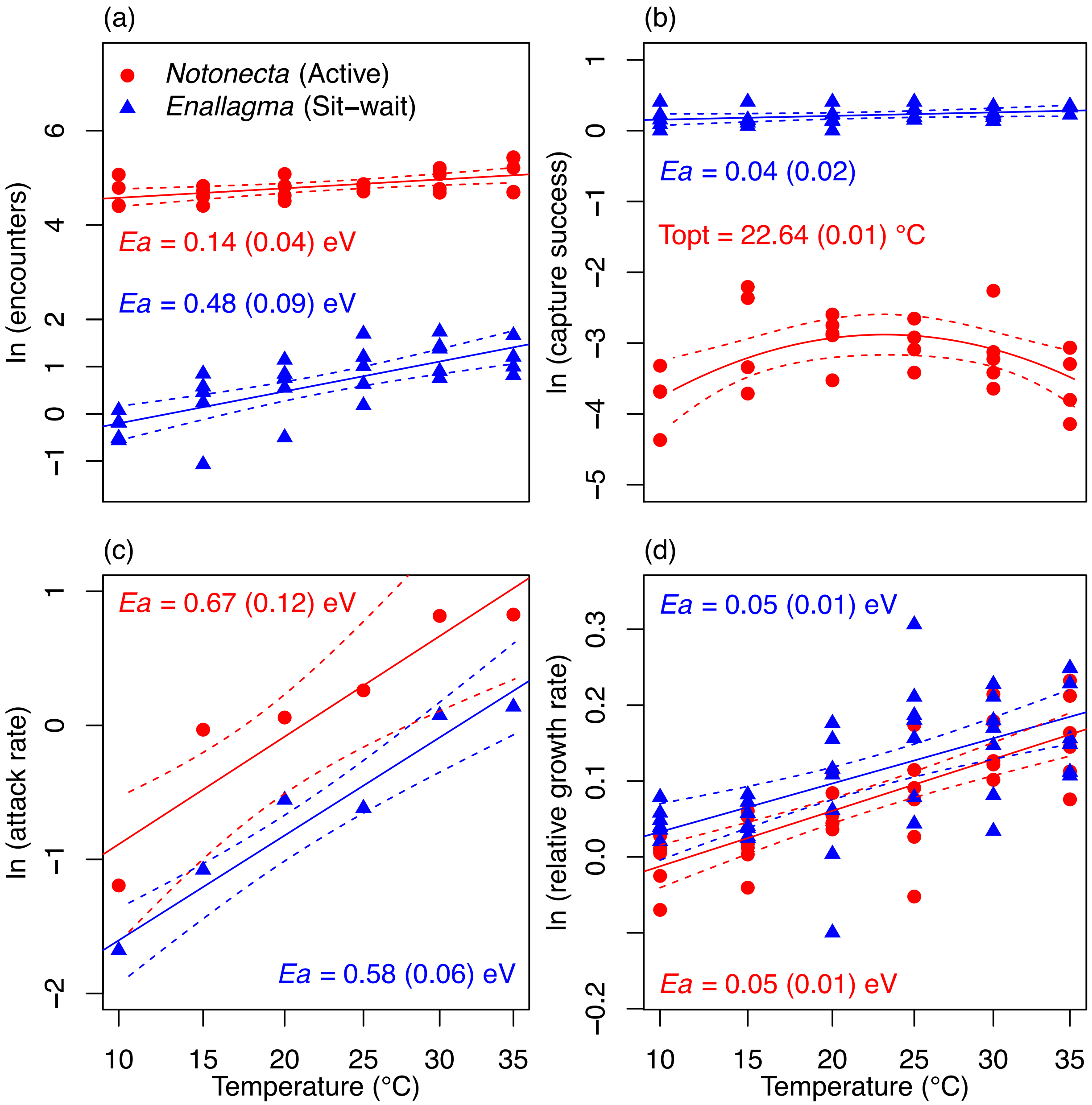

Figure 2. Relationships between temperature, functional response components, and growth rates. (a) Best fit lines of the Arrhenius equation for encounters (hr^{-1} 20 *Daphnia* L^{-1} ± 1 SE); (b) Fit of the Arrhenius equation for capture success (number of successful attacks per encounter) of *Enallagma*, and the Arrhenius-quadratic equation for *Notonecta*; (c) Arrhenius fit for attack rates (L hr^{-1}) and (d) relative growth rates (mg mg$^{0.34}$ day^{-1}). *Ea* (eV ± 1 SE) is the activation energy, and Topt (°C ± 1 SE) is the thermal optimum. Broken lines represent 95% confidence intervals.

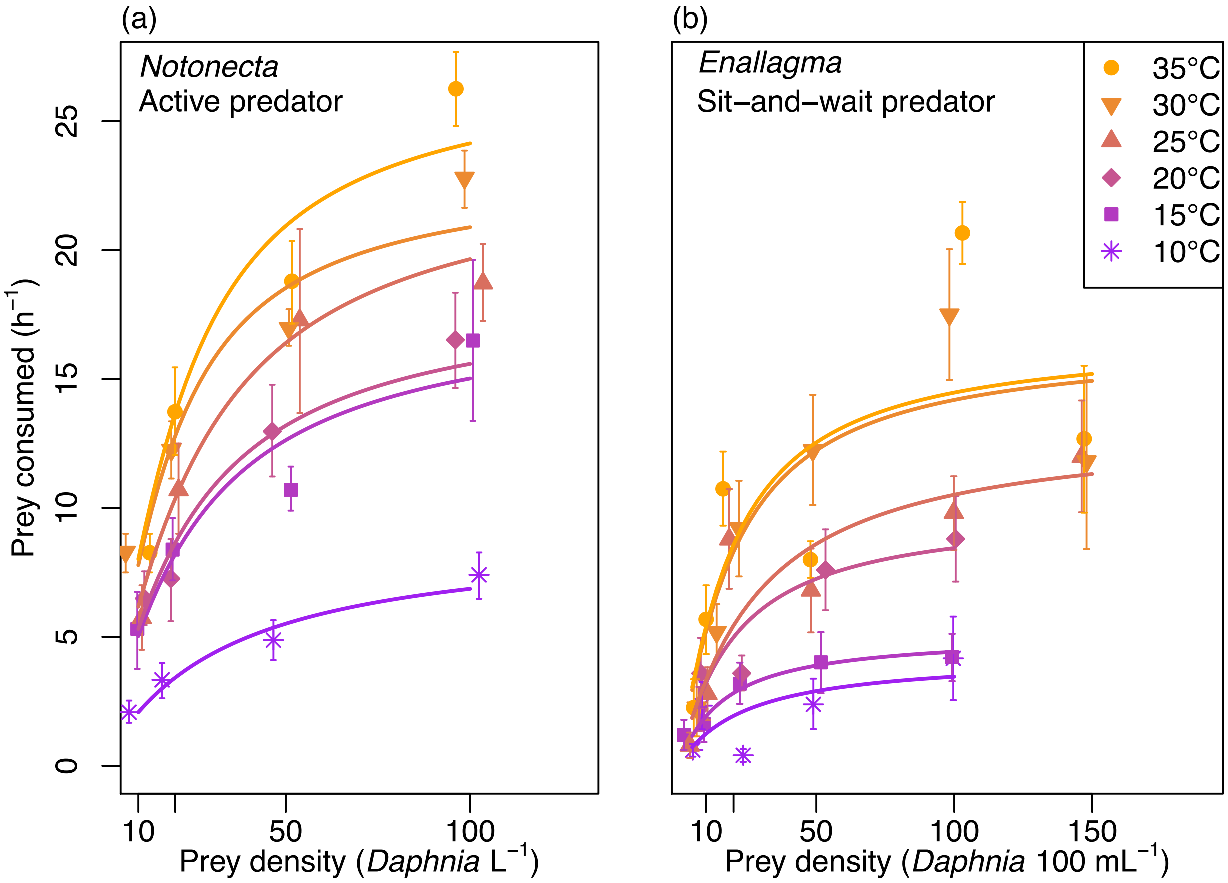

Figure 3. Functional responses at each temperature for (a) *Notonecta* and (b) *Enallagma*.

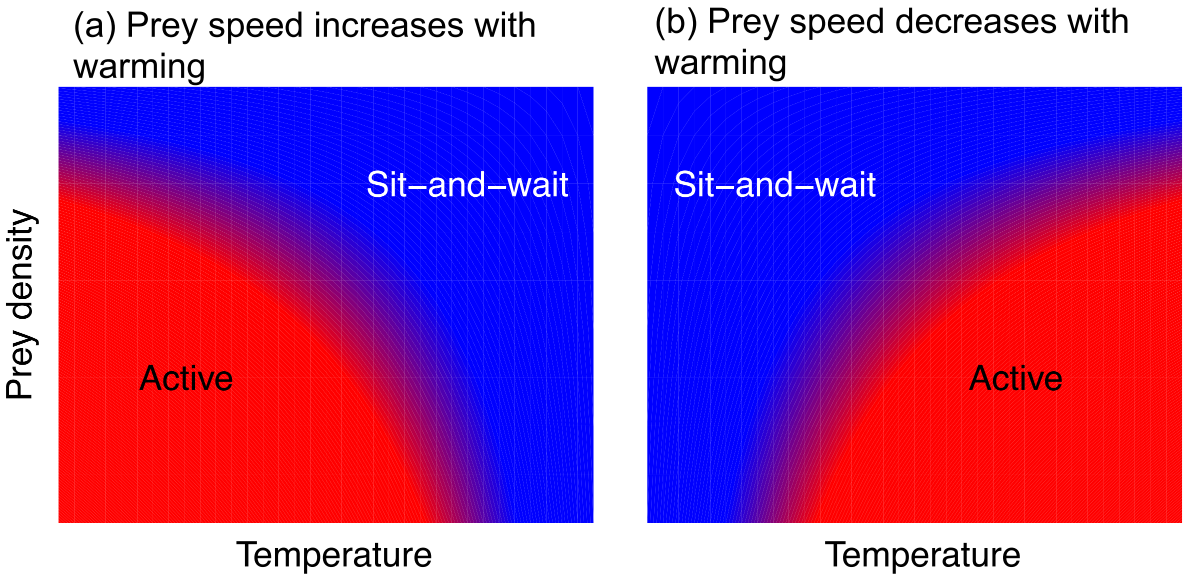

Figure 4. Hypothesized combinations of temperature and prey density that should favor active or sit-and-wait strategies. (a) When prey speed increases with warming, active strategies could be advantageous at low temperatures when prey density is low. Sit-and-wait strategies could be advantageous at high temperatures when prey density is high. (b) When prey speed decreases with warming, active strategies could be favored at high temperatures when prey density is low. Sit-and-wait strategies could be favored at low temperatures when prey density is high.

APPENDIX B: Supporting methods and results

Methods

Predator functional response experiments: predator-prey acclimation

Instar 1 *Notonecta* were collected from June 20 to July 28 and instar F-4 and F-3 *Enallagma* (F-1 is the penultimate instar) were collected from September 1 to October 7. We acclimated predators to laboratory conditions at 20 °C (mean ± 1 SD: *Notonecta* 19.1 ± 0.42, *Enallagma* 20.6 ± 0.73) under a 15:9 h L:D photoperiod for *Notonecta* and a 13:11 h L:D photoperiod for *Enallagma* (Culler et al. 2014). *Notonecta* were held in 37.8 L aquaria filled with pond water and abundant habitat structure at a density of ~1 *Notonecta* L^{-1}. *Enallagma* were held individually in 200-mL glass jars filled with pond water and an artificial plastic plant for structure. After their first molt, predators were moved to an experimental arena (Vucic-Pestic et al. 2011, Culler et al. 2014). *Daphnia* were collected from ponds in June and July 2017 and acclimated at 20 °C until experiments in filtered pond water. Preliminary acclimation periods to laboratory conditions at 20 °C were used to standardize predators to a similar size and number of days post-molt, which can affect feeding rates (Johnson 1975). Predators were then acclimated to the target experimental temperature for 24 hours prior to the food deprivation period, and *Daphnia* were acclimated for two hours at the target temperature prior to beginning trials (Thompson 1978). *Daphnia* were given a shorter acclimation period to the target temperature than predators because of their much shorter life span and to avoid natural mortality before beginning trials. Recent research has shown that shorter acclimation times are needed for shorter-lived and smaller-sized species because acclimation rate scales negatively with body size (Rohr et al. 2018).

Experimental conditions

Arenas for *Notonecta* trials were 9 x 16 x 10 cm containers filled with 1L filtered pond water, and 3 artificial plants similar in structure to *Ceratophyllum demersum*, the dominant aquatic plant in ponds in Lux Arbor Reserve. *Enallagma* trials were performed in 150-mL beakers filled with 100-mL filtered pond water, and two plants identical to those used in *Notonecta* trials suspended from the surface of the beaker. Artificial plants mimicked the habitat structure found in ponds and provided a perch for predators.

Predators were between 2 to 5 days post-molt (Johnson et al. 1975) and had been both pre-satiated and food-deprived at their target experimental temperature when trials began. *Notonecta* were food deprived for 6 hours, and *Enallagma* for 24 hours. *Notonecta* deprivation period was determined based on preliminary trials in 2016. Twenty-four hours is the optimal deprivation period to standardize hunger levels in *Enallagma* (Johnson et al. 1975). We standardized predator and prey body sizes by size-sorting under a microscope (Zeiss Stemi 508) fit with an ocular micrometer accurate to ± 0.1 mm. *Notonecta* were all instar 2 (mean ± SE length, 3.33 ± 0.01 mm) and *Enallagma* were instars F-3 and F-2 (mean ± SE head capsule width, 2.75 ± 0.02 mm). *Daphnia* used in trials were between 1 to 1.5 mm carapace length (Gergs and Ratte 2009, Culler et al. 2014)

We used experimental temperatures of 10, 15, 20, 25, 30 or 35 °C (mean ± 1 SD, 10.81 ± 0.88, 15.81 ± 1.09, 21.05 ± 1.01, 24.43 ± 1.27, 30.36 ± 0.65, 35.33 ± 0.63 °C). We used diffuse light of 610 – 725 lux inside growth chambers to avoid *Daphnia* photo-attraction toward a light source. We rotated the temperature in each chamber every other day to avoid chamber effects. *Notonecta* trials were run from July to August 2017 and *Enallagma* trials from September to October 2017.

Correction for errors in enumerating Daphnia

Notonecta digest their prey externally and leave behind *Daphnia* carapaces that are easily distinguishable from *Daphnia* that died naturally. Thus, we estimated errors in *Notonecta* trials by counting all consumed and unconsumed *Daphnia*, plus natural deaths, the sum of which was used as the actual prey density. By contrast, *Enallagma* completely consume *Daphnia*, and therefore, we estimated counting errors by running three replicate controls for each *Daphnia* density. Controls followed the same methods used in predator-prey trials, excluding the predator. The counting error was less than 1%, thus, we ignored counting errors in subsequent analyses.

Testing H1: Video analysis of predator and prey swimming speed

We used video recordings of functional response experiments to calculate *Notonecta* swimming speed (cm s^{-1}) by randomly selecting 6 movements for each video between 30 and 60 minutes, 3 of which were "burst" movements, associated with attacks on prey, lasting 1-2 seconds, and 3 were longer "patrolling" movements lasting 3-5 seconds that predators exhibited in between bouts of attacking and consuming prey. The speed of each movement was calculated using Tracker Video Software, and a mean trial speed for each respective movement was calculated as the average of the 3 swimming speeds. We also ran separate *Daphnia* swimming speed experiments following the methods from *Notonecta* functional response trials for acclimation and experimental arena. We used densities of 20 *Daphnia* L^{-1} and excluded artificial plants and predators from arenas to better observe *Daphnia*. We video-recorded these experiments for twenty minutes at each temperature of 10, 15, 20, 25, 30, 35 °C, and replicated each temperature treatment four times. Videos were analyzed for *Daphnia* swimming speed using Tracker software as follows. We quantified speed (cm s^{-1}) of the *Daphnia* individual nearest the center of the experimental arena for 20 second intervals at 5, 10, and 15 minutes of video time. Mean speed from these three observation periods was used as the overall trial velocity. To confirm that *Daphnia* were moving at a similar speed between experiments with and without predators, *Daphnia* mean speed was also calculated from videos of *Notonecta* functional response experiments at 20 and 30 °C, using the above methods. *Daphnia* speed did not differ significantly between trials with and without *Notonecta* (Welch's two-sample t-test, 20 °C, $t_2 = 0.0002$, $p = 0.999$; 30 °C, $t_{1.74} = 0.281$, $p = 0.808$).

Testing H2: Video analysis of encounter rates and capture success

We video-recorded functional response experiments with prey densities of 20 *Daphnia* L^{-1} and 20 *Daphnia* 100 mL^{-1} for *Notonecta* and *Enallagma* respectively, to test whether prey encounter rates and capture success increase more strongly for active predators with warming. Recordings were analyzed for the 60-minute duration of the experiment. We estimated total encounters as the sum of reactive and non-reactive encounters. Reactive distance was measured by taping a 4-cm grid to the back of the experimental containers.

We used video estimates of predator attack rates to quantify capture success, or the number of successful attacks per encounter (Holling 1966). Attack rates were quantified as the number of successful and unsuccessful attacks. For *Enallagma*, a successful attack was a rapid protrusion of the labium that resulted in prey capture (McPeek and Crowley 1987). For *Notonecta*, a successful attack was a rapid advance toward, and grasping of, prey (Gergs and Ratte 2009). For either predator, an attack was considered unsuccessful when the prey subsequently escaped. Before running statistical models, we calculated an hourly rate for each foraging behavior (e.g., number of encounters hr^{-1}) based on the number of seconds the predator was observable (i.e. not obscured by an artificial plant).

Testing H4: Predator growth experiments

We used a duration of four days for *Enallagma* and three days for *Notonecta* in growth experiments because preliminary trials showed that these two predators molt at different rates (it can take up to a month for *Enallagma* to molt in the lab, and days for *Notonecta*). We ran *Enallagma* growth experiments for four days to maintain consistency with methods of previous successful experiments on damselflies in the same genus (e.g., Culler et al. 2014), and because meaningful weight gain needs to be observed during the experimental duration to obtain accurate estimates of growth rates (Gordon 1968). *Notonecta* were fed for three days because of their comparatively much faster growth rate. In preliminary trials conducted over four days *Notonecta* began molting during the fourth day. Our goal was to compare intra-molt growth rates, because molting can significantly alter feeding and growth measurements (e.g., Johnson 1975). However, because we calculated relative growth rate and accounted for trial duration, we used comparable intra-molt growth rates when fitting the Arrhenius equation and running ANOVA to test for differences in the temperature scaling of growth between predator species (Fig. 2, Appendix A).

Results

Testing H3: Video analysis of predator attack rates

Total attacks (hr^{-1}) based on video observations varied significantly with temperature (ANOVA, $F_{5,44} = 14.56$, $p < 0.01$; Tukey's HSD, Table 9) and between the predators ($F_{1,44} = 821.21$, $p < 0.01$), with *Notonecta* having more attacks overall. There was not a significant interaction between temperature and predator species on total attacks ($F_{5,44} = 0.38$, $p = 0.86$). The 95% confidence intervals of *Ea* from the fit of the Arrhenius equation for attack rate (Table 2, 3) was 0.15 to 0.40 for *Notonecta*, and 0.14 to 0.33 for *Enallagma*, indicating a similar magnitude of increase in attack rates with temperature. The number of attacks per encounter did not vary with temperature (ANOVA, $F_{5,44} = 1.76$, $p = 0.14$), but did vary between predator species ($F_{1,44} = 108.14$, $p < 0.01$). There was no interaction between temperature and predator species on the number of attacks per encounter ($F_{5,44} = 0.90$, $p = 0.49$). The Arrhenius-quadratic equation provided the best fit to the relationship between temperature and number of attacks per encounter for *Notonecta*, whereas an intercept model provided the best fit for *Enallagma* (Table 2, 3). This result indicates that *Notonecta* had a hump-shaped increase in number of attacks per encounter with warming, and *Enallagma* had no increase in number of attacks per encounter with warming.

Relationship between predator handling time and warming

Although handling time was lower for *Notonecta* than *Enallagma* across all temperatures (Fig. 5), handling time of *Enallagma* decreased more with warming (Table 3). However, predators had overlapping 95% confidence intervals for *Ea* (Algorithm 3) for handling time (*Notonecta*: -0.05 to -0.36, *Enallagma*: -0.13 to -0.62), which indicates that the rate of change in handling time with warming did not differ significantly between the two predators.

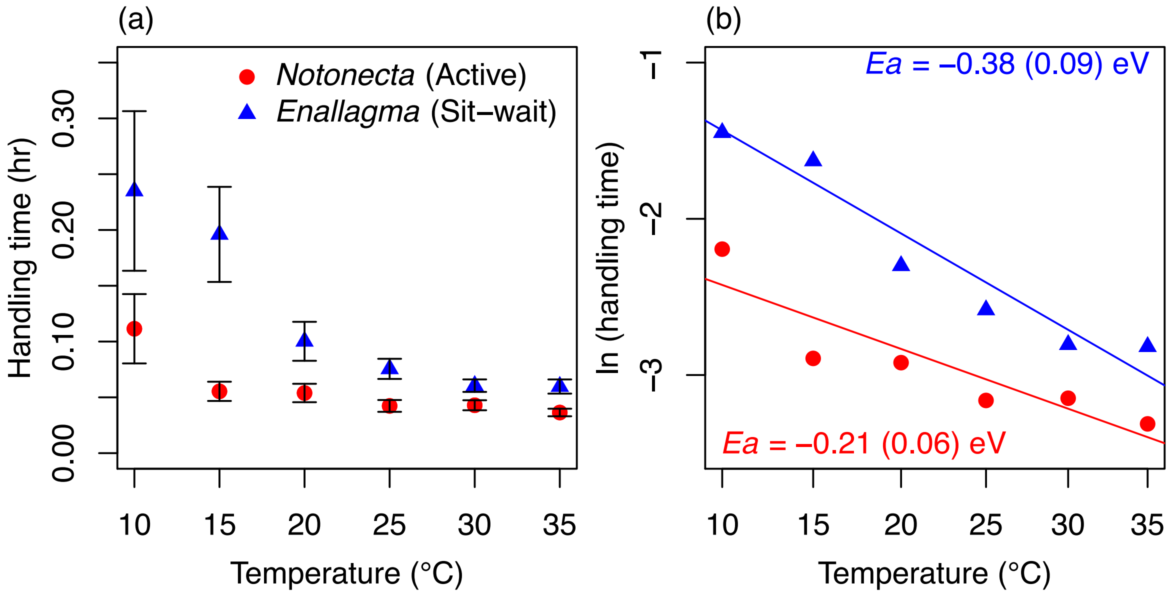

Figure 5. Relationships between temperature and handling time for *Notonecta* and *Enallagma*. (a) Handling time (hr. ± 1 SE) estimates from functional response curves, and (b) fits of the Arrhenius equation to handling times. *Ea* is the activation energy (eV ± 1 SE) estimated from the slope of the best-fit line of the Arrhenius equation, and gives the strength of the temperature response.

Table 1. Fit of Rogers Random Predator Equation to feeding data from functional response experiments at each temperature. Columns show the estimated value of a (attack rate, 100 mL^{-1} for *Enallagma* and 1 L^{-1} for *Notonecta*) and h (handling time, hr) along with their associated Z-values and P-values. P-values less than 0.05 indicate that the value for that parameter differs from zero. R^2 is the coefficient of determination.

Temperature °C	**a estimate (SE)**	**Z-value**	**P-value**	**h estimate (SE)**	**Z-value**	**P-value**	**R^2**
Enallagma							
10	0.187 (0.083)	2.262	0.024	0.235 (0.072)	3.284	0.001	0.16
15	0.340 (0.125)	2.713	<0.01	0.196 (0.043)	4.560	<0.01	0.33
20	0.573 (0.157)	3.641	<0.01	0.100 (0.017)	5.738	<0.01	0.46
25	0.539 (0.108)	4.99	<0.01	0.076 (<0.01)	8.376	<0.01	0.50
30	1.079 (0.193)	5.588	<0.01	0.060 (<0.01)	10.794	<0.01	0.48
35	1.149 (0.214)	5.370	<0.01	0.060 (<0.01)	9.378	<0.01	0.55
Notonecta							
10	0.303 (0.106)	2.857	<0.01	0.111 (0.031)	3.587	<0.01	0.67
15	0.969 (0.245)	3.948	<0.01	0.055 (<0.01)	6.427	<0.01	0.55
20	1.060 (0.273)	3.890	<0.01	0.054 (<0.01)	6.539	<0.01	0.62
25	1.299 (0.267)	4.858	<0.01	0.042 (<0.01)	7.985	<0.01	0.64
30	2.263 (0.515)	4.393	<0.01	0.043 (<0.01)	9.566	<0.01	0.87
35	2.286 (0.461)	4.957	<0.01	0.036 (<0.01)	9.979	<0.01	0.83

Table 2. Results of AICc model selection for models testing relationships between temperature and biological rates. Models fit to each response variable included intercept, Arrhenius, and Arrhenius-quadratic. The selected model is shown in bold. K is the number of model parameters. ΔAIC_c = AIC_c of the model - AIC_c minimum of all models under consideration. AICc Weight is the probability that the model provides the best fit to the data.

Response	Model	K	AICc	ΔAICc	AICc Weight	Log-likelihood
$a_{Notonecta}$	**Arrhenius**	3	19.38	0.00	0.81	-0.92
	Intercept	2	22.67	2.84	0.19	-7.34
	Arrhenius-quadratic	4	44.73	24.90	0.00	1.64
$a_{Enallagma}$	**Arrhenius**	3	10.22	0.00	0.99	3.89
	Intercept	2	20.01	9.79	0.01	-6.01
	Arrhenius-quadratic	4	38.45	28.23	0.00	4.78
$h_{Notonecta}$	**Intercept**	2	8.36	0.00	0.63	-0.18
	Arrhenius	3	9.40	1.04	0.37	4.30
	Arrhenius-quadratic	4	36.78	28.42	0.00	5.61
$h_{Enallagma}$	**Arrhenius**	3	13.75	0.00	0.54	2.12
	Intercept	2	14.11	0.35	0.46	-3.05
	Arrhenius-quadratic	4	34.79	21.04	0.00	6.60
Video attack rate$_{Notonecta}$	**Arrhenius**	3	24.55	0.00	0.64	-8.75
	Arrhenius-quadratic	4	25.66	1.11	0.36	-7.92
	Intercept	2	38.07	13.52	0.00	-16.78
Video attack rate$_{Enallagma}$	**Arrhenius**	3	9.91	0.00	0.79	-1.48
	Arrhenius-quadratic	4	12.60	2.68	0.21	-1.46
	Intercept	2	27.62	17.71	0.00	-11.58
Encounters$_{Notonecta}$	**Arrhenius**	3	4.87	0.00	0.73	1.09
	Arrhenius-quadratic	4	6.96	2.09	0.26	1.43
	Intercept	2	12.53	7.66	0.02	-4.02
Encounters$_{Enallagma}$	**Arrhenius**	3	48.22	0.00	0.64	-20.63
	Arrhenius-quadratic	4	49.39	1.17	0.36	-19.86
	Intercept	2	67.64	19.42	0.00	-31.59
Capture success$_{Notonecta}$	**Arrhenius-quadratic**	4	43.01	0.00	0.84	-16.60
	Intercept	2	46.77	3.76	0.13	-21.14
	Arrhenius	3	49.29	6.28	0.04	-21.12

Table 2. (cont’d)

Capture success$_{\textit{Enallagma}}$	**Arrhenius**	3	-38.66	0.00	0.55	22.81
	Intercept	2	-37.42	1.23	0.30	20.94
	Arrhenius-quadratic	4	-36.17	2.49	0.16	22.92
Attacks per encounter$_{\textit{Notonecta}}$	**Arrhenius-quadratic**	4	19.60	0.00	0.61	-4.89
	Arrhenius	3	20.99	1.39	0.31	-6.97
	Intercept	2	23.63	4.04	0.08	-9.57
Attacks per encounter$_{\textit{Enallagma}}$	**Intercept**	2	-22.06	0.00	0.71	13.26
	Arrhenius	3	-19.75	2.31	0.22	13.35
	Arrhenius-quadratic	4	-17.12	4.94	0.06	13.39
“Patrolling” swimming speed$_{\textit{Notonecta}}$	**Arrhenius**	3	14.19	0.00	0.72	-5.77
	Intercept	2	16.04	1.85	0.28	-3.58
“Burst” swimming speed$_{\textit{Notonecta}}$	**Intercept**	2	11.68	0.00	0.59	-3.59
	Arrhenius	3	12.43	0.75	0.41	-2.69
Growth$_{\textit{Notonecta}}$	**Arrhenius**	3	-114.38	0.00	0.75	60.55
	Arrhenius-quadratic	4	-112.16	2.22	0.25	60.70
	Intercept	2	-80.88	33.50	0.00	42.61
Growth$_{\textit{Enallagma}}$	**Arrhenius**	3	-104.22	0.00	0.77	55.43
	Arrhenius-quadratic	4	-101.77	2.45	0.23	55.43
	Intercept	2	-85.72	18.50	0.00	45.01

Table 3. Parameter estimates from best-fit models describing relationships between temperature and biological rates. Models fit to each response variable included intercept, Arrhenius, Arrhenius-quadratic, and segmented Arrhenius. P-values less than 0.05 indicate that the value for that parameter differs from zero and affects the response variable. c is the model intercept. b is the activation energy E_a from the Arrhenius equation measured in electron volts (eV). q is the quadratic term of the Arrhenius-quadratic equation.

Response	**Model**	**ln (c) ± (SE)**	**p**	**b_1* (eV) ± (SE)**	**p**	**b_2† (eV) ± (SE)**	**q (eV^2) ± (SE)**	**p**	**R^2**
$a_{\mathit{Notonecta}}$	Arrhenius	26.42 (4.88)	<0.01	0.67 (0.12)	<0.01				0.88
$a_{\mathit{Enallagma}}$	Arrhenius	21.98 (2.23)	<0.01	0.58 (0.06)	<0.01				0.96
Encounter *Notonecta*	Arrhenius	10.46 (1.66)	<0.01	0.14 (0.04)	<0.01				0.31
Encounter *Enallagma*	Arrhenius	19.66 (3.44)	<0.01	0.48 (0.09)	<0.01				0.53
Capture success *Notonecta*	Arrhenius-quadratic	- 411.15 (132.18)	<0.01	20.85 (6.75)	<0.01		-0.27 (0.09)	<0.01	0.29
Capture success *Enallagma*	Arrhenius	1.70 (0.77)	0.04	0.04 (0.02)	0.06				0.12
Swimming speed *Daphnia*	Segmented Arrhenius	38.63 (8.58)	<0.01	1.04 (0.23)	<0.01	0.11 (0.06)			0.82
Patrolling swimming speed *Notonecta*	Arrhenius	2.69 (1.92)	0.17	0.10 (0.05)	0.05				0.15
Burst swimming speed *Notonecta*	Intercept	-0.02 (0.05)	0.70						
Growth *Notonecta*	Arrhenius	2.13 (0.27)	<0.01	0.05 (0.01)	<0.01				0.62
Growth *Enallagma*	Arrhenius	1.90 (0.35)	<0.01	0.05 (0.01)	<0.01				0.39
$h_{\mathit{Notonecta}}$	Arrhenius	-11.17 (2.17)	<0.01	-0.21 (0.06)	0.02				0.78

Table 3. (cont'd)

$h_{Enallagma}$	Arrhenius	-17.07 (3.36)	<0.01	-0.38 (0.09)	0.01			0.82
Video attack rate *Notonecta*	Arrhenius	13.62 (2.39)	<0.01	0.27	<0.01			0.45
Video attack rate *Enallagma*	Arrhenius	9.94 (1.78)	<0.01	0.24 (0.05)	<0.01			0.50
Attacks per encounter *Notonecta*	Arrhenius-quadratic	-168.26 (85.67)	0.06	8.62 (4.37)	0.06	-0.11 (0.06)	0.06	0.29
Attacks per encounter *Enallagma*	Intercept	0.37 (0.03)	<0.01					

*†Note that for capture success of *Notonecta*, b_1 is the linear term of the Arrhenius-quadratic equation, and thus cannot be interpreted as the activation energy. For swimming speed of *Daphnia*, b_1 is the slope of the line segment for temperatures above the breakpoint of 28 °C, and b_2 is the slope of the line segment for temperatures below the breakpoint of the segmented Arrhenius model.

Table 4. Comparison of models fit to the relationship between temperature and swimming speed of *Daphnia*. An intercept and an Arrhenius model fit using ordinary least squares regression were compared to a segmented Arrhenius model fit using maximum likelihood estimation. Lower AIC scores indicate a better fitting model. The selected best-fitting model is shown in bold.

Model	**Residual std. error**	**R^2**	**AIC**
Intercept	0.43		30.98
Arrhenius	0.27	0.63	9.16
Segmented Arrhenius	0.20	0.82	-3.88

Table 5. P-values from Tukey's HSD testing for differences in *Daphnia* swimming speed (cm s^{-1}) between temperatures. P-values below the significance threshold of 0.05 are indicated in bold.

	10°C	**15°C**	**20°C**	**25°C**	**30°C**
15°C	0.87	-	0.99	0.98	0.20
20°C	0.92	0.99	-	0.96	0.16
25°C	0.50	0.98	0.96	-	0.51
30°C	**0.02**	0.20	0.16	0.51	-
35°C	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**

Table 6. P-Values from Tukey's HSD testing for differences in encounter rates, quantified as an hourly rate at a density of 20 *Daphnia* per liter (hr^{-1} 20 *Daphnia* L^{-1}), between temperatures. P-values below the significance threshold of 0.05 are indicated in bold.

	10°C	**15°C**	**20°C**	**25°C**	**30°C**
15°C	0.42	-	0.16	0.19	**< 0.01**
20°C	**< 0.01**	0.35	-	0.99	0.61
25°C	**< 0.01**	0.20	0.99	-	0.84
30°C	**< 0.01**	**0.01**	0.61	0.84	-
35°C	**< 0.01**	**< 0.01**	**0.04**	0.10	0.64

Table 7. P-values from Tukey's HSD testing for interactions between temperature and predator species on encounter rates, quantified as an hourly rate at a density of 20 *Daphnia* per liter (hr^{-1} 20 *Daphnia* L^{-1}). (*E* = *Enallagma, N* = *Notonecta*). P-values below the significance threshold of 0.05 are indicated in bold.

	10°C (*E*)	**15°C** (*E*)	**20°C** (*E*)	**25°C** (*E*)	**30°C** (*E*)	**35°C** (*E*)	**15°C** (*N*)	**20°C** (*N*)	**25°C** (*N*)	**30°C** (*N*)	**35°C** (*N*)
10°C (*N*)	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	1.00	1.00	0.99	0.99	0.99
15°C (*N*)	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	-	0.99	0.99	0.99	0.99
20°C (*N*)	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	0.99	-	1.00	0.99	0.99
25°C (*N*)	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	0.99	1.00	-	0.99	0.99
30°C (*N*)	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	0.99	0.99	0.99	-	1.00
35°C (*N*)	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**	0.98	0.99	0.99	1.00	-
15°C (*E*)	0.31	-	0.99	0.25	0.30	0.13	-	-	-	-	-
20°C (*E*)	**0.03**	0.99	-	0.86	0.89	0.63	-	-	-	-	-
25°C (*E*)	**< 0.01**	0.25	0.86	-	1.00	0.99	-	-	-	-	-
30°C (*E*)	**< 0.01**	0.30	0.89	1.00	-	0.99	-	-	-	-	-
35°C (*E*)	**< 0.01**	0.13	0.63	0.99	0.99	-	-	-	-	-	-

Table 8. P-Values from Tukey's HSD testing for differences in predator relative growth rates (mg mg$^{0.34}$ day^{-1}) between temperatures. P-values below the significance threshold of 0.05 are indicated in bold.

	10°C	15°C	20°C	25°C	30°C
15°C	0.99	-	0.24	**< 0.01**	**< 0.01**
20°C	**0.14**	0.24	-	0.30	**0.02**
25°C	**< 0.01**	**< 0.01**	0.30	-	0.82
30°C	**< 0.01**	**< 0.01**	**0.02**	0.82	-
35°C	**< 0.01**	**< 0.01**	**< 0.01**	0.42	0.99

Table 9. P-Values from Tukey's HSD testing for differences in total attacks (hr^{-1}) between temperatures from video observations. P-values below the significance threshold of 0.05 are indicated in bold.

	10°C	15°C	20°C	25°C	30°C
15°C	0.60	-	0.32	**0.05**	**< 0.01**
20°C	**< 0.01**	0.32	-	0.91	0.29
25°C	**< 0.01**	**0.05**	0.91	-	0.89
30°C	**< 0.01**	**< 0.01**	0.29	0.89	-
35°C	**< 0.01**	**< 0.01**	**< 0.01**	0.09	0.50

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CHAPTER 2. Life-history responses to temperature and seasonality mediate ectotherm consumer-resource dynamics under climate warming

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Abstract

Climate warming is altering life cycles of ectotherms by advancing their phenology and decreasing their generation times, which may have profound consequences for consumer-resource population dynamics and community structure. Theoretical models provide powerful tools to investigate these potential effects of climate warming on ecological communities. Yet, existing theory primarily considers communities of interacting organisms with simplified life-histories living in constant temperature environments. As such, it is difficult to predict how climate warming will affect organisms with complex life cycles living in seasonal environments. We address this knowledge gap by developing a size-structured, consumer-resource population model with seasonal temperature dependence. We parameterized our model using data from field surveys and experiments of a freshwater insect, the damselfly *Enallagma annexum*, consuming zooplankton. We use our model to simulate how climate warming in a seasonal environment could alter a key life-history trait of the consumer, the number of generations per year, and how changes in this trait mediate responses of consumer-resource population sizes and consumer persistence to increasing environmental temperature. We find that in a warming world, damselfly population sizes increase through multiple mechanisms. First, warming can decrease damselfly generation times by increasing their rates of resource ingestion and growth and/or lengthening

the growing season. Second, these life-history changes shorten the duration of the juvenile stage and "the window of vulnerability" to mortality, which increases the number of emerging adults and population-level reproduction. Unstructured models with similar assumptions found that increasing consumer rates of resource ingestion, consumer growth, and population sizes with warming destabilized consumer-resource dynamics. In contrast, our size-structured model predicts increased stability of consumer-resource populations with increased predator persistence. Our study reveals mechanisms by which climate change and latitude can alter ectotherm life-histories and populations. Overall, our study suggests that life-history changes could delay population extinctions with future warming. Life-history changes and temperature seasonality should therefore be incorporated into research investigating the impacts of climate warming on consumer-resource populations in seasonal environments.

Introduction

An outstanding question of climate change research is how rising temperatures will alter ecological communities through its effects on consumer-resource interactions. Over large temperature ranges, ectotherm biological rates, including metabolism and feeding, show concave, unimodal responses to temperature (Huey and Stevenson 1979, Englund et al. 2011), and within the rising portion of the unimodal response, ectotherm feeding rates increase exponentially with temperature as a result of increasing metabolism (Gillooly et al. 2001, Brown et al. 2004, Dell et al. 2011). Previous research has shown that higher consumer feeding rates can cause communities to become top-heavy by increasing consumer biomass (Yvon-Durocher et al. 2011, Shurin et al. 2012). However, rising temperatures can also cause declines in consumer biomass by lowering consumer energetic efficiency, the balance between biomass gains from feeding and losses from metabolism (Lang et al. 2017).

Theoretical models provide powerful tools to investigate the potential effects of climate warming on ecological communities because they allow the user to simulate population dynamics and changes in community structure over long time scales. Yet, most existing models cannot simulate realistic community outcomes, because theory has primarily considered communities of interacting organisms with simplified life histories living in constant temperature environments (Uszko et al. 2017). This contrasts with the reality that temperate species experience pronounced seasonal changes in temperature, and the majority of ectotherms have structured populations with complex life cycles that have different physiological responses to warming (Kingsolver et al. 2011, Sinclair et al. 2016). To improve our understanding of climate change effects on ecological communities, theory must consider interactions among organisms with complex life cycles in seasonally fluctuating environments.

Theory has often focused on unstructured populations of consumer-resource pairs when predicting the effects of climate warming on communities (Uszko et al. 2017). Many of these models predict that consumer biomass will decrease with warming as consumers become more susceptible to starvation extinction, and communities will become more stable (Vasseur and McCann 2005, Vucic-Pestic, Olivera et al. 2011, Binzer et al. 2012, Fussmann et al. 2014). The same models with different assumptions have predicted that consumer biomass will increase with warming and destabilize communities (Vasseur and McCann 2005, Uszko et al. 2017). These differing predictions depend, in part, on whether consumer feeding and metabolism change exponentially or in a unimodal fashion with temperature, whether consumer metabolism or feeding increases more with temperature, and whether resource carrying capacity varies with temperature (Uszko et al. 2017).

Structured population models have revealed additional mechanisms by which temperature can affect population dynamics and communities (Lindmark et al. 2019). Stage-structured models have shown that warming can alter population stage-structure (Lindmark et al. 2018) and decrease predator persistence due to feedbacks between individual performance and population stage-structure, regardless of the assumed temperature scaling of feeding and metabolism or resource carrying capacity (Lindmark et al. 2019). However, these models have focused on changes in mean temperature and have yet to include the effects of temperature seasonality. Stage-structured population models developed for insects have yielded insights into how temperature seasonality can alter life-histories of organisms with complex life cycles, including that higher mean temperatures and longer growing seasons can lead to shorter generation times (Crowley et al. 1987, Amarasekare 2019). However, these insect life history models are far more complex than simpler models derived from the familiar Rosenzweig-MacArthur model due to their use of delay differential equations to model changes in many life stages, and their results are thus difficult to compare to other theory (Uszko et al. 2017). Our aim is to provide a relatively simple and interpretable model to examine the effects of climate warming on size-structured organisms with complex life-histories in seasonal environments.

Climate warming has already altered life cycles of ectotherms by advancing their phenology and decreasing their generation times (Parmesan and Yohe 2003, Forrest 2016). All else being equal, higher environmental temperatures result in faster rates of metabolism, feeding, and growth (Gillooly et al. 2001, 2002, Brown et al. 2004, Rall et al. 2012), which also speed maturation to the adult stage (Roy and Sparks 2000, Hassall et al. 2007, Matsuda et al. 2018).

Shorter generation times could influence population dynamics by decreasing the length of time juveniles are susceptible to mortality, thereby increasing their survival to the adult stage (Benrey and Denno 1997, Anderson et al. 2001, Culler et al. 2015, Chen et al. 2019). For example, mosquitoes are predicted to have higher survival in the warming Arctic due to faster development and emergence to the adult stage, despite higher juvenile mortality from predators (Culler et al. 2015). If shorter generation times contribute to higher survivorship, then these benefits of warming may offset the negative effects of increasing mortality and result in larger consumer population sizes (Amarasekare and Savage 2012, Amarasekare 2019). Accounting for temperature effects on life cycles in consumer-resource models could modify the frequent prediction that rising temperatures increase consumer extinction risks as a result of higher metabolism and mortality (Uszko et al. 2017) and reveal new mechanisms by which warming alters population dynamics and communities.

Diverse ectotherms experience seasonally fluctuating temperatures that structure their life-history, including annual timing of feeding and growth, transitions among life stages, and adult emergence and reproduction (Wassersug 1975, Mousseau and Roff 1989, Conover 1992, Adolph and Porter 1996, Mackas et al. 2012). For these organisms, climate warming can interact with seasonality to alter their phenology and population dynamics (Kingsolver et al. 2011, Boggs 2016, Forrest 2016). For example, many insects in temperate environments have synchronized life cycles maintained by a period of winter diapause that is triggered by photoperiod and temperature (Forrest 2016). Increases in temperature and longer growing seasons have enabled insects to become active earlier in the season and produce more generations per year (Roy and Sparks 2000, Musolin 2007, Hassall et al. 2007, Matsuda et al. 2018). Yet, seasonal photoperiodic cues still induce winter diapause in many species, placing upper limits on changes in their phenology and generation times with warming (Forrest 2016). The effects of warming in seasonal environments also depend on the organisms' thermal performance: warming can have negative consequences for consumer populations by increasing summer temperatures above the thermal optimum for activity and growth, which can induce diapause, lengthen time to maturity, and increase mortality due to thermal stress (Kingsolver et al. 2011, Levy et al. 2015, 2016, Forrest 2016, Sinclair et al. 2016). The effects of warming may be most pronounced for organisms at high latitudes where warming is greater and seasonal fluctuations are more extreme (Parmesan 2007).

Theoretical models have examined the effects of seasonality on populations to understand the evolution of thermal traits (Thomas et al. 2012, Amarasekare and Johnson 2017) and species coexistence (Miller and Klausmeier 2017, Kremer and Klausmeier 2017). In addition, life cycle models have demonstrated the consequences of seasonality on life-histories and phenology for ectotherms (Taylor 1980, Gurney et al. 1992, Varpe et al. 2007). However, there are still gaps in our understanding of how climate warming affects consumer-resource population dynamics for organisms with complex life cycles living in seasonal environments. We bridge this knowledge gap by developing a size-structured consumer-resource model for ectotherms with seasonal temperature dependence. We use the framework of physiologically structured population models (PSPMs; De Roos *et al.* 2008), in which population dynamics depend on metabolism, feeding, growth in size, and transitions among life stages of the consumer, in response to resource levels and seasonally fluctuating environmental temperature. We develop and parameterize our model using field surveys and experiments of a freshwater insect, the damselfly *Enallagma annexum*, feeding on zooplankton. We use our model to simulate how climate warming in a seasonal environment could alter a key life-history trait of the consumer, the number of generations per year, and how changes in this trait mediate responses of consumer-resource population sizes and consumer persistence to increasing environmental temperature.

Methods

Life-history of Enallagma annexum

Damselflies have complex life cycles (Figs. 6-8 in Appendix) in which they spend the majority of their lifespan in an aquatic juvenile stage and emerge to a short-lived, terrestrial adult stage for reproduction. Many species have generation times that vary within and across latitudes; for species of *Enallagma*, the number of generations per year increases from less than one to two from the northern to the southern portion of their range (Corbet et al. 2006). *Enallagma* species in different locations at the same latitude also vary in their number of generations per year because environmental temperature directly increases their growth rates and decreases generation times within a latitudinal band (Corbet et al. 2006). We investigated the life-history and population dynamics of *Enallagma annexum* in southwestern Michigan, USA, where these damselflies have 11 juvenile stages (*instars*) requiring 10-11 months for development from egg to adult, and one generation per year with synchronized emergence to the adult stage that is maintained by a period of winter diapause as juveniles (McPeek1989, 1998). Adults live 1-4 weeks (Corbet 1980), during which they feed on terrestrial insects and breed, depositing their eggs in aquatic plants (Merritt and Cummins 1996).

Surveys of population dynamics of Enallagma annexum and zooplankton in ponds

We surveyed *E. annexum*, and their prey zooplankton, to characterize seasonal changes in population abundances and biomass. We sampled three ponds of Lux Arbor Reserve, southwestern Michigan, USA, twice per month from May 2016 to November 2016, and again from April to May 2017. We recorded surface water temperatures at hourly intervals using HOBO pendant temperature loggers (UA-001-64, Onset Corporation, Bourne, MA, USA). We collected *E. annexum* at three locations within each pond by sweeping a D-frame aquatic dip net (500 µm mesh, Wildco Wildlife Supply Co., Yulee, FL, USA) through macrophyte beds along a 1-m transect parallel to shore at depths of 0.25-0.75 m. We sampled zooplankton using vertical zooplankton net tows (153 µm mesh, 20.32 cm diameter opening, Wildco Wildlife Supply Co.) at water depths of 0.25 to 1.0 m and froze samples for estimation of biomass. We monitored adult emergence of *E. annexum* from May to June 2017 using floating insect emergence traps (Cadmus et al. 2016). We collected and counted all damselflies every other day and identified males to species (Westfall and May 2006). We determined that *E. annexum* is the only species of *Enallagma* in these ponds based on emergence samples and observations of adult damselflies at ponds throughout the year.

We identified *E. annexum* under a dissecting microscope (Stemi 508, Zeiss, USA) using published and online taxonomic guides (Hungerford 1933, Westfall and May 2006, Bright 2020). We tracked growth in body size of *E. annexum* by measuring the head capsule width (a common determinant of instar, Corbet 1999) of the first 20 individuals in each sample with an ocular micrometer (± 0.1 mm) and drying and weighing (± 0.01 mg) at least ten individuals of each probable instar. We estimated zooplankton biomass by sorting individuals of the Orders Diptera, Cladocera, Copepoda, and Rotifera from each sample under a dissecting microscope, drying them for 24 hours at 60°C, and weighing them (± 0.01 mg). We then calculated zooplankton biomass per liter sampled as: (dry weight)/(volume filtered by the plankton net). We estimated the volume filtered as: pi * net radius^2 * sample depth * filtering efficiency, assuming filtering efficiency of 0.5 (Benndorf 1986).

Temperature effects on feeding and growth rates of Enallagma annexum

We conducted experiments to quantify relationships between temperature and consumer functional responses and growth rates, at Kellogg Biological Station in southwestern Michigan, USA. We briefly outline methods below. For additional details, see Twardochleb et al. 2020. We collected *E. annexum* and zooplankton, *Daphnia pulex,* from ponds in Lux Arbor Reserve and acclimated them to laboratory conditions at 20 °C. We then ran experiments inside of environmental growth chambers (I36LLVL, Percival Scientific, Perry, IA, USA) set to 10, 15, 20, 25, 30 or 35 °C. These temperatures encompassed the annual mean (13 °C), the range (minimum to maximum: 10 – 31 °C) of ambient pond temperatures during the growing season, and a temperature (35 °C) above the recorded maximum (Fig. 6 in Appendix). We ran experiments with individual *E. annexum* feeding on densities of *D. pulex* ranging from 5 to 150 individuals 100 mL^{-1}. We replicated each density at every temperature at least four times for a total of 158 trials. We fit separate, Type 2 functional response curves (Holling 1959) to feeding data at each temperature using Rogers Random Predator Equation to account for prey depletion (Rogers 1972) using the ‘frair’ package in R Version 3.5.1 (Pritchard et al. 2017).

We also ran experiments to quantify relationships between temperature and *E. annexum* individual growth rates. We collected and acclimated *E. annexum* as above. Our temperature gradient included 10, 15, 20, 25, 30 or 35 °C. We replicated each temperature treatment seven times for 42 trials. We measured initial wet mass prior to trials and then fed each damselfly *D. pulex* ad libidum for four days. We then deprived damselflies of food to allow for gut evacuation, blotted them dry, weighed them for final wet mass, and then dried them for 24 hours at 60 °C, and weighed them again for final dry mass. We calculated the relationship between wet and dry mass using linear regression and used this relationship to calculate initial and final dry mass for the trials (McPeek and Anholt 2004).

We used estimates of growth and feeding rates to calculate the respiration rate of *E. annexum* at each temperature. We calculated daily mass-specific respiration μ (mg/mg/day) (Ayres and Scriber 1994, Culler et al. 2014):

Algorithm 6. Daily mass-specific respiration

$$\mu = e_c(c_{mean})(\frac{m_R}{m_C})\, t_d - \, g_{rel}.$$

Here, e_c is conversion efficiency, c_{mean} is the mean consumption rate (prey hr^{-1}) at each temperature, m_R and m_C are dry mass (mg) of an individual resource and consumer, t_d is the number of hours per day spent feeding, and g_{rel} is the relative mass gain of the consumer (mg/mg/day). We set e_c equal to 0.85 (Lawton 1970, Culler et al. 2014) and t_d equal to 8 hours (Johnson et al. 1975). We weighed *D. pulex* and *E. annexum* from experiments to determine m_R and m_C. We calculated g_{rel} (mg/mg/day) as log(initial dry mass) - log(final dry mass) from growth experiments (Ayres and Scriber 1994, Culler et al. 2014).

We fit the Arrhenius equation (Gillooly et al. 2001) to describe the temperature-dependence of biological rates (a, h, and μ):

Algorithm 7. Arrhenius model

$$Y = ce^{E_a(-\frac{1}{kT})}.$$

Here, Y is the biological rate, c is a fitted constant, E_a is the activation energy (eV) describing the strength of the temperature response, k is Boltzmann's constant (8.617 x 10^{-5} eV), and T is temperature (Kelvin). We also fit intercept-only and Arrhenius-quadratic equations (Englund et al. 2011) but found that the Arrhenius equation provided the best fit. We fit models using linear regression in R.

Size-structured consumer-resource population model (standard model)

We developed a size-structured consumer-resource model, which we refer to as the "standard model" when discussing results of simulations below. We based our size-structured consumer-resource population model on the biomass-based model of (Yodzis and Innes 1992), which is a reworking of the consumer-resource model of (Rosenzweig and MacArthur 1963). We modified this model to incorporate the complex life-history of a damselfly (Figs. 6-8 in Appendix). Using a system of ordinary differential equations, we model changes throughout the growing season in zooplankton (resource) biomass (mg/L), juvenile damselfly (consumer) body size (mg) and abundance (number of damselflies), and discrete transitions between juvenile and adult damselfly life stages (Fig. 8 in Appendix). We define the growing season as the period between when pond temperature reaches 10 ºC in spring and fall. Our lab experiments showed that damselfly and zooplankton activity was low at 10 ºC, which is consistent with previous studies demonstrating that the threshold temperature for insect development is near 10 ºC (Dixon et al. 2009). Thus, we model dynamics only during the growing season (Persson et al. 1998, Sun and de Roos 2015). We track yearly emergence from the juvenile to adult stage using discrete events within our continuous system. We track discrete, year-to-year changes in damselfly and zooplankton populations by modeling abundance and biomass at the beginning of each growing season as a function of the abundance and biomass at the end of the previous years' growing season.

To model within-season dynamics, we consider a consumer-resource pair in which biomass growth of the resource (zooplankton) Z in the absence of the consumer (damselflies) is controlled by the resource intrinsic rate of increase r and carrying capacity K. Juvenile damselflies increase their body size S by ingesting zooplankton following a saturating type 2 functional response, minus loss in growth potential due to respiration μ (Fig. 8 in Appendix). Rates of resource consumption and respiration increase linearly with juvenile body size. Changes in abundance of juvenile damselflies C is controlled by density independent, background mortality d and density dependent, interference mortality $\propto$, because cannibalism is an important source of mortality in damselflies (McPeek 1998). Abundance of adult damselflies C_a is zero when juveniles are present:

Algorithm 8. Zooplankton population growth Equation

$$\frac{dZ}{dt} = r\left(1 - \frac{Z}{K}\right) - a(T)\frac{Z}{1+h(T)a(T)Z}CS,$$

Algorithm 9. Juvenile damselfly body size

$$\frac{dS}{dt} = e_c a(T)\frac{Z}{1+h(T)a(T)Z}S - \mu(T)S,$$

Algorithm 10. Juvenile damselfly abundance

$$\frac{dC}{dt} = -d(T)C - \propto C^2,$$

Algorithm 11. Adult damselfly abundance

$$\frac{dC_a}{dt} = 0.$$

Here, a and h are the consumer attack rate and handling time on the resource, and e_c is conversion efficiency of the resource into growth in juvenile body size. The rates a, h, μ, and d are modeled as functions of temperature T using Algorithm 7 (and Algorithm 22, see below).

Our assumptions are similar to those of physiologically structured population models (PSPMs) in that rates of resource ingestion and respiration increase with damselfly body size, and ingestion is converted into growth in size. In addition, juveniles do not reproduce but they use energy for growth and development to the adult stage, and adults reproduce but do not grow (De Roos et al. 2008). Our assumptions differ from PSPMs in that we do not model energy storage or resource consumption by adults, which are terrestrial and rely on different resources. Instead, we assume that adult damselfly abundance is equivalent to the total juvenile abundance in a pond prior to emergence, and the number of new juveniles produced by adults depends on the number of adults at emergence and their fecundity. These assumptions are reasonable because the juvenile stage comprises the majority of the damselfly lifespan (see above and Corbet 1980, McPeek 1989). Therefore, to investigate the effects of temperature on life-history, we focused on modeling dynamics in the juvenile stage.

In our system (Fig. 8 in Appendix), juvenile damselflies are born at an initial body size S_{min}, develop synchronously as a cohort, and emerge to the adult stage after individual body size has reached a threshold S_{max}. To emerge to the adult stage, the temperature in ponds must also exceed 20 ºC (*emergence window*, Corbet 1999). If these *emergence conditions* are not met, then the population remains in the juvenile stage until the next emergence window (Crowley et al. 1987). Adult emergence is modeled using discrete events that are triggered by emergence conditions. Specifically, a discrete change occurs in the damselflies as all juveniles emerge from the pond and spend 30 days in the adult stage (*emergence period*), converting juvenile abundance into adult abundance. During the emergence period, damselflies are absent from the pond, enabling zooplankton biomass to recover. The discrete changes in the system at the time of emergence (t_e) are described by:

Algorithm 12. Zooplankton population biomass at time of emergence

$Z(t_e) = Z(t),$

Algorithm 13. Juvenile damselfly body size at time of emergence

$S(t_e) = S_{max},$

Algorithm 14. Juvenile damselfly abundance at time of emergence

$C(t_e) = 0,$

Algorithm 15. Adult damselfly abundance at time of emergence

$C_a(t_e) = C(t).$

Here $Z(t)$ is zooplankton biomass, and $C(t)$ is damselfly abundance just before t_e. Following emergence, adults reproduce, and adult abundance is converted into new juveniles according to their fecundity f. Upon reproduction, damselflies re-enter the pond, and the discrete changes in the system are described by:

Algorithm 16. Zooplankton population biomass after discrete event

$Z(t) = Z(t_e),$

Algorithm 17. Juvenile damselfly body size after discrete event

$S(t) = S_{min},$

Algorithm 18. Juvenile damselfly abundance after discrete event

$C(t) = f * C_a(t_e),$

Algorithm 19. Adult damselfly abundance after discrete event

$C_a(t) = 0,$

Here $Z(t)$, $S(t)$, $C(t)$, and $C_a(t)$ are state variables in Algorithms 8-11 just after the emergence period.

If juvenile size reaches S_{max} outside of the emergence window, juveniles continue to ingest zooplankton but no longer grow in size. In addition, at low prey densities, respiration may exceed resource consumption and assimilation, leading to starvation mortality for juveniles. To account for these conditions, we assume that growth in body size equals zero either when respiration exceeds resource consumption and assimilation, or $S = S_{max}$, in Algorithm 9. We model starvation mortality (De Roos et al. 2008) through Algorithm 10. Thus, juveniles are restricted to positive growth in body size as described by the piecewise equations:

Algorithm 20. Piecewise equation describing restrictions in growth in juvenile damselfly body size

$$\frac{dS}{dt} = \begin{cases} e_c a(T)\frac{Z}{1+h(T)a(T)Z}S - \mu(T)S \text{ if } \frac{e_c a(T)Z}{1+a(T)h(T)Z} > \mu(T) \text{ and } S < S_{max}, \\ 0 \text{ if } \frac{e_c a(T)Z}{1+a(T)h(T)Z} < \mu(T) \text{ or } S = S_{max} \end{cases}$$

Algorithm 21. Piecewise equation describing changes in juvenile damselfly abundance when respiration exceeds resource consumption and assimilation

$$\frac{dC}{dt}\begin{cases} -d(T)C - \propto C^2 \text{ if } \frac{e_c a(T)Z}{1+a(T)h(T)Z} > \mu(T), \\ \left(-d(T) + e_c a(T)\frac{Z}{1+h(T)a(T)Z} - \mu(T)\right)C - \propto C^2 \text{ if } \frac{e_c a(T)Z}{1+a(T)h(T)Z} < \mu(T) \end{cases}$$

Temperature seasonality influences the length of the growing season, the timing and width of the emergence window, and consumer biological rates. We vary temperature seasonally in Algorithm 7 according to a sinusoidal function,

Algorithm 22. Temperature seasonality function

$$\frac{dT}{dt} = T_{av} + T_{amp}\left(\frac{Sin2\pi(t-\phi)}{365}\right),$$

where T_{av} is average yearly temperature, T_{amp} is the amplitude giving the temperature range, t is the number of days in the year, and $t - \phi$ is the phase shift. Increasing T_{av} leads to a longer growing season and a larger emergence window.

Model parameterization

We estimated damselfly body sizes S_{min} and S_{max}, and zooplankton carrying capacity K from field data (Fig. 6 in Appendix). We assume that K is equivalent to the maximum zooplankton biomass in ponds during summer, although this is likely a conservative estimate because predation reduces standing biomass. Other biological rates were determined from the primary literature or set as needed to capture population dynamics observed in ponds (Table 10 in Appendix). We quantified parameter values for Algorithm 22 by fitting the sinusoidal function to our hourly pond temperature data using maximum likelihood estimation in R. We parameterized the temperature-dependence of biological rates a, h, and μ by fitting Algorithm 7 to our experimental data (Table 10 in Appendix). There is no clear evidence that conversion efficiency e_c or carrying capacity K vary with temperature (Peters 1983, Yvon-Durocher et al. 2011, Culler et al. 2014). We also assume that r does not vary with temperature, which is reasonable for our system in which the consumer feeds on a wide variety of zooplankton species with abundances that vary throughout the growing season, and therefore likely have different temperature-sensitivities that could not be captured by a single function.

Analysis

We analyzed our model in four forms: the "standard model" described above, and three "alternative models" in which we fixed the values of parameters or algorithms to explore their effects on the results. In alternative model 1 we fixed consumer generation time to one generation per year to demonstrate the effects of varying T_{av} in the absence of life-history changes in the consumer. In alternative model 2 we allowed growing season length and the width of the emergence window to vary with T_{av} but fixed temperature-dependent biological rates in Algorithms 8-10 to T_{av} = 13 °C. Rates still varied seasonally, but did not increase with T_{av}. In alternative model 3 we allowed biological rates to increase with T_{av}, but restricted the growing season length and the width of the emergence window to their values at T_{av} = 13 °C. These two variants together demonstrate how changing season length vs. biological rates mediate consumer life-history and population responses to T_{av}.

For all versions of our model, the long-term dynamics of the system Algorithms 8-11 are not independent of time due to temperature seasonality (Algorithm 22). We therefore simulated consumer-resource population dynamics for 100 years at T_{av} ranging from 7 to 30 °C and examined within-season population dynamics during the last ten years to compare consumer life-history (number of generations per year) among temperatures. We also examined changes in the yearly average of consumer-resource population sizes during the last twenty years of simulations. Simulations with the standard model in which we varied K or μ demonstrated that changes in either parameter did not strongly affect consumer-resource population dynamics, and therefore, we do not discuss those results further. We ran model simulations in Mathematica version 12.0 (Wolfram Research, 2019).

Results

Population dynamics of Enallagma annexum and zooplankton in ponds

We monitored consumer-resource populations during 2016-2017 in three southwestern Michigan ponds and found that T_{av} = 13 °C, and the growing season extended from April to November (Fig. 6 in Appendix). The average high temperature during July was 26 °C, corresponding to T_{amp} = 13 °C (Fig. 6a in Appendix). Zooplankton biomass varied throughout the growing season and peaked during July, with a mean across all ponds of ~ 1.5 mg/L and maximum of ~ 3 mg/L (Fig. 6b in Appendix). We detected newly hatched juvenile damselflies at the beginning of July (Fig. 6c, d in Appendix). Damselflies grew in size (Fig. 6c in Appendix) and declined in abundance (Fig. 6d in Appendix) throughout the growing season. They overwintered as juveniles, during which their average body size did not increase (Fig. 6c in Appendix). Damselflies resumed growing the following spring (Fig. 6c in Appendix) and emerged as adults in May (Fig. 6e in Appendix). We present an idealized depiction of how these consumer-resource population dynamics vary over the course of a year in Figure 7 in Appendix.

Effects of increasing temperature on consumer life-history

After 100 years of simulations of the standard model at different T_{av}, we found that damselflies persisted at $T_{av} \geq 7.5$ °C, below which, the emergence window was too narrow to permit emergence and reproduction. By examining within-season dynamics, we found that increasing T_{av} increased damselfly rates of prey ingestion and growth, lengthened the growing season, increase the width of the emergence window, and enabled shorter damselfly generation times (Fig. 9 in Appendix). At $T_{av} = 8$ °C, damselflies had one generation every two years (Fig. 9a in Appendix). At $T_{av} = 13$ °C, damselflies had one generation per year (Fig. 9b in Appendix), and at $T_{av} = 22.5$ °C, they had two generations per year (Fig. 9c in Appendix). Between these temperatures, damselflies had intermediate generation times. For example, between $T_{av} = 13$ and 22.5 °C they had one generation in some years, and two in others. Between T_{av} = 22.5 and 30 °C, damselflies continued transitioning to shorter generation times (Fig. 10 in Appendix).

Changes in consumer life-history and temperature seasonality affected consumer-resource population sizes and consumer persistence

Simulations of the standard model revealed that within a damselfly life-history strategy of one generation per year, the yearly average of juvenile and adult damselfly abundance, and zooplankton biomass, decreased with increasing temperature (Fig. 10a in Appendix). However, consumer-resource population sizes both abruptly increased when damselflies underwent a warming-induced change in life-history, for example, when transitioning from one to two generations per year at higher temperatures. Between T_{av} = 7.5 and 30 °C, the damselfly population size was above the persistence level and increased overall with warming (Fig. 10b in

Appendix). Because individual damselflies consumed more resources at higher temperatures, we found an overall decrease in zooplankton population biomass with warming (Fig. 10b in Appendix).

Simulations of alternative model 1 showed that damselfly population abundance declined with warming, and damselflies went extinct at $T_{av} = 26$ °C, when they were restricted to one generation or fewer per year (Fig. 11a in Appendix). As a result, the yearly average of zooplankton biomass remained near K across the temperature range, and above 26 °C, zooplankton biomass stabilized at K. In alternative model 2, we examined the effect of fixing temperature-dependent biological rates to T_{av} = 13 °C. Similar to our standard model, juvenile and adult damselfly abundance declined with increasing temperature within a life-history strategy of one generation per year (Fig. 11b in Appendix). The damselfly population size increased abruptly above T_{av} = 22 °C as they transitioned to more than one generation per year. This transition occurred at a lower temperature of 16.5 °C in the standard model (Fig. 10 in Appendix). In contrast to the standard model, damselflies did not transition to two generations per year below T_{av} = 30 °C (Fig. 11b in Appendix).

In alternative model 3, in which we fixed the growing season length to T_{av} = 13 °C, damselflies did not transition to more than one generation per year unless $T_{av} > 28$ °C (Fig. 11c in Appendix), whereas in the standard model, they transitioned at 16.5 °C (Fig. 10 in Appendix). Between T_{av} = 12 and 28 °C, damselfly abundance declined with increasing temperature (Fig. 11c in Appendix). Damselflies did not go extinct at very high temperatures ($T_{av} \geq 30$ °C) when season length was fixed. They continued transitioning to shorter generation times with increasing temperature as in the standard model.

Discussion

With our size-structured consumer-resource model, we demonstrate that warming results in larger consumer-resource populations, due to increased consumer rates of prey ingestion and growth, a longer growing season, and a larger emergence window that altogether enabled shorter consumer generation times. This life-history change in the consumer can offset warming-induced population declines that influence resource population biomass. In fact, high temperatures enable damselflies to transition to more than one generation per year, resulting in population persistence. In contrast, if damselflies maintain only a single generation per year, their population goes extinct at an average temperature of 26 °C. In addition, life-history transitions to more than one generation per year allowed the damselfly population size to increase at higher temperatures in our standard model. As a result, the larger damselfly population exerts stronger predation pressure, decreasing zooplankton biomass. Increasing the growing season length and consumer biological rates with temperature both contributed to these life-history changes in damselflies. Therefore, accounting for life cycle complexity and temperature seasonality modified the predicted effects of climate warming on consumer-resource population dynamics and consumer persistence.

Warming induces changes in consumer life-history

We found that warming decreased damselfly generation time through two mechanisms, increasing biological rates and increasing season length. First, warming increased damselfly rates of resource ingestion and growth, advancing their emergence to the adult stage (Fig. 9 in Appendix). Second, warming lengthened the growing season and increased the width of the emergence window, which enabled damselflies to emerge a second time in some years after reaching the emergence size. Restricting changes in resource ingestion rates or seasonality delayed life-history transitions in alternative models 2 and 3, in which damselflies did not transition to two generations per year at $T_{av} \leq 30$ °C. If damselflies cannot ingest more resources and grow faster by increasing their resource attack and handling rates, a relatively longer season is necessary to permit multiple emergence events per year. Alternatively, if season length does not vary, the damselfly growth rate must be sufficiently high to reach the emergence size twice per year. However, allowing either biological rates or season length to vary with temperature was sufficient to prevent population extinction at high temperatures (Fig. 10 in Appendix), and previous research suggests that each mechanism contributes to changes in life-history. Experimental warming increases growth rates and advances emergence in dragonflies and damselflies (Nilsson-Örtman et al. 2013, Culler et al. 2014, McCauley et al. 2018a), and their life-histories vary latitudinally (Corbet 1999, Corbet et al. 2006).

Changes in consumer life-history and temperature seasonality affect consumer persistence

Life-history transitions are necessary to offset warming-induced population declines and delay extinction. Restricting damselflies to one generation or fewer per year (alternative model 1) resulted in damselfly population declines at higher temperatures due to longer exposure to higher rates of background and respiration mortality with a longer growing season (Fig. 11 in Appendix). The key to escaping increased mortality at high temperatures is decreasing the "the window of vulnerability", by translating increased rates of resource ingestion and growth into earlier adult emergence (Benrey and Denno 1997), thereby increasing population-level reproduction. The benefits of faster transitions from juvenile to adult stages for offsetting higher mortality with warming have been demonstrated for diverse ectotherms ranging from insects to anurans and fish (Anderson et al. 2001, Taylor and Collie 2003, Culler et al. 2015, Chen et al. 2019).

Effects of life-history transitions on resource biomass and community structure

Damselflies more effectively suppressed resource biomass with warming, because their rate of resource ingestion increased with temperature through their attack rates and handling time (Table 10 in Appendix), and their population size increased as they transitioned to shorter generation times (Fig. 10 in Appendix). Therefore, our standard model predicted a top-heavy community relative to our alternative models restricting changes in consumer life-history. Our prediction of increasing consumer to resource population size is consistent with experiments (Yvon-Durocher et al. 2011, Shurin et al. 2012) and the unstructured population model of Vasseur and McCann (2005), assuming that consumer biomass gains from ingestion increase more rapidly with warming than losses from respiration, and that prey carrying capacity does not vary with temperature (also equivalent to ‘scenario a’ of Uszko et al. 2017, their Fig. 4). Vasseur and McCann (2005) predicted that this scenario would increase consumer persistence and destabilize community dynamics. Our model does not predict destabilization of community dynamics because damselflies did not overexploit their prey. Two mechanisms likely explain this difference. First, when damselflies emerged from ponds as adults, the zooplankton biomass recovered to carrying capacity as adult damselflies switched to consuming terrestrial resources (Fig. 9 in Appendix). Second, interference mortality prevented the damselfly population from becoming too large and depleting its resources (see also Crowley et al. 1987). Both mechanisms should help prevent predator-prey cycles resulting from over-exploitation of resources (Hassell and May 1973, Oaten and Murdoch 1975). Therefore, incorporating life cycle complexity, temperature seasonality, and other components of biological realism predicts alternative outcomes for consumer-resource dynamics under warming compared with simpler, unstructured models.

Model assumptions

We included simplifying assumptions in our model that may have affected the outcome of increasing temperature on population dynamics. Notably, we did not include temperature dependence of the resource intrinsic rate of increase or carrying capacity, and we modeled increases in resource ingestion rates using the Arrhenius equation, even though it is now recognized that unimodal temperature functions are more appropriate for describing changes in the attack rate and maximum ingestion rate over a large temperature range (Englund et al. 2011, Uszko et al. 2017). We used the Arrhenius equation because it provided a better fit to our experimental data than a unimodal function for temperatures at or below 35 °C (Twardochleb et al. 2020), and we restricted model simulations to $T_{av} \leq 30$ °C. This already represents an increase of 17 °C above the current average in southwestern Michigan ponds, well beyond the projected increase of 3 to 5 °C by 2100 for the high emissions scenario RCP 8.5 (IPCC 2014). Therefore, the Arrhenius equation is appropriate for modeling population responses to warming at or beyond temperatures that are realistic for our study system. Inclusion of a unimodal temperature function for resource attack and handling rates could reduce the consumer population size or cause extinction at high temperatures (Uszko et al. 2017). Temperature dependence of the zooplankton intrinsic rate of increase or carrying capacity would be unlikely to change the qualitative outcomes of our simulations, because damselflies never depleted their resources enough to induce starvation mortality. Simulations in which we varied the value of K from 0 to 100 at different temperatures showed that it had no qualitative effect on consumer-resource dynamics.

We also made simplifying assumptions about damselfly life-history to more effectively demonstrate temperature effects on populations. For example, we omitted the effects of photoperiod when defining the growing season and emergence window, despite its importance for regulating the damselfly life cycle (Ingram 1975, Ingram and Jenner 1976). We also assumed a linear increase in biological rates with body size, although the effects of temperature can vary considerably with size and life stage (Kingsolver et al. 2011). To update our model, more research is needed to quantify biological rates across a range of temperatures and damselfly body sizes. Despite these simplifying assumptions, our standard model at the reference temperature is faithful to the dynamics of damselfly populations in southwestern Michigan ponds (Figs. 6, 9 in Appendix). In addition, our model captures many of the same dynamics in the more complex damselfly population model of Crowley et al. (1987), including that damselflies fall into a stable cycle of yearly emergence within a life-history strategy and the average population size is smaller at lower temperatures (Figs. 9, 10 in Appendix). Their model was not designed to simulate climate change effects on populations, and it also differs in its depiction of transitions between life-history strategies (i.e., between one emergence every other year and one every year) by capturing “cohort-splitting”, or sub-populations that emerge in different years (Corbet 1999). Although our model cannot demonstrate the effects of temperature in producing sub-populations, it effectively captures the qualitative changes in life-history already observed in dragonflies and damselflies (Corbet et al. 2006, Braune et al. 2008, McCauley et al. 2018a) and other ectotherms in response to climate change or latitude (Roy and Sparks 2000, Musolin 2007, Hassall et al. 2007, Matsuda et al. 2018).

Conclusions

We show that changes in consumer life-history can alter the predicted outcomes of warming on populations compared to unstructured models parameterized for constant-temperature environments. Uncovering this role of life-history in mediating organism responses to warming was only possible when we incorporated life cycle complexity and temperature seasonality in our size-structured consumer-resource model. We further show that warming can induce changes in ectotherm generation times by increasing rates of resource ingestion and growth or lengthening the growing season. Moreover, life-history changes can prevent consumer population declines with warming by shortening the duration of the juvenile stage and “the window of vulnerability” to mortality, increasing the number of emerging adults and population-level reproduction. In contrast to unstructured models with similar assumptions, we find increased stability of consumer-resource populations with increased predator persistence. Our study reveals mechanisms by which climate change and latitude can affect ectotherm life-histories and populations. Overall, our study suggests that life-history transitions to shorter generation times could delay population extinctions with warming. Life cycle complexity and temperature seasonality should therefore be incorporated into research investigating the impacts of climate warming on consumer-resource populations in seasonal environments.

APPENDIX

Table 10. Model variables and default parameter values. *Average fecundity is 618 eggs female damselfly^{-1} or 309 damselfly^{-1}. †Selected such that modeled dynamics match population dynamics observed in field measurement. ‡Measurements from our experiments closely match those for *Enallagma* from (Culler et al. 2014).

Parameter	Value	Unit	Interpretation	Source
r	1	day^{-1}	Resource intrinsic rate of increase	
K	3	mg L^{-1}	Resource carrying capacity	Field measurements
a	1.8756	L day mg damselfly^{-1}	Mass-specific consumer attack rate	Twardochleb et al. 2020
h	3.75	days mg damselfly mg Daphnia^{-1}	Consumer handling time	†
μ	0.19	mg mg day^{1}	Consumer respiration rate	Twardochleb et al. 2020 ‡
e_c	0.85	unitless proportion	Consumer conversion efficiency	Culler et al. 2014, Lang et al. 2017
d	0.006	day^{-1} damselfly^{-1}	Per capita consumer background mortality	McPeek and Peckarsky 1998
$\propto$	0.01	day^{-1} damselfly^{-1}	Per capita interference mortality coefficient	Anholt 1990
S_{min}	0.01	mg	Initial consumer mass	Field measurements
S_{max}	4.5	mg	Maximum juvenile consumer mass	Field measurements
f	309	eggs damselfly^{-1}	Total per capita consumer fecundity	Bots et al. 2010*
Ea	0.55	eV (electron volts)	Activation energy of attack rate	Twardochleb et al. 2020
Eh	-0.37	eV (electron volts)	Activation energy of handling time	Twardochleb et al. 2020
$E\mu$	0.36	eV (electron volts)	Activation energy of respiration	Twardochleb et al. 2020‡
Ed	0.57	eV (electron volts)	Activation energy of background mortality	McCoy and Bolker 2008
T_{av}	13	ºC	Average yearly temperature	Field measurements
T_{amp}	13	ºC	Amplitude of yearly temperature	Field measurements
ϕ	365/4	days	Phase shift of temperature function	Field measurements

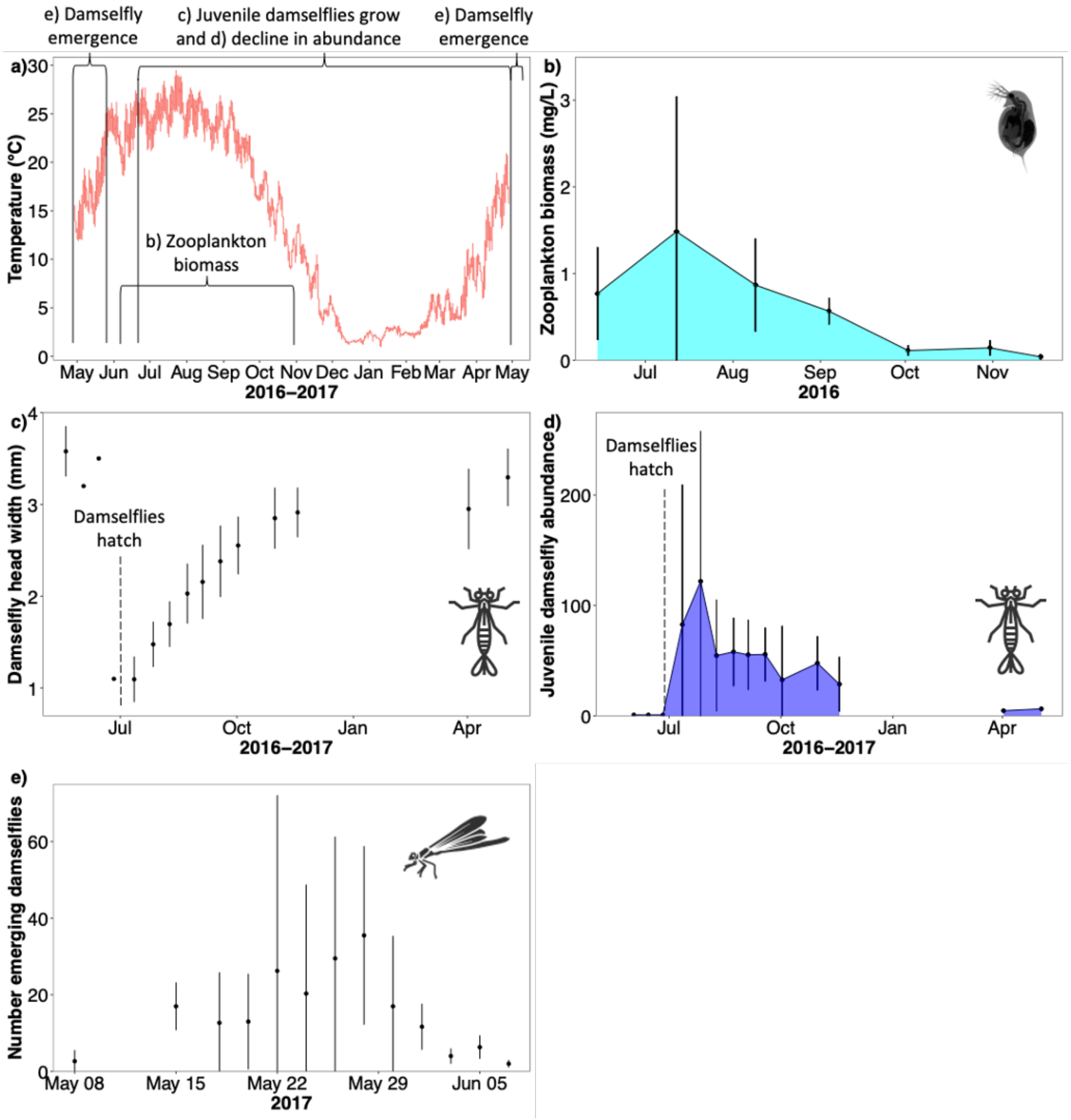

Figure 6. Average seasonal dynamics in three ponds. a) temperature from May 2016 to May 2017 overlayed with vertical lines indicating the time period for consumer-resource population dynamics shown in panels b-e); b) zooplankton biomass during the 2016 growing season (pond temperature > 10 ºC); c) juvenile damselfly head capsule width (a proxy for body size) increased after juvenile damselflies hatched in July 2016 and reached its maximum in April and May 2017

Figure 6. (cont'd) just before damselflies emerged to the adult stage; d) juvenile damselfly abundance was highest in the summer of 2016 after juvenile damselflies hatched and declined throughout the growing season. Abundance was at a minimum in May-June 2017 when damselflies emerged from the pond to the adult stage; e) damselfly emergence to the adult stage occurred throughout May 2017. In b), c), and d) no samples were taken December 2016 through March 2017. Zooplankton icon courtesy of Integration and Application Network.

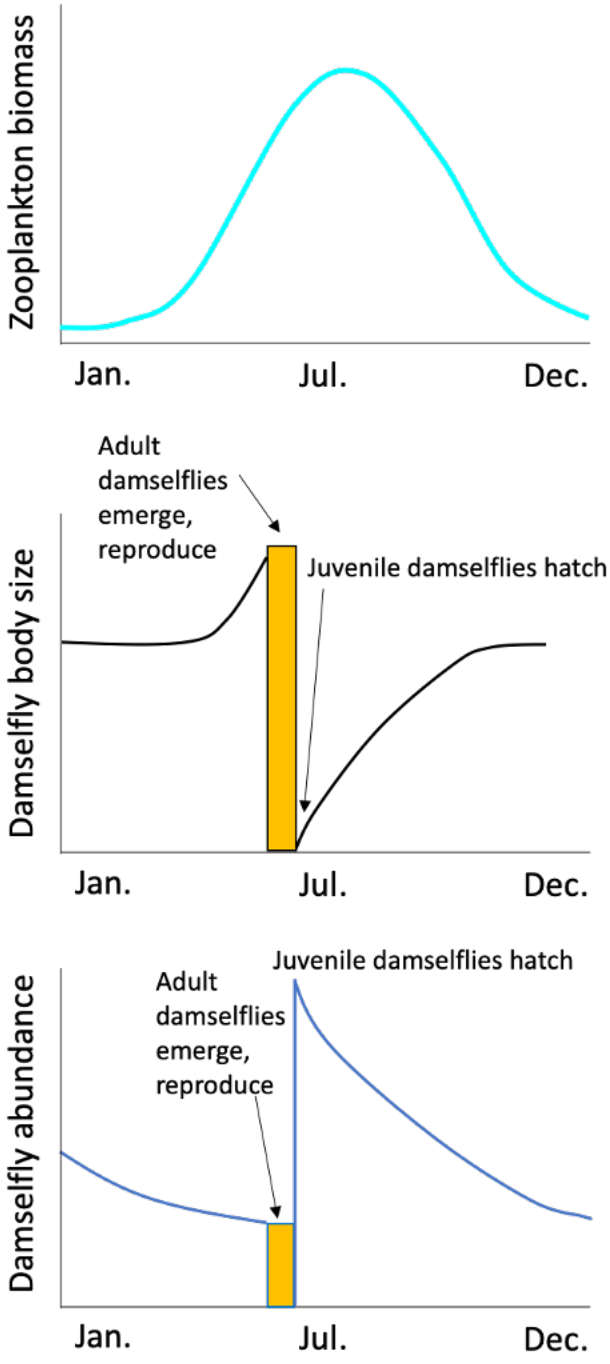

Figure 7. Idealized depiction of consumer-resource population dynamics in Michigan ponds throughout a year. Here damselflies have one generation per year. Top panel: zooplankton biomass varies seasonally and peaks in mid-July. Middle panel: damselfly body size

Figure 7. (cont'd) is at its minimum after juvenile damselflies hatch in early summer. Juvenile damselflies grow in size (black lines) over the course of the year and overwinter in a state of reduced activity and growth. They resume growth the following spring and emerge to the adult stage. Adult damselflies spend ~30 days outside of ponds foraging and reproducing (orange bars depict adult size and duration outside of ponds). Bottom panel: juvenile damselfly abundance (blue lines) is high after they hatch in early summer and declines due to background and respiration mortality throughout the year. In spring of the following year, damselflies emerge to the adult stage (orange bars depict adult abundance and duration spent outside of the pond) and reproduce. Juvenile abundance is high in ponds after juvenile damselflies hatch.

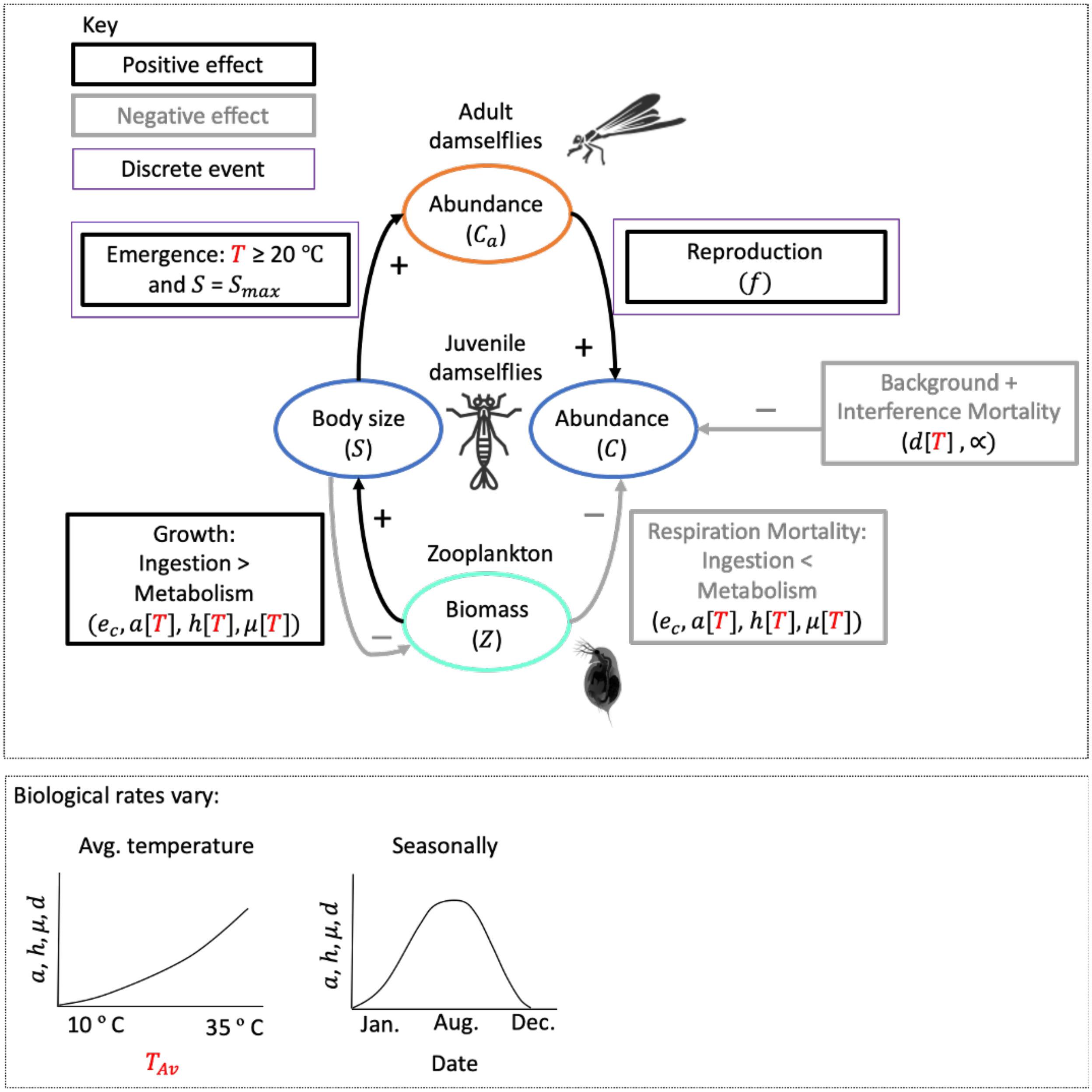

Figure 8. Conceptual diagram of the size-structured consumer-resource model. Top panel: within a growing season, juvenile damselflies grow in size when their ingestion of zooplankton exceeds metabolism, which reduces zooplankton biomass. Juvenile damselfly abundance is reduced through background and interference mortality, and also through respiration mortality when metabolism exceeds ingestion. Damselflies emerge to the adult stage when their body size is equal to S_{max} and temperature exceeds 20 ºC, which is represented by a discrete event within

Figure 8. (cont'd) otherwise continuous dynamics. In a second discrete event, reproduction by adult damselflies produces the following cohort of damselflies. State variables are shown in ovals, biological events affecting state variables are enclosed in rectangles, and biological rates affecting each event are in parentheses. Temperature-dependent parameters are shown with a bracketed *T*. Bottom panel: biological rates vary in our model with both average temperature (Algorithm 7) and seasonal changes in temperature (Algorithm 22). Zooplankton icon courtesy of Integration and Application Network.

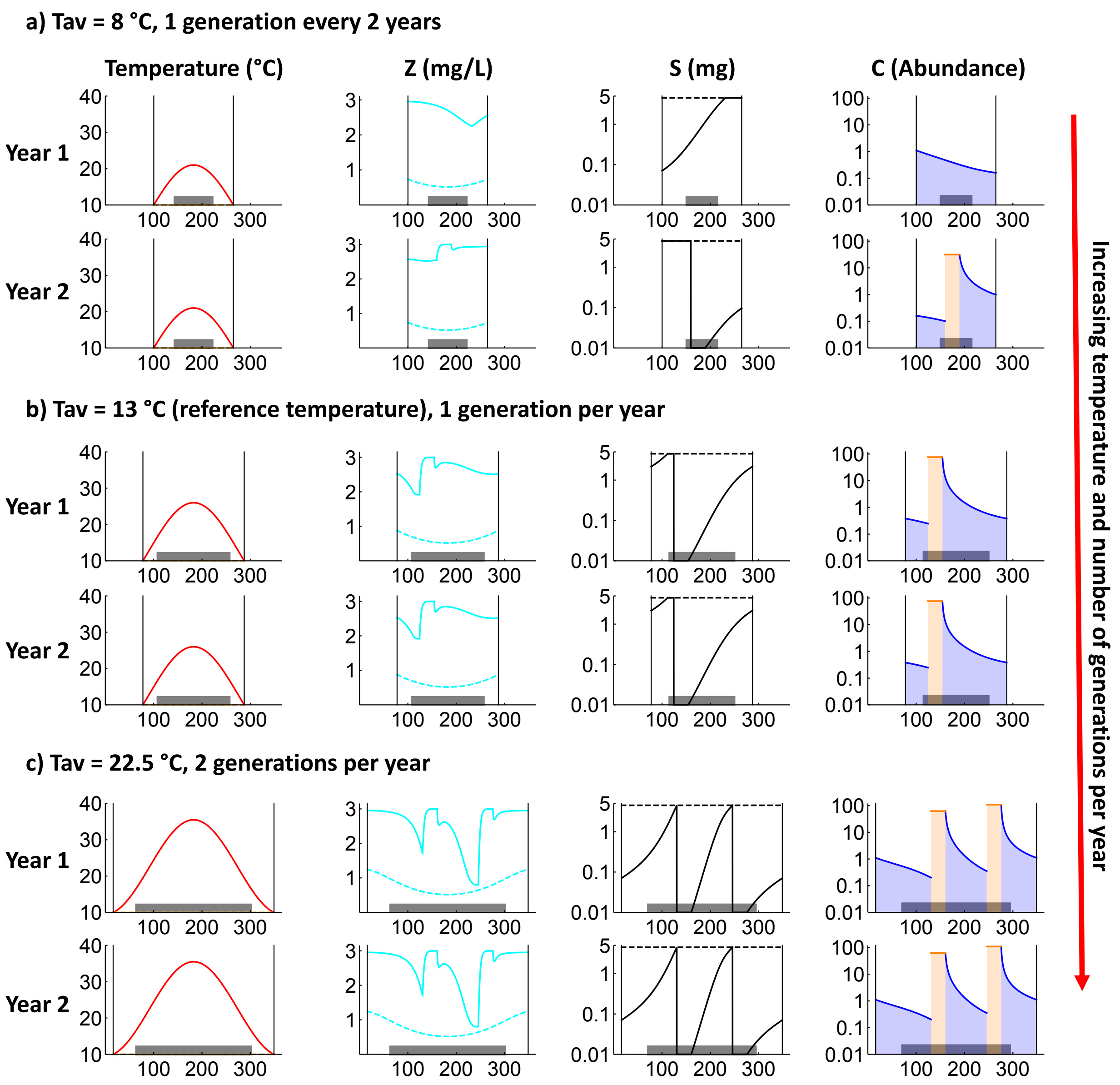

Figure 9. Modeled within season dynamics. Modeled for a) average temperature of 8 ºC, damselflies have one generation every other year; b) 13 ºC (reference temperature), one generation per year; and c) 22.5 ºC, two generations per year. From left to right: solid lines represent temperature, zooplankton biomass, damselfly body size, and damselfly abundance during one growing season. Broken lines on plots of zooplankton biomass indicate the break-even zooplankton biomass required for damselflies to avoid starvation mortality. Broken lines on plots of damselfly body size indicate S_{max}, the threshold size for emergence to the adult stage.

Figure 9. (cont'd) Blue fill on plots of damselfly abundance give the juvenile abundance, and orange bars are the number of emerging adults multiplied by their fecundity. This gives the number of new damselflies produced during the emergence window. Black vertical lines on plots indicate the duration of the growing season (temperature ≥ 20 ºC) and gray bars at the bottom of plots indicate the width of the emergence window (temperature ≥ 10 ºC).

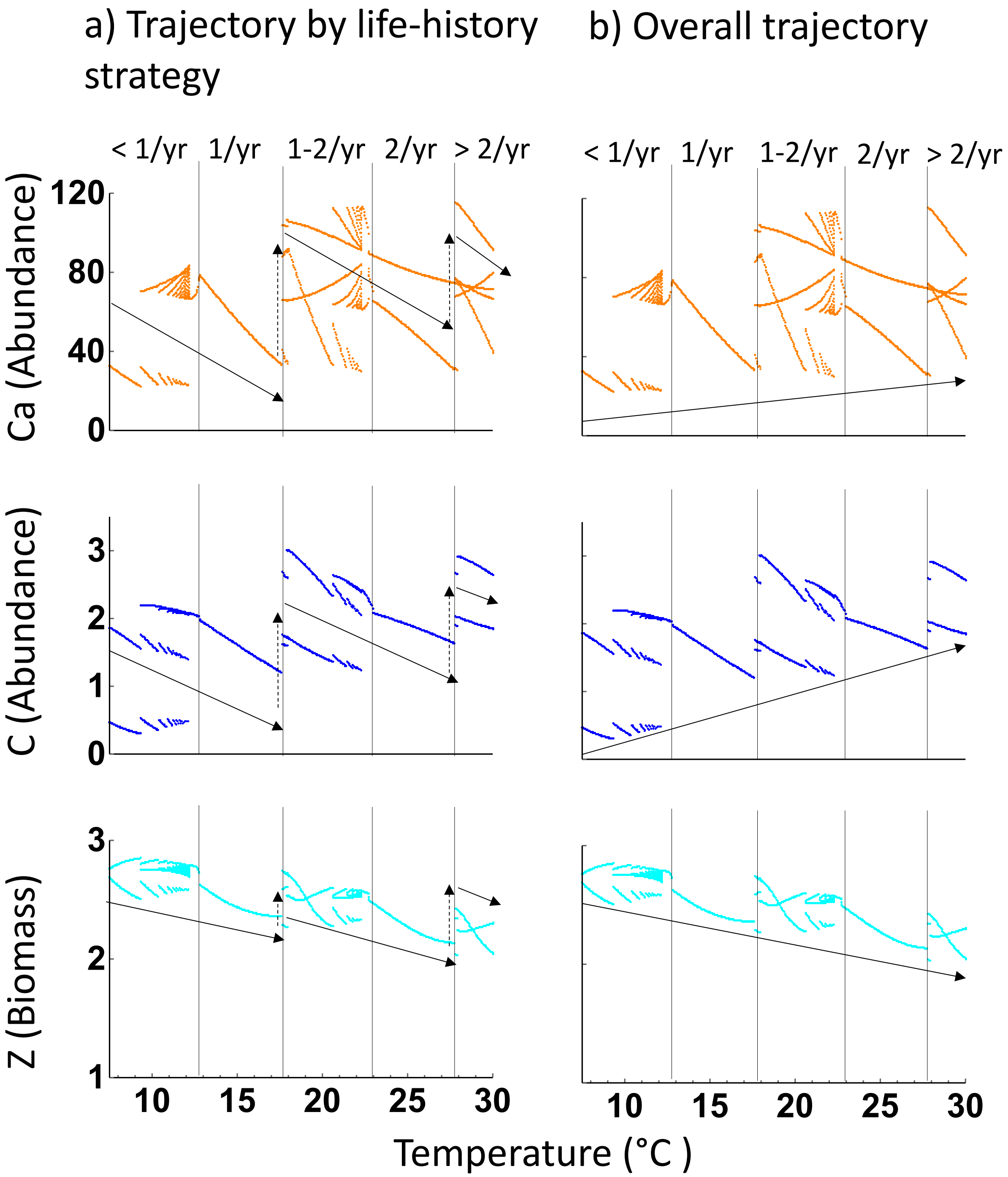

Figure 10. Bifurcation diagrams demonstrating effects of temperature in our standard model. Yearly average zooplankton biomass (bottom panel), juvenile damselfly abundance (middle panel), and the number of emerging adult damselflies multiplied by their fecundity,

Figure 10. (cont'd) giving the number of new damselflies produced during the emergence window (top panel). Vertical lines and annotations at the top of each panel divide the diagram according to the damselflies' qualitative life-history dynamics. From left to right, as temperature increases damselflies transition from fewer than one generation per year to more than two generations per year. Solid arrows in panel a) show population trajectories within a damselfly life-history strategy (e.g. one or fewer generations per year). Broken arrows track abrupt shifts in population size at major life-history transitions (e.g. when damselflies transition from one to more generations per year). Solid arrows in panel b) demonstrate overall population trajectories across the temperature range.

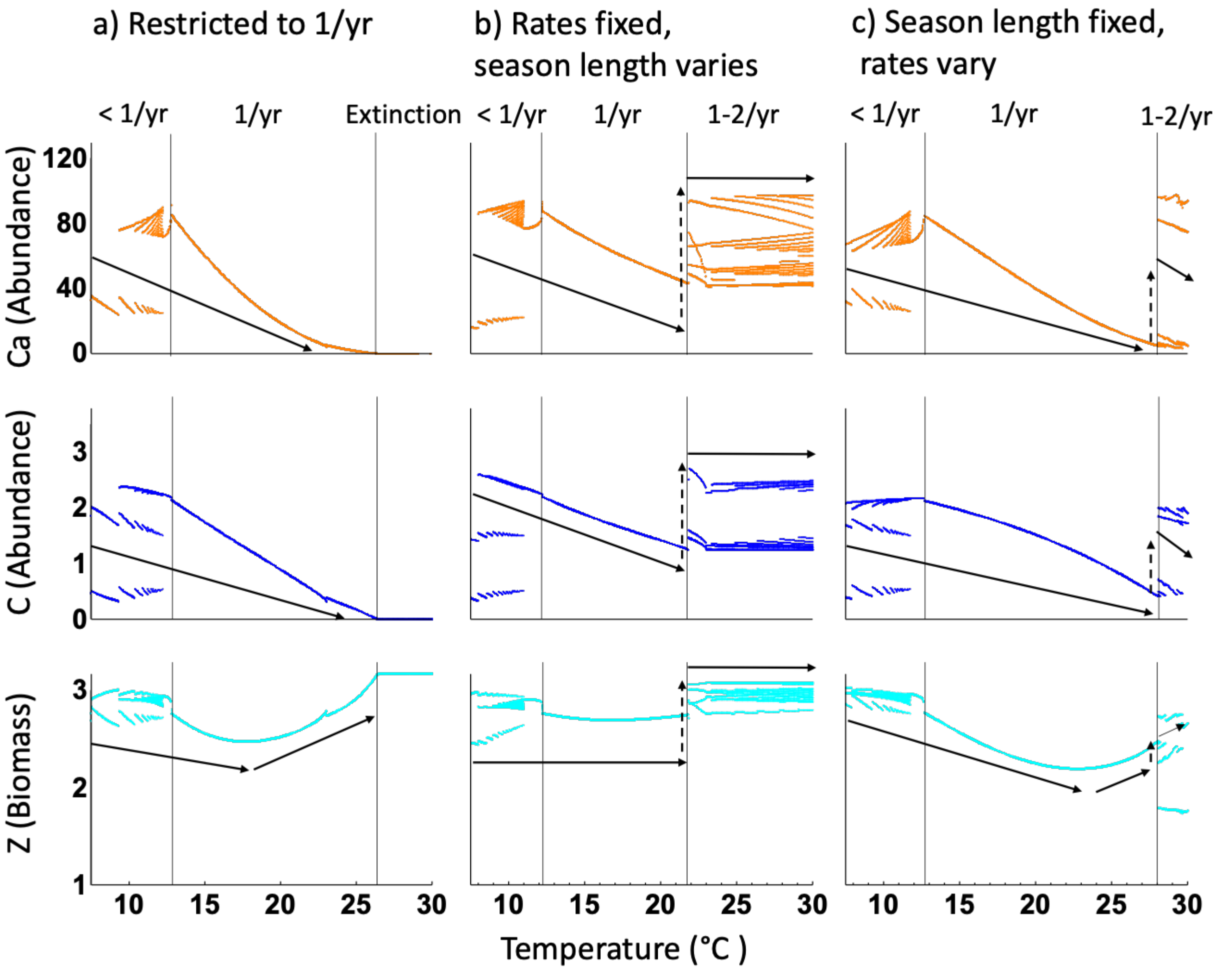

Figure 11. Bifurcation diagrams demonstrating effects of temperature in our alternative models. a) alternative model 1, b) alternative model 2, c) alternative model 3. Panels and annotations are as in Figure 10. From left to right in each panel, as temperature increases damselflies transition from fewer than one generation per year to more than one generation per year, except in a) damselflies go extinct at high temperatures. Solid arrows show population trajectories within a damselfly life-history strategy (e.g. one or fewer generations per year). Broken arrows track abrupt shifts in population size when damselflies transition from one to more generations per year.

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CHAPTER 3. Freshwater insect occurrences and traits for the contiguous United States

†The following co-authors contributed to this chapter: Laura Twardochleb, Ethan Hiltner, Matthew Pyne, and Phoebe Zarnetske

††Upon publication of this chapter, the data package referenced herein will be openly accessible through Environmental Data Initiative with the following citation:

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Abstract

Freshwater insects are essential indicators of ecosystem health. They comprise 60% of freshwater animal diversity, are widely used to assess water quality, and provide prey for numerous freshwater and terrestrial taxa. Our knowledge of the distribution of freshwater insect diversity in the United States is incomplete because we lack comprehensive, standardized data on their distributions and traits at the scale of the contiguous US (CONUS). We fill this knowledge gap by presenting a database of 2.05 million occurrence records for 932 genera in the major freshwater insect orders, at 51,044 stream locations sampled between 2001 and 2018 by federal and state biological monitoring programs. We tripled the number of occurrence records and locations, and added records for 118 genera, over existing open-access databases. We also present life history, dispersal, morphology, and ecology traits and trait affinities (analogous to fuzzy-coded traits) for 1,007 stream insect genera, assembled from existing databases, reference books, and the primary literature. We nearly doubled the number of traits for 11 trait groups and added traits for 180 genera that were not available from open-access databases. Our database,

Freshwater insect occurrences and traits for the contiguous United States (Freshwater insects CONUS), facilitates mapping freshwater insect taxonomic and functional diversity, and when paired with environmental data, will provide a powerful resource for quantifying how the environment shapes stream insect diversity and taxon-specific distributions.

Introduction

Understanding the distribution of biological diversity at continental scales is a key goal of biogeography, community ecology, and conservation research (Ricklefs et al. 1993, Wiens and Donoghue 2004, Pereira et al. 2013). Species occurrence records and functional traits are needed to quantify and map taxonomic and functional diversity for monitoring and assessing environmental influences on populations, ecological communities, and ecosystem functioning (Pereira et al. 2013, Jetz et al. 2019). Taxon-specific distribution data are also essential for predicting geographic ranges and species responses to global change, important facets of conservation planning (Rodríguez et al. 2007, Serra-Diaz and Franklin 2019). Ecologists have made progress toward assembling taxonomic occurrence and trait datasets that enable mapping broad-scale biodiversity patterns of terrestrial organisms (e.g., Belmaker and Jetz 2011, Butler et al. 2017), marine organisms (Grady et al. 2019), and freshwater fish (Comte and Olden 2017). Despite this progress, open-access biodiversity datasets for freshwater insects, such as the USEPA Freshwater Biological Traits database (USEPA database, U.S. EPA 2012, Poff et al. 2006, Vieira et al. 2006), and the Water Quality Portal (WQP, waterqualitydata.us) are not easily combined for biodiversity mapping, because they contain outdated taxonomic names and trait terminology, and have gaps in trait assignment for many taxa. In addition, they do not provide fuzzy traits commonly used by researchers in European and other regions that would facilitate cross-continental comparisons and assembly of global trait databases (Schmera et al. 2015). Below we briefly describe the history, uses, and limitations of existing databases, and the need for integrated, comprehensive, and standardized trait and occurrence datasets for mapping taxonomic and functional diversity and taxon-specific distributions of freshwater insects in the contiguous US.

Freshwater insects are indicators of ecosystem health, and changes to their biodiversity can signal wider shifts in biodiversity of other taxonomic groups and ecosystem functioning (Covich et al. 1999, Bonada et al. 2006, Suter and Cormier 2015). Despite the importance of freshwater insects in both aquatic and terrestrial realms (Covich et al. 1999, Baxter et al. 2005), there are significant gaps in our knowledge of their biodiversity patterns (Balian et al. 2008). Without data on their occurrences and traits, it is difficult to map distributions of freshwater insect taxonomic and trait diversity, especially at broad scales (Balian et al. 2008 Troia and McManamay 2016). It is critical that we fill these knowledge gaps because freshwater insects are used to assess water quality (Barbour et al. 2000, Bonada et al. 2006) and provide prey for numerous taxa, including freshwater fish, riparian birds, bats, and lizards (Baxter et al. 2005).

Systematic surveys of ecological communities provide some of the highest quality occurrence data for assessing biodiversity, but few of these datasets have been integrated over large spatial scales, especially for freshwater insects (Troia and McManamay 2016, Jetz et al. 2019). Incidence data and range maps are also limiting for freshwater insects. For example, insect occurrence records from the Global Biodiversity Information Facility (GBIF), derived primarily from museum collections, are sparse (Troia and McManamay 2016), and expert range maps from the International Union for the Conservation of Nature (IUCN) are only available for one of the nine major freshwater insect orders, damselflies and dragonflies (Odonata), (IUCN, 2020). Because ecologists still lack a dataset of systematically surveyed, freshwater insect occurrence records covering the major freshwater insect orders and spanning the contiguous US, previous studies have mapped stream insect diversity only for a subset of insect orders (e.g., Ephemeroptera, Plecoptera, Trichoptera, Vinson and Hawkins 2003, Shah et al. 2014) or for regions of the US (Poff et al. 2010, Pyne and Poff 2017). One of our goals is to integrate systematically surveyed community data for freshwater insects into occurrence datasets for biodiversity mapping.

Environmental agencies in the United States and in countries throughout the world use macroinvertebrates in bioassessment of stream condition in compliance with mandates to protect the ecological integrity of surface waters (Barbour et al. 2000, Bonada et al. 2006). In the US, local, tribal, state, and federal agencies have monitored macroinvertebrate community composition at georeferenced stream locations since the passage of the Clean Water Act in 1972 (Barbour et al. 2000). These systematic community surveys provide a rich source of information about stream insect occurrences. Some of these data are already publicly available online through the Water Quality Portal (WQP, waterqualitydata.us), including data from the US Geological Survey (USGS) National Water Quality Assessment and the US Environmental Protection Agency (USEPA) National Aquatic Resource Surveys. However, additional monitoring data from state agencies have yet to be integrated and released as open-access datasets. A database is needed that collates and standardizes the biological monitoring data from these disparate sources and integrates it with trait databases using consistent and updated trait terminology (Schmera et al. 2015) and up-to-date taxonomy. It is important to standardize and integrate traits with freshwater insect occurrence records, because trait distributions are needed to assess biodiversity patterns and monitor the ecological integrity of surface waters (Statzner and Bêche 2010, Schmera et al. 2017). An integrated database of occurrence records and functional traits will facilitate mapping of stream insect diversity in the US.

There is a long history in stream ecology of using functional traits of stream macroinvertebrates to measure aquatic community and ecosystem responses to environmental stressors (Dolédec et al. 1999, Statzner and Bêche 2010). The composition of insect traits, such as body size, functional feeding group, and morphology, is influenced both by instream habitat measures, including velocity and timing of stream flow (the habitat template, Townsend and Hildrew 1994), and landscape filters, including climate and human activity (Poff 1997). Therefore, trait composition of stream insect communities is often used to infer impacts of human disturbance (Bonada et al. 2006), and traits are widely incorporated into indicator analyses by state and federal agencies for assessing stream condition (e.g., Stoddard et al. 2008, Mazor et al. 2016). Previous efforts to standardize and document traits of stream insects for the US have resulted in a widely used, publicly available dataset, the USEPA Freshwater Biological Traits database (U.S. EPA 2012). The initial data for the USEPA database were compiled for USGS by Vieira et al. (2006) and subsequently reclassified by Poff et al. (2006) to reflect functional trait niches of lotic insects. However, there remain significant gaps in trait coverage. Many insect taxa were never assigned traits, and many more have assignments for just a single trait, such as body size. The USEPA database also contains limited data on trait variation within genera, either by species, literature source, or geographic region, and the database does not summarize this variation using fuzzy trait assignments commonly used by researchers in Europe and other regions (Schmera et al. 2015). Moreover, the trait assignments are not consistent with a recently proposed unified terminology for traits of stream organisms (Schmera et al. 2015). Therefore, the US traits are not compatible with those used in Europe and other regions.

Expanding on the USEPA traits database by increasing the number of trait assignments, standardizing taxonomy and trait terminology, and providing trait variation in the form of fuzzy traits would facilitate continental and global mapping of stream insect trait composition and functional diversity.

We present a database, *Freshwater insect occurrences and traits for the contiguous US (Freshwater insects CONUS)*, for genera from the major freshwater insect orders: Coleoptera, Diptera, Ephemeroptera, Hemiptera, Lepidoptera, Megaloptera, Neuroptera, Odonata, Plecoptera, and Trichoptera. Our occurrence dataset contains over 2.05 million occurrence records for 932 genera sampled from 51,044 stream locations between 2001 and 2018. Our trait dataset includes dispersal, ecology, life-history, and morphology traits (Table 11 in Appendix) assigned at the genus level for 1,007 freshwater insect genera, including the 932 genera in our occurrence dataset. Our occurrence records are primarily from wadable streams, and our trait dataset is primarily for stream insects, although some occurrence records are from larger rivers, and some insects assigned traits also occur in ponds, lakes, or rivers. We build upon the foundational occurrence and trait databases described above by integrating occurrence records from state agencies that were not accessible online and providing updated, standardized taxonomy and trait terminology. We also greatly expanded the number of insect genera with trait assignments and provided fuzzy traits to facilitate integration and comparison with trait databases in other regions of the world. Together, these datasets enable mapping both taxonomic and functional diversity of stream insects, as well as distributions of individual insect genera and traits.

Methods

We implemented five sequential steps of compiling data sources, digitalizing data, data cleaning, taxonomic harmonization, and trait assignment (Fig. 12 in Appendix). We detail these steps below.

Data sources

We compiled our freshwater insect occurrence dataset by downloading records from the WQP in February 2017 as follows. We selected “All” for Location, Site, and Sampling parameters. We selected “Invertebrates” and “Benthic macroinvertebrates” for the Assemblage and “All” for the Taxonomic Name under Biological Sampling parameters. This resulted in a dataset of 2,738,480 records for macroinvertebrate taxa identified to order, family, genus, or species from 66,356 sampling locations, before data cleaning and taxonomic harmonization. To fill spatial gaps in occurrence records, we requested biomonitoring data from 30 state agencies, and downloaded or received records from 19 agencies. This added 6,067,204 records from 55,791 locations, some of which were duplicates of the WQP data.

We began assembling the freshwater insect trait dataset by downloading records from the USEPA database in September 2017. The USEPA database contains trait information from 967 publications and government reports spanning 2005 to 2017, but primary data sources are Vieira et al. (2006) and Poff et al. (2006). The database includes habitat, life history, mobility, morphology and ecology trait data for 1,343 North American macroinvertebrate genera, including freshwater insects, mollusks, and arachnids. We subset the USEPA database to include only insect taxa, which resulted in a dataset of traits for 908 insect genera before harmonizing genus names with the latest taxonomic designations. We cross-referenced genera between the USEPA database and our occurrence dataset to search for taxa without trait assignments, identified the needed traits for those taxa, and filled the gaps in trait data through systematic literature review (outlined below). We also added trait data for taxa already in the USEPA database that were missing assignments for some traits.

We began by merging unpublished trait data compiled in 2014 by M. I. Pyne for a California project on stream hydrology (Mazor et al. 2016, Stein et al. 2017). This dataset focused on macroinvertebrates found in California streams that were not represented in the Poff et al. (2006) trait database and included trait assignments for 73 insect genera not in the USEPA database. The 2014 trait data were compiled using a systematic search of 1) the Vieira et al. (2006) and USEPA trait databases, 2) freshwater entomology books and taxonomic identification manuals, and 3) peer-reviewed articles of each taxon (mostly at genus level) that contained life-history information (Usinger 1963, Edmunds et al. 1976, Menke 1979, Brigham et al. 1982, McAlpine and Wood 1989, Hilsenhoff 1995, Westfall and May 1996, Wiggins 1996, McCafferty 1998, Needham et al. 2000, Smith 2001, Stewart and Stark 2002, Ward et al. 2002, Epler 2006, Merritt et al. 2008, Thorp and Covich 2009, Epler 2010, Tachet et al. 2010). If there were remaining gaps in trait information, an expert taxonomist was consulted to fill in the gaps (Boris Kondratieff, personal communication).

After merging the unpublished trait data, we conducted an initial search of the freshwater insect trait literature in the contiguous US. We began by searching freshwater entomology books and published and online taxonomic identification manuals. We then followed established guidelines for conducting a systematic search of the primary literature (Pullin and Stewart 2006). We searched Web of Science, Google Scholar, and the Michigan State Library Catalog to identify peer-reviewed papers containing information on the ecology of freshwater insects. This search was conducted from September 2017 to December 2018 and referenced papers from 1911 to 2018. We used the following search terms: genus AND Emergence synchron* OR emergence season* OR feed mode* OR dispersal* OR flight strength OR flying strength OR voltinism OR thermal preference OR rheophil* OR respir* OR body size OR habit OR larvae OR gill OR tegument OR plastron OR depositional OR erosional. We retained sources published in English with one or more of these search terms in the abstract, title, or key words, and that contained dispersal, ecology, life history, or morphology information for freshwater insects of North America. In addition to published trait sources, we used iNaturalist citizen science data (iNaturalist 2018) to assign emergence season. These data consist of time-stamped occurrence records submitted by commercial and recreational fisherman since May 2013 in order to track emergence dates of freshwater insects across North America. Sources for trait data are provided in the final data tables (Fig. 13 in Appendix).

Data digitalization

We digitized details about sampling methodology that were absent from state datasets. We requested the geodetic datum of horizontal coordinates for sampling locations through E-mails with agencies. We also recorded the sampling equipment and area of stream bottom sampled by requesting methodology directly from agencies or by digitizing information in state field sampling manuals. These details could potentially be used to estimate sampling effort across sites. Yet, many agencies did not record their sampling methods for some samples, and thus gaps remain in the documentation of sampling methodology.

When digitizing trait information, we focused on a subset of the traits originally documented in the USEPA database that should be influenced by environmental gradients of climate, land use, topography, and base flow that are important predictors of stream insect functional composition at broad spatial extents (Bonada et al. 2007, Díaz et al. 2007, Lawrence et al. 2010, Poff et al. 2010, Statzner and Bêche 2010, Pyne and Poff 2017). We organized traits following recommendations for a global, unified trait terminology for stream ecology (Schmera et al. 2015), by creating “trait groups” of closely related traits (e.g. “small”, “medium”, and “large” are traits of the trait group “maximum body size”), and summarizing related trait groups into “grouping features” of life history, dispersal, morphology, or ecology (Table 11 in Appendix).

When digitizing traits from entomology books and taxonomic guides we reviewed each source for all genera in our database with missing traits. When pulling information from the primary literature, we systematically searched for traits one genus at a time. Where possible, we also converted trait textual descriptions in the “comments” column of the USEPA database into trait assignments. We recorded traits at the genus or the species level using accepted trait definitions (Table 11 in Appendix). We documented trait variation within each genus by separating sources by row when compiling traits from multiple literature sources for a single genus. In addition, traits for the same genus from different geographical regions, and traits for different species within a genus were separated by row. Thus, each genus could have a different trait recorded for each row based on the species, region, and literature source. If, for any source (within a row) there were two or more possible traits from the same trait group (Table 11 in Appendix), we recorded the most commonly occurring trait documented by the source while also noting all other possible traits as “trait comments”. Although the traits recorded as “comments” did not influence final trait assignments, they are provided with the final datasets as additional natural history information. We summarized trait variation across rows within a genus (across species, regions, and literature sources) into trait affinity scores (analogous to fuzzy-coded traits; see *Assigning traits*, below).

One of the limitations of both the USEPA database and our database is that traits are not well defined by life-history stage for certain taxa. Most freshwater insects have an obligate aquatic larval stage transitioning to a terrestrial adult stage, and traits for these insects are assigned for the aquatic larval stage. However, many insects in the orders Coleoptera and Hemiptera are aquatic in both the larval and adult stages and have traits that differ by stage (Merritt et al. 2008). Most trait entries for these taxa in the USEPA database are for the adult stage. Similarly, we found during our systematic search of the trait literature that adult traits for Coleoptera and Hemiptera were more commonly available than larval traits. Therefore, there is a bias toward traits for adult stages of Coleoptera and Hemiptera in our database.

Data cleaning

During the first step of data cleaning, we removed duplicate occurrence records and those with missing coordinates. Next, we visually examined records for georeferencing errors by mapping all occurrence locations for each insect family and comparing maps of their distributions to GBIF range maps (GBIF 2020). This represents an independent assessment of range, as most GBIF records are from museum collections. In addition, we searched data providers and datasets in GBIF for the agencies that provided our occurrence records and found no records of data contributions to GBIF from those providers. We removed obvious geographical outliers (e.g., points in the ocean), corrected transposed latitude and longitude coordinates, and coordinates with an incorrect sign on the decimal degrees of latitude or longitude. We also mapped data by state to asses georeferencing errors (records falling outside state bounds). In total, we removed 5,325,297 duplicate records and 836,310 records that were missing sampling coordinates or contained georeferencing errors. We then removed an additional 211,627 records during taxonomic harmonization (see below) either because records were for non-insect taxa, or misspellings or other errors rendered the taxa unidentifiable. This resulted in a dataset of 2,432,450 occurrence records of insects identified to order, family, genus, or species, from 55,791 sampling locations. We performed data cleaning and taxonomic harmonization in R version 3.5.3 (R Core Team 2019). Scripts with R code for data cleaning are provided through GitHub and the Environmental Data Initiative (see *Data accessibility*, below).

Taxonomic harmonization

After data cleaning, we verified and harmonized taxonomic names between the occurrence and trait datasets using the 'taxize' package version 0.9.92 in R (Chamberlain et al. 2019). We used 'taxize' to search the database of the Integrated Taxonomic Information System (ITIS) to extract updated genus names, taxonomic serial numbers, and upstream names (Family, Order) for each taxon. Some names were not found in the ITIS database due to misspellings, missing data in ITIS (e.g. for recently identified taxa), or because names were invalid, and ITIS contained no valid synonyms. For these cases, we verified names by manually searching other online sources, including GBIF (GBIF 2020), IUCN (IUCN 2020), and the primary literature. We accepted names that were listed as valid US taxa by a majority of sources. Although we assigned an accepted name for those taxa, we could not assign an accepted taxonomic serial number from ITIS. In addition, some names could not be verified using any source. For those taxa, we assigned the valid upstream name (Family or Order) from ITIS. In total, we re-assigned names for 413 taxa in the trait dataset, including 58 changes to the genus name. In the occurrence dataset, we re-assigned 704 names, including 177 genus names, 96 of which were combined into 36 genera.

Assigning traits

We assigned traits at the genus level in two ways, as modal traits and trait affinities (Fig. 12 in Appendix). We assigned modal traits as the most frequently occurring trait in a trait group (Table 11 in Appendix) across all species, geographic regions, and literature sources (rows) for a genus. Affinity scores account for trait variation within a genus by species, geographic region, or literature source, and are analogous to fuzzy-coded traits used by researchers in Europe and other regions (Usseglio-Polatera et al. 2000, Schmera et al. 2015). Trait affinities differ from fuzzy-coded traits in that they are assigned as proportions, whereas fuzzy-coded traits are assigned using an ordinal scale (Usseglio-Polatera et al. 2000, Schmera et al., 2015). They were assigned by computing the proportion of rows for each genus that were assigned to each trait in a trait group, such that each row counted as a single trait contribution. Thus, each species, geographic location, and literature source for a genus contributed a single value toward the affinity score. Affinity scores sum to 1 across all traits in a trait group for each genus.

Results

Data organization and usage

The freshwater insects CONUS database is organized as nine relational data tables with associated metadata (Fig. 13, Table 12 in Appendix). Metadata accompanying the dataset includes information on project funding, contributors, geographic and temporal scope, variable names, descriptions, measurement scales, missing values, and trait codes. We provide our data tables as .csv files with metadata through Environmental Data Initiative (EDI). The R scripts that we used for data cleaning are also available through EDI and GitHub. We encourage submissions of occurrence and trait records for future updates to the database. A template and instructions for data submission is available at GitHub. See *Data accessibility*, below, for links to the EDI and GitHub repositories.

Here, we describe a few of the many uses for our database. In its "raw" form users can extract trait data for insects identified to order, family, genus, or species by merging the Raw_Traits table with the Ancillary_Trait and Ancillary_Taxonomy tables (Fig. 13 in Appendix). We recorded trait variation in Raw_Traits, with each row for a genus presenting trait data for a different species, location, or literature source. Users can thus extract traits by state ("Study_location_state") or literature source ("Study_citation"), or summarize trait variation within a genus, family, or order when the Raw_Traits table is merged with the Ancillary_Taxonomy table (through the "Submitted_name" column). In addition, users can merge the Raw_Community_Data and Ancillary_Taxonomy tables (Fig. 13 in Appendix) to find occurrence records for insect species and map their distributions across the US (as in Fig. 14 for insect genera, in Appendix). Searching columns in the Raw_Community_Data table enables users to extract and map insect records for each state ("Study_state" column), monitoring organization ("Monitoring_organization" column), or type of water body ("Location_description" column). Moreover, merging the Raw_Community_Data and Ancillary_Sample_Method tables will enable users to isolate records that were sampled using a particular equipment or protocol, such as a Hess sampler, D-frame aquatic dipnet, or Hester-Dendy sampler by searching the "Sample_method" column.

In the "cleaned" form of the database, the Genus_Occurrences table enables users to map genus richness (Fig. 14a in Appendix) and the distributions of individual insect genera. In addition, when merged with the Ancillary_Taxonomy table, records in the Genus_Occurrences table enable mapping distributions of genus richness and individual insect genera by family or order (Fig. 14b in Appendix). A great strength of the “cleaned” data tables comes from merging the Genus_Occurrences table with the Genus_Traits or Genus_Trait_Affinities table using the “Genus” columns. This enables users to examine the spatial distributions of insect traits and trait affinities by genus (Fig. 15 in Appendix), and family or order when also combined with the Ancillary_Taxonomy table. More nuanced mapping of trait distributions is also possible. For example, users could map trait distributions for a particular state, monitoring organization, water body type, or sampling methodology, as described above for the “raw” data tables.

Biodiversity patterns in data

We mapped insect genus richness by location using data in Genus_Occurrences (Fig. 14 in Appendix). By merging Genus_Occurrences and Genus_Traits, we also mapped distributions of freshwater insect functional traits for the contiguous US (Fig. 15 in Appendix). These maps reveal some obvious sampling biases (see *Bias in occurrence and trait records*, below) and interesting patterns in the distributions of functional traits. For example, insect genera with bivoltine or multivoltine life cycles, corresponding to short generation times, and genera that prefer warm eurythermal habitats are concentrated in warm, low-lying regions, including southern California and Florida (Fig. 15 in Appendix). We see opposite patterns for some rheophily and respiration traits, where gilled insects and those preferring erosional habitats are concentrated in mountain regions of the Western and Northeastern US. Previous studies suggest that gilled insects and those with adaptations to life in erosional habitats should be found in cool, well-oxygenated, and fast-flowing waters, such as are found in high elevation streams (Poff et al. 2010, Statzner and Bêche, 2010). These hypotheses could be tested definitively by combining our database with environmental data.

Bias in occurrence and trait records

Our maps of genus richness (Fig. 14 in Appendix) clearly illustrate spatial bias in occurrence records. These biases are partly due to the fact that some state agencies have not digitized their biological monitoring data. Moreover, sampling effort, including the number of samples, and the area sampled, varied within and among datasets. These sources of bias resulted in sparse genus occurrence records in several states in the Midwest, mountain West, and Southeastern US (Fig. 14a in Appendix). There are also obvious gaps in occurrences and traits for the insect orders Hemiptera, Lepidoptera, Megaloptera, and Neuroptera (Fig. 14b, Table 13 in Appendix). Fewer aquatic insect genera reside within these orders compared to the obligate aquatic orders Ephemeroptera, Plecoptera, and Trichoptera, or the other well-represented aquatic orders Coleoptera and Diptera. Their relative rarity could have resulted in training biases in which aquatic ecologists and taxonomists are less likely to accurately identify uncommon taxa, or targeted sampling biases in which sampling methodology is designed to capture genera from common orders.

Another common source of bias originated when identifying specimens in the laboratory. Some state agencies identify all macroinvertebrate specimens to family, whereas others use inconsistent methodology by identifying some taxonomic groups (e.g., Dipterans) to family or order, and other groups to genus. We removed all records for insects identified to family when producing our Genus_Occurrences table, which effectively excluded whole state datasets. However, records for insects identified to family or order are still available in Raw_Community_Data.

Biases in occurrence records could be corrected by aggregating records using a larger spatial unit and then applying coverage-based rarefaction (Chao and Jost 2012) to down-weight the influence of well-sampled areas on spatial patterns of genus richness. For example, one could aggregate occurrence records by watershed (e.g., USGS hydrologic units), treating each occurrence location as a spatial replicate, and then compute the sample coverage in each watershed. One would then rarefy or interpolate genus richness for equal levels of sample coverage across watersheds. Coverage-based rarefaction can be performed with the ‘iNext” package in R (Hsieh et al. 2016). The R packages “biogeo” and “dismo” can also assist with assessing bias in occurrence records and modeling genus distributions (Hijmans et al. 2017, Robertson et al. 2016).

Trait coverage was most complete for the ecology trait groups feeding style, habit, and rheophily, and the morphology trait group maximum body size (Fig. 16a in Appendix). The trait groups with the fewest genera with assignments included the following life history and dispersal trait groups: synchronization of emergence, emergence season, female dispersal, and adult flying strength (Fig. 16a in Appendix). Approximately half of the insect genera in our database are still missing assignments for these four trait groups. In addition, there are gaps in coverage for all traits; no trait group contains a trait assignment for every genus in our database. These gaps highlight the need for more trait measurements of freshwater insects, especially insects in the orders Hemiptera, Lepidoptera, Megaloptera, and Neuroptera (Fig. 14b, Table 13 in Appendix). Moreover, there is bias toward adult stage traits for Coleoptera and Hemiptera (see *Data digitalization*, above), which indicates that more trait measurements are also needed for larval stages of insects in these orders. We expect that additional trait data is available in books and scientific articles that has yet to be digitized and standardized, and many research programs have trait datasets that are not published in any form. We encourage submission of these unpublished datasets to future updates of our database (see *Data accessibility*, below).

Data sources for most of our 11 trait categories were from every state in the contiguous US. However, there are geographic biases in trait assignments for certain trait groups, including female dispersal and emergence synchrony, which we derived from studies conducted in fewer than 30 states. Another source of geographic bias arises from the USEPA database, which contains a large amount of trait information from insects in Maine, North Carolina, and Utah. In addition, the trait data from the USEPA database were compiled by researchers from Colorado State University (Poff et al. 2006, Vieira et al. 2006), and the traits for our database, *Freshwater insects CONUS*, were compiled by researchers at Michigan State University. Each of these components could bias the assignment of modal traits or affinities for certain trait groups, such as thermal preference, that are spatially influenced by environmental variables. Overrepresentation of trait information for certain species within a genus could also skew trait assignments toward values for those species. Trait affinities (analogous to fuzzy-coded traits) help to account for these sources of bias by quantifying trait variation for each trait group within a genus across species, geographic areas, and literature sources. Data users should compare modal traits to trait affinities and the data in Raw_Traits to gain insight into the sources of trait variation and biases for each genus. These sources of bias are not unique, and future updates to our database will improve the geographic scope and resolution of traits across species within each genus.

Comparison to other datasets

We tripled the number of occurrence records and locations from what was available in the WQP, and we added occurrence records for 118 genera that were not previously available in open-access databases. The WQP contained 677,005 genus occurrence records from 18,705 locations and 814 genera, after data cleaning and taxonomic harmonization. Our *Freshwater insects CONUS* database contains over 2.05 million genus occurrence records for 932 genera at

51,044 stream locations (Fig. 16 in Appendix). Of the occurrence records, 565,376 are repeat detections of the same taxa over time.

We nearly doubled the number of trait records available for the 11 trait groups we considered, from 24,655 traits in the USEPA database to 47,000 in our CONUS database (Raw_Traits, Fig. 13 in Appendix). As a result, we increased the number of genera assigned a modal trait (Fig. 16 in Appendix). After taxonomic harmonization and data cleaning, the USEPA database contained traits for 827 insect genera, to which we added traits for 180 genera, for a total of 1,007 insect genera with trait assignments (Fig. 16, Table 13 in Appendix). We also updated taxonomic names to reflect the most current genus designations and trait assignments to align with the unified trait terminology for stream organisms (Schmera et al. 2015). Finally, we added trait affinity scores (Genus_Trait_Affinities, Fig. 13 in Appendix), which were not included in the USEPA database, in order to facilitate conversion of US traits to the European system of fuzzy coding and account for trait variation within genera.

Conclusions

Our *Freshwater insects CONUS* database provides the most comprehensive datasets of freshwater insect occurrence records and traits for the contiguous US by including records for a majority of the estimated 1,160 freshwater insect genera in North America (Balian et al. 2008). Our occurrence dataset provides good spatial coverage of occurrence records for most of the major freshwater insect orders because our data derive from systematic community surveys. Another strength of our database is that our trait data are more comparable to datasets used by researchers in Europe and other regions of the world by including trait variation as trait affinities, analogous to fuzzy-coded traits, and utilizing unified trait terminology (Schmera et al. 2015). These components are included to facilitate linking our database to those in other countries for cross-continental analyses of functional composition and diversity in freshwater insects. We identified regions of the US and taxa for which more occurrence and trait data are needed, and we encourage data submissions for future updates to our database. Our database can be used to map freshwater insect taxonomic and functional diversity, and when paired with environmental data, will provide a powerful resource for quantifying how the environment shapes diversity patterns, as well as taxon-specific distributions, across the contiguous US.

Data accessibility

The database is available as .csv files through the Environmental Data Initiative (EDI) with the following citation:

Twardochleb, L.A., E. Hiltner, M. Pyne, P. Bills, and P.L. Zarnetske. 2020. Freshwater insect occurrences and traits for the contiguous United States, 2001 - 2018 ver 5. Environmental Data Initiative. https://doi.org/10.6073/pasta/8238ea9bc15840844b3a023b6b6ed158.

We also provide the R scripts used for data cleaning through EDI and GitHub at https://github.com/aquaXterra/freshwater_insects_CONUS. We invite data submissions for future updates to the database. Instructions and a data submission template are available through GitHub: https://github.com/aquaXterra/freshwater_insects_CONUS. To submit data or ask questions about the data submission process, email Dr. Phoebe Zarnetske, Michigan State University SpaCE Lab (Spatial & Community Ecology Lab) plz@msu.edu. A link to this repository with updated information can be found at the MSU SpaCE Lab website: www.communityecologylab.com.

APPENDIX

Table 11. Functional traits of freshwater insects. To be consistent with the unified trait terminology for stream organisms proposed by Schmera et al. (2015), we have reorganized traits by grouping feature and trait groups. A definition for each trait and literature citation for that definition are provided.

Grouping Feature	**Trait Group**	**Trait**	**Definition**	**Definition Citation**
Life history	Number of generations per year	Semivoltine	< 1 generation per year	Poff et al. 2006
		Univoltine	1 generation per year	Poff et al. 2006
		Bi_multivoltine	> 1 generation per year	Poff et al. 2006
	Synchronization of emergence	Well	Emergence occurs within a matter of days	Poff et al. 2006
		Poorly	Emergence occurs within a matter of weeks or months	Poff et al. 2006
	Emergence season	Spring	Emergence between the months of March and May	
		Summer	Emergence between the months of June and August	
		Fall	Emergence between the months of September and November	
		Winter	Emergence between the months of December and February	
Dispersal	Female dispersal	Low	< 1 km flight before laying eggs	Poff et al. 2006
		High	> 1 km flight before laying eggs	Poff et al. 2006
	Adult flying strength	Weak	Taking frequent breaks while flying, or flight is low to the ground	Poff et al. 2006
		Strong	Able to fly into a light breeze or fly for several miles without breaks	Poff et al. 2006

Table 11. (cont'd)

Morphology	Maximum body size	Small	< 9 mm	Poff et al. 2006
		Medium	9-16 mm	Poff et al. 2006
		Large	> 16 mm	Poff et al. 2006
	Respiration mode	Tegument	An outer covering, outer enveloping cell layer, or membrane used to acquire oxygen	Merritt et al. 2008
		Gills	A thin walled structure with trachea, used for the absorption of oxygen	Arnett Jr. 2000
		Plastron, spiracle	Oxygen is absorbed from the atmosphere, from aquatic plants, or from a temporary air store, such as an air film or bubble on the surface of the body, or a permanent air store (a plastron).	Merritt et al. 2008
Ecology	Rheophily	Depo	Occupies running-water pools or margins with fine sediments (sand and silt)	Merritt et al. 2008
		Depo_eros	Occupies both erosional and depositional habitats	Merritt et al. 2008
		Eros	Occupies running-water riffles with coarse sediments (cobbles, pebble, gravel)	Merritt et al. 2008
	Thermal preference	Cold stenothermal	< 5 °C	Vieira et al. 2006
		Cold-cool eurythermal	0-15 °C	Vieira et al. 2006
		Cool-warm eurythermal	5-30 °C	Vieira et al. 2006
		Warm eurythermal	15-30 °C	Vieira et al. 2006
		Hot eurythermal	> 30 °C	Vieira et al. 2006

Table 11. (cont'd)

Habit	Crawler	Adapted for crawling on the surface of floating leaves of vascular hydrophytes or fine sediments on the bottom of water bodies	Merritt et al. 2008
	Burrower	Inhabiting the fine sediment of streams and lakes	Merritt et al. 2008
	Clinger	Representatives have behavioral and morphological adaptations for attachment to surfaces in stream riffles and wave-swept rocky littoral zones of lakes	Merritt et al. 2008
	Skater	Adapted for skating on the surface where they feed as scavengers on organisms trapped in the surface film	Merritt et al. 2008
	Swimmer	Adapted for "fishlike" swimming in lotic or lentic habitats	Merritt et al. 2008
	Sprawler	Inhabiting the surface of floating leaves of vascular hydrophytes or fine sediments	Merritt et al. 2008
	Climber	Adapted for living on vascular hydrophytes or detrital debris with modifications for moving vertically on stem-type surfaces	Merritt et al. 2008
	Planktonic	Inhabiting the open water limnetic zone of standing waters	Merritt et al. 2008
Feeding Style	Predator	Insects that ingest prey whole or in parts (engulfers), or pierce prey tissues and suck fluids (piercers)	Merritt et al. 2008
	Collector-gatherer	Insects that collect and consume decomposing organic matter	Cummins 1973

Table 11. (cont'd)

	Collector-filterer	Insects that collect and filter living algal cells or detritus	Merritt et al. 2008
	Herbivore	Insects that scrape algae, or shred or pierce living aquatic plants	Merritt et al. 2008, Poff et al. 2006
	Shredder	Insects that shred decomposing vascular plant tissue (detritivores)	Poff et al. 2006
	Parasite	Parasites that consume living animal tissue	Merritt et al. 2008

Table 12. Contents and relationships among data tables (Fig. 13). "Links to Other Tables" indicates which columns can be used to join related tables. The database assembly steps (Fig. 12) involved in creating each table are also provided.

Data Table Name	Content	Links to Other Tables	Database Assembly Steps
Raw_Traits	Cleaned trait data using R scripts for each taxonomic name ("Submitted name_trait", usually genus, occasionally species or family) recorded in datasets from the WQP, state agencies, or USEPA. There are multiple trait entries separated by row for each taxon, with each row presenting trait data recorded from a different location, species, or literature source.	Ancillary_Taxonomy through "Submitted_name" column.	1,2,3,4
Genus_Traits	Modal traits for each genus assigned from data in Raw_Traits using R scripts.	Genus_Trait_Affinities and Ancillary_Trait through "Trait" column. Genus_Occurrences and Ancillary_Taxonomy through "Genus" column.	5
Genus_Trait_Affinities	Trait affinities for each genus assigned from data in Raw_Traits using R scripts.	Linkages are the same as for Genus_Traits, above.	5
Ancillary_Trait	Information about traits contained in Table 1.	Genus_Traits and Genus_Trait_Affinities through "Trait" column.	1
Genus_Occurrences	Genus occurrence records produced from Raw_Community_Data using R scripts.	Genus_Traits through "Trait" column and Ancillary_Taxonomy through "Genus" column.	3,4

Table 12. (cont’d)

Ancillary_Taxonomy	Data from taxonomic harmonization, including taxonomic names (“Submitted_name”) recorded in the WQP, state data, and USEPA database, and the corresponding accepted names, taxonomic serial numbers, and higher taxonomic designations. Users can search on any column in Ancillary_Taxonomy and find corresponding occurrence and trait records in other tables.	Raw_Traits and Raw_Community_Data through “Submitted_name” column. Genus_Traits, Genus_Trait_Affinities, and Genus_Occurrences through “Genus” column.	4
Data_Sources	Information about source data files, state agency websites, and agency contacts.		1
Raw_Community_Data	Cleaned occurrence data from the WQP and state agencies using R scripts. Includes records for taxa identified to species, genus, family, or order.	Genus_Occurrences through “Unique_ID”. Ancillary_Taxonomy through “Submitted_name”. Ancillary_Sample_Method through “Sample_method”.	2,3,4
Ancillary_Sample_Method	Detailed methodology for sample methods in Raw_Community_Data.	Raw_Community_Data through “Sample_method” and Data_Sources through “Data_source”.	2

Table 13. Number of genus occurrence and trait records by insect order.

Order	Number of genus occurrence records	Number of genus occurrence locations	Number of genera with occurrence records	Number of genera with trait records
Coleoptera	210,077	44,669	145	160
Diptera	862,826	49,572	335	363
Ephemeroptera	381,077	46,737	93	100
Hemiptera	22,528	10,231	48	56
Lepidoptera	3,756	2,956	18	4
Megaloptera	25,056	15,302	9	8
Neuroptera	389	362	3	2
Odonata	73,760	23,102	66	73
Plecoptera	137,377	29,155	97	99
Trichoptera	338,460	46,682	127	145

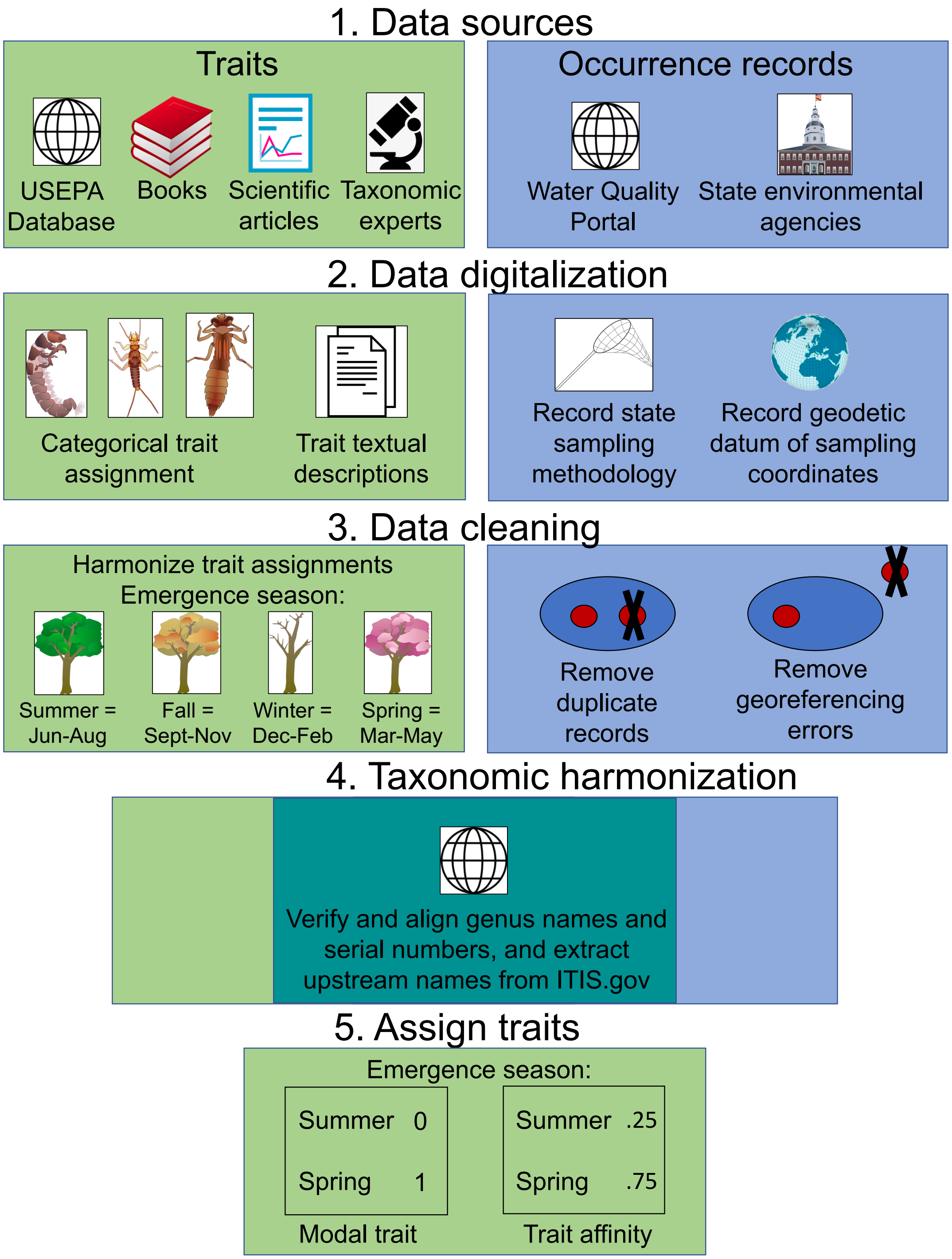

Figure 12. Database assembly steps. Steps for traits are shown in green boxes and occurrence records in blue boxes. We assembled our trait dataset from the USEPA Biological Traits Database, taxonomic guides and entomology texts, scientific articles, and with the help of

Figure 12. (cont'd) taxonomic experts. The occurrence dataset was assembled from data from the Water Quality Portal and requests to state environmental agencies. We recorded trait data following definitions in Table 11 and recorded state sampling methodology based on state agencies' field sampling manuals. We digitized data in Microsoft Excel. We then performed data cleaning and taxonomic harmonization in R, using the package "taxize" version 0.9.92. Finally, we assigned modal traits as the most commonly occurring trait in a trait group for each genus, and a trait affinity, or the percentage affinity of a genus toward each trait in a trait group. Icons courtesy of the Integration and Application Network.

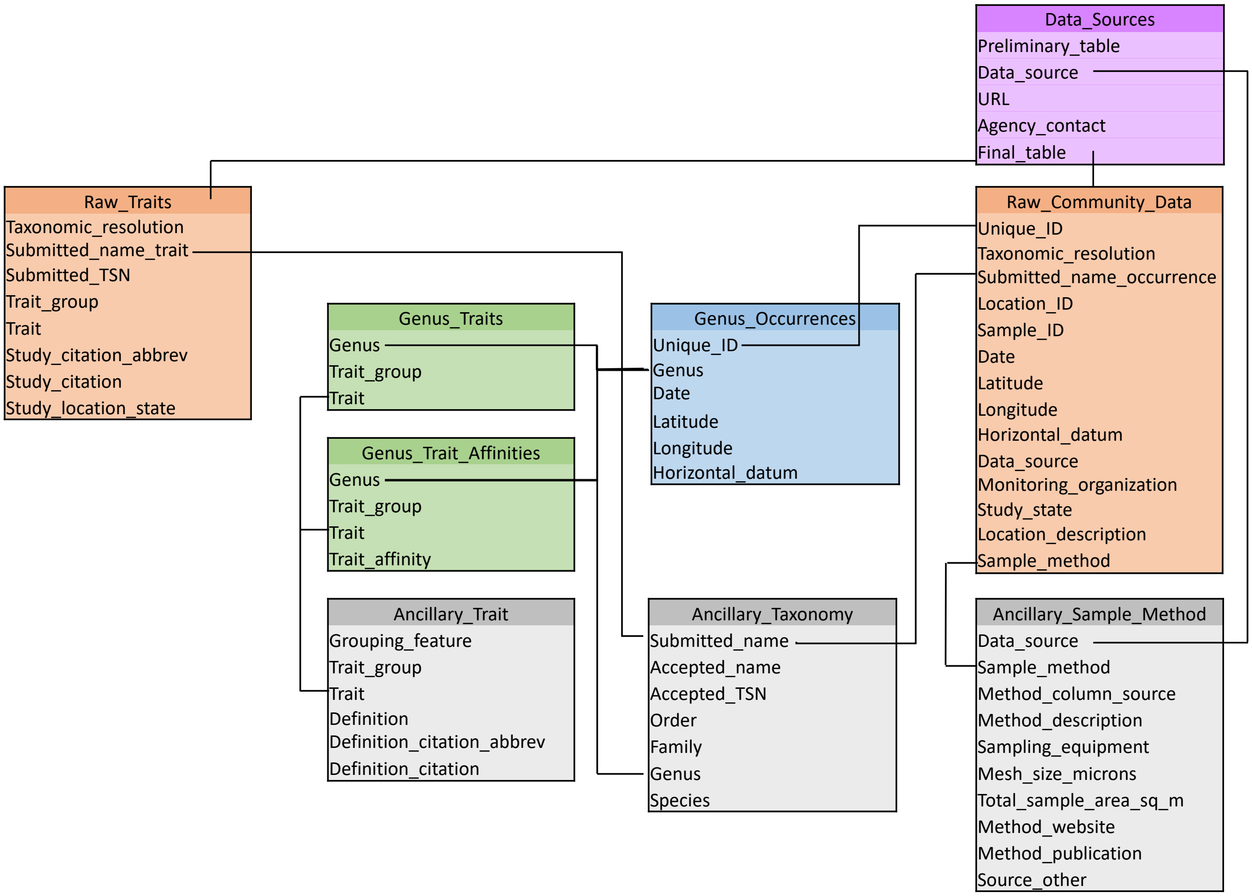

Figure 13. Database layout with connecting lines indicating relations among tables. Orange boxes are the "raw" community and trait datasets cleaned from data in the Data_Sources table (purple) using R scripts. "Cleaned" trait tables are shown in green and occurrence records in blue. Tables of ancillary information are in gray. From left to right: Raw_Traits contains data for each genus varying by location, species, and literature source, that we digitized and cleaned during steps 2, 3, and 4 of database assembly (Fig. 12). Genus_Traits and Genus_Trait_Affinities contain modal traits and trait affinities that we produced from Raw_Traits using R scripts during step 5. Ancillary_Trait contains information about each trait (Table 11). Genus_Occurrences contains occurrence records that we produced from Raw_Community_Data using R scripts in database assembly steps 3 and 4. Ancillary_Taxonomy contains taxonomic names recorded in

Figure 13. (cont'd) the WQP, state data, and USEPA database, and their corresponding accepted names, taxonomic serial numbers, and higher taxonomic designations obtained during step 4. Raw_Community_Data contains occurrence data from the WQP and state agencies supplied in data tables listed in Data_Sources. We recorded additional data about state sampling methodology in Ancillary_Sample_Method during step 2. We cleaned the data files in Data_Sources using R scripts during steps 3 and 4.

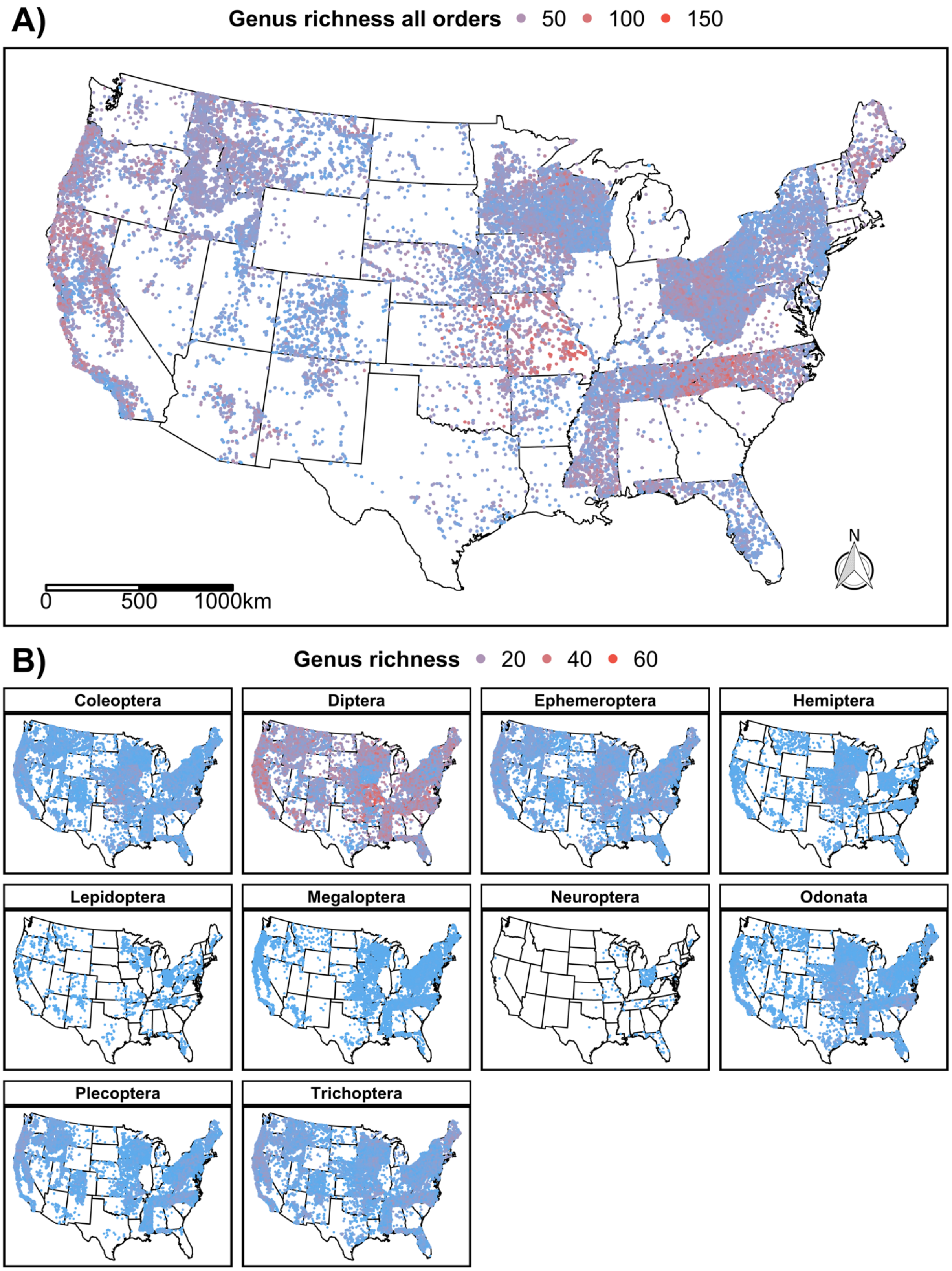

Figure 14. Genus richness by occurrence location. For all orders A), and B) for each order

Figure 14. (cont'd) individually. Blue points indicate low genus richness, and red indicate high richness. Note that genus richness has not been corrected for sampling bias.

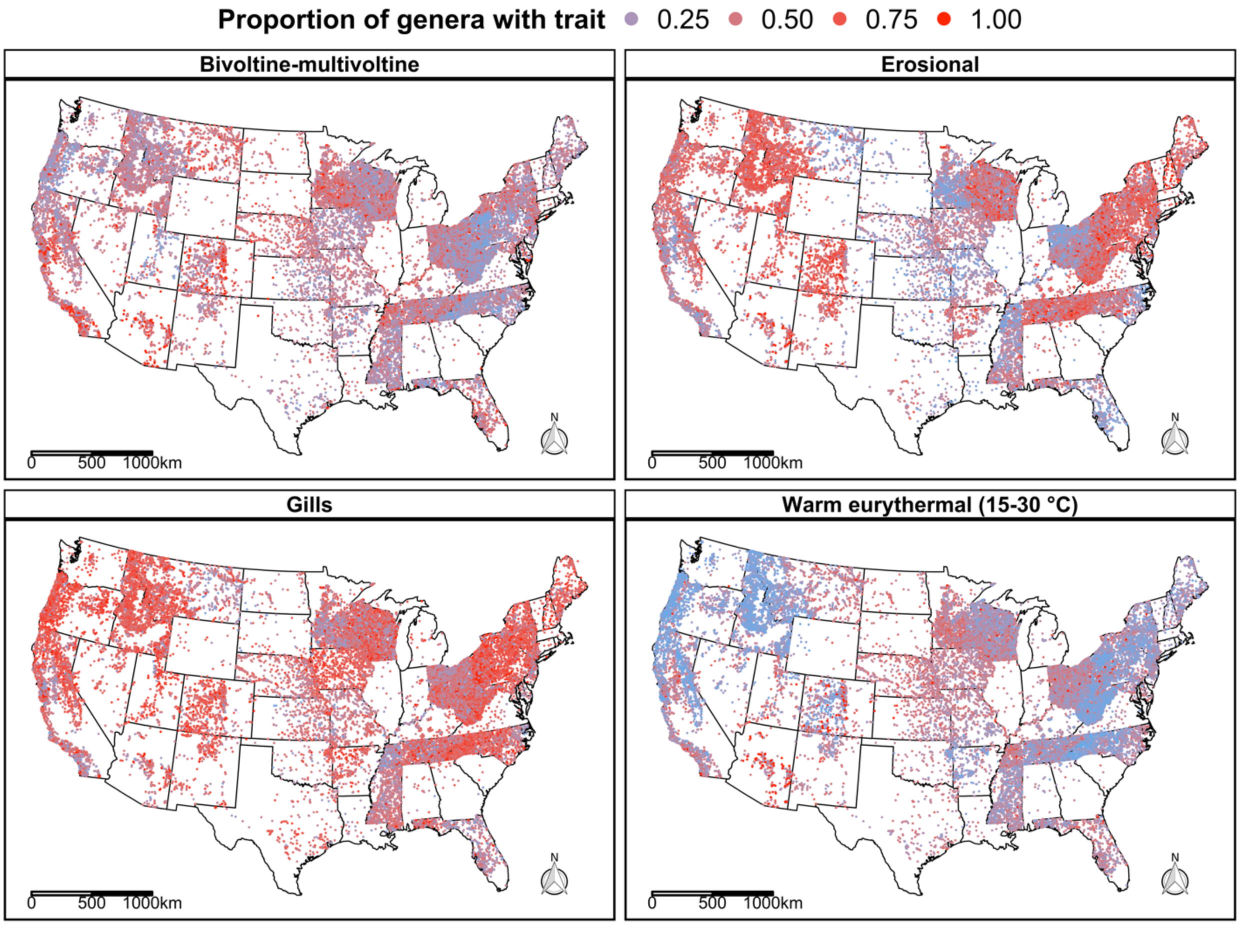

Figure 15. Proportion of genera at each occurrence location assigned a modal trait of bivoltine-multivoltine (number of generations per year), erosional (rheophily), gills (respiration mode), and warm eurythermal (thermal preference). Blue points are sites where a low proportion of genera have the trait, and red points indicate that a high proportion have the trait.

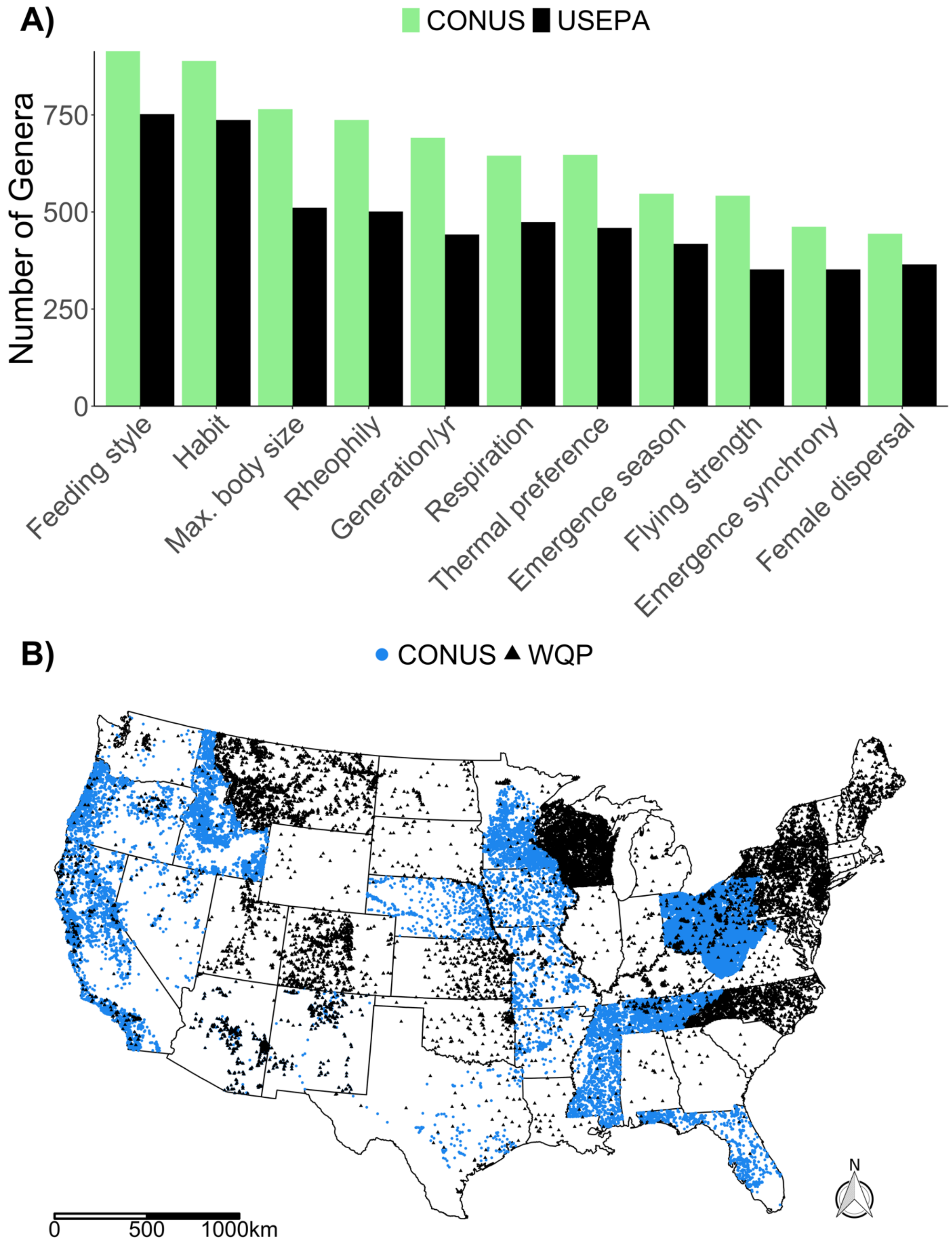

Figure 16. Numbers of trait and occurrence records in the database.

Figure 16. (cont'd) a) Traits: number of genera assigned a modal trait for each trait group after data cleaning and taxonomic harmonization with data originating from the USEPA traits database (black bars; USEPA) vs. our database (green bars; CONUS). b) Occurrence Records: locations after data cleaning originating from the WQP (black points) vs. our database (blue points; CONUS).

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CHAPTER 4. A trait-based assessment of climate sensitivity of freshwater insects in the contiguous United States

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Abstract

Climate change threatens freshwater biodiversity in the United States. As a result of thermal stress and changes in stream flows, many freshwater organisms are likely to experience geographic range shifts and population extinctions in the future. Stream insects control many ecosystem processes, fuel terrestrial food webs, and are widely used as bioindicators. However, their vulnerability to climate change has been largely overlooked. We examined the *climate-sensitivity* of freshwater insects, or the degree to which their geographic distributions are strongly determined by climatic variability, using the relationships between climate and measures of freshwater insect biodiversity. These measures included insect genus richness, Ephemeroptera, Plecoptera, and Trichoptera (EPT) genus richness, functional richness, Rao's quadratic entropy, and individual traits and trait profile groups (i.e., clusters of interrelated traits). Among the 11 traits and 3 trait profile groups we considered, erosional flow preference, cold-cool eurythermal preference, and univoltine life-history traits were the most sensitive to climate. We used these traits to assess and map the climate-sensitivity of stream insect genera. Using these trait-based indicators, we found that insects in the orders Plecoptera and Trichoptera are likely the most sensitive to climate change. Insect species within these orders should be prioritized for protection in order to avoid population extirpations and species extinctions with climate change. Our trait-

based indicators of climate sensitivity also showed that watersheds with the highest proportions of sensitive taxa are throughout the mountain West and Appalachian regions of the United States. These regions should be prioritized for further research and protection of their watersheds to guard against losing freshwater biodiversity due to climate change. By providing biological indicators of climate change vulnerability, our research enhances efforts by scientists and managers to prioritize watersheds and taxa for restoration and conservation in the United States.

Introduction

Climate change is altering freshwater habitats in the United States by increasing water temperatures and changing stream flows (Poff et al. 2002). These habitat changes can directly impact survival and geographic ranges of ectothermic freshwater organisms through thermal stress and habitat loss (Ficke et al. 2007, Heino 2009), and indirectly through changes to life-history traits such as dispersal and reproduction (Braune et al. 2008, Poff et al. 2012, McCauley et al. 2018b). Thus, we expect that many freshwater organisms will be vulnerable to extinction under climate change, and many more will undergo distributional shifts to track their climatic niche, driving large-scale alterations in freshwater biodiversity in the United States (Poff et al. 2012). Despite this expectation, the *climate change vulnerability* of stream insects, or the extent that their biodiversity will be lost due to climate change (Foden and Young 2016), has not been assessed at the extent of the contiguous United States. Assessing the vulnerability of stream insects across watersheds in the contiguous US would identify vulnerable insect taxa and enhance efforts by scientists and managers to prioritize watersheds for restoration and conservation, by identifying biological indicators of climate change vulnerability.

Insect vulnerability to climate change is under-assessed (Pacifici et al. 2015), and the relationship between freshwater insect diversity and climate is poorly understood (Vinson and Hawkins 1998, Heino 2009). These knowledge gaps are especially troubling given that a biodiversity crisis is already underway for terrestrial insects (Hallmann et al. 2017, Lister and Garcia 2018). The few studies that have examined stream insect vulnerability to climate change have been conducted in Europe or in smaller regions of the United States or for a limited subset of insect taxa (Poff et al. 2010, Conti et al. 2014, Shah et al. 2014, Pyne and Poff 2017). One study predicted losses of up to 80% of genus richness for freshwater insects in the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) in some regions of the US by 2080 (Shah et al. 2014). However, these taxa represent just 353 (30%) of the estimated 1,160 freshwater insect genera in North America (Balian et al. 2008). Freshwater insects comprise the majority of freshwater animal diversity (Balian et al. 2008), are used in the assessment and regulation of water quality (Barbour et al. 2000, Bonada et al. 2006), and provide a prey base for freshwater fish, riparian birds, amphibians, bats, and lizards (Covich et al. 1999, Baxter et al. 2005, Suter and Cormier 2015). Thus, losses of stream insect diversity due to climate change could threaten critical freshwater ecosystem functions (Dudgeon et al. 2006).

Stream macroinvertebrates serve as indicators of ecosystem health, and changes to their biodiversity can signal wider shifts in ecosystem functioning and biodiversity of other taxonomic groups (Bonada et al. 2006, Suter and Cormier 2015). Thus, US state and federal environmental agencies use stream macroinvertebrate taxonomic and trait diversity measures for bioassessment of stream condition in compliance with the Clean Water Act’s mandate for protecting the ecological integrity of surface waters (Barbour et al. 2000). However, standard bioassessment metrics are designed as indicators of generalized ecosystem stress and may underestimate the impacts of climate change (e.g., Lawrence et al. 2010). Identifying stream insect taxa that are vulnerable to climate change, and the mechanistic drivers of their vulnerability (e.g., biological traits), would provide new tools that researchers and managers could use to measure ecosystem responses to climate-specific stressors.

Climate change vulnerability is commonly assessed using correlative or trait-based approaches (Foden and Young 2016). Correlative approaches typically use a species distribution model (SDM) to estimate a species' current climatic niche and apply climate projections to predict the species' future range (Pacifici et al. 2015, Foden and Young 2016). This is effective for identifying exposure to climate change by revealing which taxa may undergo range contractions (Foden and Young 2016). However, this approach does not provide information about *why* species are vulnerable, because SDMs do not identify the components of species' biology that influence their responses to climate change (Thomas et al. 2011). Trait-based assessment approaches can identify *why* species are vulnerable (Garcia et al. 2014, Pacifici et al. 2017), because traits are organismal features that impact fitness via their effects on growth, reproduction and survival (Violle et al. 2007) and provide the mechanistic link between the environment and fitness of freshwater insects (Poff 1997). Climate-responsive traits could include preference for cold, fast-flowing water (cold-cool eurythermal preference and erosional rheophily traits; Table 1 in Appendix). In addition, voltinism (number of generations per year) could be responsive to climate because insect development rates increase with temperature (Table 1 in Appendix, see also Chapter 2 and the review by Ward and Stanford 1982). Traits are already used in bioassessment metrics (e.g., Gerritsen 1995, Mazor et al. 2016), and knowledge of which traits are responsive to climate change would help scientists and managers develop trait-based indicators to assess watershed vulnerability to climate change.

Climate change is occurring in tandem with increases in human land use, such as developed and cultivated land cover (Martinuzzi et al. 2014), which are often considered the largest threats to biodiversity because they cause habitat loss and pollution (Sala 2000). At the watershed scale, the aggregate effects of land use, geography, and climate likely explain patterns of freshwater biodiversity (Poff 1997, McCluney et al. 2014), and thus watershed attributes such as land cover, topography, and flow could modify observed relationships between climate and stream insect taxonomic and trait diversity (Table 14 in Appendix). Although these relationships are well studied at local, within-stream scales (Allan 2004, Bonada et al. 2007, Lawrence et al. 2010, Herbst et al. 2019), there remains a significant gap in our understanding of how these relationships scale up to larger, continental extents (Vinson and Hawkins 1998, Heino 2009). Quantifying the relationships between stream insect taxonomic and trait diversity and climate within the context of other watershed attributes across the contiguous US will enhance our understanding of the vulnerability of stream insects to climate change.

One of the first steps to understanding stream insect and watershed vulnerability to climate change is identifying *biological indicators of climate sensitivity*, such as the insect taxa, biological traits, and biodiversity measures (e.g., taxonomic and functional diversity) whose geographic distributions are strongly determined by climatic variability or change (Foden and Young 2016). An assessment of the climate sensitivity of stream insects for the contiguous US is needed to facilitate conservation planning by identifying taxonomic and trait-based biological indicators of climate change vulnerability, that would enhance existing efforts to prioritize watersheds for restoration and conservation. Here, we present an assessment of the climate-sensitivity of stream insects in the contiguous US at the watershed scale. Our goals are to: 1) investigate how relationships with climate and other watershed attributes differ among taxonomic and trait diversity measures, 2) compare these measures as indicators of stream insect climate sensitivity, 3) identify insect genera that are sensitive to climate change based on their traits, and 4) identify watersheds containing a high proportion of climate-sensitive taxa. We quantify the climate-sensitivity of stream insect taxonomic and trait measures relevant for bioassessment and understanding relationships between the environment and biodiversity (Statzner and Bêche 2010, Pereira et al. 2013, Mazor et al. 2016, Schmera et al. 2017). These include insect genus richness, Ephemeroptera, Plecoptera, and Trichoptera (EPT) genus richness, functional richness, Rao's quadratic entropy, and individual traits (Table 14 in Appendix) and trait profile groups (i.e., clusters of interrelated traits; Verberk et al. 2013). Using relationships between climate and traits and trait profile groups, we assess the sensitivity of individual insect genera to climate change and map distributions of sensitive taxa in watersheds across the contiguous US. In Table 14 we provide expected relationships between traits, climate, and other watershed attributes.

Methods

Climate, land cover, and other watershed attributes

We compiled environmental watershed attributes of bioclimatic predictors, hydrology, land cover, and topography summarized at the grain size of U.S. Geological Survey (USGS) Hydrologic Unit Code 4 (HUC4) and only within the political boundaries of the contiguous US using R version 3.6.2 (R Core Team 2019). These watershed boundaries were equivalent to LAGOS watersheds (Soranno et al. 2017), hereafter, "watershed". Climatic predictors were summarized by watershed for the contiguous US for 2001-2018, comprising 19 annual bioclimatic variables (Hijmans et al. 2005). Modeled climate data were obtained from the

PRISM Climate Group (PRISM 2020). We downloaded the version labelled "M3" in January 2020. From the 4 km PRISM monthly projections from 2001-2018 we included modeled precipitation (PRISM variable 'ppt') and modeled temperature ranges (PRISM variables 'tmin', 'tmean', 'tmax'). We calculated 19 Annual Bioclimatic variables for our 18 years of PRISM data using the 'dismo' R package version 1.1-4 (Hijmans et al. 2017) and saved to the Open Geospatial Consortium (OGC) geotiff format. We reprojected the rasters to Albers Equal Area Conic projection (origin 23 N 96 W; standard parallels 29.5 N and 45.5 N; datum NAD83; ellipse GRS80; EPSG = 5070) to match the watersheds. The gridded annual bioclimatic variables (raster layers) were then divided into watershed polygons by first using the R package 'raster' version 3.0-12 (Hijmans et al. 2020) with the 'crop' followed by 'mask' functions. We then summarized the bioclimatic values in each watershed with annual mean using standard functions in R. We then calculated the mean and standard deviation of annual mean values across all years in order to obtain a mean and standard deviation for each bioclimatic predictor for 2001-2018 by watershed.

We calculated percentage perennial, intermittent, and ephemeral stream length, and baseflow in each watershed by downloading shapefiles for the USGS National Hydrography Dataset for hydrology variables (NHD; USGS 2016) between November 2017 and January 2018. Percentage stream length data comprise stream length at the grain size of watershed polygons across the contiguous US. Stream data were obtained from the NHD (USGS 2016). For each polygon, we calculated the total stream length by categories (e.g., natural stream) and permanence, as well the percentage of total polygon stream length that is within each permanence category. We downloaded the 1 km resolution gridded dataset based on interpolations of baseflow index values from stream gauges between 1951-1980 on October 5, 2018 (USGS 2003). Metadata for the dataset is included in the data download location. We reprojected the rasters to Albers Equal Area Conic projection and summarized the raster within watershed polygons using R. All raster cells touched by the polygons were included in the watershed summaries by using the ‘mask’ function in the ‘raster’ package version 3.0-12 in R (Hijmans et al. 2020). We summarized baseflow index values to means at the watershed grain size across the contiguous US. A website URL for R scripts and detailed methods for calculating these variables are in Bibliography. We summarized the files by category and permanence within watershed polygons.

For watershed topography, we downloaded the 30 m National Elevation Dataset (NED, USGS 1999) on June 22, 2016. We merged all 1x1 degree tiles to generate a raster reprojected to Albers Equal Area Conic projection. We computed slope and aspect from the elevation raster, using a 9x9 pixel kernel. The digital elevation model, slope, and aspect (raster layers) were summarized to hydrologic unit polygons using the ‘rasterstats’ module in python (Perry 2015), specifically the ‘zonal_stats’ function, to calculate summary statistics. Pixels were considered inside the watershed polygon if their center point was within the polygon (all_touched = False). For each polygon, we computed the mean, minimum, maximum, and standard deviation of elevation, and the mean of slope and mean of aspect.

For land cover classes, we downloaded the National Land Cover Dataset (NLCD, see Bibliography) and summarized the mean spatial percent of land cover class by watershed for each year using Google Earth Engine. We used the 30 m NLCD for 2001, 2004, 2006, 2008, 2011, 2013, and 2016, obtained via the Google Earth Engine Catalog (see Bibliography), which uses automatic reprojection. We used Google Earth Engine API (GEE) data manipulation functions to extract annual NLCD land class values, and the reducer function 'frequency histogram' (see Bibliography) to obtain counts of pixels for each NLCD land cover class within each watershed polygon, per year. We then derived the percentage of area covered by each land cover class within each NLCD per year with the simple division counts dividing the land cover class pixels by total pixels in all land cover classes in the watershed. Note the denominator does not include those pixels within the watershed that were not assigned a land cover class by NLCD, so this is an approximation of the total area of the watershed. The GEE reducer functions include a pixel if at least ~50% of the pixel is in the region. We then calculated the mean and standard deviation of percentage of each of the 20 land cover classes across years (temporal mean) by watershed (See Bibliography). We further summarized NLCD land cover into the following categories: percentage barren land (31), percentage cultivated land (81 + 82), percentage forested land (41 + 42 + 43), percentage wetland (90).

Stream insect occurrence and trait database

We compiled a database, *Freshwater insects CONUS (see Chapter 3, Freshwater insect occurrences and traits for the contiguous United States),* of freshwater insect occurrences and functional traits for the contiguous US, for genera from the major freshwater insect orders: Coleoptera (beetles), Diptera (midges), Ephemeroptera (mayflies), Hemiptera (true bugs), Lepidoptera (moths), Megaloptera (fishflies), Neuroptera (net-winged insects), Odonata (dragonflies and damselflies), Plecoptera (stoneflies), and Trichoptera (caddisflies). We compiled and standardized over 2.05 million genus occurrence records for 932 genera, at 51,044 stream locations sampled between 2001 and 2018 by federal and state biological monitoring programs. We also assembled life history, dispersal, morphology, and ecology traits from existing databases, scientific books, and the primary literature, and assigned traits and trait affinities for 1,007 insect genera. The traits in *Freshwater insects CONUS* are all categorical and were assigned using standardized terminology and definitions for traits of stream organisms, such that related traits were grouped into the following categories: adult flying strength, body size, emergence season, emergence synchrony, feeding style (similar to functional feeding group), female dispersal, habit (mode of living), respiration mode, rheophily (flow preference), thermal preference, and voltinism (number of generations per year) (Poff et al. 2006, Schmera et al. 2015).

Taxonomic and trait diversity measures

We quantified taxonomic and trait diversity measures at the watershed scale by treating each sampling location within a watershed as a replicated incidence sample and using coverage-based rarefaction to correct for differences in sample coverage among HUCs. We calculated coverage-based rarefaction curves (genus richness by sample coverage) for each HUC from sample size-based rarefaction curves (genus richness by number of samples) and sample completeness curves (sample coverage by number of samples), using the 'iNext' R package version 2.0.2 (Chao and Jost 2012, Hsieh et al. 2016). Eighty five percent of watersheds were sampled at 90% or higher coverage, and 4% had no samples. We removed watersheds with less than 90% sample coverage from further consideration in analyses. We then randomly resampled the sites in each of the remaining watersheds with replacement down to 90% sample coverage before calculating diversity measures. We repeated resampling 100 times, and then calculated the mean overall genus richness and EPT richness, as the mean number of unique genera across all resampled sites within a watershed.

We quantified two complementary measures of functional diversity (FD) by watershed using sites resampled to 90% coverage as above and the 'FD' R package version 1.0-12 (Laliberté and Legendre 2010). We calculated functional richness, the number of unique trait combinations in a community when traits are categorical, and Rao's quadratic entropy (Rao's Q), a measure of pairwise functional distances between taxa that is similar to functional dispersion (Botta-Dukát 2005, Villéger et al. 2008, Laliberté and Legendre 2010). To calculate functional richness and Rao's Q, the FD package requires a pairwise distance or dissimilarity matrix, with distances among genera that can be represented in Euclidean space, and then analyzes the distance matrix with principal coordinate analysis (PCoA). The resulting PCoA axes are then used as traits to compute FD indices (Laliberté and Legendre 2010).

We used all available traits from 10 of the 11 trait groups. We excluded emergence season because many taxa were missing a trait assignment for that group, and there is no strong evidence that this trait's distribution is clearly linked to climate, land cover, or other environmental attributes. We equally weighted traits across groups to avoid biasing FD indices toward groups containing many traits (e.g., body size has three traits and habit has eight) (Laliberté and Legendre 2010). The raw trait by genus matrix contains categorical traits for 1,007 genera, many with missing values. We first removed genera that were missing values for over half of the traits, then we calculated Gower dissimilarity and applied a square root transformation (Legendre and Legendre 2012). However, we found that the resulting dissimilarity matrix was not representative of Euclidean space. Therefore, we repeated this procedure by sequentially removing taxa with missing traits and recalculating the square root corrected, Gower dissimilarity matrix. After removing all genera that were missing traits, we found that the resulting matrix was approximately Euclidean. This resulted in a dissimilarity matrix for 363 genera.

As alternatives to removing genera with missing traits, we compared two imputation methods. In the first, we assigned the most commonly occurring trait by insect family. In the second, we used the missForest package version 1.4 in R, which uses a nonparametric imputation method that does not require phylogenetic relationships among taxa (Stekhoven and Buhlmann 2012). For both methods, we compared the resulting trait composition of insect families and the spatial distributions of traits to values from the raw dataset using all 1,007 insect genera. The imputation methods strongly homogenized traits spatially and across insect families. Therefore, we proceeded with unimputed data and excluding genera with missing traits.

We calculated functional richness and Rao's Q from the square root corrected Gower dissimilarity matrix. When calculating functional richness, we retained the first 9 PCoA axes, representing 80% of the information in the dissimilarity matrix. To facilitate comparison of diversity values across watersheds and indices, we standardized functional richness by the global value across all watersheds, and scaled Rao's Q by its maximal value (Villéger et al. 2008). We also rescaled genus and EPT richness using min-max normalization. This constrained all taxonomic and functional diversity measures to values between 0 and 1.

Relationships between climate and stream insect diversity

We selected environmental predictor variables with low multicollinearity for quantifying relationships with stream insect diversity, by examining correlation matrices among all possible predictor and response variables. We also mapped distributions of environmental predictors (Fig. 17 in Appendix) and taxonomic and functional diversity (Fig. 18 in Appendix) to examine potential relationships. We selected the following as continuous, fixed effects predictors for regression models: the mean of maximum temperature of the warmest month (bio 5, temperature), mean baseflow, mean slope, mean precipitation of the warmest quarter (bio 18, precipitation), mean percent intermittent stream length, mean percentage of the watershed with barren land cover, mean percentage cultivated land cover, mean percentage forested land cover, and mean percentage wetland cover, and a first order interaction between temperature and forested land cover. We scaled all predictor variables to zero mean and unit variance using the ‘decostand’ function in the ‘Vegan’ R package version 2.5-6 (Oksanen et al. 2017). We then fit multiple linear regression models separately for each response variable.

We found evidence for significant spatial autocorrelation in the residuals for all models using Moran’s I statistic. Therefore, we fit each model as a spatial autoregressive model using a Bayesian framework with the ‘CarBayes’ R package version 5.2 (Lee 2013). We modeled the influence of spatial autocorrelation on diversity measures with spatially correlated random effects conditioned on a weighted neighborhood matrix specifying the adjacency structure of watersheds. We specified a Gaussian error structure for the response variables and used conditional autoregressive priors of Leroux et al. (2000). We assessed convergence of models using Geweke diagnostics and trace plots (Hobbs and Hooten 2015). We defined important predictors as having 95% credible intervals not overlapping zero. We compared full models to reduced models omitting less important predictors, using the Deviance Information Criterion (DIC) and the Watanabe-Akaike Information Criterion (WAIC) (Hobbs and Hooten 2015). Models with lower DIC or WAIC scores are considered more parsimonious (Table 15 in Appendix). To compare different biological indicators of climate sensitivity, we also used a consistent set of reduced models across all diversity and trait measures (see fourth corner analyses, described below) that included six predictors with consistently strong relationships with biodiversity: temperature, precipitation, baseflow, slope, cultivated land cover, forested land cover, and spatial random effects (Fig. 17, Tables 14, 15 in Appendix).

Identifying trait profile groups

Trait profile groups are traits that co-occur within organisms due to trait interrelationships (trade-offs, spinoffs, and synergies between traits, Verberk et al. 2013) and phylogenetic constraints (Poff et al. 2006), and have been proposed as an alternative to individual traits for understanding how climate influences distributions of stream insects (Hamilton et al. 2020). We identified trait profile groups using cluster analysis of insect genera and the same traits as FD indices, in the 'cluster' R package version 2.1.0 (Maechler et al. 2019). We removed genera that were missing over half of the traits and calculated Gower dissimilarity for the remaining 689 genera in the trait by genus matrix. We then used partitioning around medoids to identify clusters of 2 to 10 trait profiles and selected the optimal number of clusters using three methods: average silhouette width, the total within-cluster sum of squares, and the gap statistic (Tibshirani et al. 2001, Borcard et al. 2011, Legendre and Legendre 2012). Each of these methods suggested that three clusters were optimal for grouping genera by traits.

Fourth corner analysis to identify trait sensitivity to climate

We tested individual trait-environment and trait profile-environment relationships using fourth corner analysis in the 'ade4' R package version 1.7-15 (Dray et al. 2007). We combined three data matrices: genus occurrence by watershed, environment by watershed, and traits or trait profiles by genus, using watersheds that were resampled to 90% sample coverage and the same environmental predictors as above, except for spatial random effects, as there is currently no method for incorporating spatial effects into fourth corner analysis with categorical traits (Braga et al. 2018). We selected a subset of traits in trait groups that are most responsive to climate, including rheophily, thermal preference, and voltinism (Table 14, Fig. 19 in Appendix). We omitted genera with missing values, which are not permitted in fourth corner analysis, resulting in a trait matrix for 488 genera. We then used permutation model 2, randomly permuting the rows of the genus by watershed matrix to test the hypothesis that the environment controls genus assemblages. This permutation procedure has a correct type I error rate when traits are fixed (Legendre and Legendre 2012). We repeated this permutation 999 times and used the 'holm' method of adjusting p-values to test for significance of trait-environment relationships.

Identifying climate-sensitive stream insects and mapping climate sensitivity

We quantified the climate-sensitivity of biodiversity measures using the median coefficient estimates for temperature and precipitation from the set of reduced spatial models including temperature, precipitation, baseflow, slope, percent cultivated land cover, percent forested land cover, and spatial random effects (Table 16 in Appendix). We estimated the climate-sensitivity of individual traits and trait profiles using the correlation coefficient 'r' for temperature and precipitation from fourth corner analyses (Tables 17-20 in Appendix). For each measure, we calculated climate sensitivity as the mean of the absolute value of model coefficients (Figs. 22-25 in Appendix). We then assigned two sensitivity scores for each insect genus, using the mean sensitivity of rheophily, thermal preference, and voltinism traits, and the sensitivity score for its trait profile (Fig. 25 in Appendix). We examined separate histograms of mean scores for individual traits and trait profiles for a breakpoint to categorize genera into 'high', 'medium', and 'low' sensitivity categories. We selected breakpoints for each sensitivity category, where taxa classified as highly sensitive to climate based on their traits had a mean score above 0.08, taxa with medium sensitivity had a score between 0.05 and 0.075, and taxa with low sensitivity had a score below 0.05. For trait profiles, highly sensitive taxa had scores above 0.008, and taxa with low sensitivity had scores below 0.005. Histograms did not show an intermediate group of scores based on trait profile groups. These methods for assigning categories are similar to methods of Pritt and Frimpong (2010) for determining rarity of freshwater fish. However, rather than using expert opinion to assign trait scores (Foden et al. 2013, Spencer et al. 2019), we used observed relationships of traits with climate to determine sensitivity (Pacifici et al. 2017, 2018, Jarić et al. 2019). We mapped the proportion of taxa with high climate sensitivity in each watershed separately for scores based on traits and trait profiles.

Results

Spatial patterns of stream insect diversity and traits

Genus and EPT genus richness were relatively high in watersheds of the US West coasts and Southeast, the Ozark plateau of Arkansas and Missouri, the upper Midwest, and Maine (Fig. 18 in Appendix). EPT richness was relatively higher in the Appalachian region and intermountain West compared with other diversity measures. Functional richness was highest along the Southwest and East coasts and in the Ozark region. Rao's quadratic entropy showed somewhat different spatial patterns than other measures and was highest in the Southeast and Midwest. We also mapped the proportion of genera in each watershed possessing rheophily, thermal preference, and voltinism traits that were previously shown to be climate-sensitive (i.e., with spatial distributions that are strongly determined by climate, Fig. 19 in Appendix). Watersheds in the Western US and Appalachian region had the highest proportions of genera with cold-cool eurythermal preference and erosional flow preference (rheophily). These regions also had relatively high proportions of taxa with univoltine life-histories, although this trait was more widely distributed across the US than sensitive rheophily and thermal preference traits.

Trait profile groups

We identified three trait profile groups from cluster analysis (Fig. 20 in Appendix). Trait profile 1 is composed primarily of genera in the EPT and Diptera orders with small to medium juvenile body sizes, low female dispersal, well synchronized emergence, mixed feedings styles, weak adult flying strength, clinging and sprawling habits, gill and tegument respiration, depositional and depositional-erosional rheophily traits, cold-cool eurythermal (0 - 15 ºC) to cool-warm eurythermal (5 - 30 ºC) preferences, and univoltine life-histories. A representative genus for this group is Doroneuria (Plecoptera). Taxa in trait group 1 dominate the genus richness in mountainous watersheds of the western and northeastern US (Fig. 21 in Appendix). Trait profile 2 is dominated by genera in the orders Diptera and Coleoptera (Fig. 20 in Appendix). These taxa have primarily small body sizes, low female dispersal, poorly to well synchronized emergence, collector-gatherer and predatory feeding styles, strong to weak adult flying strength, burrowing, clinging, sprawling, or swimming habits, tegument respiration, mixed rheophily, primarily cool-warm eurythermal (5 - 30 ºC) and warm eurythermal (15 - 30 ºC) preferences, and bi-multivoltine life-histories. A representative genus for this group is Aedes (Diptera). Taxa in this group have a cosmopolitan distribution (Fig. 21 in Appendix). Trait profile 3 is representative of taxa in the orders Coleoptera, Diptera, and Odonata (Fig. 20 in Appendix) that have primarily medium to large body sizes and high female dispersal, poorly to well synchronized emergence, and are predators with strong adult flying strength, gilled respiration, depositional rheophily, a mix of habits, cool-warm eurythermal (5 - 30 ºC) to warm eurythermal (15 - 30 ºC) preference, and univoltine and semivoltine life-histories. A representative genus for this group is Anax (Odonata). Overall, taxa from this group are most dominant in watersheds of the Midwest, and they comprise a smaller proportion of the genus richness in watersheds across the US relative to taxa in trait groups 1 and 2 (Fig. 21 in Appendix).

Relationships between climate and stream insect diversity

Results of model selection for conditional autoregressive models are given in Table 15 in Appendix. We found that models including only spatial random effects were the most parsimonious according to DIC and WAIC for explaining patterns in genus richness and functional richness across the contiguous US. However, two alternative models, including temperature, slope, percent forested land cover, and spatial random effects, or temperature, precipitation, and spatial effects, performed nearly as well for predicting functional richness. In addition, an alternative model including temperature, precipitation, and spatial random effects performed nearly as well for genus richness as the selected model. The best-performing model for EPT genus richness included temperature, precipitation, and spatial random effects, although an alternative model including baseflow, percent forested land cover, and spatial random effects, and one including only temperature and spatial effects performed nearly as well as the selected model. Two models performed nearly equally for explaining Rao's quadratic entropy, one including temperature, precipitation, slope, and spatial random effects, and one including temperature, baseflow, slope, percent cultivated and forested land cover, and spatial effects.

Among our selected best-performing models, we found that maximum temperature of the warmest month was positively correlated with all diversity measures, and mean precipitation of the warmest quarter was negatively correlated with genus and EPT richness (Fig. 22, Table 16 in Appendix). Forested land cover was positively correlated with genus, EPT, and functional richness, and cultivated land cover was positively correlated with functional richness. Baseflow was positively correlated with EPT richness and negatively correlated with Rao's Q. Slope was negatively correlated with all diversity measures except EPT richness. In addition, spatial autocorrelation had a strong, positive effect on all four biodiversity measures (Fig. 22, Table 16 in Appendix).

Relationships between climate, traits, and trait profile groups

Temperature and precipitation were not strong predictors of distributions of trait groups (Fig. 23, Table 17 in Appendix). In contrast, baseflow, cultivated land cover, and slope explained the majority of variation in trait profile distributions. Insect genera in trait profile 1 (EPT and Diptera) more commonly occurred in watersheds with high baseflow, slope, and percentage of forested land cover. Watersheds with a high percentage of cultivated land cover had lower occurrences of taxa in trait profile 1, and higher occurrences of taxa in trait profile 3 (Coleoptera and Odonata). Insect genera in trait profile 2 (Diptera and Coleoptera) were more common in watersheds with lower baseflow and slope.

Insect genera with erosional, cold-cool eurythermal, and univoltine traits occurred less commonly in watersheds with higher temperatures (Fig. 24, Tables 18-20 in Appendix). In addition, insects with erosional rheophily and cold-cool eurythermal preference were less common, and insects with univoltine traits were more common, in watersheds with high precipitation in the warmest quarter. Overall, the strongest relationship between climate and individual traits was the negative relationship between precipitation in the warmest quarter and taxa with cold-cool eurythermal preference. We detected other strong relationships between the environment and traits. Baseflow, slope, and forested land cover were positively correlated with occurrences of erosional, cold-cool eurythermal, univoltine, and semivoltine taxa, and cultivated land cover was negatively correlated with the same traits. In general, we detected opposite relationships between these environmental variables and taxa with depositional or depositional-erosional traits, cool-warm eurythermal, warm eurythermal, and hot euthermal preferences, and bi-multivoltine life-histories. Occurrences of taxa with cold stenothermal preference were not strongly correlated with environmental variables.

Indicators of climate sensitivity in stream insects

We compared the climate sensitivity of stream insect diversity measures, individual traits, and trait profile groups (Fig. 25 in Appendix). Overall, individual traits were more sensitive to climate than trait profile groups or diversity measures. Among the diversity measures, genus richness and EPT genus richness responded more strongly to climatic predictors than functional richness or Rao's quadratic entropy. Among the traits, cold-cool eurythermal preference was the most sensitive to climate, followed by erosional flow preference and other rheophily traits, thermal preference, and voltinism traits. Trait profile groups were weakly responsive to climate.

Distributions of climate-sensitive taxa in US watersheds

More than half of highly sensitive insect genera are in the orders Plecoptera and Trichoptera according to sensitivities of individual traits, whereas sensitive genera according to trait profiles are primarily in the orders Coleoptera, Diptera, and Odonata (Table 21 in Appendix and Electronic Appendix 1). Approximately one third of insect genera within the orders Plecoptera and Trichoptera are highly sensitive to climate according to their individual traits, whereas the majority of Coleoptera and Odonata are highly sensitive according to their trait profiles (Table 22 in Appendix). Watersheds in the US with the highest proportions of climate-sensitive taxa according to individual traits are in the mountain west and Appalachian regions, whereas the highest proportions of sensitive taxa according to trait profiles are in the Southeast and Midwest (Fig. 26 in Appendix).

Discussion

Our objectives were to assess the sensitivity of individual insect genera to climate change and map distributions of sensitive taxa in watersheds across the contiguous US. We assessed climate sensitivity using traits and trait profile groups and found that the distributions of insect genera with erosional flow preference, cold-cool eurythermal preference, and univoltine life-histories were most sensitive to climate. This finding is congruent with our expectations based on previous, smaller-scale studies (Table 14 in Appendix). Our study is unique in providing the first evidence that these traits are strong indicators of climate sensitivity at a national scale and for a very large number (488) of insect genera. We also found that trait profile groups were poor indicators of climate sensitivity, contrary to suggestions that trait profiles could outperform individual traits in detecting relationships between geographic distributions of freshwater insects and the environment (Hamilton et al. 2020). Using our trait-based indicators of climate sensitivity, we found that insects in the orders Plecoptera and Trichoptera are likely the most sensitive to climate change, and watersheds with the highest proportions of sensitive taxa are found throughout the mountain West and Appalachian regions of the United States. We also examined relationships of freshwater insect diversity measures of genus richness, EPT genus richness, functional richness, and Rao's quadratic entropy, with climate and other watershed attributes across the contiguous United States. Overall, we found that spatial effects had a stronger influence on the geographic distribution of diversity measures than climate variables (Table 16, Fig. 22 in Appendix).

Relationships between climate and stream insect diversity

Genus richness and EPT richness showed stronger relationships with temperature and precipitation than functional richness and Rao's Q among models including climatic predictors of taxonomic and trait diversity (Figs. 22 and 23, Table 16 in Appendix). However, the best performing model for genus richness and functional richness included only spatial random effects (Table 15 in Appendix). Strong spatial effects could result from historical biogeography constraining regional patterns of genus and functional richness (Wiens and Donoghue 2004), and other studies have found similarly strong spatial effects at the extent of the contiguous US for other taxonomic groups (e.g., Read et al. 2020). Alternative models for genus richness and functional richness including temperature and precipitation performed nearly as well as the best performing models including only spatial random effects, and the best model for Rao’s quadratic entropy also included climate variables (Table 15 in Appendix). Temperature had a strong positive effect on all measures, but precipitation strongly affected only genus richness (Fig. 22, Table 16 in Appendix). Each of these diversity measures was relatively high in watersheds of the Southeast and West coasts where temperatures are warmer (Figs. 17 and 18 in Appendix). Positive correlations between temperature and taxonomic and functional diversity are common at broad spatial extents, because warmer regions receive more energy, fueling primary production that supports diverse niche opportunities and higher numbers of taxa (Hawkins et al. 2003). Lower temperatures in mountain streams could explain why diversity was relatively low in mountainous regions (McCain and Grytnes 2010). In addition, genus richness and functional richness were higher in watersheds with forested land cover (Figs. 17, 18, 22 in Appendix), consistent with a large body of research demonstrating the importance of forest cover for providing instream habitat, shading, and food resources for freshwater organisms (Naiman and Decamps 1997, Sponseller et al. 2001, Allan 2004).

The positive association of EPT genus richness with temperature at the continental scale is consistent with relationships detected at the extent of Europe and could explain the global pattern of decreasing EPT genus richness with latitude (Vinson and Hawkins 2003, Shah et al. 2015). Our findings that EPT richness was highest in mountainous regions of the US, and that richness increased with watershed slope and forested land cover are also consistent with these studies (Vinson and Hawkins 2003, Shah et al. 2015). Thus, at the continental scale, high EPT genus richness may be indicative of habitat conditions of mountain streams, including low human land use and cold, fast-flowing, well-oxygenated waters. Although the best-performing model for EPT genus richness included temperature and precipitation (Table 15 in Appendix), this diversity measure is likely not an effective indicator of watershed vulnerability to climate change at the extent of the contiguous US, because its positive correlation with maximum temperature of the warmest month suggests that warming could increase watershed EPT genus richness. Yet, previous research has projected that EPT genus richness will decrease at the extent of the contiguous US (Shah et al. 2014), and individual EPT genera will be locally extirpated (Pyne and Poff 2017) due to increasing temperature and altered stream flows. In addition, long-term studies have detected local declines in EPT richness due to increasing temperature and drought (Hamilton et al. 2010, Herbst et al. 2019), although one long-term study found equivocal responses of EPT richness to climate change (Lawrence et al. 2010). Occurrences of individual EPT genera may provide better indicators of climate change vulnerability for watersheds than EPT genus richness (see below). Although taxonomic and trait diversity measures reveal important relationships between stream insects and the environment, we did not consider them further as indicators of climate change vulnerability.

Relationships between climate and stream insect traits

Distributions of insect genera with erosional flow preference, cold-cool eurythermal preference, and univoltine life-histories were most strongly determined by climate (Fig. 24 in

Appendix). Many of the genera possessing these traits are distributed in mountainous watersheds of Western states (Fig. 19 in Appendix) that are relatively cool, receive little precipitation, and baseflow contributes the majority of their permanent stream flow (Fig. 17 in Appendix). Climate change in the Western US is expected to increase stream temperatures and alter habitat availability by advancing snowmelt and increasing summer stream drying (Barnett et al. 2008, Wu et al. 2012, Seager et al. 2013, Reynolds et al. 2015), which could directly affect distributions and survival of taxa with cool-cool eurythermal and erosional flow preferences (Chessman 2012). Climate change could also indirectly reduce their survival, because many of these taxa have gilled or tegument respiration (Fig. 20 in Appendix) requiring high levels of dissolved oxygen that are likely to decline with stream warming and drying. For these reasons, an earlier vulnerability assessment of freshwater insects in the Western US used rheophily and thermal preference to assess climate sensitivity (Poff et al. 2010). In addition, studies have already shown that drought shifts the trait composition of stream macroinvertebrate communities from taxa with cold thermal preference and erosional flow preference to taxa with warm eurythermal preference and depositional flow preference (Aspin et al. 2019, Herbst et al. 2019). Our findings are consistent with previous research and suggest that cold-cool eurythermal preference and erosional flow preference are good indicators of climate vulnerability for stream insects.

Relationships between climate and trait profile groups

Trait profile groups have been proposed as a metric for understanding how the environment shapes freshwater biodiversity and community composition, that could outperform individual traits by accounting for trait interrelationships (Poff et al. 2006, Verberk et al. 2013, Hamilton et al. 2020). Compared to individual traits, we found that distributions of trait profiles were less affected by the environment overall (compare coefficient estimates in figures 23 and 24 in Appendix), and less sensitive to climate (Fig. 25 in Appendix). Their relative insensitivity to environmental variables is likely due to their geographic distributions being more spatially dispersed than individual traits (Figs. 19 and 21 in Appendix). Previous studies have found stronger relationships between climate and trait profile groups (but see Pilière et al. 2016), but these did not use trait profiles for insects at a continental extent (Poff et al. 2010, Aspin et al. 2019). There are currently no guidelines for computing trait profiles, and although most studies use the traits of Poff et al. (2006), each study has used a different clustering method or assembled profiles based on expert knowledge, and computed numbers of profiles ranging from three (Poff et al. 2010) to eight or more (Verberk et al. 2008, Pilière et al. 2016, Aspin et al. 2019). The trait profiles that we computed are similar to the three trait profiles of Poff et al. (2010) that were indicative of streams with different thermal and flow regimes in the western US. Trait profile 1 is similar to their cold, stable trait group, and trait profiles 2 and 3 each have trait combinations overlapping with their warm, unstable group. These groups are widely distributed across the US (Poff et al. 2010). Our research indicates that these trait profiles are not as effective as individual traits for identifying climate sensitivity of stream insects at the watershed scale and at the extent of the contiguous US. They may be more effective for understanding climate vulnerability of stream insects at finer grains and smaller extents (e.g., Poff et al. 2010, Aspin et al. 2019). Standardizing the methodology for computing trait profiles would facilitate evaluations of their effectiveness for different applications.

Distributions of climate-sensitive taxa in US watersheds

Assigning sensitivity by rheophily, thermal preference, and voltinism traits suggests that the highest concentrations of climate-sensitive stream insects are in the Western US and the

Appalachian region (Fig. 26 in Appendix). Trait profiles suggest the opposite pattern, that the highest concentrations are in the Southeast and Midwest. Moreover, the individual traits indicate that sensitive genera are primarily in the Plecoptera and Trichoptera orders, whereas trait profiles indicate that sensitive taxa are in the Coleoptera, Diptera, and Odonata. These latter orders dominate trait profiles 2 and 3, which are similar to the warm, unstable trait group that Poff et al. (2010) suggested are resistant to population declines and range contractions with climate change because of their adaptations to warm, fluctuating environmental conditions. Moreover, climate vulnerability assessments for stream insects conducted in the mountainous regions of the US suggest that EPT taxa and those with cold thermal preference and erosional rheophily traits are most vulnerable to climate change (Hamilton et al. 2010, Poff et al. 2010, Pyne and Poff 2017). Therefore, individual rheophily and thermal preference traits provide reliable indicators of climate change vulnerability.

Moving forward: using climate-sensitive traits as indicators of climate change vulnerability

We assessed the climate-sensitivity of stream insect genera in watersheds across the contiguous US and showed that the distributions of insect genera with erosional flow preference, cold-cool eurythermal preference, and univoltine life-histories are most strongly affected by climate. Climate sensitive traits could be used to assess the vulnerability of individual taxa (see Electronic Appendx 1 for climate-sensitive genera) and incorporated into multi-metric indices for assessing climate change vulnerability of stream sites, similar to those already in use for assessing stream condition (e.g., Lawrence et al. 2010, Mazor et al. 2016). For example, many multi-metric indices incorporate the percentage of shredder taxa in assessment of ecosystem condition, and percentage of erosional and cold-cool eurythermal taxa could similarly be incorporated as indicators of climate change vulnerability. Moreover, the percentage of climate-sensitive genera (Electronic Appendix 1) could be incorporated into metrics, similar to the percentages of EPT taxa and intolerant taxa (Mazor et al. 2016).

Using our trait-based indicators of climate sensitivity, we found that insects in the orders Plecoptera and Trichoptera are likely the most sensitive to climate change. Insect species within these orders should be prioritized for protection in order to avoid population extirpations and species extinctions with climate change. Our trait-based indicators of climate sensitivity also showed that watersheds with the highest proportions of sensitive taxa are throughout the mountain West and Appalachian regions of the United States. These regions should be prioritized for further research and protection of their watersheds to protect against losing freshwater biodiversity due to climate change. By providing biological indicators of climate change vulnerability, our research enhances efforts by scientists and managers to prioritize watersheds and taxa for restoration and conservation in the United States.

APPENDIX

Table 14. Hypothesized relationships between climate and other watershed attributes and stream insect taxonomic and trait indicators.

Env. variable	Env. state	Taxonomic and trait diversity	Responsive trait groups	Dominant traits	Evidence
Temperature	High	High or low	Rheophily, Thermal preference, Voltinism	Depositional, Cool-warm eurythermal (5 - 30 ºC), Warm eurythermal (15 - 30 ºC), Bi-multivoltine (> 1 generation per year)	Vinson and Hawkins 1998, Bonada et al. 2007, Poff et al. 2010, Lawrence et al. 2010, Domisch et al. 2011, Chessman 2012, Kuemmerlen et al. 2015
Precipitation	High	High	Rheophily, Thermal preference, Voltinism	Erosional, Depositional-erosional, Cool eurythermal (0 - 15 ºC), Semivoltine (< 1 generation per year)	Chessman 2012, Aspin et al. 2019, Herbst et al. 2019
Baseflow	High	High	Rheophily, Thermal preference, Voltinism	Erosional, Depositional-erosional, Cold stenothermal (< 5 ºC) or cool eurythermal (0 - 15 ºC), Univoltine (1 generation per year), Semivoltine (< 1 generation per year)	Poff et al. 2010, Aspin et al. 2019, Herbst et al. 2019
Elevation	High	Low	Thermal preference, Voltinism	Cold stenothermal (< 5 ºC) or cool eurythermal (0 - 15 ºC), Univoltine (1 generation per year) or Semivoltine (< 1 generation per year)	Rahbek 1995, Vinson and Hawkins 2003, Díaz et al. 2007
Cultivated land cover	High	Low	Thermal preference, Voltinism	Warm eurythermal (15 - 30 ºC), Bi-multivoltine (> 1 generation per year)	Díaz et al. 2007, Dolédec et al. 2006, Dolédec et al. 2011, Kuemmerlen et al. 2015, Zuellig and Schmidt 2012
Forested land cover	High	High	Thermal preference, Voltinism	Warm eurythermal (15 - 30 ºC), Bi-multivoltine (> 1 generation per year)	Vinson and Hawkins 2003, Zuellig and Schmidt 2012,Twardochleb and Olden 2016

Table 15. Results of spatial model selection. Models in bold are the best model according to the deviance information criterion (DIC) and widely applicable information criterion (WAIC). Models with lower DIC and WAIC scores are considered more parsimonious. The number of effective parameters for each model is the p.d. (DIC) or p.w. (WAIC). Underlined models were those used in further analyses comparing climate sensitivity of different biodiversity and trait indicators. Ephemeroptera, Plecoptera, and Trichoptera richness is abbreviated as EPT richness, and Rao's quadratic entropy as Rao's Q. The full model included a spatial autocorrelation parameter and the following fixed effects parameters: climate (temperature and precipitation), baseflow, slope, barren land cover, cultivated land cover, forested land cover, and wetland cover, percent intermittent stream length, and an interaction term between temperature and forested land cover.

Biodiversity measure	Model	DIC	p.d.	WAIC	p.w.
Genus richness	Full	-226.51	85.84	-205.72	83.24
Genus richness	Climate + baseflow + slope + cultivated + forest + space	-248.41	95.41	-233.05	85.20
Genus richness	Climate + slope + space	-253.65	99.01	-241.40	85.23
Genus richness	Climate + space	-266.17	104.92	-257.80	86.26
Genus richness	Temperature + space	-265.85	103.58	-255.72	86.70
Genus richness	**Space**	**-269.67**	**105.83**	**-262.35**	**86.04**
EPT richness	Full	-221.71	70.11	-194.16	78.69
EPT richness	Climate + baseflow + slope + cultivated + forest + space	-249.34	85.98	-229.39	82.60
EPT richness	Climate + baseflow + forest + space	-251.19	88.97	-233.59	82.63
EPT richness	Baseflow + forest + space	-264.46	95.91	-251.98	83.38
EPT richness	**Climate + space**	**-266.72**	**105.58**	**-258.29**	**86.88**
EPT richness	Temperature + space	-265.18	106.65	-258.11	78.14
EPT richness	Space	-262.68	106.09	-255.51	86.25
Functional richness	Full	-363.27	60.56	-358.20	53.38
Functional richness	Climate + baseflow + slope + cultivated + forest + space	-365.64	58.80	-360.72	52.15
Functional richness	Climate + slope + cultivated + forest + space	-362.25	55.93	-357.27	50.26
Functional richness	Temperature + slope + cultivated + forest + space	-366.21	59.37	-360.83	52.86
Functional richness	Temperature + slope + forest + space	-369.77	64.00	-365.91	54.71
Functional richness	Climate + space	-369.31	66.92	-365.07	57.01
Functional richness	Temperature + space	-368.49	65.11	-364.68	55.49

Table 15. (cont'd)

Functional richness	**Space**	**-371.57**	**68.73**	**-368.26**	**57.50**
Rao's Q	Full	-668.12	58.91	-677.00	40.22
Rao's Q	Climate + baseflow + slope + cultivated + forest + space	-669.22	56.94	-677.96	39.03
Rao's Q	**Climate + slope + space**	**-669.99**	**62.49**	**-680.80**	**41.39**
Rao's Q	Temperature + slope + space	-668.63	60.72	-679.05	40.49
Rao's Q	Climate + space	-644.99	65.81	-656.54	43.29
Rao's Q	Temperature + space	-646.30	65.49	-657.84	43.08
Rao's Q	Space	-631.74	71.99	-644.12	46.96

Table 16. Parameter values for reduced spatial models. Posterior medians, and lower (2.5%) and upper (97.5%) 95% credible intervals for each parameter. The term 'space' refers to the spatial autocorrelation parameter. Other parameters are fixed effects.

Biodiversity measure	Parameter	Median	2.5%	97.5%
Genus richness	Intercept	0.5082	0.4921	0.5248
	Temperature	0.0837	0.0259	0.1359
	Precipitation	-0.0718	-0.1385	-0.0020
	Baseflow	0.0318	-0.0146	0.0783
	Slope	-0.0837	-0.1335	-0.0345
	Cultivated land	0.0316	-0.0208	0.0831
	Forested land	0.1482	0.0819	0.2143
	Space	0.4895	0.1952	0.8697
EPT richness	Intercept	0.5226	0.5063	0.5388
	Temperature	0.0538	0.0042	0.1034
	Precipitation	-0.0837	-0.1406	-0.0229
	Baseflow	0.0826	0.0397	0.1256
	Slope	-0.0446	-0.0905	0.0008
	Cultivated land	0.0246	-0.0211	0.0694
	Forested land	0.1857	0.1256	0.2437
	Space	0.3666	0.1167	0.7794
Functional richness	Intercept	0.195	0.1841	0.2061
	Temperature	0.0460	0.0210	0.0705
	Precipitation	-0.0228	-0.0519	0.0065
	Baseflow	0.0147	-0.0073	0.0367
	Slope	-0.0347	-0.0588	-0.0109
	Cultivated land	0.0316	0.0074	0.0549
	Forested land	0.0591	0.0294	0.0884
	Space	0.4919	0.1159	0.9072
Rao's Q	Intercept	0.8408	0.8362	0.8454
	Temperature	0.0133	0.0025	0.0239
	Precipitation	0.0144	-0.0007	0.0286
	Baseflow	-0.0112	-0.0202	-0.0024
	Slope	-0.0168	-0.0269	-0.0066
	Cultivated land	0.0055	-0.0047	0.0156
	Forested land	-0.0066	-0.0196	0.0065
	Space	0.8706	0.5723	0.9875

Table 17. Fourth-corner analysis results for trait profile groups. The column 'r' provides the correlation coefficient between each watershed environmental variable and the trait profile group and 'r std. error' is the standard error of the correlation coefficient. The 'P-value' is the probability value for the correlation coefficient before adjustment for multiple pairwise comparisons, and 'P-value adj.' is the P-value adjusted for multiple comparisons.

Trait profile	Environmental variable	r	r std. error	P-value	P-value adj.
1	Temperature	-0.001	-0.100	0.982	1
1	Precipitation	-0.008	-1.635	0.091	0.637
1	Baseflow	0.018	3.387	0.001	0.018
1	Cultivated land	-0.024	-4.832	0.001	0.018
1	Forested land	0.013	2.479	0.012	0.108
1	Slope	0.022	4.424	0.001	0.018
2	Temperature	0.014	2.698	0.006	0.072
2	Precipitation	0.004	0.701	0.483	1
2	Baseflow	-0.021	-4.143	0.001	0.018
2	Cultivated land	0.012	2.291	0.021	0.168
2	Forested land	-0.014	-2.823	0.010	0.1
2	Slope	-0.017	-3.414	0.001	0.018
3	Temperature	-0.012	-2.665	0.006	0.072
3	Precipitation	0.006	1.127	0.260	1
3	Baseflow	0.001	0.190	0.837	1
3	Cultivated land	0.016	3.230	0.003	0.039
3	Forested land	0.000	-0.080	0.947	1
3	Slope	-0.008	-1.675	0.099	0.637

Table 18. Fourth-corner analysis results for rheophily (flow preference) traits.

Trait	Environmental variable	r	r std. error	P-value	P-value adj.
Depositional	Temperature	0.093	4.371	0.001	0.066
Depositional	Precipitation	0.102	5.108	0.001	0.066
Depositional	Baseflow	-0.133	-6.460	0.001	0.066
Depositional	Cultivated land	0.156	7.590	0.001	0.066
Depositional	Forested land	-0.130	-5.940	0.001	0.066
Depositional	Slope	-0.165	-8.160	0.001	0.066
Depositional-erosional	Temperature	0.039	4.550	0.001	0.066
Depositional-erosional	Precipitation	0.055	6.463	0.001	0.066
Depositional-erosional	Baseflow	-0.044	-5.081	0.001	0.066
Depositional-erosional	Cultivated land	0.0184	2.276	0.02	0.360
Depositional-erosional	Forested land	0.002	0.192	0.856	1
Depositional-erosional	Slope	-0.048	-5.588	0.001	0.066
Erosional	Temperature	-0.112	-5.165	0.001	0.066
Erosional	Precipitation	-0.135	-6.580	0.001	0.066
Erosional	Baseflow	0.148	6.963	0.001	0.066
Erosional	Cultivated land	-0.141	-6.797	0.001	0.066
Erosional	Forested land	0.100	4.544	0.001	0.066
Erosional	Slope	0.177	8.615	0.001	0.066

Table 19. Fourth-corner analysis results for thermal preference traits.

Trait	Environmental variable	r	r std. error	P-value	P-value adj.
Cold stenothermal (< 5 ºC)	Temperature	0.020	2.859	0.005	0.105
Cold stenothermal (< 5 ºC)	Precipitation	-0.028	-4.031	0.001	0.066
Cold stenothermal (< 5 ºC)	Baseflow	-0.002	-0.258	0.81	1
Cold stenothermal (< 5 ºC)	Cultivated land	-0.023	-3.023	0.002	0.066
Cold stenothermal (< 5 ºC)	Forested land	-0.009	-1.321	0.186	1
Cold stenothermal (< 5 ºC)	Slope	0.011	1.549	0.123	1
Cold-cool eurythermal (0 - 15 ºC)	Temperature	-0.097	-3.959	0.001	0.066
Cold-cool eurythermal (0 - 15 ºC)	Precipitation	-0.2016	-8.320	0.001	0.066
Cold-cool eurythermal (0 - 15 ºC)	Baseflow	0.179	7.290	0.001	0.066
Cold-cool eurythermal (0 - 15 ºC)	Cultivated land	-0.150	-6.088	0.001	0.066
Cold-cool eurythermal (0 - 15 ºC)	Forested land	0.056	2.232	0.025	0.391
Cold-cool eurythermal (0 - 15 ºC)	Slope	0.220	8.680	0.001	0.066
Cool-warm eurythermal (5 - 30 ºC)	Temperature	-0.003	-0.254	0.799	1
Cool-warm eurythermal (5 - 30 ºC)	Precipitation	0.114	9.137	0.001	0.066
Cool-warm eurythermal (5 - 30 ºC)	Baseflow	-0.065	-4.963	0.001	0.066
Cool-warm eurythermal (5 - 30 ºC)	Cultivated land	0.045	3.650	0.001	0.066
Cool-warm eurythermal (5 - 30 ºC)	Forested land	0.058	4.697	0.001	0.066
Cool-warm eurythermal (5 - 30 ºC)	Slope	-0.070	-5.229	0.001	0.066
Warm eurythermal (15 - 30 ºC)	Temperature	0.078	5.071	0.001	0.066
Warm eurythermal (15 - 30 ºC)	Precipitation	0.056	3.726	0.001	0.066
Warm eurythermal (15 - 30 ºC)	Baseflow	-0.083	-5.604	0.001	0.066
Warm eurythermal (15 - 30 ºC)	Cultivated land	0.084	5.527	0.001	0.066
Warm eurythermal (15 - 30 ºC)	Forested land	-0.104	-6.495	0.001	0.066
Warm eurythermal (15 - 30 ºC)	Slope	-0.115	-7.533	0.001	0.066
Hot euthermal (> 30 ºC)	Temperature	0.045	4.643	0.001	0.066
Hot euthermal (> 30 ºC)	Precipitation	0.048	5.131	0.001	0.066
Hot euthermal (> 30 ºC)	Baseflow	-0.042	-4.311	0.001	0.066
Hot euthermal (> 30 ºC)	Cultivated land	0.036	3.795	0.001	0.066
Hot euthermal (> 30 ºC)	Forested land	-0.037	-3.750	0.001	0.066
Hot euthermal (> 30 ºC)	Slope	-0.054	-5.791	0.001	0.066

Table 20. Fourth-corner analysis results for voltinism (number of generations per year). Bi-multivoltine is two or more generations per year, univoltine is one generation per year, and semivoltine is fewer than one generation per year.

Trait	Environmental variable	r	r std. error	P-value	P-value adj.
Bi-multivoltine	Temperature	0.027	2.073	0.038	0.456
Bi-multivoltine	Precipitation	-0.018	-1.461	0.147	1
Bi-multivoltine	Baseflow	-0.028	-2.301	0.031	0.403
Bi-multivoltine	Cultivated land	0.028	2.313	0.023	0.391
Bi-multivoltine	Forested land	-0.079	-6.213	0.001	0.066
Bi-multivoltine	Slope	-0.038	-2.981	0.004	0.088
Univoltine	Temperature	-0.024	-2.532	0.016	0.304
Univoltine	Precipitation	0.024	2.723	0.007	0.140
Univoltine	Baseflow	0.016	1.706	0.088	0.968
Univoltine	Cultivated land	-0.019	-2.161	0.027	0.391
Univoltine	Forested land	0.058	6.337	0.001	0.066
Univoltine	Slope	0.014	1.528	0.132	1
Semivoltine	Temperature	-0.004	-0.477	0.649	1
Semivoltine	Precipitation	-0.010	-1.188	0.235	1
Semivoltine	Baseflow	0.019	2.315	0.023	0.391
Semivoltine	Cultivated land	-0.013	-1.560	0.121	1
Semivoltine	Forested land	0.031	3.582	0.001	0.066
Semivoltine	Slope	0.035	4.086	0.001	0.066

Table 21. Proportion of climate-sensitive genera that are in each insect order according to individual traits and trait profile groups.

Order	<u>Traits</u>	<u>Trait profiles</u>
	Proportion of sensitive genera	Proportion of sensitive genera
Coleoptera	0.115	0.201
Diptera	0.159	0.345
Ephemeroptera	0.097	0.063
Hemiptera	0.053	0.075
Lepidoptera	0.000	0.000
Megaloptera	0.000	0.005
Neuroptera	0.000	0.003
Odonata	0.027	0.155
Plecoptera	0.257	0.043
Trichoptera	0.292	0.109

Table 22. Proportion of genera in each insect order that are climate-sensitive according to individual traits and trait profile groups.

Order	Traits Proportion highly sensitive	Trait profiles Proportion highly sensitive
Coleoptera	0.224	0.714
Diptera	0.138	0.632
Ephemeroptera	0.157	0.259
Hemiptera	0.231	0.634
Lepidoptera	0.000	0.000
Megaloptera	0.000	0.400
Neuroptera	0.000	0.500
Odonata	0.060	0.871
Plecoptera	0.330	0.167
Trichoptera	0.303	0.295

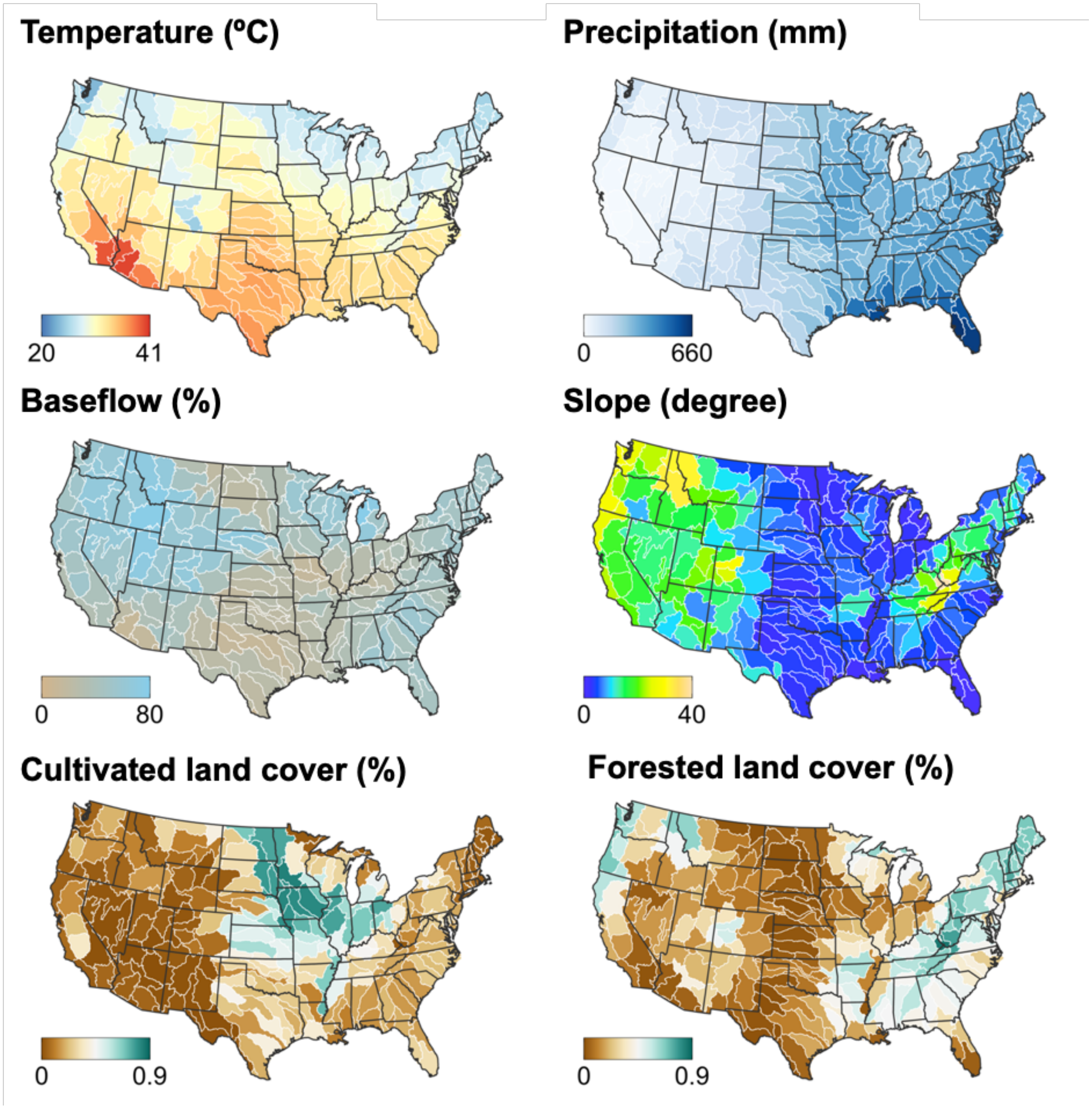


Figure 17. Environmental variables summarized by watershed. These include mean of the maximum temperature of the warmest month (ºC) and mean precipitation of the warmest quarter (mm) for 2001-2018 (PRISM 2018), baseflow mean (percent) for 1951-1980 (USGS 2003), slope mean (degree) (NED, USGS 1999), and mean cultivated land cover (percent) and mean forested land cover (percent) for 2001, 2004, 2006, 2008, 2011, 2013, and 2016 (NLCD).

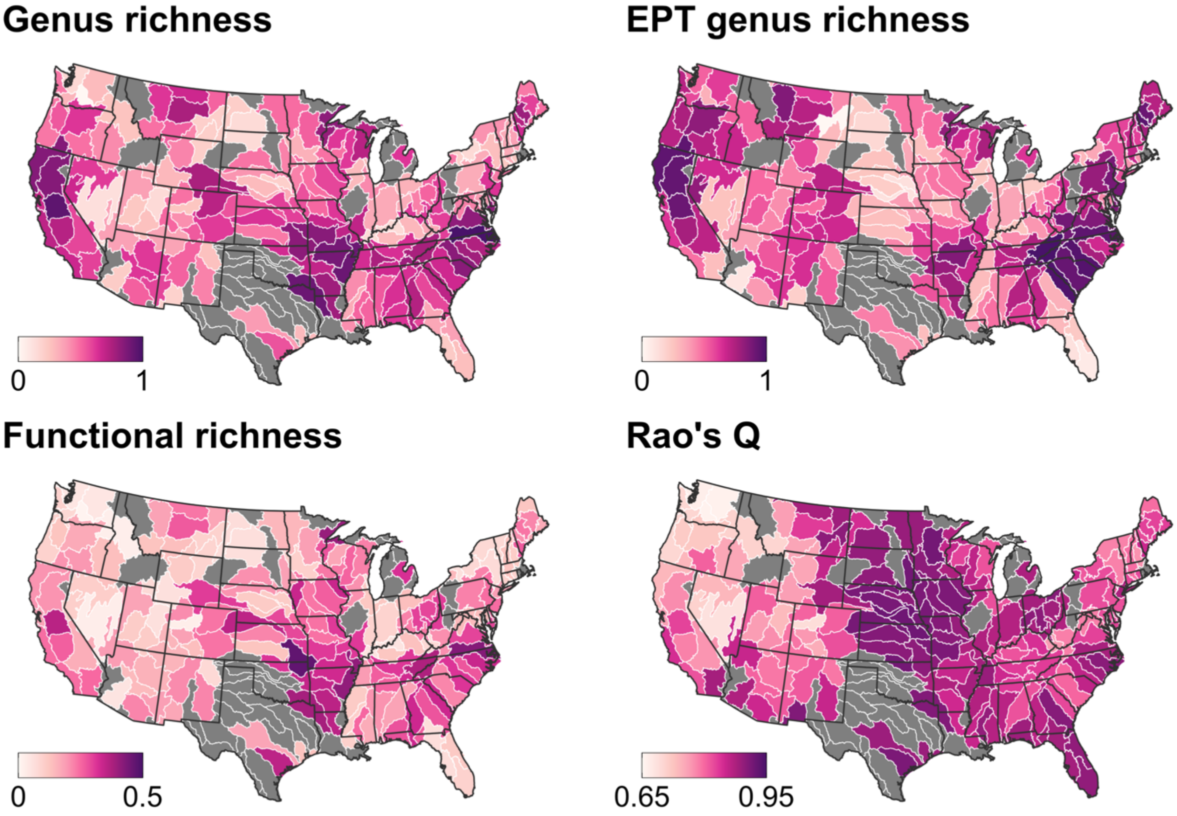
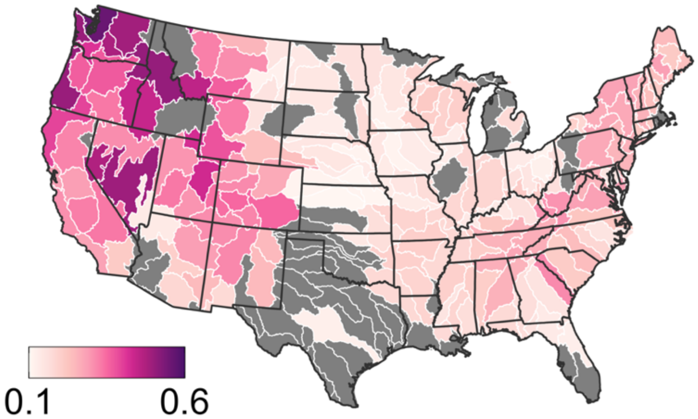
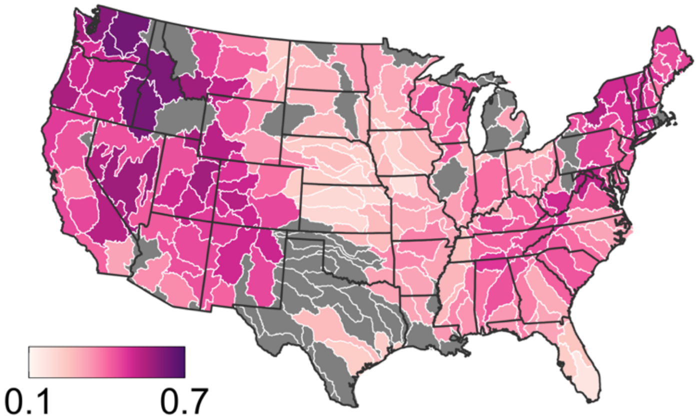


Figure 18. Freshwater insect diversity measures summarized by watershed. Measures have been scaled to facilitate comparison.

Cold-cool eurythermal



Erosional



Univoltine

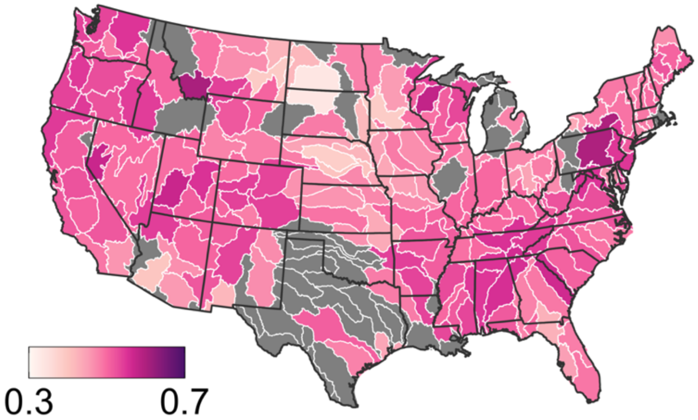


Figure 19. Proportion of genera in each watershed with climate-sensitive traits.

Figure 19. (cont'd)

These include cold-cool eurythermal preference (0-15 ºC), associations with erosional habitats (rheophily), and univoltine life-history (one generation per year).

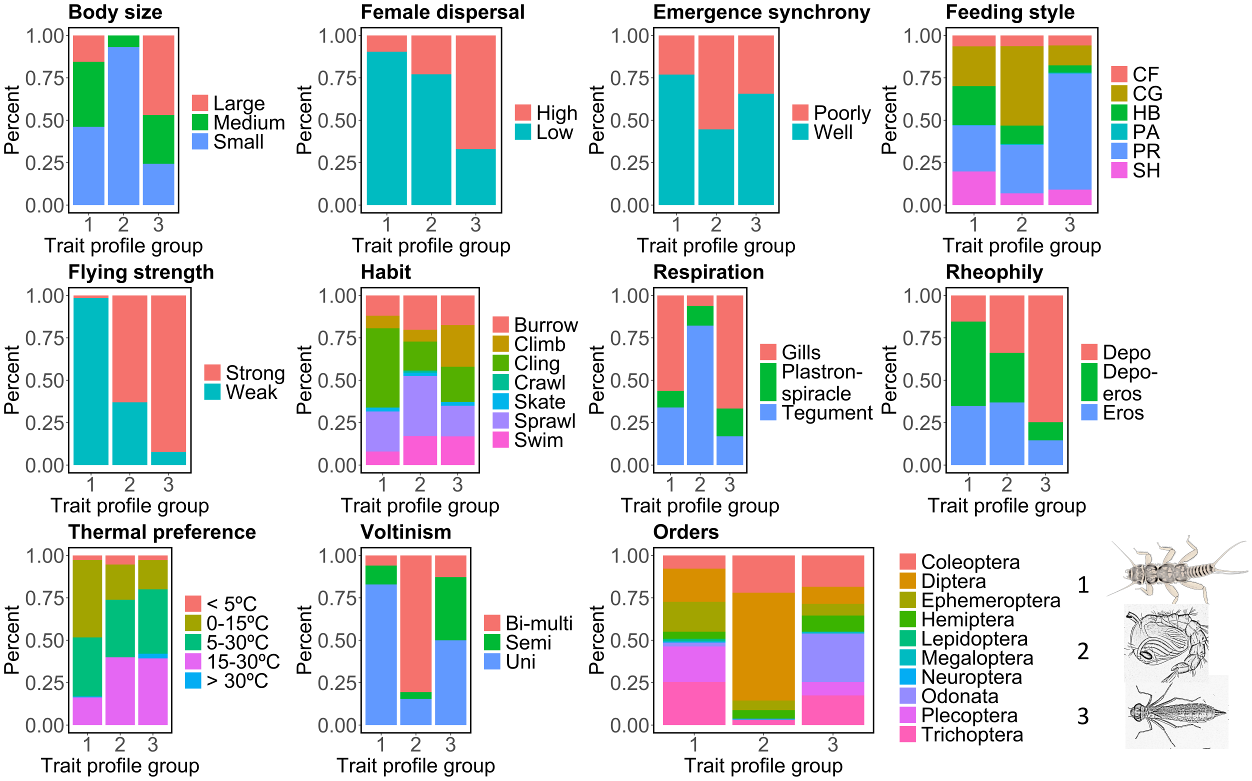


Figure 20. Composition of each trait profile group. The first ten panels provide the trait composition, and the final panel gives the composition of insect orders. Representative genera for each profile are: 1) Doroneuria (Plecoptera), 2) Aedes (Diptera), and 3) Anax (Odonata). Trait abbreviations are: Feeding style, CF=collector-filterer, CG=Collector-gatherer, HB=herbivore, PA=parasite, PR=predator, SH=shredder; Rheophily, Depo=depositional, Depo-eros=depositional-erosional, Eros=erosional; Voltinism, Bi-multi=bi-multivoltine (>1 generation per year), Uni=univoltine (1 generation per year), Semi=semivoltine (<1 generation per year).

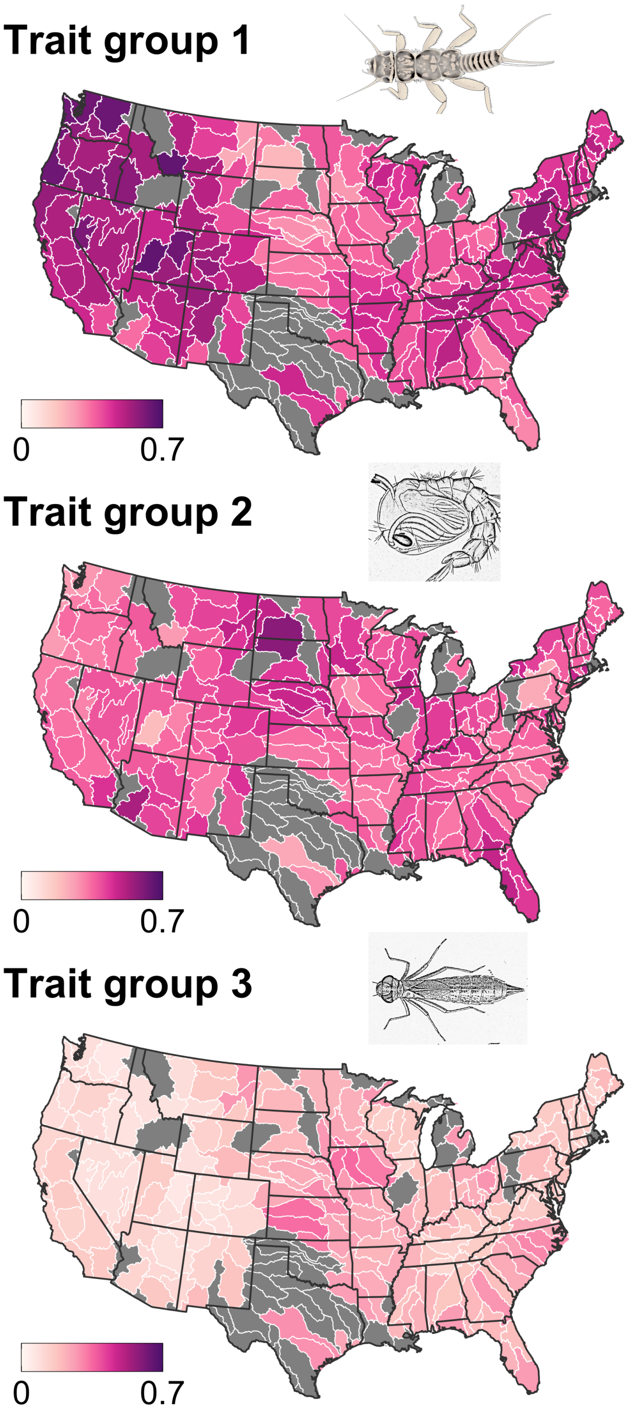


Figure 21. Proportion of genera in each watershed belonging to each trait profile group. Trait profile 1 is composed primarily of genera in the EPT and Diptera orders with small to medium juvenile body sizes, depositional and depositional-erosional rheophily traits, cold-cool eurythermal (0 - 15 ºC) to cool-warm eurythermal (5 - 30 ºC) preferences, and univoltine life-

Figure 21. (cont'd) histories. Trait profile 2 is dominated by genera in the orders Diptera and Coleoptera. These taxa have primarily small body sizes, mixed rheophily, primarily cool-warm eurythermal (5 - 30 ºC) and warm eurythermal (15 - 30 ºC) preferences, and bi-multivoltine life-histories. Trait profile 3 is representative of taxa in the orders Coleoptera, Diptera, and Odonata that have primarily medium to large body sizes, depositional rheophily, cool-warm eurythermal (5 - 30 ºC) to warm eurythermal (15 - 30 ºC) preference, and univoltine and semivoltine life-histories.

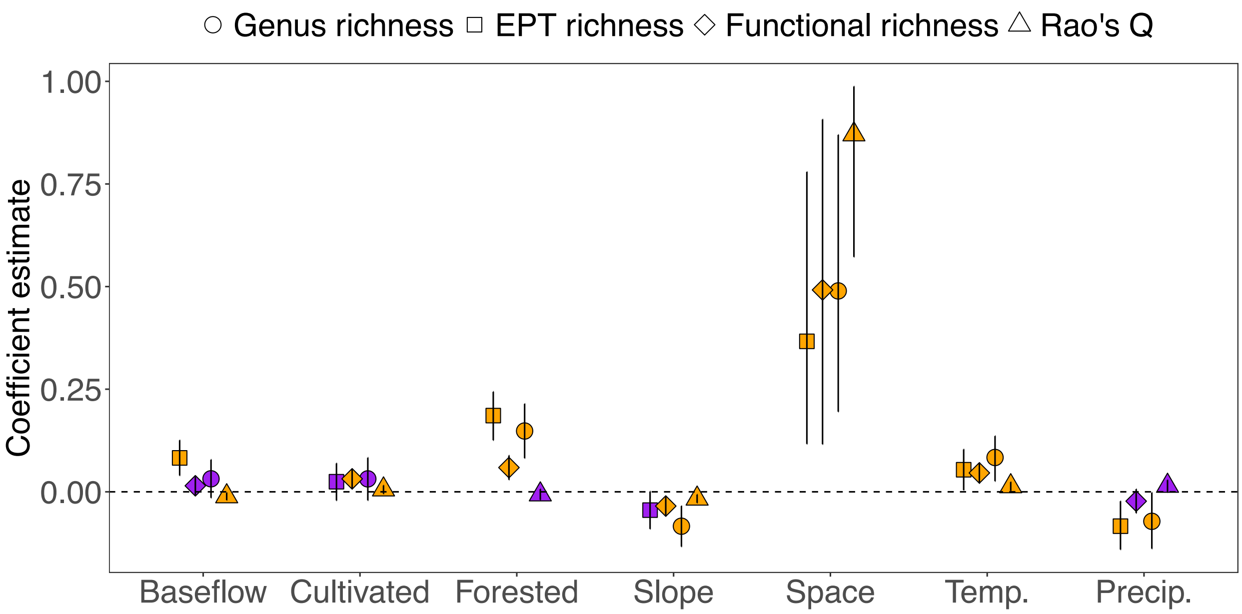


Figure 22. Coefficient estimate (median ± 95% credible intervals) for each predictor variable in spatial models with freshwater insect diversity measures. Orange points indicate coefficients with 95% credible intervals not overlapping zero.

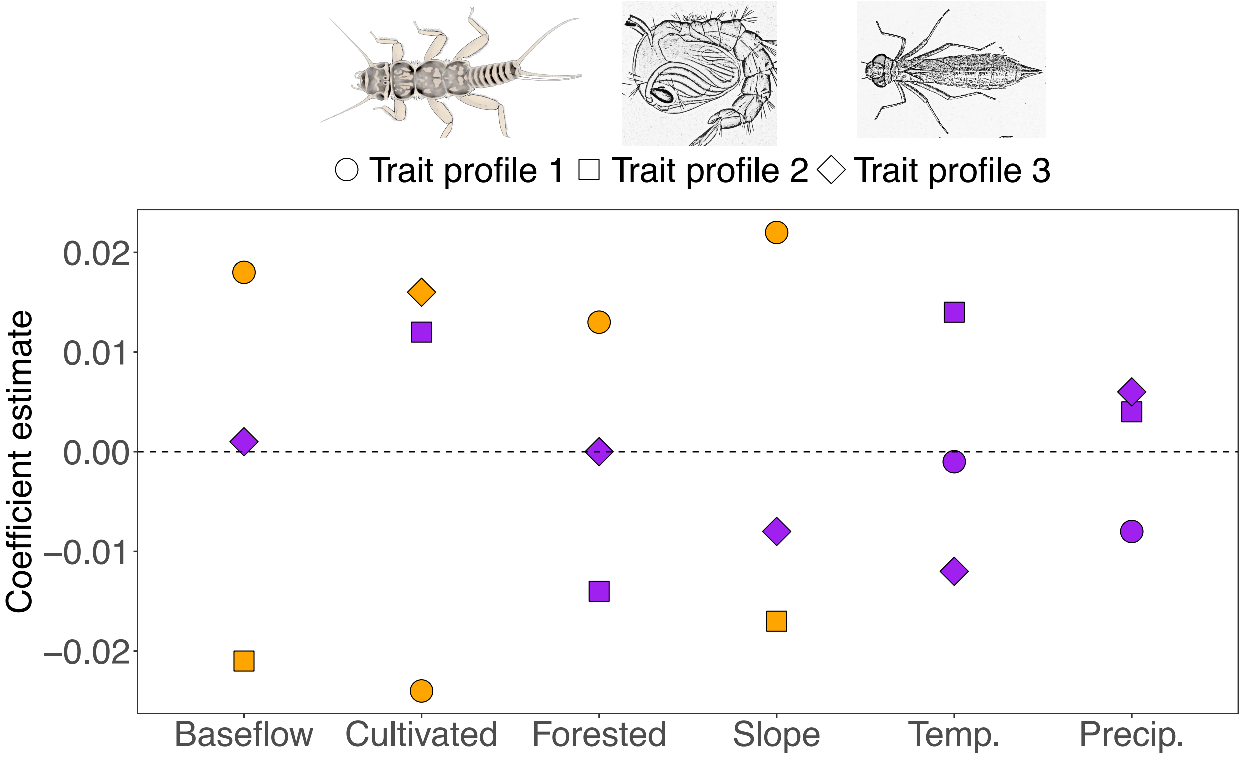


Figure 23. Correlation coefficients for predictor variables from fourth corner analysis with trait profile groups. Orange points indicate coefficients that were significant after adjustment of p-values. Purple points indicate coefficients that are not significant. Error bars are omitted for clarity.

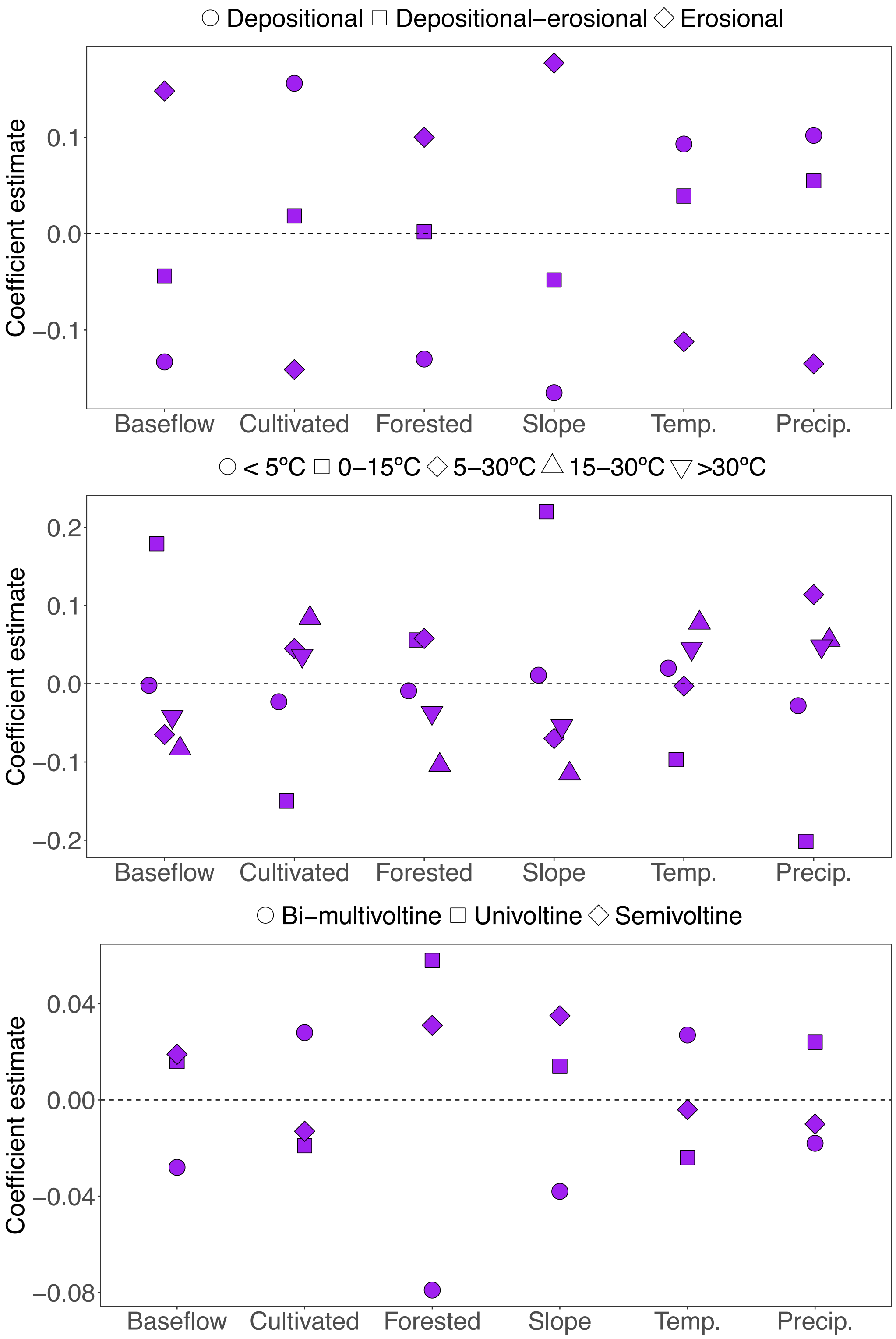


Figure 24. Correlation coefficients for predictor variables from fourth corner analysis with individual traits. From top to bottom: rheophily, thermal preference, and voltinism (number of generations per year). Bi-multivoltine is two or more generations per year, univoltine is one

Figure 24. (cont'd) generation per year, and semivoltine is fewer than one generation per year. Error bars are omitted for clarity.

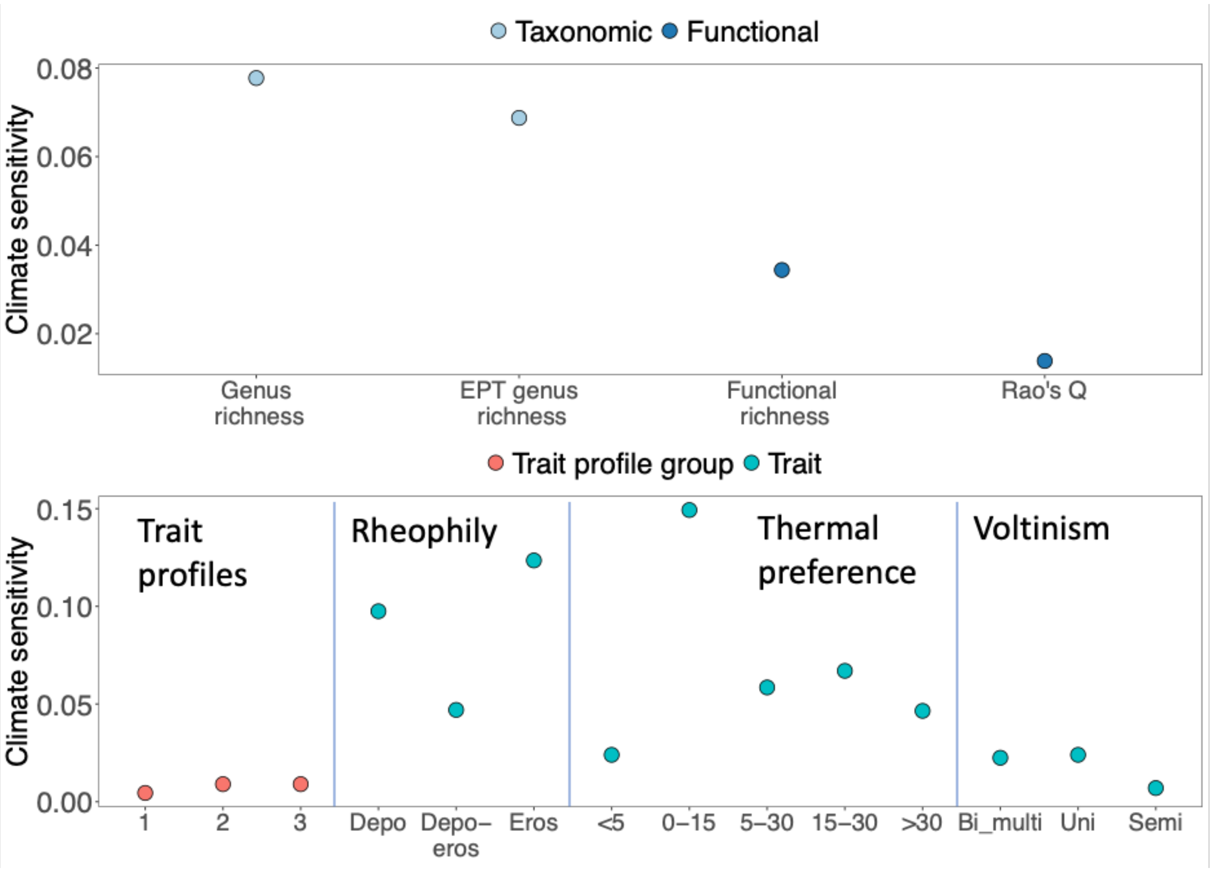


Figure 25. Mean climate sensitivity score for insect diversity measures (top) and traits and trait profile groups. Trait abbreviations are: rheophily, Depo=depositional, Depo-eros=depositional-erosional, Eros=erosional; Voltinism, Bi-multi=bi-multivoltine (>1 generation per year), Uni=univoltine (1 generation per year), Semi=semivoltine (<1 generation per year).

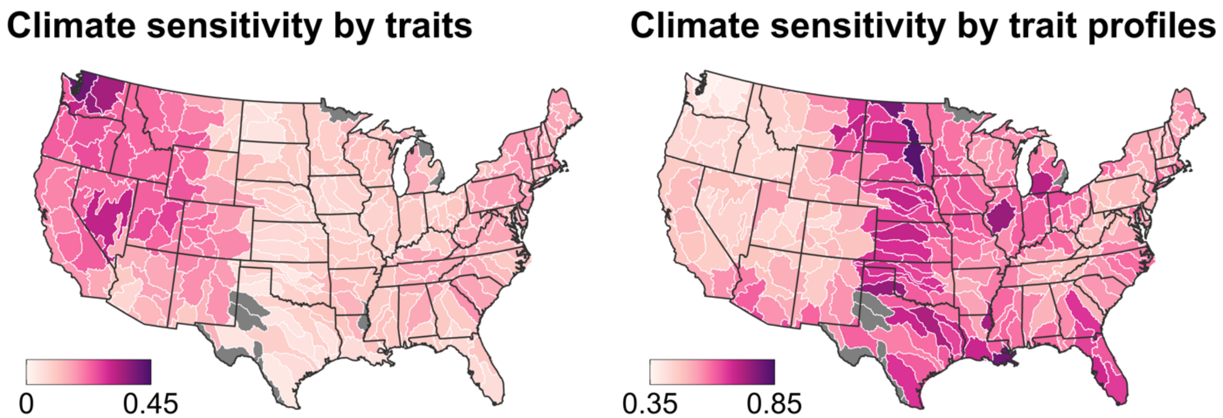


Figure 26. Proportion of genera in each watershed classified as highly sensitivity to climate. Climate sensitivity is calculated according to sensitivity of each genus' rheophily, thermal preference, and voltinism traits (left) or trait profile groups (right).

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CHAPTER 5. Conclusions, and recommendations for managers and conservation practitioners

Introduction

I addressed two, important knowledge gaps that have hindered efforts to conserve freshwater biodiversity in a changing climate, including understanding 1) physiological and demographic consequences of increasing temperatures for freshwater organisms and 2) relationships between climate and freshwater organisms across large geographic areas. In this chapter, I briefly describe the need for research addressing these two knowledge gaps and how each of my dissertation chapters advances our knowledge of these topics for freshwater insects. I also provide recommendations for how managers and conservation practitioners can use the results of my research to prioritize freshwater organisms and watersheds for protection in a changing climate.

1) Quantifying physiological and demographic consequences of increasing temperatures was identified as a key knowledge gap in recent frameworks for assessing the effects of climate change on species (Foden and Young 2016, Paukert et al. 2017). In particular, we need research demonstrating how climate change will alter individual physiology through changes to biological rates of metabolism, feeding, growth, and reproduction, to accurately forecast changes in populations and geographic distributions of freshwater organisms (Ficke et al. 2007, Paukert et al. 2017). We can use relationships between climate and physiology to forecast changes in populations with physiologically structured population models (De Roos and Persson 2001), and changes in geographic distributions using mechanistic species distribution models (Kearney and Porter 2009). Predictions from such models inform conservation and management of freshwater organisms by identifying species that are likely to experience population declines, geographic range contractions, or extinctions (Foden and Young 2016). Those species can then be prioritized for protection and further research.

For many freshwater organisms, we still lack knowledge of key physiological responses to temperature that are needed to forecast changes in populations and geographic distributions with climate change (Dell et al. 2011, Whitney et al. 2016). These include upper thermal tolerance limits and thermal performance curves for biological rates such as metabolism, swimming speed, and feeding and growth rates (Deutsch et al. 2008, Dell et al. 2011). We can fill these knowledge gaps by conducting experiments to understand physiological changes with increasing temperature for a large number of freshwater organisms. Knowing that we cannot measure physiological responses of all species, we can also utilize trait-based approaches for prediction. For example, we can predict responses of species to climate change by grouping them into thermal tolerance trait categories (Poff et al. 2010) or thermal guilds (Comte and Grenouillet 2013). Either approach has its advantages and disadvantages. Using detailed thermal relationships from experiments can provide more precise predictions of demographic responses to climate change than trait-based approaches, but these experimental data are more difficult to obtain (Foden and Young 2016). Alternatively, trait-based approaches can help us generalize physiological and population responses of organisms with similar traits without directly measuring their thermal responses (Foden et al. 2013, Pacifici et al. 2017, 2018). When possible, we should utilize these complementary approaches to identify freshwater species to prioritize for conservation in a changing climate (Pacifici et al. 2015, Foden and Young 2016).

With my research, I used detailed experiments and a trait-based approach to understand physiological and demographic consequences of increasing temperature for freshwater organisms. In Chapter 1 (Twardochleb et al. 2020), I investigated how changes in biological rates with temperature are mediated by foraging traits of freshwater insects. These foraging traits are ubiquitous and include active predation whereby predators patrol for prey (e.g., sharks) and sit-and-wait, where predators remain in a fixed location and attack prey that move within their pursuit distance (e.g., snakes) (Preisser et al. 2007). Using a series of experiments across a temperature gradient of 10 to 35 ºC, I demonstrated that for two predatory freshwater insects, the sit-and-wait damselfly *Enallagma annexum* and the active backswimmer *Notonecta undulata*, metabolism, feeding, and growth rates increased with warming when predators consumed crustacean zooplankton prey *Daphia pulex*. In particular, feeding rates more than doubled for both predator species between 10 and 35 ºC.

Despite apparent similarities in their responses to temperature, the active backswimmer may be more likely than the sit-and-wait damselfly to starve as a result of climate warming if prey populations decline (Twardochleb et al. 2020). Because the backswimmer has a higher metabolic rate due to its active feeding style, it has to eat more prey than the sit-and-wait damselfly. Warming further increases the active predator’s hunger, requiring that it eat even more prey to avoid starvation. By contrast, the sit-and-wait damselfly uses little energy to capture prey and may be able to withstand periods of low prey availability even at high temperatures. More research is needed that investigates how active vs. sit-and-wait foraging traits mediate physiological and demographic responses to temperature for freshwater organisms living in environments with variable prey availability. If sit-and-wait predators generally outperform active predators in warm environments with low prey availability, then these predators could proliferate with climate warming in environments where prey populations have declined. With future research, we may be able to categorize predator species according to their feeding traits and predict where their populations will increase or decline with climate warming.

In Chapter 2, I used physiological data from experiments in Chapter 1 (Twardochleb et al. 2020) to parameterize a physiologically structured population model and predict the population-level consequences of climate warming for the predatory damselfly *Enallagma annexum* consuming zooplankton *Daphnia pulex*. I used this model to simulate how climate warming in a seasonal environment could alter a key life-history trait of the damselfly, generation time, and how changes in this trait mediated responses of predator-prey population sizes and predator persistence to increasing environmental temperature. I found that with warming, the damselfly population size increased due to higher feeding and growth rates, and a longer growing season, that enabled damselflies to emerge faster to the adult stage and reproduce more times per year. These life-history changes prevented extinction of the damselfly population at very high temperatures. Overall, the results of Chapters 1 and 2 demonstrate that by increasing predator feeding and growth rates with temperature, climate warming can result in shorter generation times and larger population sizes of predators. Species that decrease their generation times in response to warming may benefit from climate change with larger populations and reduced extinction risks. Damselflies and dragonflies have generation times that vary with latitude (Corbet et al. 2006), and thus more research is needed to understand how these and other freshwater organisms with variable generation times respond to climate change throughout their geographic range. Such knowledge could help us better identify the geographic locations where species are vulnerable to extinction or are likely to benefit from climate change.

2) Identifying relationships between climate and freshwater organisms across large geographic areas will improve our understanding of which species are most *vulnerable* to range contraction and extinction and which regions will likely lose freshwater biodiversity (Heino 2009). Such studies have already shown promise for identifying climate change-induced range shifts (Hickling et al. 2006), regional changes in species traits (Bonada et al. 2007), and regions that are susceptible to biodiversity loss (Poff et al. 2010, Pyne and Poff 2017). Further, these studies are used in climate change vulnerability assessments, which rely on relationships between climate and species' traits and geographic distributions to quantify vulnerability and prioritize species and regions for protection (Foden and Young 2016). More studies are needed that identify climate change vulnerability of freshwater insects in the United States, because the few vulnerability assessments for freshwater organisms have focused on fish (Comte and Olden 2017) and those for freshwater insects have been regional or for a limited number of taxa (Poff et al. 2010, Shah et al. 2014, Pyne and Poff 2017).

Datasets of species occurrences and traits covering large geographic areas are central to conducting climate change vulnerability assessments (Foden and Young 2016, Jetz et al. 2019). Existing open-access biodiversity datasets for freshwater insects, such as the USEPA Freshwater Biological Traits database (U.S. EPA 2012, Poff et al. 2006, Vieira et al. 2006) and the Water Quality Portal (waterqualitydata.us) omit many occurrence records available from state biological monitoring programs, contain outdated taxonomic names and trait terminology, and have gaps in trait assignment for many taxa. To conduct climate change vulnerability assessments for freshwater insects in the United States, we need updated biodiversity datasets of occurrence records and traits.

In Chapter 3, I filled this knowledge gap by creating a database of occurrence records and functional traits for freshwater insects in the contiguous United States. I compiled over 2.05 million genus occurrence records for 932 genera in the major freshwater insect orders, at 51,044 stream locations sampled between 2001 and 2018 by federal and state biological monitoring programs. I assembled life history, dispersal, morphology, and ecology traits for 1,007 insect genera. I provide updated, standardized taxonomy and trait terminology, and greatly expanded the number of insect genera with trait assignments and occurrence records over previous open-access databases. My database, *Freshwater insect occurrences and traits for the contiguous US (Freshwater insects CONUS)*, can be used to map freshwater insect taxonomic and trait distributions. When paired with environmental data, my database will enable quantification of relationships between climate and freshwater insect geographic distributions across the contiguous US.

In Chapter 4, I used my database, *Freshwater insect occurrences and traits for the contiguous US (Freshwater insects CONUS,* Chapter 3), to assess the *climate-sensitivity* of freshwater insects in the United States, or the degree to which their geographic distributions are strongly determined by climatic variability. This is a first step toward assessing their climate change vulnerability (Foden and Young 2016). I assessed the strength of relationships between temperature, precipitation, and the geographic distributions of freshwater insect traits and trait profile groups (i.e., clusters of interrelated traits). Among the 11 traits and 3 trait profile groups that I considered, erosional flow preference, cold-cool eurythermal preference, and univoltine life-history traits (one generation per year) were the most sensitive to climate. I used these traits to assess and map the climate-sensitivity of stream insect genera. Using these trait-based indicators, I found that insect genera in the orders Plecoptera and Trichoptera are likely the most sensitive to climate change, indicating that their geographic distributions are strongly correlated with climate. Insect genera with high climate sensitivity may be more likely to experience changes in their geographic ranges, including range contractions, local extirpations, or extinctions, as a result of climate change. I also found that watersheds with the highest proportions of sensitive taxa are throughout the mountain West and Appalachian regions of the

United States. These watersheds may be more likely to lose biodiversity with climate change, as a high proportion of their resident insect genera may undergo range contractions and extinctions. By providing biological indicators of climate change vulnerability, my research can enhance efforts by managers and conservation practitioners to prioritize watersheds and taxa for restoration and conservation in the United States.

Recommendations for managers and conservation practitioners

With knowledge of freshwater insects' traits in local watersheds, managers and conservation practitioners can use traits identified in Chapter 4 (rheophily, thermal preference, and voltinism) to rapidly assess the sensitivity of individual insect species, genera (see Electronic Appendix 1 in Chapter 4 for climate-sensitive genera), and families. If trait information is lacking for freshwater insects in a particular area, managers and conservation practitioners can use the trait database of Chapter 3 to first assign traits, and then assess freshwater insect sensitivity to climate change using the climate sensitive traits of Chapter 4 (Electronic Appendix 1). Taxa with high sensitivity to climate based on these traits should then be prioritized for protection, because they are likely to undergo population declines or range contractions with climate change. By combining these sensitivity assessments with downscaled climate projections, managers and conservation practitioners can then conduct a full vulnerability assessment (Foden and Young 2016).

Climate sensitive traits should also be incorporated into multi-metric indices for assessing climate change vulnerability of stream sites, similar to those already in use for assessing stream ecological condition (e.g., Lawrence et al. 2010, Mazor et al. 2016). For example, many multi-metric indices incorporate the percentage of shredder taxa in assessment of ecosystem condition, and percentage of erosional and cold-cool eurythermal taxa could similarly be incorporated as indicators of climate change vulnerability. Moreover, the percentage of climate-sensitive genera (Chapter 4) could be incorporated into metrics, similar to the percentages of EPT taxa and intolerant taxa (Mazor et al. 2016). Stream sites with high climate sensitivity index scores are those that should be restored and protected to prevent losses of biodiversity due to climate change.

My trait-based indicators of climate sensitivity showed that insects in the orders Plecoptera and Trichoptera are likely the most sensitive to climate change. Insect species within these orders should be prioritized for protection in order to avoid population extirpations and species extinctions with climate change. Without further knowledge of insect species' traits, percentage of an insect assemblage in the Plecoptera and Trichoptera orders could be used as an indicator of climate change vulnerability for streams, whereby streams with large percentages of Plecoptera and Trichoptera species in their insect assemblages are considered more vulnerable to biodiversity loss with climate change than streams with low representation of species in these orders. Further, my trait-based indicators of climate sensitivity showed that watersheds with the highest proportions of sensitive taxa are throughout the mountain West and Appalachian regions of the United States. These watersheds should be prioritized for conducting full vulnerability assessments and protection against losing freshwater biodiversity due to climate change.

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