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EXPERIMENTAL APPROACHES TO UNDERSTANDING TEMPERATURE RESPONSES
OF SELECT NORTH CAROLINA MACROINVERTEBRATES

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Executive Summary:

The objectives of this work were to better understand the physiological basis for thermal requirements of North Carolina aquatic macroinvertebrates. Macroinvertebrates are the primary faunal group used by the North Carolina Division of Water Resources (NC DWR) and municipal entities in ecological monitoring programs because they predominate in aquatic ecosystems, and are responsive to environmental change. Because the thermal limits or realized niches for NC invertebrates were largely unknown, a major goal of this project was to determine how respiratory patterns of aquatic macroinvertebrates aligned with expert judgement by NC DWQ staff. Specifically, DWQ staff provided a list of 57 species thought to belong to one of the following groups: cold stenotherms, cold eurytherms, wary eurytherms and warm stenotherms. Our task was to compare respiratory characteristics of species belonging in these different groups such that a more extensive listing of species' thermal preferences could be generated on the basis of this fundamental biology. While undertaking this task, we began collaboration with Dr. Charles Hawkins (Utah State University) who generated thermal niche models (inferred temperature minima, optima and maxima) for 656 NC taxa. Our major findings include:

- There was strong agreement between NC DWQ expert judgement and quantitative niche model estimates.
- There appears to be an ideal range of oxygen consumption rates for aquatic insects regardless of inferred thermal optima (respiration rates of species at their inferred thermal optima were similar across species).
- Changes in oxygen consumption rates across temperatures were more pronounced in cooler adapted species than in warmer adapted species.

In addition to temperature, we intended to investigate flow requirements for North Carolina macroinvertebrates using respirometry. Due to limitations of our equipment, we instead examined several fundamental aspects of aquatic insect thermal biology with a lab-reared mayfly, *Cloeon dipterum*. Rearing this species in the lab allowed us to examine the impact of rearing temperature on respiration rates, and whether rearing temperature influenced sensitivity to thermal manipulation (thermal ramping to warmer temperatures). We also analyzed how body weight influenced respiration rates across temperatures for this species. Our major findings include:

- Oxygen consumption rates increased with increasing temperature, although respiration rates at and around the performance optimum for the species were similar suggesting a metabolic 'sweet spot' may exist.
- Metabolic Q_{10} s (fold change in metabolic rate over a 10°C temperature increase) were no different across rearing temperatures, meaning that a species' Q_{10} s are stable across a range of temperature conditions.
- Critical thermal maximum (CT_{max}) values were determined to be ecologically irrelevant
- It is metabolically more expensive to maintain a given body weight at warmer temperatures. This may drive smaller body sizes under warmer conditions.

The linkage of niche models to life history and physiological work that was stimulated by this project became the basis of a successful grant application to the National Science Foundation. It

is anticipated that refined niche models for NC taxa will result from this future effort. Other products generated from WRI support include:

- ¹Quantitative thermal niche models for 656 NC macroinvertebrate taxa
- Three peer reviewed research articles (in preparation)

¹Note: This dataset will be made publically available after it has been published in a peer-reviewed journal.

Acknowledgments:

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Background

Temperature is a major determinant of aquatic macroinvertebrate geographical distributions on both global and regional scales. Globally, certain species can only survive in tropical latitudes, others are only found in temperate zones, and some are restricted to extremely cold waters (Pörtner 2002). Even within temperate zones, there are a wide range of habitat temperatures that partition species into different distributions. Vannote and Sweeney (1980) describe how latitude, altitude and stream order (i.e. size) influence the temperature of a given body of water within the United States, creating a variety of thermal regimes. For instance, within a given drainage, temperatures may vary enough that there are distinct aquatic macroinvertebrate communities as you move from headwaters to lower reaches. Aquatic macroinvertebrate populations will also vary temporally (different community compositions throughout the year) based on seasonal differences in daylight and the duration of growing seasons (Sweeney & Vannote 1981). Vannote and Sweeney hypothesized that the driving force behind each species' distribution is how temperature drives resource allocation between growth (somatic and reproductive tissues) and metabolic maintenance costs (respiration). Species are most abundant at thermal regimes that are closest to optimal for growth and development (maximized size and fecundity) (Vannote & Sweeney 1980).

Researchers have examined the impacts of temperature on a variety of life history endpoints for aquatic macroinvertebrates (e.g. insects) and several major trends have emerged. One such observation has been termed the Temperature-Size Rule (TSR), which describes the negative relationship between temperature and body size (Atkinson 1994). Generally, within a given species, relatively cooler temperatures produce larger individuals, while warmer temperatures produce smaller individuals. This trend tends to be especially robust amongst aquatic organisms (Atkinson 1995; Forster et al. 2012). The TSR can be explained by two other life history endpoints influenced by temperature: development time and growth rate. As temperatures increase, development time shortens and growth rate increases (Sweeney 1978). However, growth rates do not increase enough to offset the much shorter development time, resulting in small individuals (van der Have & de Jong 1996; Walters & Hassall 2006). Fitness is also impacted by temperature. Larger body sizes (associated with cooler temperatures) often correspond to increased fecundity in females (Angilletta, Michael J et al. 2004; Honek 1993; Vannote & Sweeney 1980; Harvey et al. 1980). As follows, individuals developing under warmer conditions will produce fewer, and possibly smaller, eggs (Ernsting & Isaaks 2000). Additionally, temperature also impacts metabolic rates. For a given species, metabolic rate (i.e. respiration rate) will increase with increasing temperatures (Gillooly et al. 2001; Kauffman 1969; Sweeney & Schnack 1977). Overall, nearly every aspect of aquatic insect development is influenced by temperature.

Multiple researchers have hypothesized that basic physiological processes are the underlying drivers determining an insects optimal temperature (Chown & Gaston 1999; Vannote & Sweeney 1980; Pörtner 2002). Pörtner (2002) posited that thermal tolerance ranges may be due to an inability to deliver adequate oxygen to tissues at sub-optimal temperatures. For instance at sub-optimally warm temperatures, an organism's demand for oxygen may not be commensurate with oxygen delivery to tissues, thus creating a mismatch between oxygen supply and demand. Vannote and Sweeney (1980) also hypothesized that changes in metabolic rate are important in determining thermal preferences in aquatic insects. They speculated that suboptimal temperatures (i.e. too warm) necessitated changes in energy allocation within an

organism (Pörtner 2010; Pörtner 2002; Vannote & Sweeney 1980). For example, an insect developing under warm conditions may allocate more energy toward metabolism (respiration rate) to maintain basic processes while investing less energy in growth and reproduction (Vannote & Sweeney 1980). This theory provides a possible explanation for the life history trends observed: under warmer temperatures, if more energy is partitioned to respiration and less is available for growth, the resulting individuals would be smaller. In both of these hypotheses, metabolic rates (respiration rates) are central to thermal preference. Further, the degree to which a given species alters its metabolic rate when faced with suboptimal temperatures may be indicative of its tolerance to temperature perturbations. Since our understanding of aquatic ectotherm physiology is limited, these hypotheses remain largely untested.

While each species theoretically has its own preferred thermal range, a given ecosystem will support a community of species with differing thermal optima. As such, some species may be at the edge of their thermal tolerance and susceptible to small changes in temperature. Therefore, changes in stream temperature may result in loss of that species, independent of other stressors or factors (Ward & Stanford 1982). Aquatic macroinvertebrates are heavily relied upon in biomonitoring programs conducted by North Carolina Division of Water Resources and similar programs in other states due to their abundance in freshwater ecosystems and their sensitivity to environmental stressors (Lenat 1993). Decreased abundance and/or species richness within biomonitoring data for a given site traditionally signifies that anthropogenic stressors have degraded a habitat, however, unsuitable temperatures may have the same effect (Barbour et al. 1999; Klemm et al. 1990; Ward & Stanford 1982). Given global climate trends, as well as localized changes in land use patterns (increasing impervious surfaces, agriculture, industrial practices) rising surface water temperatures are a growing concern (Manciocco et al. 2014). With a poor understanding of aquatic macroinvertebrate thermal biology, we are unable to disentangle whether stressful temperatures or anthropogenic factors are the major cause of decreasing or disappearing species at a given site. Ultimately, our ability to interpret biomonitoring data is limited in the context of temperature, which may lead to ‘false positives’ when evaluating water quality.

Overview

In this study, we had two primary objectives. Our first goal was to investigate whether physiological measures (respiration rates) were fundamentally distinct in species with different thermal preferences. NCDENR provided an initial list of North Carolina aquatic insect taxa that belonged to different thermal preference categories that helped guide our field-collection efforts. We utilized our state-of-the-art respirometry equipment (Loligo Systems, Tjele, Denmark) to measure respiration rates of individual insect larvae in real time across a range of temperatures. During experiments we measured standard metabolic rates (resting respiration rates at a given temperature), as well as metabolic Q_{10s} (fold change in metabolic rate over a 10°C temperature increase) to examine sensitivity to thermal change. During the course of our research, we found it difficult to contextualize our respirometry results without any knowledge of the thermal optima of our test species. To address this issue, we collaborated with Charles Hawkins at Utah State University who generated a thermal niche model for North Carolina aquatic invertebrates. From the model, thermal optima as well as upper and lower thermal limits were estimated for 209 species. With this information we were able to consider our respirometry data in the context of inferred thermal preference and relate metabolic endpoints to thermal preference.

For our second set of experiments, we originally aimed to explore flow requirements for NC macroinvertebrates to determine a $flow_{crit}$ value (lowest flow tolerated by a species) to further inform biomonitoring interpretation. We discovered that our equipment did not function with high enough precision at low flow rates to answer this question. Instead, we conducted a series of experiments to gather a basic understanding of how aquatic insect physiologies (i.e. respiration rates) were influenced by rearing temperature and by thermal manipulation. We conducted these experiments with a laboratory-reared mayfly, *Cloeon dipterum*, where we were able to raise hatchlings at different temperatures and assay them over the course of development. We measured the same respirometry endpoints (SMRs, Q_{10S}) as with the NC macroinvertebrates. Additionally, we investigated the usefulness of CT_{max} measurements and closely evaluated the role of body weight in the context of temperature. Our work with *C. dipterum* allowed us to validate basic assumptions underlying our first set of experiments and also answered fundamental questions within aquatic insect physiology.

Methods

Part 1: North Carolina insects

Sample collection

Aquatic macroinvertebrates were collected by Buchwalter lab personnel throughout the year, with four main sampling sites across the state: Cataloochee Creek (Great Smoky Mountains National Park), Yadkin River (Happy Valley, NC), Basin Creek (near Abshers, NC), and Eno River (Hillsborough and Durham, NC). Insects were transported back to the laboratory at North Carolina State University where they were held at their collection temperature for 2 days prior to experimentation. During the acclimation time, insects were gradually transitioned to ASTM Artificial Soft Water (ASW).

Respirometry

To assess metabolic rates, individual insects were placed in respirometry chambers that serially measured oxygen consumption of each insect over time. Insects were acclimated to respirometry chambers for 4 hours at their rearing temperature prior to thermal ramping to establish a standard metabolic rate (SMR). Initial data points were omitted from analyses to reduce the influence of handling stress on SMR measurements. ASW was used in all respirometry experiments. Water bath temperature was controlled by a heater/chiller (Cole Palmer, Vernon Hills, IL) that was programmed to administer an environmentally relevant thermal ramp rate of 1°C/hour. Each ramp began from the temperature at which we collected that species, so starting temperatures varied by location and season (range: 6-22°C). Upon completion of thermal ramping experiments, insects were weighed to obtain a wet weight and stored in ethanol for taxonomic identification. Samples were identified by NCDENR. Detailed methods can be found in Camp, et al 2014 (Camp et al. 2014).

Flow experiments proved to be challenging with our equipment, thus $flow_{crit}$ values could not be determined. The respirometry chambers require continuous, thorough mixing for accurate oxygen consumption measurements, thus decreasing flow rates low enough to calculate a $flow_{crit}$ resulted in measurements with high uncertainty.

Niche model

Thermal niche models were generated by collaborators at Utah State University. The models combined aquatic insect biomonitoring data across 209 sites throughout North Carolina and modeled mean summer stream temperature (MSST) estimates at the same sites. Hill et al (2013) described the statistical methodology involved in determining modeled mean summer stream temperature (Hill et al. 2013). The model yields probability of capture for a given species at each site based on its predicted mean summer stream temperature (for an example, see Figure 1). Next, they estimated the thermal optimum for each species as the weighted average of MSST, with a weighting factor of relative abundance at a given site (Hill & Hawkins 2014). In addition to thermal optima, upper and lower thermal limits, as well as tolerance (range from lower to upper thermal limits) were estimated. We used this data to contextualize our respirometry data.

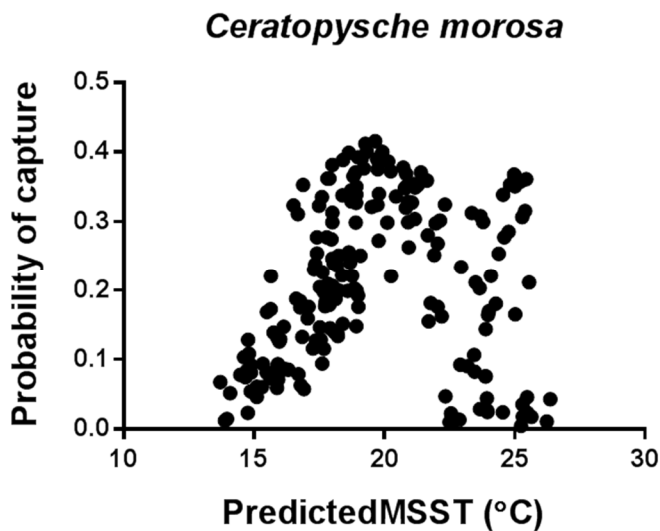


Figure 1. Example of the thermal niche model data for a caddisfly *Ceratopsyche morosa*. The plot shows predicted mean summer stream temperature against the probability of capture for each site. The predicted thermal optimum for this species is 19.8°C.

Data analysis

We employed a mass scaling correction ($\log^{0.75}$) to our NC insect respiration rate data to make more meaningful comparisons of species with different body weights (Brown et al. 2004). This allometric scaling was used because respiration rates are described on a mass specific basis and size highly influences metabolic rate. In our data set, insect wet weights ranged from 1.8-57.5 mg wet weight. All statistical tests were performed using GraphPad Prism (version 5.01, GraphPad Software, La Jolla, California).

Part 2: *Cloeon dipterum*

Rearing

C. dipterum mothers were obtained from Stroud Water Research Center (Avondale, PA) and eggs were hatched at North Carolina State University (Raleigh, NC) into ASW. Upon hatching, larvae were moved to 1 L mason jars containing periphyton plates as a food source and reared at either 17, 19.5, 22, 24.5, or 27°C. Temperature was held constant and a photoperiod of 12:12 light/dark was used. Stroud Water Research Center determined 22°C to be a performance optimum for *C. dipterum* based on full life-cycle studies, thus we chose temperatures at and

around this temperature (Funk et al. in prep) (Figure 2). Full life-cycle studies are arduous and expensive, so this type of data is very rare.

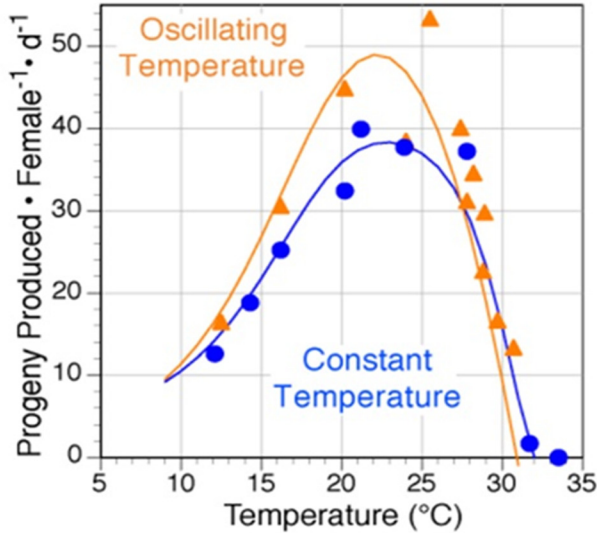


Figure 2. Full life-cycle data for *C. dipterum* generated by researchers at Stroud Water Research Center. A performance optimum exists at 22°C, and a full life-cycle cannot be completed beyond 33°C.

Respirometry

To assess metabolic rates, larvae from a subset of rearing temperatures (17, 19.5, 22, 24.5, or 27°C) were sub-sampled throughout development for respirometry experiments (same methods as Part 1). Thermal ramps varied in length from 10-17°C depending on rearing temperature. CT_{max} experiments also utilized a 1°C/hr ramp and were concluded when larvae were moribund. Upon completion of the thermal ramp, insects were weighed to obtain a wet weight.

Results and Discussion

Part 1: North Carolina insects

The NCDENR list included aquatic insects in 4 thermal preference categories: cold stenotherms (restricted to cold water), cold eurytherms (prefer cold water but can tolerate warmer temperatures), warm eurytherms (cosmopolitan distribution), and warm stenotherms (restricted to warm water sites) (Table 1). In our initial examination of the thermal niche model, we analyzed the tolerances and optima of the aquatic insects in the NCDENR list. We focused on the upper thermal limit since warm temperatures are typically of more concern than cool temperatures. We compared the difference in the upper thermal limit and optimum temperature (ΔT °C) for each species against their optimum temperature. Figure 7 illustrates the relationship between the tolerance and optima data for the insects in the thermal preference categories. Interestingly, the niche model predicts both stenothermic groups tend to have narrower thermal tolerances, while the two eurythermic groups tend to have broader tolerances. This is consistent with the general thermal preference trends associated with each category. Overall, there is a strong agreement between the qualitative thermal preference grouping by NCDENR and the thermal niche model data.

Table 1: North Carolina insect thermal preference list by NCDENR

Cold Stenotherms	Cold Eurytherms	Warm Eurytherms	Warm Stenotherms
<i>Arctopsyche irrorata</i>	<i>Ceratopsyche bronta</i>	<i>Acroneuria abnormis</i>	<i>Asioplax dolani</i>
<i>Ceratopsyche alhedra</i>	<i>Ceratopsyche sparna</i>	<i>Caenis</i> spp	<i>Dolania americanum</i>
<i>Ceratopsyche slossonae</i>	<i>Dolophilodies</i> spp	<i>Cheumatopsyche</i> spp	<i>Ephemerella argo</i>
<i>Cinygmula subequalis</i>	<i>Drunella walkeri</i>	<i>Chimarra</i> spp	<i>Haploperla fleeki</i>
<i>Epeorus pluralis</i>	<i>Epeorus dispar</i>	<i>Hydropsyche betteni</i>	<i>Hydropsyche incommada</i>
<i>Fattigia pele</i>	<i>Epeorus vitreus</i>	<i>Hydroptila</i> spp	<i>Hydropsyche phalerta</i>
<i>Isoperla slossonae</i>	<i>Heptagenia marginalis</i>	<i>Leucotrichia pictipes</i>	<i>Nectopsyche pavida</i>
<i>Maccaffertium merrivulanum</i>	<i>Isoperla holochlora</i>	<i>Maccaffertium modestum</i>	<i>Neoperla</i> spp
<i>Parapsyche apicalis</i>	<i>Leuctra</i> spp	<i>Nectopsyche exquisita</i>	<i>Neureclipsis</i> spp
<i>Parapsyche cardis</i>	<i>Malirekus hastatus</i>	<i>Oecetis persimilis</i>	<i>Oecetis cinarescens</i>
<i>Peltoperla</i> spp	<i>Micrasema rickeri</i>	<i>Paraclodes fleeki</i>	<i>Tricorythodes robacki</i>
<i>Rasvena</i> spp	<i>Micrasema wataga</i>	<i>Perlesta</i> spp	
<i>Remenus</i> spp	<i>Neophemera purprea</i>	<i>Seratella deficiens</i>	
<i>Stenacron carolina</i>	<i>Neophylax consimilis</i>	<i>Stenacron interpunctatum</i>	
<i>Tallaperla</i> spp	<i>Stenacron pallidum</i>	<i>Tricorythodes</i> spp	
<i>Viheoperla ada</i>			

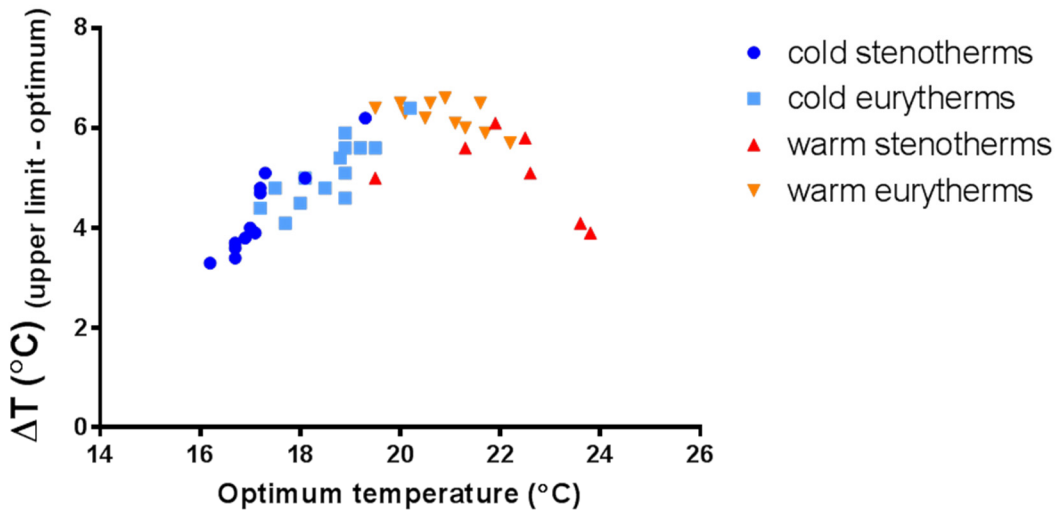


Figure 3: A comparison of ΔT (the °C difference between the upper thermal limit and optimum) and optimum temperatures for each species present in a thermal preference list created by NCDENR.

Next, we conducted the same analysis (optima vs. upper thermal tolerances) for the entire North Carolina insect data set (656 aquatic invertebrate species) and the same general trend persisted. Insects with low and high optima tended to have narrower tolerances and mid-range optima tend to have a broader tolerance (Figure 8). The majority of species from our field-collection fall within the left arm of the parabola (i.e. optima lower than 20°C). Our initial analyses of the thermal niche model suggested that the differences in upper thermal tolerances (ΔT) and optima may be a useful avenue to explore when investigating the underlying differences in physiology for our field collected species.

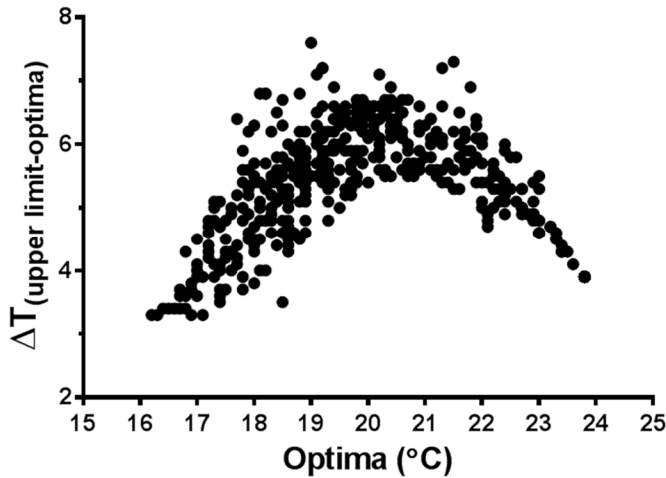


Figure 4: A global analysis of the thermal niche model data for North Carolina insects. Each point represents a different species. The plot depicts thermal tolerance (ΔT (°C) upper thermal limit – optima) vs. optima. Overall, species with low and high optima tend to have narrower thermal tolerance windows, while species with mid-range optima tend to have broader tolerances.

During our sampling we collected 26 species, 18 species of which could be identified accurately and were also present in the thermal niche model data set. Figure 9 shows overlays of the thermal niche data with mass-scaled respiration rate data for a subset of our experimental species. Table 2 contains a list of all species we used in our metabolic rate analyses.

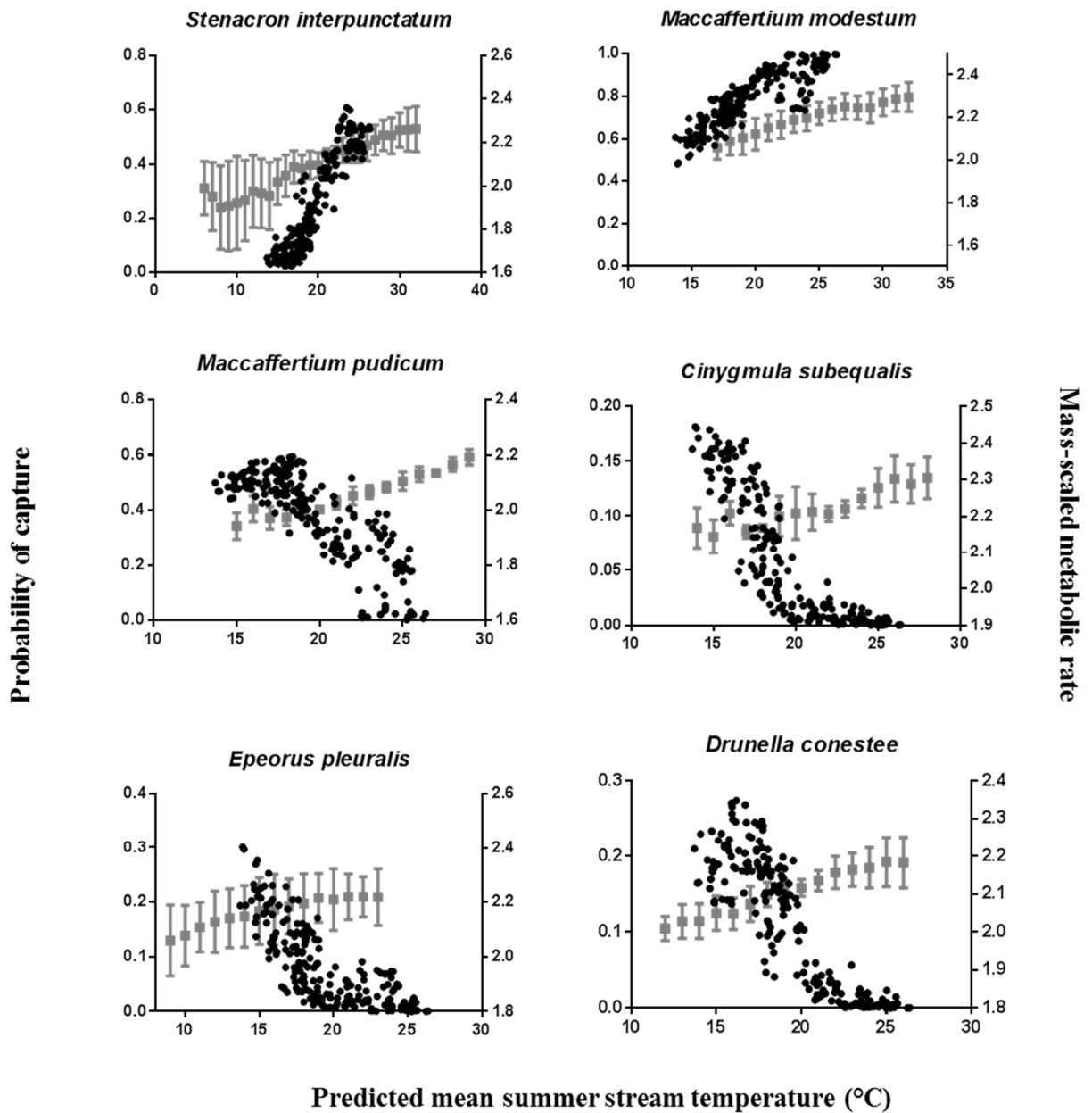


Figure 5: Six representative overlays of thermal niche model data and respirometry data for North Carolina field-collected insects. Left y-axes are probability of capture, x-axes are predicted mean summer stream temperature, and right y-axes are mass-scaled metabolic rates.

Table 2: Field-collected taxa list

<i>Ceratopsyche morosa</i>	<i>Hydropsyche venularis</i>
<i>Ceratopsyche sparna</i>	<i>Isonychia</i> spp
<i>Cinygmula subaequalis</i>	<i>Isoperla namata</i> gr.
<i>Drunella conestee</i>	<i>Maccaffertium modestum</i>
<i>Epeorus pleuralis</i>	<i>Maccaffertium pudicum</i>
<i>Ephemerella catawba</i>	<i>Paraleptophlebia</i> spp
<i>Ephemerella dorothea</i>	<i>Stenacron interpunctatum</i>
<i>Ephemerella invaria</i> gr.	<i>Sweltsa</i> spp
<i>Hydropsyche betteni</i>	<i>Tallaperla</i> spp

We first examined metabolic rates of each species at its thermal optimum to determine whether there was a relationship between metabolic rate and optimum temperature (Figure 10). There was no significant linear relationship ($p > 0.5$), thus the mass-scaled respiration rates at thermal optima were approximately the same. Two species, *Certaopsyche sparna* and *Ephemerella invaria* gr. had metabolic rates slightly lower than the rest of the species tested. Overall, this observation may be useful for approximating the optimum temperature for a novel species with respirometry methods alone. By conducting thermal ramping experiments on a novel species, we could determine the temperature for which their metabolic rate approaches the metabolic rate of other species at their optimum. Further studies would be needed to confirm this approach.

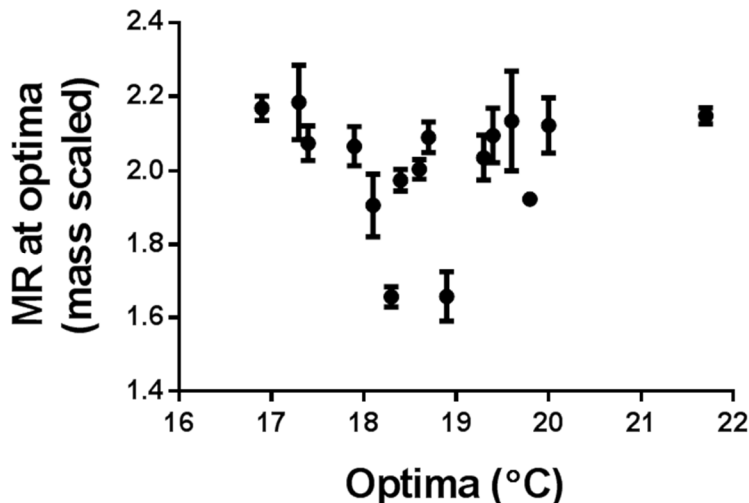


Figure 6: Optimum temperatures compared to mass-scaled metabolic rate (MR) of each species at its optimum. There is no significant linear relationship.

Next, we analyzed our respirometry data in the context of the niche model to determine whether changes in respiration rates were indicative of thermal preference. We calculated a metabolic ratio that accounted for differences in the upper span of thermal tolerance for each species:

$$\frac{(\text{Respiration rate at upper thermal limit temperature}/\text{respiration rate at optimum temperature})}{\Delta T (\text{°C})_{(\text{upper-optimum})}}$$

This term allowed us to investigate how much each species' respiration rate was influenced by temperature in its upper thermal span. We examined this term against thermal optima and found a significant negative linear relationship ($p = 0.011$, $R^2 = 0.496$) (Figure 11). Thus, insects with more mid-range thermal optima (generally more eurythermic) were not 'pushed around' metabolically by temperature as much as those with lower optima (generally more stenothermic). This finding provides evidence that eurythermal species may be able to tolerate a wide range of temperatures due to a basic physiological difference—their metabolic rates do not vary widely with changing temperatures. Conversely, stenothermic insects may be limited to a narrow temperature range because their metabolic rate changes more drastically as temperatures change. Taken together, we have evidence to support the notion that differences in thermal tolerance between aquatic insect species are driven by fundamentally different respiratory physiologies.

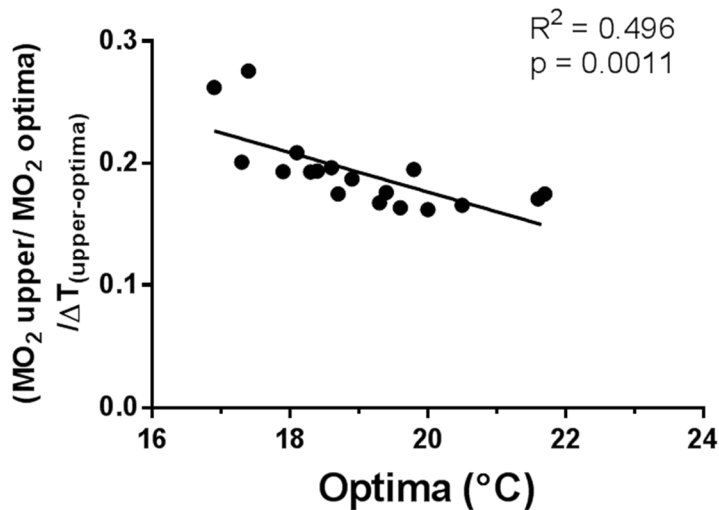


Figure 7: Relationship between changes in respiration rate over upper thermal span vs. optimum temperature for 19 species. Temperature perturbs metabolic rate to a lesser extent for species with more moderate optima.

Part 2: *C. dipterum*

Thermal ramps were conducted for 98 *C. dipterum* larvae during the course of the study. Figure 3 shows an example of the respirometry data collected during a typical thermal ramping experiment. Standard metabolic rates for each rearing temperature were calculated by averaging several hours of respirometry data at that temperature. SMRs across rearing temperatures were compared with a One-Way ANOVA with Tukey's adjustment for multiple comparisons. The metabolic rates at the performance optimum and adjacent temperatures (22 ± 2.5 °C) were no different, while the 17 and 27°C metabolic rates were significantly different than the optimum (22°C) ($p < 0.001$, $p < 0.001$, respectively) (Figure 4). Although metabolic rates increased with warmer temperatures, there appears to be a metabolic 'sweet spot' around the performance optimum where metabolic rate does not dramatically change with small temperature fluctuations.

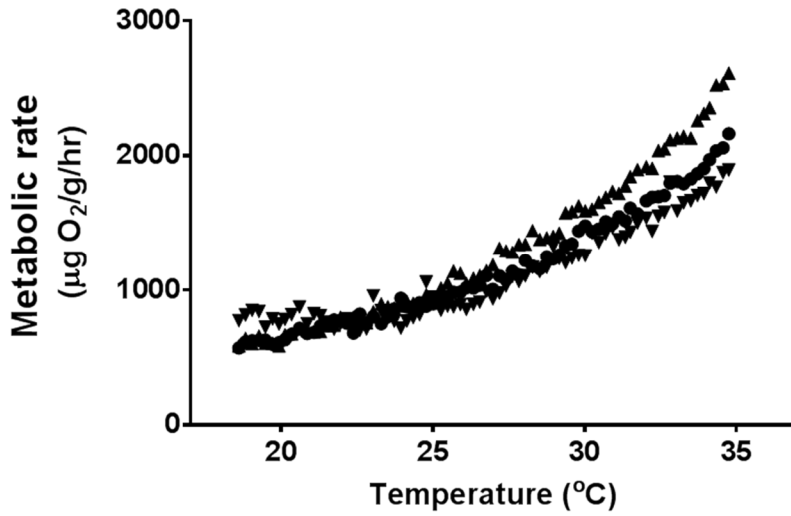


Figure 8. An example of respirometry data collected from *C. dipterum* during a 17 °C thermal ramp of 1 °C/hr. Each symbol represents serial measurements from the same larvae over time.

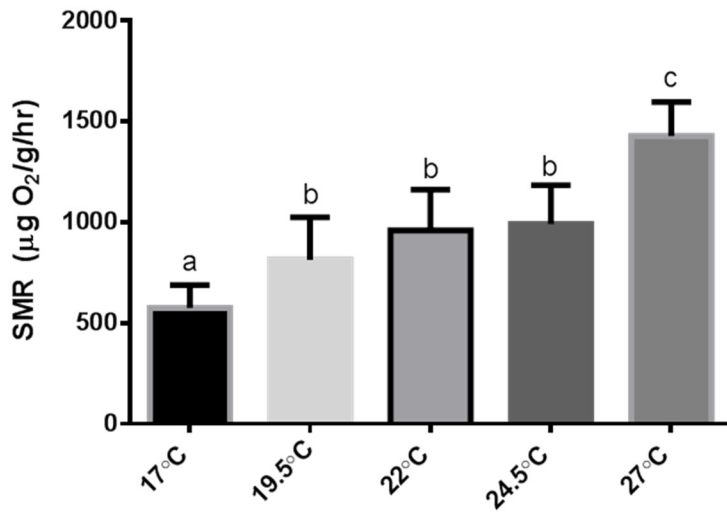


Figure 9: Standard metabolic rates (SMRs) for *C. dipterum* across the 5 rearing temperatures. Letters denote significant differences between groups.

We also compared Q_{10} s (fold change in metabolic rate across a 10°C temperature increase) across rearing temperatures and found that there were no significant differences (One-Way ANOVA, $p > 0.05$) (Figure 5). This suggests Q_{10} remains stable for a given species across thermal conditions, and is not influenced by rearing temperature.

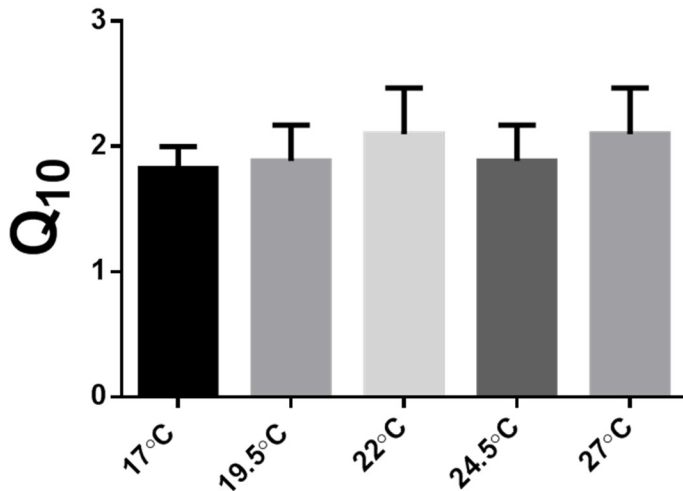


Figure 10: Metabolic Q_{10} s for each rearing temperature. All Q_{10} s were near 2 and no significant differences were found.

CT_{\max} experiments were performed to determine a ‘knock down’ temperature in *C. dipterum* (Figure 11). Based on the full-cycle data from Stroud Water Research Center, it was clear that CT_{\max} experiments were not ecologically relevant because respiration rates steadily increased past temperatures for which this species fails to complete a life cycle (Figure 2). This is consistent with our Q_{10} data, in that Q_{10} s were not smaller in the 27°C rearing group despite experiencing thermal ramps well beyond the maximum full life-cycle temperature. Taken together, we did not observe a tapering off of consumption or a lower Q_{10} value suggesting that oxygen consumption capacity is not a limiting factor at high temperatures unsuitable for survival. Thus, our results do not support the theory by Pörtner (2002) that a mismatch between oxygen supply and demand determines thermal limits.

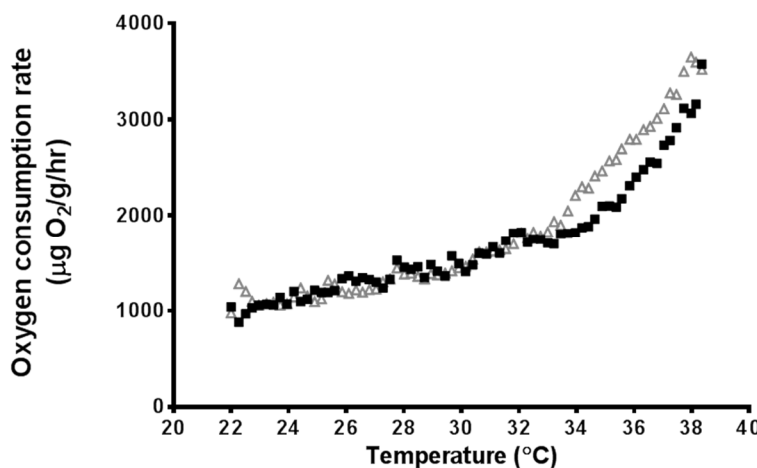
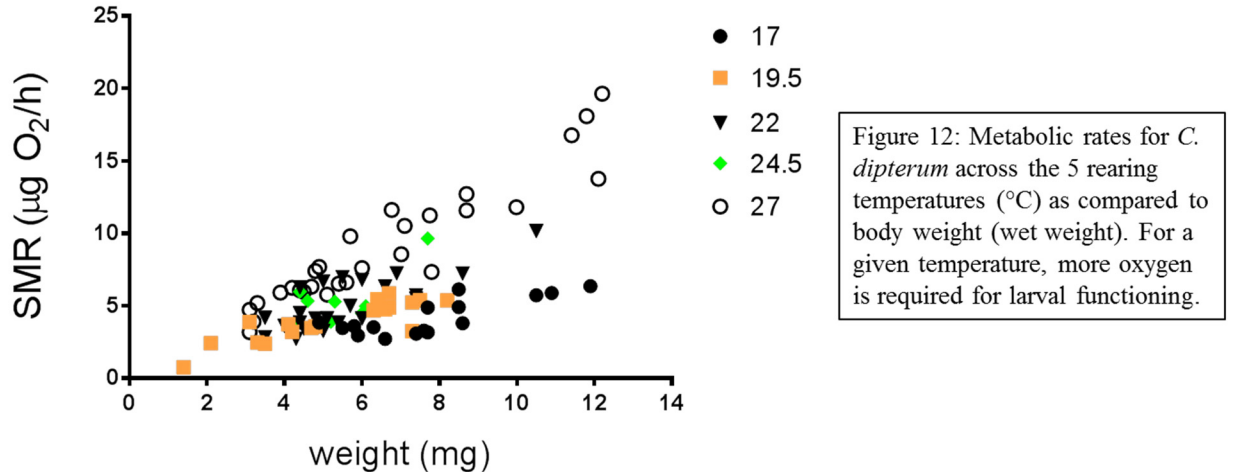


Figure 11: CT_{\max} thermal ramping with *C. dipterum*. Respiration rates did not plateau during the experiment despite surpassing the temperature for which this species can successfully complete a life cycle (see Figure 2). Ramping rate is 1°C/hr .

Next, we asked whether warmer rearing temperatures created a higher metabolic demand on a per weight basis. We assessed this by examining individual SMRs ($\mu\text{g O}_2/\text{hr}$) versus body weight (mg) for all 5 rearing temperatures. Figure 6 shows that for a given larval wet weight, a higher rearing temperature necessitates higher oxygen consumption. The relationship becomes more pronounced as larvae grow larger (compare 4 vs. 10 mg). This provides evidence to support the hypothesis by Vannote and Sweeney (1980) that increased temperatures, especially sub-optimally warm temperatures, demand alterations in energy allocation towards respiration.



Overall, we can glean several important lessons from the *C. dipterum* rearing study. First, we saw that metabolic data trends with a performance optimum, as illustrated in our standard metabolic rate data at and around the optimum of 22°C for *C. dipterum*. Secondly, we determined that Q_{10} is stable across rearing temperatures, suggesting that is a consistent value for a given species independent of the temperature from which a thermal manipulation originates. The constancy of Q_{10} values across temperatures was an important finding as we transitioned to our North Carolina insect experiments, because our field-collected insects developed under a variety of temperatures. Further, we determined that CT_{max} values are not a useful endpoint for this species in the context of full life-cycle data. Finally, we present evidence that supports the theory that increasing temperatures force alterations in energy allocation, and we observed an intensification of this trend with increasing body weight.

Summary

Part 1: North Carolina insects

With the North Carolina thermal niche model, we learned that insects with different thermal preferences (stenothermal vs eurythermal) also display differences in the breadth of their thermal tolerance. We observed that stenothermal insects tended to have narrower tolerance limits, which helps explain their more specific thermal preference. In contrast, eurythermal insects tended to have broader tolerances, and they typically had relatively moderate thermal optima. The thermal niche model also allowed us to contextualize our North Carolina insect respirometry data. By comparing changes in respiration rate over the course of a thermal ramp for each species, we determined that insects preferring colder temperatures are impacted by changes in temperature more than species with moderate optima, demonstrating that basic

physiological parameters (respiration rate) are influential in determining an insects' thermal tolerance.

Part 2: *C. dipterum*

The research with *C. dipterum* yielded several new and important findings for the field of thermal biology. We demonstrated that in *C. dipterum* there appears to be a metabolic 'sweet spot' near the temperature at which performance is optimized. We also discovered that warmer temperatures demand higher oxygen consumption on a per mass basis, supporting the hypothesis that energy allocation shifts towards respiration with increasing temperatures (Vannote & Sweeney 1980). We also found that rearing temperature does not influence Q_{10} , suggesting that for a given species, the malleability of its metabolic rate is consistent. This information confirmed an underlying assumption critical for our North Carolina insect study: that a given species would respond in a consistent way to temperature change despite different developmental conditions (geographical location, rearing temperature). Thus, we confidently could pool data for each of our field-collected species.

Finally, it is important to note that the performance optimum generated for *C. dipterum* by Stroud Water Research Center is not equivalent to the niche model thermal optima. The optimum for *C. dipterum* was calculated under controlled laboratory conditions (ample food, no predators) and therefore likely overestimates the upper thermal bounds. The niche model data, in contrast, inherently integrates the effects a variety of habitat challenges by virtue of utilizing biomonitoring data.

Conclusions

This research demonstrated that there are basic physiological differences between aquatic insect species with different thermal preferences. This has major implications for the field of thermal biology and other related fields such as ecotoxicology. This research has also highlighted the utility of niche models in interpretation of biomonitoring data. For instance if a sampling site is experiencing increased thermal pollution, the niche model could be consulted to determine whether the water temperatures have surpassed upper thermal limits of any species that have disappeared from the site (see Appendix 1 for complete niche model data). Further, the niche model could be used to determine whether additional species may be at the edge of their tolerance, thus at risk of disappearing. The niche model provides an additional means of interpreting biomonitoring data with considerations of both chemical stressors and temperature. Ultimately this research is an important first step in understanding aquatic insect thermal biology and paves the way for future research in the field that will further improve our ability to interpret biomonitoring data.

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