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PHYSIOLOGICAL ECOLOGY – ORIGINAL RESEARCH

Lifetime eurythermy by seasonally matched thermal performance of developmental stages in an annual aquatic insect

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Abstract

Organisms with annual life cycles are exposed to life stage specific thermal environments across seasons. Seasonal variation in thermal environments can vary across years and among sites. We investigated how organisms with annual life cycles respond to predictable seasonal changes in temperature and unpredictable thermal variation between habitats and years throughout their lives. Field surveys and historical records reveal that the spatially and temporally heterogeneous thermal environments inhabited by the annual mayfly *Ephemerella maculata* (Ephemerellidae) shift the date for transition to the next, life stage, so that the thermal phenotype of each life stage matches the thermal environment of the specific habitat and year. Laboratory studies of three distinct life stages of this mayfly reveal that life stage transitions are temperature dependent, facilitating timing shifts that are synchronized with the current season's temperatures. Each life stage exhibited specific thermal sensitivity and performance phenotypes that matched the ambient temperature typically experienced during that life stage. Our study across the whole life cycle reveals mechanisms that allow organisms to achieve lifetime eurythermy in a dynamic seasonal environment, despite having narrower thermal ranges for growth and development in each life stage.

Keywords Aquatic insect · Temperature · Life cycle · Season · Stream

Introduction

In nature, organisms can be exposed to a wide range of temperatures throughout their lives. Within days and across seasons, habitat temperatures vary predictably. However, unpredictable temperature changes occur between days due to extremes of weather and among years due to changes in global circulation patterns (e.g., El Niño-Southern Oscillation and Pacific decadal Oscillation). Within a species' distribution, temperatures vary across different spatial scales, from broad regional patterns to local microhabitats. Physiological responses of organisms to predictable and unpredictable temperature variation are a critical determinant for maximizing fitness (Angilletta 2009).

At optimal temperatures, organisms have maximal reproductive fitness, with idealized growth, longevity and reproduction. While even short-term exposure to extreme temperatures can be lethal to many organisms, prolonged exposure to sub-optimal temperature can also reduce the organisms' success by different factors such as bioenergetic costs (Somero et al. 1996; Dallas and Ross-Gillespie 2015). As the ambient temperature changes seasonally in most places, an optimal temperature in one season may be sub-optimal or extreme in another season. Two strategies are well known for adjustment for the seasonal temperature change: a eurythermy strategy and a stenothermy and diapause strategy. In the eurythermy strategy, organisms maintain high performance across a wide range of habitat temperatures. Yet, eurythermy comes at a cost to reduced maximal performance at preferred temperatures (Kristensen et al. 2008; Willett 2010). In contrast, organisms with a stenothermy strategy exhibit high performance in a narrow range of temperature. Thus, stenotherms have elevated performance in one season, while for the rest of the year, they tolerate sub-optimal temperatures in behaviorally and reproductively inactive states.

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Many organisms have environmentally tolerant states (e.g., hibernation and estivation) that include metabolic suppression (e.g., diapause) during seasons when the thermal environment would be detrimental to other life stages (Tauber and Tauber 1976; Denlinger 2002).

Here, we propose a third potential life cycle strategy of annual organisms: a strategy that shifts thermal phenotype between life stages. By shifting thermal ranges between life stages in concert with the seasonal temperature change, annual organisms may achieve lifetime eurythermy in a seasonal environment. Recent studies have shown that some organisms exhibit different optimal temperatures between life stages, such that they match predictable changes in ambient temperature (Kingsolver et al. 2011; Radchuk et al. 2013; Miller et al. 2013). For example, leaf miner moths shift their temperature tolerance as they metamorphose from larvae to adult in correspondence with the microclimate of their habitats (Pincebourde and Casas 2015). While most such studies have focused on organisms that are exposed to different temperatures as they metamorphose and change habitat, organisms with annual life cycles are also exposed to different temperatures in the same habitat as they grow. Life stages that occur in summer time may have a different thermal range from life stages occurring in winter and spring.

When organisms shift thermal performance phenotypes between life stages, it is critical that those match changes in habitat temperature, so that organisms live at physiologically preferred habitat temperatures throughout their lives (Gu et al. 2008; Ragland and Kingsolver 2008; Charmantier et al. 2008; Williams et al. 2015). Because the thermal environment can vary unpredictably between years and habitats, optimal annual timings for life stage transitions should also vary with habitat conditions. Temperature-dependent life stage shifts can synchronize life stages to particular thermal environments: during warm spring seasons, plants extend their leaves at an earlier date (Menzel and Fabian 1999), and insects grow faster and mature earlier in the season (Wilson and Barnett 1983). In contrast, during warm autumn seasons, leaf senescence occurs at later dates (Menzel and Fabian 1999) and bird migrations are delayed and occur later in the season (Van Buskirk et al. 2009). Particularly for univoltine organisms, such temperature-dependent phenological shifts allow organisms to adjust when life stage transitions occur, so that each life stage is exposed to environmental temperatures within a preferred range.

Here, we test the hypothesis that organisms with an annual life cycle respond to predictable and unpredictable thermal environments as eurytherms by temperature-dependent timing of stenothermic life cycle stages, and in doing so survive across a broader thermal environment than would be expected from thermal performance of any single life stage. We use a mayfly species, *Ephemerella maculata* (Ephemerellidae), to study how an organism with an annual life cycle cope with predictable and unpredictable temperature variation in space and time. Many Ephemerellidae mayflies mature and emerge faster in warmer environments (Sweeney and Vannote 1978), as is common for various ectotherms (Wilson and Barnett 1983). However, the life stage specific correlations between thermal habitat preference and thermal performance ranges and optima have not been described. To examine the relationships among seasonal and spatial variation in habitat temperature, phenology, and thermal physiology, we examined *E. maculata*'s geographical distribution, thermal heterogeneity, and adult emergence time in each habitat type used by the species. To determine the effect of temperatures on the timing of life stage transitions and describe the thermal performance of each life stage, we conducted lab rearing experiments over a range of temperatures.

Materials and methods

Study system and sample collection for experiments

Ephemerella maculata is a stream dwelling mayfly, widely distributed in California (Allen 1968; Meyer and McCafferty 2008) that has a well-described annual (univoltine) life cycle (Uno and Power 2015; Uno 2019). Adults oviposit in early summer in small, cool tributary creeks, and embryos develop there. Hatching occurs in autumn, and early instar nymphs migrate to mainstem rivers during late fall and winter where they complete their nymphal life during the spring and early summer when mainstem rivers become warmer. Late instar nymphs emerge as adults from the warm mainstem rivers in early summer and mate. Female adults fly to adjacent small cool tributaries, oviposit, and die. The migration of adult *E. maculata* provides a critically important seasonal trophic input for predators in tributaries (Uno and Power 2015; Uno 2016).

Study subjects for the lab rearing experiments were collected from the South Fork Eel River watershed, in and around the Angelo Coast Range Reserve in Mendocino County, California (39° 44′ 17.7″ N, 123° 37′ 48.8″ W). In this watershed, adults oviposit eggs in Fox Creek, a cool tributary, and first or second instar nymphs migrate to the mainstem South Fork Eel River. Late instar nymphs (> 5 mm body length, excluding final instars identified as having colored wing pads) were collected from the mainstem South Fork Eel River and Fox Creek and transported at the collection temperature to a field laboratory within an hour. Eggs and first instar nymphs were collected from Fox Creek and transported at the collection temperature to the University of California Berkeley campus within 5 h of collection. All the specimens were collected within a few days before experiments were initiated. Our field observations have shown that

the development time of individual *E. maculata* can vary by up to 1 month (Uno 2016). Because we collected study subjects in the field, there was a range of embryonic developmental stage or instar number at each collection time. Each experiment was conducted with individuals collected at a time, and for each experiment, we randomized individuals among treatments to avoid confounding initial developmental stage with experimental treatments.

Natural variation in temperature and nymph emergence times

We investigated natural variation in summer water temperature and *E. maculata* emergence time at a range of spatial (regional-to-local habitat) and temporal (year-to-year) scales (Fig. 1). Habitat scale variation and year-to-year scale variation of emergence time were measured by direct observations of adults (see Uno 2016 for detail) at the South Fork Eel River study site, and we used an existing database for analyses across a larger spatial scale, as described below. Data precision and resolution of emergence time was unavoidably lower for large spatial scale analyses due to limitations of available data. The summer water temperature used for respective analysis was different in resolution and terms as described below due to the limited access to data. For each spatial and temporal scale, we tested for an association of emergence time with summer water temperature using linear regression analysis.

Regional scale emergence times were retrieved from the California Environmental Data Exchange Network (CEDEN; https://www.ceden.org/) and from museum records (Allen 1968) (Fig. 1a). For each site, we defined emergence time as the latest date when nymphs were observed, and we used linear regression to examine the relationship between emergence time and the summer water temperature. Summer water temperature was represented by the predicted August 2013 mean water temperature based on NorWeST stream temperature modeling (https://www.fs.fed.us/rm/boise/AWAE/projects/NorWeST/ModeledStreamTemperatureScenarioMaps.shtml).

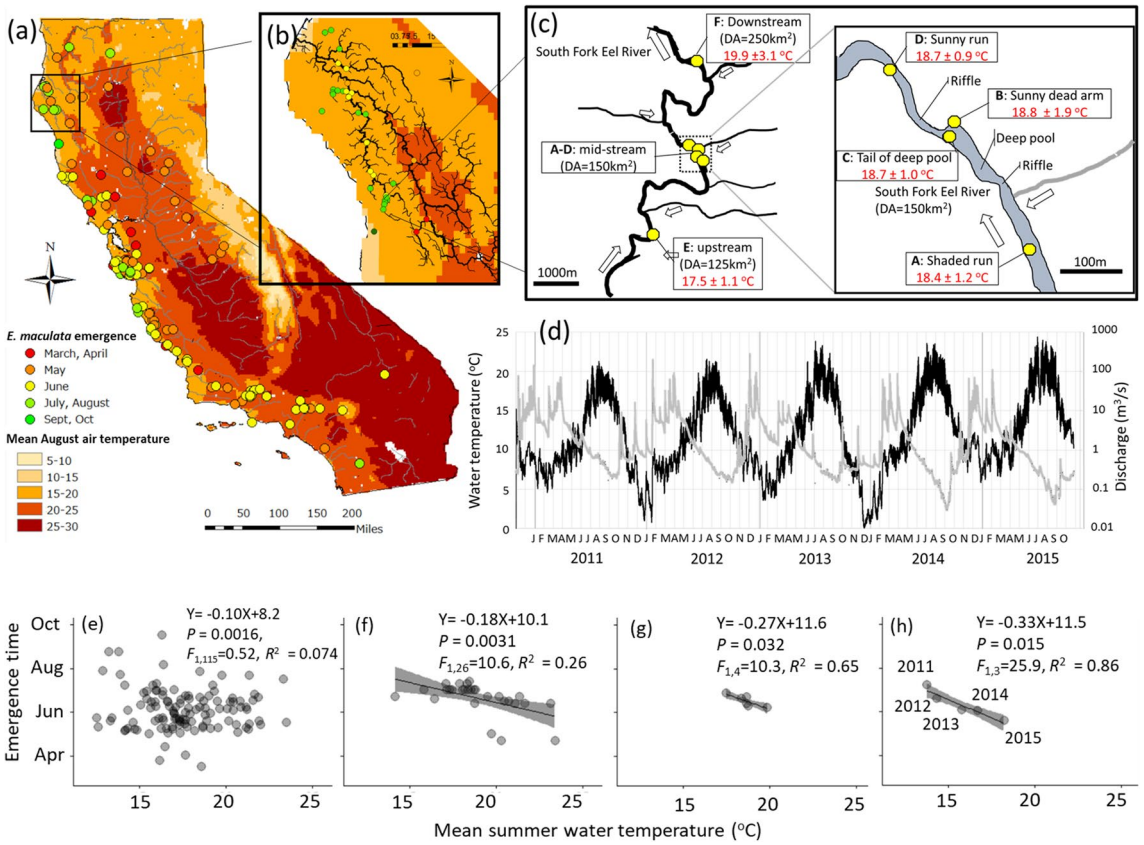

Fig. 1 **a–d** Spatial and temporal variation of ambient temperatures in nature. See supplemental information for data source. **a** Regional scale variation of water temperature in California. **b** Landscape scale variation of water temperature in the Eel River watershed. **c** Habitat scale variation of water temperature in the South Fork Eel River. **d** Temporal variation of water temperature in the South Fork Eel River. **e–h** Relationship of ambient temperature and emergence date of *E. maculata*. See methods for how *E. maculata* emergence date and summer water temperature at each scale was estimated. The line and the gray zone in figures (**e–h**) indicate the regression and 95% confidence interval. Dots represent sites in (**e–g**) and years in (**h**)

Landscape scale emergence times were collected by broad field surveys in the Eel River Watershed in Northern California in 2013 (Fig. 1b). We visited 29 sites within the watershed every 2 weeks and we assessed presence or absence of late instar nymphs. The presence–absence of nymphs at each site was determined by an exhaustive survey of all habitats with kick nets for at least 30 min by at least three trained aquatic biologists (see Uno 2019 for details). For this analysis, we defined emergence time as the latest date that nymphs were found. Summer water temperature was represented by the predicted August 2013 mean water temperature based on NorWeST stream temperature modeling.

Habitat scale emergence times were assessed by intense weekly field surveys in 2014 (Fig. 1c). Six sites representing a range of habitats including riffles, pools, and runs with different thermal conditions were selected for this study in the South Fork Eel River (see Uno 2016 for details). For this study, median date of the entire *E. maculata* population emergence from each habitat was used as the emergence date of the site. As summer water temperature, mean June 2014 water temperature was calculated from temperature logger (iButton, Dallas Semiconductor, Dallas, TX, USA) data collected for the month at 30 min intervals.

Effects of interannual variations in water temperature on the emergence time were assessed by 5 years of direct observation of *E. maculata* adults in Fox Creek, a tributary of the Eel River (Fig. 1d). Flight periods of adults was determined by daily visual field observations and sweepings with butterfly nets from mid-May to early-August each year from 2011 to 2015. The median date of the flight period each year was used as the emergence date of the year. The emergence dates were compared to the summer water temperature of the same year. Water temperature and discharge of the South Fork Eel River during that period was recorded at retired USGS Gauge #11475500 (South Fork Eel River at Branscomb) located about 5 km upstream of the study site. In the summertime, water temperatures were 1–2 °C warmer at study sites than at the gauge (H. Uno, pers. obs.). As summer water temperature, we used mean June water temperature of the gauge data.

Temperature sensitivity of development and time of life stage transitions

We reared eggs, first instar nymphs, and late instar nymphs at a range of temperatures reflecting annual river temperature variation across the complete life cycle of *E. maculata* in the study area, and examined their responses. Eggs were reared until they hatched, and the time until hatching and the hatching rate were recorded. First instar nymphs were reared for 10 days and their survival rates were recorded. Late instar nymphs were reared until they emerged, and the time until emergence and the successful emergence rates were recorded. Because the winged stage of this mayfly (including subimago and imago stage) lives only for 3–4 days after emergence (Uno unpublished), the timing of emergence is a good proxy for date of oviposition. Temperatures were maintained within ± 1 °C of a range of temperatures that occur in nature for eggs (5, 9, 13, 14.5, 16.5, and 19 °C), first instar nymphs (3, 4.5, 14.5, 16.5, 19, and 22 °C), and late instar nymphs (5, 9, 13, 17, 21, and 25 °C). The temperature in each water bath was monitored with a temperature logger (iButton, Dallas Semiconductor, Dallas, TX, USA). At each temperature and for each life stage, we maintained three replicate containers containing 1 L of continuously aerated COMBO, a freshwater culture medium (Kilham et al. 1998). Specimens were acclimated to the incubation temperature over 2 h by slowly changing the temperature of each replicate. The average development time and mortality of individuals within each of three replicate containers was used in statistical analysis. We acknowledge that our experiment measures are slightly different from standardized physiological measures (Dallas and Ketley 2011), yet in this study, our priority was to simulate the natural thermal stress rather than the standard method to examine the effect of temperature on the natural population of aquatic insects.

For eggs, the hatching experiment was performed over 2 years. On June 28, 2013, we set up the rearing experiment at 14.5, 16.5, and 19 °C under ambient light condition. On September 20, 2014, we set up the rearing experiment at 5, 9, and 13 °C with the same setting but in a dark walk-in cold room. For all the experiments, three egg masses were placed in each of the three containers at each temperature. Using a dissecting scope at 10 × and 30 × magnification, eggs were monitored at 5-day intervals until all hatched or died. We counted the number of non-viable eggs (distinguished with its cloudy color), viable eggs (embryos), and empty egg shells. The proportion of eggs that hatched successfully was calculated for each container.

For first instar nymphs, survival rate was measured in two experiments on October 26, 2013 and October 13, 2015. We reared between three and five individuals in each of three replicate containers per temperature treatment. Survival of the nymphs was estimated as the proportion of individuals still alive in each container after 10 days.

For late instar nymphs, survival rate to emergence was determined using between 6 and 11 individuals in each of three replicate containers at each temperature. We provided three periphyton covered cobbles (~ 10 cm diameter, from South Fork Eel River) in each container as a food source; old cobbles were removed, and fresh ones were added every 5 days. We initiated the experiment on June 22, 2014 and assessed survival at 5-day intervals until July 30, 2014 when all nymphs had emerged as adults or died. The emergence rate was calculated for each container as the proportion of individuals that emerged successfully in each container.

Development times of eggs to hatch and nymphs to emerge were estimated for each container as median date of the egg hatch and nymph emergence in the experiment. To examine the relationship of water temperature and development time, we conducted regression analysis (linear for nymph emergence and power curve for egg hatch). Levene's test was applied prior to each analysis to confirm homogeneity of variances. Furthermore, hatching rate, survival, and emergence rates were plotted against temperature to determine the temperature range where > 50% of individuals survived or progressed to the next stage, which we call as the "viable temperature for development". A logistic curve was fit for the survivorship of eggs and first instar nymphs as they exhibited monotonic relationships. A second-order polynomial regression was fit for the late instar nymphs as their survival was non-linear with respect to temperature.

Short-term exposure to high temperatures

We measured the effects of short-term exposure to high temperatures on the survival of late instar nymphs and eggs, and life stages that naturally occur in spring and summer when the ambient temperature is high. Here, we simulated mid-day summer temperature variation experienced in hot locations. Dissolved oxygen was saturated in the natural habitats of the species at mid-day (Uno unpublished data). Therefore, water used in these experiments was aerated to exclude the potential effects of oxygen level on heat tolerance (Verberk et al. 2011). We placed three late instar nymphs and two egg masses in each container (17 cm diameter, 15 cm high). A total of 12 containers were held at the control temperature of 20 ± 2 °C for 48 h. Then, to assess tolerance to mid-day heat spikes, we shifted three containers to one of four temperature treatments [20 (control), 29, 33, and 37 °C] for 2 h. Water temperature in the containers equilibrated to the treatment temperature within 30 min. At the end of the 2-h incubation, survival was assessed. Nymphs were determined dead if gill tissue ventilation ceased and animals were unresponsive to physical stimuli. Oxygen consumption was used to score egg survivorship using a Loligo Systems Sensor Dish Reader (PreSens, Germany) at 20 °C (see below for details). Mortality was assigned to eggs with respiration rates less than 50% of control eggs incubated at 20 °C. Survival rates were plotted against temperature to determine temperature range yielding > 50% survival, which we call the "viable temperature for short-term exposure".

Mass-specific respiration rate

To examine if the eggs were in diapause, we measured the mass-specific respiration rate of eggs and two other life stages used for the rearing experiments in comparison. Eggs, first instar nymphs, and late instar nymphs were acclimated for at least 2 weeks to a range of temperatures that typically occur in the South Fork Eel River (5, 9, 13, 17, 21, and 25 °C). Respiration rates were assessed at the acclimation temperature for ten first instar nymphs placed together in a 200 µl sealed cell, 0.2–0.5 mg of egg masses in a 200 µl sealed cell, and one individual late instar nymph in a 750 µl sealed cell. Nine replicates of each life stage were measured along with two blank cells (water only) with a Loligo Systems Sensor Dish Reader in dark conditions. Respiration rates were measured for 15 min, after which time the lowest dissolved oxygen observed was 65%. To assess biomass, specimens were dried at 60 °C for > 10 h and weighed. To examine the effect of life stage and the water temperature on the respiration rate, we conducted ANCOVA.

Results

Emergence from natural rivers

Emergence records for *E. maculata* in CEDEN (73 sites) and museum records (63 sites) ranged from March to October over a wide range of California (Fig. 1a). On a broad regional scale, the relationship of the emergence time and the August water temperature was not significant (Fig. 1e). Within the Eel River watershed, emergence was earlier in downstream habitats where the water temperature is usually warmer, and later in cooler tributaries (Fig. 1b). The emergence time was inversely associated with mean August water temperature, such that emergence was delayed in colder water (Fig. 1f). Emergence time also varied among habitats within the same segment of river corresponding to the habitat temperature (Fig. 1c, g).

Emergence time varied year-to-year and was inversely correlated with the year-to-year variations in water temperature. Summer water temperature gradually increased in the South Fork Eel River from 2011 to 2015, as California experienced severe drought in 2014 and 2015 (Fig. 1d). Correspondingly, emergence of *E. maculata* shifted to earlier in the season. Ovipositing swarms of female adult *E. maculata* were observed July 7 (or earlier)–July 22 in 2011, June 18–July 15 in 2012, June 2–July 5 in 2013, June 1–July 6 in 2014, and May 18–June 24 in 2015 (Fig. 1h).

Temperature dependence of life stage transitions

The time required for life stage transitions was temperature dependent (Fig. 2). Eggs hatched earlier at cooler temperatures (Fig. 2a, b). At each temperature, egg hatching was synchronized, but more so at lower temperature (Fig. 2a). The relationship of the water temperature and the median hatch timing at each container was best fit with a power curve, hatch date (Julian) = $0.327e^{0.313 \times \text{Temp}} + 295.0$

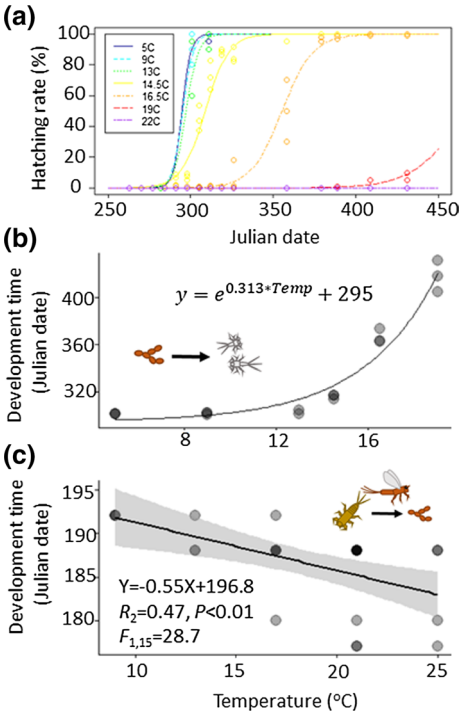

Fig. 2 Influence of water temperature on time to life stage transition. **a** Egg hatching rate in relation to temperature. **b** Relationship between water temperature and median egg hatching time. **c** Relationship between water temperature and the nymph emergence time

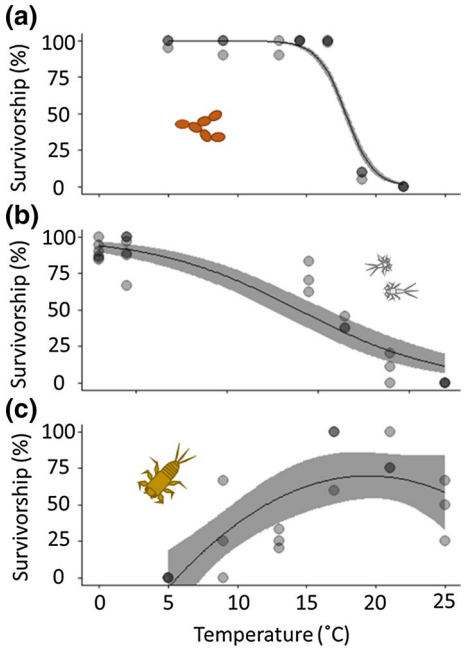

Fig. 3 Thermal responses of *E. maculata* in the rearing experiments. **a** Egg hatching. **b** 10 day survivorship of first instar nymphs. **c** Emergence rate of late instar nymphs. The line and the gray zone in the figures indicate the regression and 95% confidence intervals of the fit

(Fig. 2b). In contrast, late-stage nymphs emerged as adults earlier at warmer temperatures (Fig. 2c; $F_{1,15} = 28.7$, $R^2 = 0.47$, $P < 0.01$). On average, the time until emergence since the start of the rearing experiment was 19 days at 9 °C and 10 days at 25 °C (Fig. 2c).

Viable temperatures for development and short-term exposure to high temperature

The long-term rearing experiment showed that thermal performance varied between life stages (Fig. 3). Egg hatching rate (Fig. 3a) and survival of first instar nymphs (Fig. 3b) were higher at cooler temperatures in the range of temperatures we tested (3–25 °C). The egg survivorship was best fit with a logistic curve, $S = 1/1 + \exp(-17.7 + 0.9 \times \text{Temp})$, while the first instar nymph survivorship was best fit with a logistic curve, $S = 1/1 + \exp(-3.53 + 0.25 \times \text{Temp})$. In contrast, the survivorship of late instar nymph was highest at an intermediate temperature (21 °C) (Fig. 3c). The survivorship of late instar nymph was bet with a second-order polynomial regression, $S = -0.0037 \times \text{Temp}^2 + 0.14 \times \text{Temp} - 0.69$. The viable temperature for development (temperature range of > 50% hatching or survivorship) was 3–18 °C for eggs, 3–14 °C for first instar nymphs, and 12–27 °C for late instar nymphs.

After short-term exposure to high temperature, survival was higher for eggs than for late instar nymphs (Fig. 4). Late instar nymphs exhibited 100% survival at 29 °C, but 100% mortality at temperatures > 33 °C. In contrast, 0% egg mortality occurred after exposure to temperatures as high as 37 °C. For short-term exposures, eggs tolerated much higher temperature than the optimal temperature for the egg hatching documented in the lab rearing experiment (18 °C, see below).

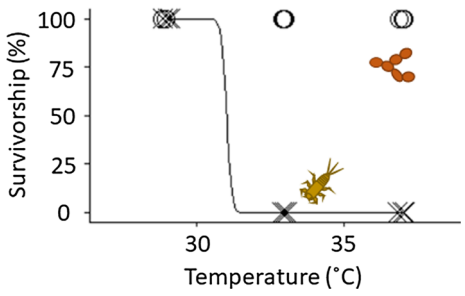

Fig. 4 Thermal responses of *E. maculata* following 2-h heat exposure. Crosses indicate survivorship of eggs and circles indicate survivorship of late instar nymphs

Respiration rates

Mass-specific respiration rates significantly varied between life stages ($F_{2,107}=43.0$, $P<0.001$), but did not vary significantly with temperature ($F_{2,107}=0.944$, $P=0.33$) (Fig. 5).

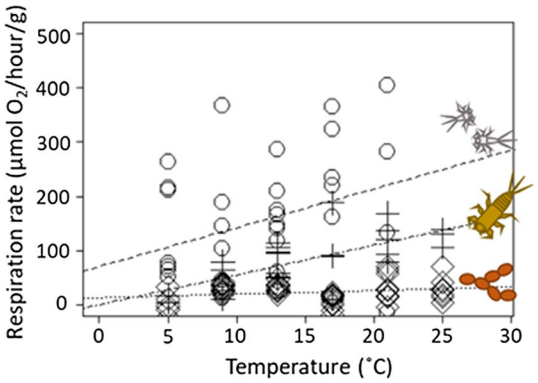

Fig. 5 Mass-specific respiration rate of *E. maculata* at three life stages. Circles: embryos (eggs), Crosses: first instar nymphs. Diamonds: late instar nymphs

There was a significant interaction between life stage and the temperature ($F_{2,107}=3.7$, $P<0.05$)). The association of respiration rate with temperature was significant for nymphs ($\text{respiration}_{\text{nymph}}=0.55\times\text{temperature}+0.87$, $F_{1,26}=25.5$, $R^2=0.50$, $P<0.001$) but not for eggs ($F_{1,48}=2.2$, $R^2=0.024$, $P=0.14$) and young instar nymphs ($F_{1,33}=3.6$, $R^2=0.07$, $P=0.07$).

Discussion

This study demonstrates that a univoltine mayfly, *Ephemerella maculata*, maintains a eurythermal phenotype by adjusting the thermal performance of each stenothermal life stage to match the corresponding seasonal change in thermal habitat (Fig. 6). Though a few studies have examined organisms' responses to temperature across life stages (Kim et al. 2017), no prior studies have combined lab experiments and field data. Hence, the life-history strategy of *E. maculata* to achieve eurythermy may occur in other mayflies and other annual organisms.

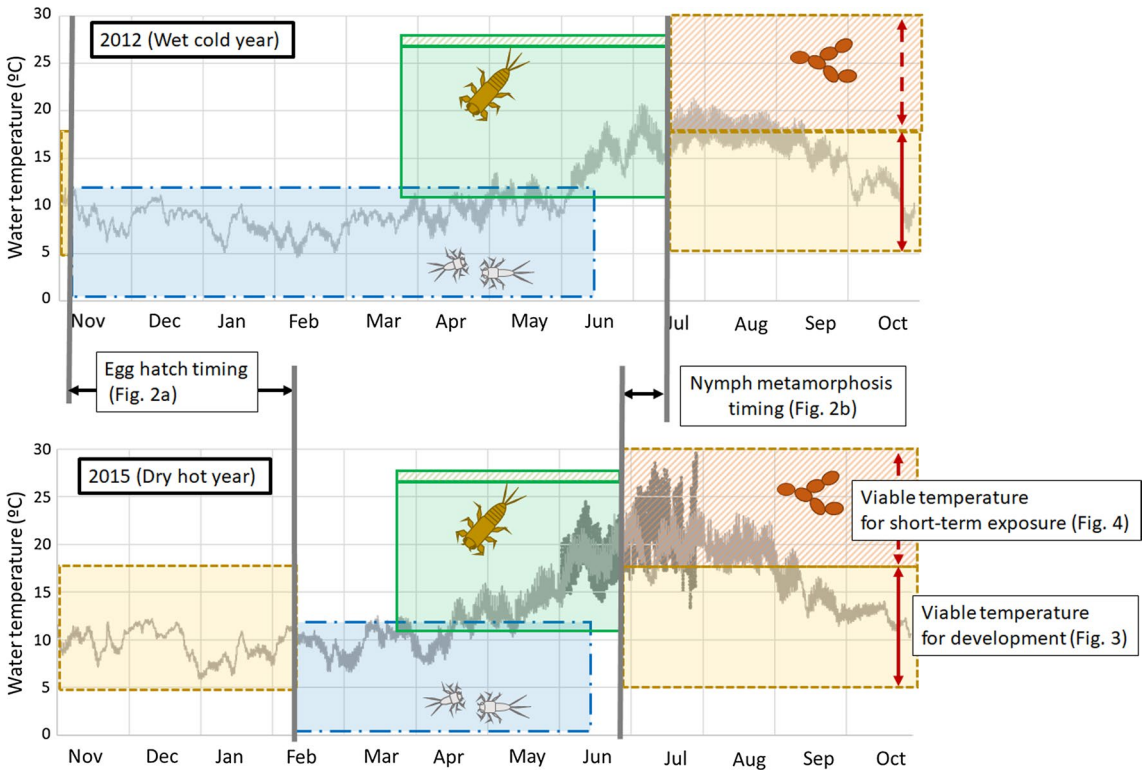

Fig. 6 Observed variation in seasonal thermal regimes in warm and cold habitats and years, and expected responses of *E. maculata* based on the rearing experiments of this study. Color shaded boxes in the figure indicate the estimated occurrence periods and viable temperature ranges of different life stages; blue dot dashed box: young instar nymphs, green solid line box: late instar nymphs, and orange dashed line box: eggs (embryos). Viable temperature ranges in the figure indicate temperature ranges where *E. maculata* exhibited > 50% survivorship in long-term and short-term experiments. Light gray: thermal regime of cool habitats in a cool year (2012). Dark gray: thermal regime of cool habitats in a warm year (2015). Black: thermal regime of warm habitats in a warm year (June and July, 2015)

Survivorship following short-term heat exposure was higher for eggs (embryos) compared to other life stages. Extremely high water temperatures (as high as 34 °C in shallows near the shore) can occur in habitats of *E. maculata* when stream flows decline during the summer dry season (Uno personal observation). The elevated thermal tolerance of eggs after short-term exposure to high temperature may allow the species to survive unpredictable temperature spikes. The low respiration rate of eggs indicates that eggs suppress metabolism and cease their development under sub-optimal thermal conditions, which is commonly known as diapause or quiescence (Tauber and Tauber 1976). A sister taxa of *E. maculata*, *Serratella ignita* can also diapause, although its diapause was averted by warm temperature in contrast to *E. maculata* (Elliott 1978). Diapause of eggs prevents energy loss during high temperatures and is an effective strategy for this mayfly to endure summer thermal conditions beyond active tolerance thresholds.

The time required for life stage transitions was temperature-dependent, as revealed by lab experiments which suggested that warm conditions sped up the development from nymphs to adults and consequently to eggs in spring, and the warm condition also delayed the hatch of eggs to nymphs in fall. Early maturation of nymphs to adults under high temperatures in spring, allowing individuals to enter a heat-tolerant egg diapause before the arrival of stressful temperatures, has been documented in terrestrial (Pruess 1983) and aquatic insects (Sweeney and Vannote 1978) as well as other ectotherms (Atkinson 1994). In contrast, delayed hatching of diapausing eggs under high temperature, ensuring that eggs hatch when habitat temperatures are more favorable, has been observed in aquatic insects (Bohle 1972; Elliott 1978; Pritchard et al. 1996) (but see Ross-Gillespie et al. 2018). The inverse temperature-dependent responses of the two life stage transitions increase the likelihood that individuals will be at the thermally resistant diapause state during hot weather, which can minimize the duration of diapause.

The viable temperature for development of each observed *E. maculata* life stage matched the typical temperature of the development season, given that in the natural environment, eggs are laid in summer, hatch in fall, first instar nymphs rear over winter, and late instar nymphs occur during the spring. Variation in thermal responses across life stages have been documented in diverse organisms with complex life cycles as responses to habitat changes across life stages (Kingsolver et al. 2011; Miller et al. 2013; Pincebourde and Casas 2015). In *E. maculata,* shifts in thermal range also occur in response to seasonal temperature changes. A seasonal shift in viable temperature for development allows annual species to achieve lifetime eurythermy despite the limited thermal range of each life stage.

Integrating temperature variation at the study site with thermal responses of *E. maculata* allows for a seasonal perspective on life-history shifts in thermal physiology (Fig. 6). While seasonal temperature variation differs by years and locations, temperature-dependent phenology synchronizes *E. maculata* physiology with seasonal changes in habitat temperature. Overall, *E. maculata* maximize the nymphal growing season while ensuring that they tolerate unpredictable thermal variations. Phenological variations across broader spatial scales observed in our study may also be represented by their genetic adaptations (Ross-Gillespie 2014) in addition to the phenotypic thermal responses shown in our lab experiments. Yet as responses to the temperature variations at small spatial and temporal scales, the phenotypic responses of phenological shift observed in our lab experiment should play large roles in allowing annual organisms to adjust to the ambient environment and survive.

This study provides a strong case that an organisms' entire life cycle needs to be evaluated to understand their actual responses to changing environments, especially for univoltine or annual organisms (Radchuk et al. 2013; Rivers-Moore et al. 2013). Studies that examine all life stages are few despite increasing numbers of investigations that focus on a single life stage (Kingsolver et al. 2011; Dallas and Rivers-Moore 2012). This study shows that a shift in thermal responses between life stages combined with temperature-dependent transitions between life stages represents a strategy to increase resistance to unpredictable thermal variation despite relative stenothermy at each life stage. While this study only examined the effect of temperature on survivorship, growth rate and fecundity are also likely to have thermal sensitivity differences among life stages (Sweeney et al. 2018). Overall, whole life cycle responses to temperature need more consideration in annual organisms that experience seasonal thermal fluctuations.

Large spatial and temporal variation in temperature occurs in the natural environment and impacts of climate change on organisms are likely to differ from those measured in thermally homogeneous lab experiments (Sheldon and Dillon 2016). Spatial heterogeneity in temperature can increase the resistance of organisms to climate change by allowing them to behaviorally avoid high-temperature habitats (Helmuth and Hoffman 2001; Nielsen et al. 1994). This study shows that organisms can also take advantage of temporal heterogeneity in temperature (i.e., seasonal temperature change) in response to year-to-year temperature change by seasonally shifting the time of life stage transitions. Additional thermal response studies of multiple life stages may reveal that many annual organisms are more resistant to a changing environment than previously documented. In addition, the thermal responses described in this study are based on predictable seasonal patterns of temperature changes. Extreme environmental irregularities could misalign the thermal range of the life stage with the ambient temperature and expose a particular life stage to unfavorable temperature.

In streams, for example, reduced seasonal temperature fluctuations by water releases from dams (Stanford et al. 1996; Olden and Naiman 2010) may impact populations of such annual insects. Conserving or restoring natural seasonal fluctuations in temperature may help organisms to survive in a changing world with their innate life cycle responses to thermal variations.

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