Thermal limits across life stages do not predict contemporary geographic distributions

Sarah Ouimette

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Signed by the final Examining Committee:

_____ Chair

Ian Ferguson

_____ Examiner

Pedro Peres-Neto

_____ Examiner

Emma Despland

_____ External Examiner

Grant Brown

_____ Co-supervisor

Piero Calosi

_____ Supervisor

Jean-Philippe Lessard

Approved by _____

Robert Weladji

_____ 2018 _____

André Roy

ABSTRACT

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Rapid and ongoing climate change is causing a complete redistribution of life on Earth. To predict species' geographic responses to climate change, it is critical that we establish the role of species' thermal tolerances in shaping their climatic envelopes. Using experimentally-derived measures of thermal limits and a database of georeferenced occurrence records, we test whether thermal limits can predict the hottest and coldest temperatures experienced within the geographic distributions of 13 North American odonate species. We measure thermal limits in both odonate larvae and adults to account for potential life stage-related differences. Lastly, we use a time-calibrated phylogeny of North American odonates to estimate the effects of evolutionary history on the relationship between species' thermal and climatic limits. We find that, even after accounting for ontogenetic differences and phylogeny, thermal limits do not translate into climatic limits. Further, we determine that species' thermal limits are constrained by phylogeny, while climatic limits appear to have evolved free from phylogenetic associations. This suggests that the evolvability of odonates' thermal limits is limited and that currently, species are in disequilibrium with their environment. Additionally, other traits or processes, such as biotic interactions, are potentially shaping odonates' geographic distributions. In the face of climate change, odonates are unlikely to adapt to novel environmental conditions and thus will likely have to continue to shift their geographic distributions in order to track their ancestral thermal niches. Further, purely climate-based models will likely be insufficient for predicting odonates' geographic responses to climate change.

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INTRODUCTION

Rapid climate change brings new urgency for understanding how species' thermal tolerances shape their geographic distributions. The global redistribution of organisms is one of the most prominent biotic responses to ongoing climate change (Parmesan and Yohe 2003, Sunday et al. 2012). The geographic distributions of countless species have shifted and/or contracted (Thomas et al. 2004, Chen 2012, Kerr et al. 2015), and considerable variation exists in both the direction and magnitude of these shifts (Parmesan et al. 1999, Ott 2010, Sunday et al. 2011, Lenoir and Svenning 2015). To understand species' geographic responses to climate change, we need to establish the processes shaping the margins of species' geographic distributions. Dispersal ability (Arribas et al. 2012), biotic interactions (Wisz et al. 2013, Araújo and Rozenfeld 2014), glacial history (Pinkert et al. 2017b), and physiological thermal tolerances (Calosi et al. 2008, 2010, Bozinovic et al. 2011) are all key factors which can shape species' distribution. However, species' responses to increasing temperatures will largely depend on the role of species' thermal limits in shaping their distributions (Pither 2003, Sunday et al. 2014, 2015). Evolutionary history and adaptive capacity jointly determine species' thermal limits (Grigg and Buckley 2013). Further, it is well established that species' thermal limits and their adaptability are not constant throughout ontogeny (Bowler and Terblanche 2008). Despite this, the effects of evolutionary history and ontogenetic variation on species' thermal limits is often overlooked in the field of macroecology.

Thermal tolerances often predict the species' climatic envelopes, defined here as the climatic conditions experienced within species' geographic distributions (Gaston and Spicer 2001, Calosi et al. 2008, 2010, Bozinovic et al. 2011, Slatyer et al. 2013). Studies also show direct links between species' thermal breadths and the climatic variability experienced within their distributions (Addo-Bediako et al. 2000, Gutiérrez-Pesquera et al. 2016). Fewer studies investigate the relationship between species' thermal limits and the limits of their climatic envelopes (Kimura 2004, Huey et al. 2009, Duarte et al. 2012, Kellermann et al. 2012b, Sunday et al. 2012, Andersen et al. 2015). Here, we define the upper and lower limits of species' climatic envelopes as species' climatic heat and cold limits. Studies assessing the relationship between species' thermal tolerances and their climatic envelopes often focus on ectothermic species. The results from these studies are equivocal, some finding direct links between thermal and climatic

limits (Duarte et al. 2012, Andersen et al. 2015), while others do not (Kimura 2004, Kellermann et al. 2012b, Gouveia et al. 2014). A potential explanation for this lack of consistency in the relationship between climatic envelopes and thermal tolerances could be that these studies almost always focus on one life stage - the adult. Considering that life stages differ in their morphology and physiology, life-stage specific estimates of thermal tolerance may not always be representative of the species' entire thermal niche (Bowler and Terblanche 2008, Kingsolver et al. 2011).

Ontogenetic variation in thermal tolerances is a crucial aspect of ectotherm thermal biology, yet studies rarely account for it in the context of macroecology and macrophysiology (Radchuk et al. 2013, Levy et al. 2015, MacLean et al. 2016). Most ectotherms have complex life cycles with ecologically and morphologically distinct life stages (Kingsolver et al. 2011, Stoks et al. 2012). Each life stage can inhabit completely different habitats (*i.e.* terrestrial *versus* aquatic) and experience different levels of seasonal and diurnal environmental variation (Coyne et al. 1983). Numerous studies demonstrate marked life stage-related differences in ectotherms' physiological responses to high and low temperatures (Vernon and Vannier 1996, Terblanche et al. 2005, Rinehart et al. 2006, Marais et al. 2009, MacLean et al. 2016). As an example, in the tropical butterfly *Bicyclus anynana,* the pupal stage's upper thermal limit is 4 °C higher than the adult stage (Sinclair 1999). Macroecological studies rarely integrate these stage-related differences in species' thermal tolerances, the majority focussing on the more conspicuous adult stage (Addo-Bediako et al. 2000, Kingsolver et al. 2011, Radchuk et al. 2013, Chiu et al. 2015). Overlooking the sensitivities of earlier life stages can result in erroneous estimates of species' thermal tolerances, especially when variation throughout ontogeny is substantial (Addo-Bediako et al. 2000). It may also result in inaccurate conclusions regarding the relationship between species' thermal tolerances and their climatic envelopes (Klockmann et al. 2017). More alarmingly, this can lead to gross over- or underestimates of species' sensitivities to climate change (Kingsolver et al. 2011, Levy et al. 2015). Given that species' thermal tolerances in different life stages are underpinned by the different physiological mechanisms they likely differ in the level to which they are evolutionarily conserved.

Conservatism in species' thermal tolerances across their life cycle can influence the relationship between thermal and climatic limits (Kellermann et al. 2009). Thus, understanding the extent to which thermal limits are phylogenetically conserved can have important

implications for predicting species' distributions and responses to climate change (Tingley et al. 2009). Closely related species can have more similar traits, either because they are locally adapted to similar environments or because they share an evolutionary history (Freckleton and Jetz 2009). Phylogenetic inertia refers to the case when species' traits are representative of past evolutionary history rather than adaptations to current climatic conditions (Hansen 1997, Blomberg and Garland 2002, Labra et al. 2009). If a species' thermal limits demonstrate phylogenetic inertia, they may reflect ancestral rather than current thermal regimes (Wiens and Graham 2005). This can cause species to be in disequilibrium with current environmental conditions (Kellermann et al. 2012a). Alternatively, species' may track their ancestral thermal niches throughout their geographic distributions (Wiens and Graham 2005). Generally, upper thermal limits tend to be more conserved across phylogeny than lower thermal limits (Addo-Bediako et al. 2000, Araújo et al. 2013, Grigg and Buckley 2013). Resultantly, species, particularly from higher latitudes, tend to under fill the warmest parts of their potential environmental niches (Sunday et al. 2011). In contrast, species' cold thermal limits, which tend to be less conserved, are often more closely related with current environmental temperatures (Kimura 2004, Sunday et al. 2012, Araújo et al. 2013, Andersen et al. 2015). Since these results are from studies focused on adult life stages, it remains unclear whether these trends are consistent in earlier life stages.

Evolutionary history can also have a significant impact on species' climatic envelopes (Wiens and Donoghue 2004). Species' traits, such as thermal tolerance, ultimately shape their climatic envelopes; these traits are determined by species' evolutionary history and their adaptive capacity (Wiens and Donoghue 2004). Phylogenetic niche conservatism (PNC) is the tendency of phylogenetically-related species to retain similar traits over time due to a limited adaptive capacity or a lack of selective pressure (Harvey and Pagel 1991, Wiens and Graham 2005). If the traits that determine species' distributions are highly conserved this can cause closely related species to display more similar geographic distributions. Consequently, species' climatic envelopes would also show evidence of evolutionarily conserved. The strongest drivers of PNC in species' climatic envelopes are likely those related to physiological traits since physiology likely plays a predominant in shaping these envelopes (Wiens and Donoghue 2004). Integrating PNC and evolutionary history into the relationship between species' thermal tolerances and their

climatic envelopes requires a biological model with a well-characterized evolutionary history and known geographic distributions.

In this sense, odonates are an ideal model for investigating whether thermal tolerances shape species' distributions whilst concurrently accounting for both ontogenetic variation and phylogenetic relationships. Odonates originated in the tropics during the Carboniferous (Pritchard and Leggott 1987, Misof and et al. 2014). However, several adaptations to cold temperatures allowed younger lineages to persist in temperate regions (May 1976). Species from the families *Coenagrionidae* and *Libellulidae* are among the youngest of the group (Rehn 2003, Turgeon et al. 2005). Having evolved under drastically different climatic regimes, odonates are an ideal group to assess the role of evolutionary history in shaping the relationship between thermal and climatic limits. Further, odonates are also particularly sensitive to temperature. Dragonflies and damselflies are poikilothermic ectotherms, meaning their body temperatures closely match their environment (Corbet 1980). Several aspects of odonate physiology and ecology relate to temperature, including egg-laying rates (Mcvey 1984, Martens 1993), egg and larval development rates (Pritchard et al. 1996, Suhling et al. 2015), adult flight (May 1981, Marden 1995), emergence time (Richter et al. 2008, McCauley et al. 2015), voltinism (Braune et al. 2008), and production of pigmentation for thermoregulation (Pinkert et al. 2017a). Temperature also shapes to odonate community structure (Burgmer et al. 2007) and is positively correlated with species richness (Eversham and Cooper 1998). Odonates pronounced geographic responses to climate change further exemplify their sensitivity to temperature is (Hickling et al. 2005, Flenner and Sahlén 2008). Odonate species, particularly from lentic habitats, show pronounced poleward distributional shifts in order to track shifting isotherms (Hickling et al. 2005, Flenner and Sahlén 2008, Grewe et al. 2013).

In addition to strongly responding to climate, odonates have marked life stage-related differences in nearly all aspects of their biology (Corbet 1980). Odonates transition from an aquatic larval stage to a terrestrial adult (Corbet 2004). Due to this extreme habitat shift, stage-related differences in odonates' thermal tolerances are thought to be particularly pronounced (Kingsolver et al. 2011). Lentic odonate species inhabiting permanent water bodies can survive for years as larvae (McPeek 2008). In North America, these larvae must endure partial or complete freezing of the water bodies they live in. In contrast, the adult stage is ephemeral, living on average for a few days or weeks depending on the species (Corbet 2004). Adults

emerge during the summer; they experience higher average temperatures and substantially greater diurnal variation than the larval stage (Corbet 1999). Adults and larvae experience distinct climatic conditions; thus, different physiological mechanisms likely drive their thermal tolerances. For example, oxygen limitations can drive the upper thermal limits of odonate larvae (Verberk and Calosi 2012, Chown et al. 2015). In contrast, adults have much higher oxygen delivery capacities and respiratory control, and thus their upper thermal limits are probably related to processes such protein denaturation, loss of membrane function, and dehydration preventing evaporative cooling (Pörtner 2001, Chown and Terblanche 2006, Bowler and Terblanche 2008). If odonate species' thermal limits determine their distributions, we expect that the limits of both the adult and larval stage will play a role in shaping this relationship. As a consequence, integrating the thermal tolerance of both larvae and adults is deemed important for improving predictions of species' climatic heat and cold limits based on their thermal limits.

In this study, we determine whether odonate species' thermal limits shape their climatic envelopes. Further, we investigate the role of ontogenetic variation and evolutionary history in shaping this relationship. To achieve this, we used controlled laboratory experiments to estimate the thermal limits of 13 odonate species in both their larval and adult life stages. We then estimated their climatic envelopes using a database containing over 19,000 georeferenced occurrence records for which we extracted climatic variables. To investigate the influence of evolutionary history, we used a time-calibrated phylogeny of North American odonates (Arrowsmith et al. in press) to perform phylogenetic regressions and estimate the phylogenetic signal in species' thermal tolerances and climatic envelopes. Specifically, we tested, (1) thermal limits shape climatic limits; (2) life stages differ in their physiological thermal tolerances; (3) accounting for stage-related differences improves predictions of species distributional limits, and (4) closely related species demonstrate more similar thermal and climatic limits than distantly related species. Taken together, these hypotheses will elucidate the importance of considering life-stage related differences in species' thermal tolerances, and the influence of evolutionary history, when predicting species' distributions.

METHODS

Study System and Site

Thermal tolerance experiments were framed in the context of a common garden, whereby all individuals were collected from the same locality, during the same season. We acknowledge the potential for intraspecific variation in species' thermal tolerances throughout their geographic distributions; although, it is not directly assessed here due to the logistic constraints of obtaining comparable measures of species' thermal tolerances throughout each of their distributions (Clusella-Trullas and Chown 2014). Consequently, we must rely on the assumption that interspecific differences in species' thermal tolerances exceed intraspecific differences between populations (Spicer and Gaston 1999).

All odonate larvae and adults used for thermal tolerance experiments were collected from the *Réserve Faunique Duchénier* (Rimouski, QC, Canada, 48.287, -68.337). This nature reserve, which spans over 272 km^2 and includes over 130 lakes, is located in the lower Saint-Lawrence region in the boreal forest. Its forest is dominated mainly by sugar maple (*Acer saccharum*), yellow birch (*Betula alleghaniensis*), balsam fir (*Abies balsamea*), black spruce (*Picea mariana*), and northern white cedar (*Thuja occidentalis*). Lakes within the reserve are well vegetated; their riparian zones are dominated by feathered reed grasses, mosses and low shrubs. The main predators within these lakes are fish, predominantly brook trout (*Salvelinus fontinalis*) and arctic char (*Salvelinus alpinus*). These lakes also serve as breeding grounds for many dragonfly and damselfly species. The average temperature and mean annual precipitation from the nearest weather station are 4.4 °C and 686.5 mm, respectively (Environment Canada 2017).

To select target species for our study we compiled a list of the most abundant dragonfly and damselfly species in Rimouski, QC, using publicly available inventories (Abbott 2006, Savard 2011) and published range maps (Paulson 2011). All species included in the list of candidate species were lentic, non-migratory and could be found in well-vegetated or forested lakes in their larval stage. To determine which lakes within the reserve had the highest abundances of odonate larvae from our candidate species list, we conducted preliminary sampling in over 20 lakes. We chose to focus this assessment on the larval stage since larval and adult abundances are typically tightly correlated (McCauley 2006, McPeek 2008). We also selected for species with distinct geographic distributions (see Figure 1) to maximize differences

in their climatic envelopes. Based on abundances obtained during preliminary sampling and species' geographic distributions, we selected 13 target species for our study (see Table A1). Of the 20 different lakes visited, we selected 10 as sampling sites for specimen collection (see Table A2). Sampling sites were chosen based on abundance of our target species, accessibility, canopy openness in the riparian zone, and minimal anthropogenic disturbance (Arrowsmith et al. in press).

Specimen collection and maintenance

Larvae

We sampled odonate larvae from June to September 2017 and May to August 2018. Odonate larvae abundances fluctuate seasonally (Wissinger 1989), therefore two field seasons were required in order to collect and test a sufficient number of individuals of each of our target species to estimate their physiological thermal limits. Larvae were collected in the mid-afternoon from the riparian zones of the lakes found at our 10 different sampling sites using D-framed aquatic nets. Given the obvious differences in sizes and wing pad development, we concluded that individuals used for thermal tolerance testing had not all reached the same instar in their larval development. The identification of instar number is not well established for the species included in our study, as it is for some other species (Leggott and Pritchard 1985). Further, individuals needed to be collected throughout the entire summer in order to attain sufficient sample sizes for thermal tolerance measures. For these reasons, we were unable to control for instar number when estimating species' thermal limits. Despite this, given that each species was sampled throughout the summer, we estimate that we obtained a similar range of instars for each species. We sampled larvae by passing a D-framed aquatic net on the surface layer of the lake sediment or along submerged vegetation. After collection, larvae were placed in individual polyethylene cups with water from the sampled lake and transported back to the laboratory in an insulated Styrofoam box to reduce temperature variation during transport. Upon arrival to the laboratory, larvae were transferred into large round polyethylene containers (diam. = 11.5 cm, depth = 10.0 cm, vol. = 900 mL) filled with pre-aerated dechlorinated tap water at room temperature. A rectangular piece of mesh was placed inside each container to provide a surface for gripping and emerging if individuals reached this stage (Apodaca and Chapman 2004). Before conducting thermal tolerance tests, larvae were placed in temperature-controlled

incubators (MLR-352H-PA, Panasonic Healthcare, Wood Dale, IL, USA) at constant temperature for 7 to 10 d to reduce the effects of previously experienced thermal history (Terblanche et al. 2007). Acclimation temperatures were chosen within the range characterizing each life stages' natural habitat, while still employing the same temperature differential between subgroups of each life stage (Δ = 10 °C).

Larvae used for physiological cold and heat limit estimates were exposed to 10 and 20 °C, respectively. Incubators were set to a relative humidity of 85 % and a 12 D : 12 L light regime for 7 to 10 d. Individuals used for CT_{max} were placed into incubators (MLR-352H-PA, Panasonic Healthcare) set to 20 °C, while those used for SCP testing were ramped from 20 to 10 °C at 0.5 °C h^{-1} and then kept constant at 10 °C for the remainder of the exposure period. Containers were bubbled regularly to ensure that oxygen levels remained near saturation (Sesterhenn et al. 2013). In order to avoid high mortality rates during the exposure period, larvae used for CT_{max} experiments were fed *Daphnia sp.* daily throughout the exposure period except on the day they were tested. Larvae used for SCP testing were not fed, since mortality rates at 10 °C were relatively low, and because food content in the gut can introduce additional variability in SCP (Sinclair et al. 2015).

Adults

Adult sampling was carried out on clear, sunny days from mid-June to early-September 2017 and mid-June to late-August 2018, since this coincides with the emergence and peak activity period of adult odonates in Quebec (Paulson 2011). We conducted sampling over two field seasons in order to collect a sufficient number of individuals for thermal tolerance testing; this was especially critical for species where the adult emerges only for a relatively short period of time (ex: *Cordulia shurtleffii*). We sampled adults at the same sites as larvae in order to maximize the chances of capturing the same species in both life stages (McCauley 2006, McPeek 2008). Adults were collected using butterfly nets; once captured individuals were placed in square mesh cages (mesh size = 0.001 cm, height = 15 cm, length = 15 cm, depth = 15 cm). Cages were built out of mesh so that temperatures remained near the outside temperature and so that specimens had a surface for gripping. Adults were transported back to the laboratory within 4 h of capture to reduce the amount of time spent in potentially stressful conditions. Upon arrival back to the

lab, cages containing adults were placed directly inside temperature-controlled incubators (MLR-352H-PA, Panasonic Healthcare).

Adults used for physiological cold and heat limit estimates were exposed to 15 and 25 °C, respectively. The relative humidity of the incubators was set to 60 %, and the lighting was on a 1 D : 1-5 L light regime for 2 to 6 h. Individuals used for CT_{max} testing were ramped from 20 °C to 25 °C at 3 °C h^{-1} , while individuals used for SCP testing were ramped from 20 °C to 15 °C at the same rate. We exposed adults to higher acclimation temperatures than larvae since on average they experience higher temperatures in their natural habitats (Corbet 2004). Adults were exposed for 2 to 6 h, which is much shorter than the larval exposure period which lasted 7 to 10 d. The length of the exposure periods was chosen relative to the length of time spent in each life stage. On average odonates' larval stage lasts for several months whereas the adult stage lasts only for a few days or weeks (Paulson 2011). Further, adults were much more sensitive to captivity than larvae; conserving adults in incubators (MLR-352H-PA, Panasonic Healthcare) for over 12 h resulted in mortality rates above 50 %.

Determination of heat tolerance

Larvae

To determine larvae's critical thermal maxima (CT_{max}) we used the dynamic ramping method, which involves increasing the temperature at a set rate until a set of predefined endpoints are observed in all individuals (see Lutterschmidt and Hutchison 1997). All CT_{max} were carried out in a computer-controlled water bath (F32 HL, Julabo, Allentown, PA, USA). Experiments on larvae commenced at 20 °C; following a 10 min recovery period, the temperature of the bath was ramped at 0.5 °C min^{-1}. Ramping rates can influence species' thermal tolerances (Terblanche et al. 2007); if they are too slow species may become acclimated resulting in overestimates of their thermal range, but if they are too fast there may be a mismatch between species body temperature and the temperature of the water bath (Ernst et al. 1984). We ramped larvae at 0.5 °C min^{-1} since previous experiments have shown that similar rates are suitable for aquatic macroinvertebrates (Dallas and Rivers-Moore 2012). Within the water bath (F32 HL, Julabo) larvae were placed in individual aluminum wells (diam = 7.5 cm, depth = 3.0 cm, vol = 100 mL) filled with pre-aerated, dechlorinated tap water. The temperature of the wells was monitored directly using a thermocouple (K type, Omega, Laval, QC, Canada) connected to a calibrated

digital thermometer (HH802U, Omega). The thermocouple (K type, Omega) was placed in one of the six wells, preliminary tests confirmed that the temperature did not differ significantly between wells. During preliminary experiments, the following behavioral endpoints were identified in odonate larvae: 1) *loss of muscular control*, attempts to crawl or swim result in uncoordinated movement of individual's limbs; 2) *onset of muscular spasms*, individual exhibits involuntary rapid and repeated muscular contractions; 3) *no response to stimuli*, individual no longer exhibits voluntary nor involuntary movements and they do not respond to three consecutive rapidly executed sprays with a pipette. *No response to stimuli* was selected to define species' CT_{max} in the larval stage since it was readily observed in all individuals and the least variable behavioral endpoint within species. Further, this endpoint is ecologically relevant since it represents a state in which the larva would no longer be able to escape conditions that would lead to its death in nature (Lutterschmidt and Hutchison 1997). Following CT_{max} tests larvae were preserved in 70 % ethanol and identified to the species level.

Adults

Dynamic ramping experiments on adult odonates were performed in air, within a custom-made thermal chamber connected to a computer-controlled water bath (F32HL, Julabo). Preliminary trials confirmed that the temperature within the chamber was homogeneous throughout ramping experiments. We employed a faster ramping rate for adults, 1 °C min^{-1}, since they experience more rapid fluctuations in temperature within their terrestrial habitats than do larvae in their aquatic habitat (Corbet 2004). Thus, since our goal was to obtain ecologically relevant estimate of thermal tolerances rather than comparable estimates across life stages we used a faster ramping rate for adults compared to larvae. Although, we used differential rates for adult and larvae, we ensured that both rates were within the range typically employed for thermal tolerance experiments (0.1 – 1 °C min^{-1}; Terblanche et al. 2007). Once individuals were placed in the thermal chamber, they were kept at 25 °C for 10 mins before commencing the ramping experiment. This 10 min period allowed individuals to recover from being transferred from their mesh cages into the thermal chamber. The following behavioral endpoints were identified in adult odonates during ramping experiments: 1) *first attempt to fly*, the first time the individual deliberately beats its wings and either lifts completely or partially off the bottom on the chamber, 2) *last attempt to fly*, the last instance where the individual deliberately beats its wings and lifts

completely or partially off the bottom of the chamber, 3) *abdomen curl*, the last instance where the tip of the individual's abdomen curls downwards to its thorax, 4) *onset of spasms*, the first instance where the individual exhibits uncoordinated muscular contractions of its legs and/or its wings, 5) *no movement*, when the individual's breathing becomes indiscernible and it no longer displays any voluntary or involuntary movement. *No movement* was chosen to define adult odonate species' CT_{max} since it was consistently observed in all individuals and it represents a state that would lead to death in nature (Lutterschmidt and Hutchison 1997). All adult specimens used for CT_{max} tests were preserved in glassine envelopes and identified to the species level.

Determination of supercooling points

Larvae

As a result of limited mobility and water freezing before larvae reach a consistent endpoint, it was impossible to reliably measure critical thermal minima (CT_{min}) in odonate larvae. Therefore, we used supercooling points (SCP) as proxies for species' cold tolerances (Sinclair 1999). The SCP is defined as the temperature at which an organisms internal body fluids begin to freeze (Sinclair et al. 2015). It is detectable due to latent heat produced upon the induction of the phase change (Sinclair et al. 2015; see Figure 1A). For measurements, an individual that was blotted dry and (as in Frisbie & Lee 1997) equipped with a precision fine wire thermocouple (type T, 2 m length, 0.13 mm diameter, 36-gauge, Omega), which was first threaded through a small hole in a 5 mL Eppendorf cap (as in Sinclair et al. 2015). A small piece of blue tack was then applied just below the beaded end of the thermocouple, which was secured to the top of the individual's abdomen using superglue (modified from Sinclair et al. 2015). The individual was then placed inside the Eppendorf, and the top was sealed using cling wrap and laboratory parafilm. The larvae could not be submerged in water for SCP measurements because the thermocouples (type T, Omega) do not function in ice and because inoculative freezing can alter species' SCP. (Frisbie and Lee 1997). SCP measurements were carried out inside a computer-controlled water bath (F32 HL, Julabo) filled with Ethylene glycol (70%). A maximum eight individuals were measured *per* test. Once each individual was equipped with a thermocouple and secured into a sealed Eppendorf, it was lowered into the bath. Eppendorfs were submerged to the lid and held secure within the water bath (F32 HL, Julabo) by aluminum wells with holes in the bottom. The computer-controlled water bath was programmed to decrease at 0.5 °C min^{-1} from either 10 or 20

°C depending on the acclimation temperature of the given subgroup. Each thermocouple was connected to a high precision data logger (CR1000, Campbell Scientific, Edmonton, AB, Canada), which logged the individual's body temperatures every second using the Campbell Scientific PC400 software on a laptop computer. SCP tests were terminated once a clear upward spike in temperature was observed (see Figure A1).

Adults

The method described above was also used to measure SCP in adult odonates, with certain modifications to adjust for the differences in individual morphology between the life stages. Due to their larger size, the adult individuals were placed in aluminum wells, instead of inside Eppendorfs within the water bath (F32 HL, Julabo). The aluminum wells were not sealed because they were not completely submerged in the water bath (F32 HL, Julabo), and were immersed such that the Ethylene glycol solution (70%) would not flood the wells. Further, the temperature of the water bath (F32 HL, Julabo) was decreased from 15 or 25 °C based on the subgroup's acclimation temperature. Since adults are terrestrial and thereby experience more rapid declines in temperature than do aquatic larva, we used a faster cooling rate (1 °C min^{-1}) for adult SCP measurements. All individuals used for SCP testing were preserved in glassine envelopes and identified to the species level. Species' thermal limits were estimated based on the average values of SCP and CT_{max} obtained for the given species following physiological testing (see Table A3).

Estimation of species' climatic envelopes

We used known occurrence records and associated climatic variables to define the climatic heat and cold limit of each of our target species. We compiled publicly available occurrence records from OdonataCentral (Abbott 2006), georeferenced vouchers from the Ouellet-Robert entomological collection at the University of Montreal Biodiversity Centre, and field data previously collected by Arrowsmith et (in press). To improve the databases reliability, records without an associated georeference, locality or which provided an unreliable georeference (*i.e.* in the ocean) were removed. Duplicate records were also removed to ensure that the dataset was representative of species' distributions rather than relative abundances or sampling biases. The final database contained 19,111 records. We extracted climatic variables from WorldClim

(Hijmans et al. 2005) to the coordinates of each of the filtered occurrence records for our target species. To estimate species' climatic heat and cold limits, we used the average of the 95th percentile of the hottest temperatures of the warmest month (BIO5) and 5th percentile of the coldest temperature of the coldest month (BIO6) experienced within each species' geographic distributions, respectively. The average values of these extractions were calculated for each species (see Table A4). All geographic information system (GIS) operations were performed in R studio version 1.1.383 (R Core Team 2017) using the *raster* package (Hijmans 2017).

Statistical Analysis

We tested the hypothesis that species' thermal limits determine their climatic limits using a series of ordinary least squares (OLS) regressions. More specifically, we modelled species' climatic heat and cold limits, respectively using CT_{max} and SCP as predictor variables. The full model predicting species' climatic heat limits included CT_{max} of the adult and larval stage, and their interaction. Similarly, the full model predicting species' climatic cold limits included adult and larval SCP, and their interaction. Each full model was compared to a reduced model which excluded the interaction and two other reduced models which either additionally excluded the thermal limit of the adult or larval stage (see Table 1). To conclude whether odonate species' thermal limits translate into climatic limits we determined the significance of each model at the 0.05 level and assessed their Akaike's Information Criterion (AIC) weights. The AIC weights provide the likelihood of the model given the data. Assumptions of normality and homoscedasticity were assessed using Shapiro-Wilks test and the studentized Breusch-Pagan test, respectively. All OLS models met both the assumptions of normality (minimum $W = 0.893$, $p = 0.127$) and homoscedasticity (maximum $BP = 4.663$, minimum $p = 0.324$).

To determine whether species' thermal tolerances in their adult and larval stage are coupled we calculated Pearson's correlation coefficient for both CT_{max} and SCP. Normal distributions were found for species' CT_{max} (minimum $W = 0.918$, $p = 0.239$) and SCP (minimum $W = 0.847$, $p = 0.034$) as well as in the sample sizes (minimum $W = 0.899$, $p = 0.128$). We avoided direct comparisons between the thermal limits of the adult and larval stage due to methodological differences in their measurement. To further investigate whether accounting for stage-related differences of species' thermal tolerances improved predictions of their climatic envelopes we used AIC-based model selection. Using the above-mentioned set of

OLS models, we determined whether models which included the thermal tolerances of both life stage had a better fit, than those which included only one life stage. Model fit was compared using AIC scores. The models with the lowest AIC scores were classified as the best fit models; if models differed by less than 2 AIC scores they were considered statistically equivalent (Johnson and Omland 2004).

Species' traits are likely to be more similar among closely related species (Wiens and Graham 2005). To account for potential phylogenetic non-independence in our dataset we ran all OLS models within a phylogenetic generalized least squares (PGLS) framework (Grafer 1989, Symonds and Blomberg 2014). To conduct this analysis we trimmed a previously assembled phylogenetic tree (Arrowsmith et al. in press) using the R package *ape* (Paradis et al. 2004) to include only our target species. PGLS models were constructed assuming that thermal tolerance traits evolved under Brownian motion (Symonds and Blomberg 2014). If the phylogenetic signal in the full model's residuals was strong, PGLS rather than OLS models were used for model selection (Revell 2010). Assumptions of normality and homoscedasticity were verified using Shapiro Wilks test and residual versus fitted diagnostic plots, respectively. All PGLS models met the assumptions of normality (minimum $W = 0.895$, $p = 0.136$) and homoscedasticity (visual assessments).

To estimate the degree to which thermal and climatic limits are phylogenetically conserved we estimated the phylogenetic signal in each set of traits (Wiens et al. 2010). Using the trimmed phylogenetic tree, we estimated Blomberg's K for SCP and CT_{max} in the larval and adult life stages and for species' climatic heat and cold limits. Blomberg's K is a commonly implemented test used for detecting phylogenetic signal (Munkemuller et al. 2012). It is based on a model of trait evolution *via* Brownian motion (Blomberg et al. 2003). Values of K were estimated in using the R package *ape* (Paradis et al. 2004). A K value equal to zero indicates that traits are evolving independently from phylogeny. If K is less than one, this indicates that a phylogenetic signal is present, but it is less strong than it would be if the traits were evolving under Brownian motion. A value of K equal to one means that the trait is evolving in complete accordance with Brownian motion. A value of K above one indicates that traits are even more similar between species that if the trait were evolving under Brownian motion, which would indicate evolutionary conservatism (Blomberg et al. 2003). Values of K near or above one would support PNC (Wiens et al. 2010). To assess similarities between species' thermal limits we

performed a series of permutational multivariate analyses of variance (PERMANOVA) modelling differences in species' CT_{max} or SCP. If the term *Species* was significant, we employed post-hoc Dunn's tests to determine which species differed significantly from each other. Non-significant differences between the thermal tolerances of closely related species would further support for PNC. All statistical analyses were performed in R studio version 1.1383 (R Core Team 2017).

RESULTS

Odonate species' climatic envelopes could not be predicted based on their physiological thermal tolerances (Table 1). The model for predicting climatic heat limits which included only the critical thermal maxima (CT_{max}) of the adult life stage had the lowest Akaike's Information Criterion (AIC) score but was non-significant ($p = 0.392$; Table 1). Similarly, the model for predicting climatic cold limits with the lowest AIC included only the supercooling points (SCP) of the larval stage and was also non-significant ($p = 0.155$; Table 1). Species' with the highest physiological tolerances for heat and cold did not correspond with those experiencing the highest and lowest temperatures within their geographic distributions (Figure 3).

We found no significant correlation between adult and larval CT_{max} (Pearson's correlation coefficient = 0.234, n = 13, $p = 0.441$; Figure 2) nor SCP (Pearson's correlation coefficient = 0.272, n = 12, $p = 0.369$; Figure 2). The number of individuals used to calculate species' average CT_{max} and SCP varied between 4 and 34 (Table A3), however we did not find that averages were biased by sample sizes (Table A5). Contrary to our expectation, the integration of both life stages into models predicting species' climatic limits did not improve model fit (Table 1). Models which included the thermal tolerances of only one life stage were statistically equivalent (Δ AIC < 2) to those that included both life stages (Table 1). This was true for both models predicting climatic heat limits (maximum Δ AIC = 1.722; Table 1) and those predicting climatic cold limits (maximum Δ AIC = 1.895; Table 1).

The phylogenetic signal in the residuals of all full models, estimated within a phylogenetic generalized least squares (PGLS) framework using Pagel's lambda (λ), was weak (maximum $\lambda = 0.248$; Table 1). This indicates that the relationship between species' thermal and

climatic limits is relatively unconstrained by phylogeny. Thus, ordinary least squares (OLS) models rather than PGLS models were used for model selection and comparison. This weak phylogenetic signal could be, at least partially related, to the lack of a phylogenetic signal in species' climatic limits (Figure 3).

Values of Blomberg's K for climatic heat and cold limits were low indicating that the trait is not evolving under Brownian motion. *Enallagma ebrium* had the highest physiological capacity to tolerate cold temperature, yet *Aeshna eremita* demonstrated the lowest climatic cold limits (Figure 3). In addition to this, closely related species such as *Lestes disjunctus* and *Lestes congener* show pronounced differences in both their climatic heat and cold limits, despite having similar thermal tolerances (Figure 3). Although species' climatic limits showed weak phylogenetic associations, species' thermal tolerances, particularly cold tolerance, were strongly constrained by phylogeny.

Odonate species' SCP in both their adult and larval life stages show strong phylogenetic conservatism (Figure 3). Estimates of Blomberg's K for SCP were close to one in the larval stage and equal to one in the adult stage (Figure 3), indicating that cold tolerance has evolved in accordance with Brownian motion. As a result, closely related species have very similar cold tolerances in both their larval ($F_{12,140} = 9.686$, $p < 0.01$; Figure 2A) and adult stage ($F_{11,172} = 15.89$, $p < 0.01$; Figure 3A). Across both life stages species from the suborder *Zygoptera* (damselflies) had lower cold tolerances than those from the suborder *Anisoptera* (dragonflies; Figure 3). In contrast, the phylogenetic signal in species' heat tolerances was relatively weak (Figure 3). However, most species did not differ significantly in terms of their heat tolerances in the larval ($F_{12,224} = 1.598$, $p = 0.09$; Figure 2A) nor adult life stage ($F_{12,205} = 2.877$, $p < 0.01$; Figure 3A). The conservatism of thermal but not climatic limits complements the results from the AIC-based model selection, which also indicate that species' thermal limits do not translate into their climatic limits (Table 1).

DISCUSSION

Understanding the role of thermal tolerances in shaping species' geographic distributions is pivotal for predicting species' sensitivities to ongoing climate change. One of the most widely

recorded biotic responses to climate change is the global redistribution of the Earth's biota (Parmesan and Yohe 2003). Researchers frequently use climatic niche models to forecast species' geographic responses to climatic shifts; these models rely on the assumption that climate and species' thermal tolerances determine their geographic distributions (Guisan and Zimmermann 2000). Here, we ask if odonate species' thermal tolerances shape their climatic envelopes and whether ontogenetic variation in thermal limits and constraints imposed by evolutionary history modify this relationship. Contrary to our initial expectation, odonate species' thermal limits do not translate into the climatic limits experienced within their distributions. Further, we find that adult and larval thermal limits are decoupled, but that the integration of the thermal limits of both life stages does not improve predictions of species' climatic limits. Lastly, we show stronger phylogenetic signal in SCP than CTmax, suggesting that cold tolerance evolved more recently than heat tolerance in odonates. We find only a weak phylogenetic signal in species' climatic limits derived from their geographic distributions. Overall, these results suggest that odonates' thermal tolerances are decoupled throughout ontogeny and constrained by evolutionary history, but that they do not determine species' climatic envelopes.

Given that ectotherms rely on their external environment to regulate their body temperature (Hofmann and Todgham 2010), we expected that odonates' thermal limits would be related to their climatic limits. However, previous studies also report a low degree of correspondence between ectothermic species' thermal tolerances and current climatic conditions (Kimura 2004, Arribas et al. 2012, Kellermann et al. 2012b, Munguía et al. 2012). In addition, the link between species' thermal tolerances and climatic envelopes is often especially weak in the Northern hemisphere (Sunday et al. 2011). Our results add to a large body of evidence which suggests that purely climate-based models may be insufficient for predicting ectothermic species' geographic distributions and responses to climate change (Davis et al. 1998).

The mismatch between odonate species' thermal and climatic limits emerges even after accounting for life stage-related differences in species' thermal tolerances. Adult and larval critical thermal maxima (CT_{max}) and supercooling points (SCP) are poor predictors of species' climatic heat and cold limits, respectively. The mismatch in the larval stage could be the result of using macroclimatic air temperatures rather than lake temperatures to estimate species' climatic limits. Nonetheless, although we find that it is not the case for North American odonates, we

still support the claim that ontogenetic differences in species' thermal limits have the potential to explain previously observed discrepancies between species' thermal tolerances and climatic envelopes (Levy et al. 2015). Here, we find that both species' upper and lower thermal limits are decoupled between the larval and adult life stages. This result aligns with previous research, which demonstrates substantial variation in species' heat and cold tolerances throughout their life cycles (Bowler and Terblanche 2008, Marais et al. 2009, Klockmann et al. 2017, Zhao et al. 2017). The decoupling of heat and cold tolerances indicates that different physiological mechanisms underpin these traits (Kingsolver et al. 2011). For macroinvertebrates, that transition from an aquatic to a terrestrial habitat, oxygen limitation likely drives upper thermal limits in the larval stage (Verberk and Calosi 2012). Adults do not share this limitation since they have a higher capacity for oxygen delivery (Stevens et al. 2010). A previous study suggests that life stages differ in their supercooling points primarily due to differences in their body masses and lipid contents (Vernon and Vannier 1996). The decoupling of both heat and cold tolerances throughout ontogeny suggests that measuring a single life stage to estimate the thermal limits of a species with a complex life cycle will result in unrepresentative estimates (Addo-Bediako et al. 2000). Thus, our results still highlight the importance of considering life stage-related differences in species' thermal tolerances, even though they do not improve predictions of geographic distributions in this study (Kingsolver et al. 2011, Levy et al. 2015). We suggest that future studies should evaluate the effects of ontogenetic variation in taxa where thermal tolerances are known to have a significant impact on species' distributions (Calosi et al. 2008, 2010, Duarte et al. 2012, Andersen et al. 2015). A potential explanation for the lack of improved predictive power following the integration of both life stages could be due to the constraints imposed by evolutionary history.

In the following study, we find evidence of phylogenetic conservatism in species' thermal limits, but not their climatic limits. Since we find a mismatch between species' thermal and climatic limits, we propose that evolutionary history rather than local adaptation is shaping species' thermal tolerances. Although we find only a weak phylogenetic signal in species' CT_{max}, species across the phylogeny generally show no significant differences in their larval or adult heat tolerances. The common ancestor of all odonates evolved under tropical conditions during the Carboniferous (Corbet 2004). Thus, homogeneity in species' CT_{max} could be the result of phylogenetic conservatism at a higher taxonomic level than those measured here; *i.e.* beyond the

genus, family, and suborder level. Retaining their ancestral heat tolerances likely results in niche underfilling of odonates climatic heat limits (Sunday et al. 2011). Since we collected all specimens from the same locality, an alternative explanation could be that all measured species are locally adapted to the climate of this region (Castañeda et al. 2004). We expect that this alternative is unlikely, given the high level of conservatism and limited adaptability generally reported for ectothermic species' upper thermal limits (Terblanche et al. 2006, Araújo et al. 2013).

In terms of cold thermal limits, we found a strong phylogenetic signal in both adult and larval SCP. Although odonates originated in the tropics, a few younger lineages developed adaptations to cold that allowed them to expand into temperate climates (May 1976, Rehn 2003). The strong phylogenetic signal in species' SCP indicates that species retain these ancestral adaptations to cold. Further, the fact that the phylogenetic signal is stronger in SCP compared to CT_{max}, indicates that species evolved their cold tolerances later in the phylogeny. This result is consistent with odonates evolutionary history; they originated from a tropical ancestor and later radiated into temperate climates (Rehn 2003, Turgeon et al. 2005). *Coenagrionidae* is among the youngest odonate families (Rehn 2003, Turgeon et al. 2005). Here, we find that species from this family, such as *Enallagma ebrium,* have particularly low SCP. Facing climate change, species with phylogenetically constrained thermal tolerances tend to have a higher propensity to shift their geographic distributions, rather than to adapt to novel environmental conditions (Wiens and Graham 2005, Soberon and Nakamura 2009, Wiens et al. 2010). Studied attribute this response to the low evolvability of these species' thermal limits (Wiens and Graham 2005, Soberon and Nakamura 2009, Wiens et al. 2010). In response to rapid climate change, odonates are shifting their geographic distributions poleward, potentially to track their ancestral thermal niches (Hickling et al. 2005). These strong phylogenetic relationships in odonate species' thermal tolerances did not translate into their climatic envelopes, which appear to be evolving almost free from phylogenetic associations.

The lack of phylogenetic signal in species' climatic limits indicates that the traits or processes that shape them are likely not evolutionarily conserved. Dispersal ability and biotic interactions both have the potential to modify the extent to which species "fill their thermal niches (Araújo and Luoto 2007, Duncan et al. 2009, Arribas et al. 2012). Studies show that lentic species, such as those included in this study, generally have high dispersal capacities. (Hof et al.

2006, Grewe et al. 2013, Pinkert et al. 2017b). Further, closely-related species such as *Lestes congener* and *Lestes disjunctus* display large differences in both their geographic distributions and climatic envelopes. Their morphologies are nearly identical; thus, they likely display very similar flight performances. For this reason, we do not expect that their dispersal abilities drive said differences in their distributions. Recent poleward shifts in odonates geographic distributions suggest that even weaker dispersers within the clade are still capable of reaching newly suitable habitats (Hassall and Thompson 2008). In contrast to dispersal, biotic interactions often play a significant role in shaping odonate community structure, richness and distributions (McPeek 2008). More specifically, predation by fish in the larval stage can prevent specific species from persisting within a given area, while facilitating the persistence of other species (Crowder and Cooper 1982, Mcpeek 1990, Johansson and Brodin 2003). Generally, fish tend to exclude larger dragonflies, allowing smaller dragonflies and damselflies to persist within these lakes and ponds (McPeek 2008). It has yet to be determined whether these interactions scale up to the continental level to affect the distributions of North American odonates.

Our study suggests thermal tolerances are not the main determinants of North American odonates' geographic distributions. Species adult and larval thermal limits could not predict the hottest and coldest temperatures experienced within each species' distribution. Despite the decoupling of adult and larval thermal limits, their integration did not improve predictions of species' climatic limits. The lack of phylogenetic signal in species' climatic limits indicates that traits or processes unconstrained by phylogeny likely shape these limits. Biotic interactions, although not evaluated here, have the potential to influence the large-scale distributions of odonates. In contrast to climatic limits, thermal limits appear to be closely associated with phylogeny. This suggests that odonates have a limited ability to shift their upper and lower thermal limits in response to environmental change. Thus, we expect that in response to climate change odonates will continue to shift their geographic distributions, rather than adapt to new climatic conditions.

REFERENCES

Abbott, J. C. 2006. OdonataCentral: an online resource for the distribution and identification of Odonata. www.odonatacentral.org.

Addo-Bediako, A., S. L. Chown, and K. J. Gaston. 2000. Thermal tolerance, climatic variability and latitude. Proceedings of the Royal Society B: Biological Sciences 267:739–745.

Andersen, J. L., T. Manenti, J. G. Sørensen, H. A. Macmillan, V. Loeschcke, and J. Overgaard. 2015. How to assess Drosophila cold tolerance: Chill coma temperature and lower lethal temperature are the best predictors of cold distribution limits. Functional Ecology 29:55–65.

Apodaca, C. K., and L. J. Chapman. 2004. Larval damselflies in extreme environments: Behavioral and physiological response to hypoxic stress. Journal of Insect Physiology 50:767–775.

Araújo, M. B., F. Ferri-Yanez, F. Bozinovic, P. A. Marquet, F. Valladares, and S. L. Chown. 2013. Heat freezes niche evolution. Ecology Letters 16:1206–1219.

Araújo, M. B., and M. Luoto. 2007. The importance of biotic interactions for modelling species distributions under climate change. Global Ecology and Biogeography 16:743–753.

Araújo, M. B., and A. Rozenfeld. 2014. The geographic scaling of biotic interactions:406–415.

Arribas, P., J. Velasco, P. Abellán, D. Sánchez-Fernández, C. Andújar, P. Calosi, A. Millán, I. Ribera, and D. T. Bilton. 2012. Dispersal ability rather than ecological tolerance drives differences in range size between lentic and lotic water beetles (Coleoptera: Hydrophilidae). Journal of Biogeography 39:984–994.

Arrowsmith, J., S. Prakash, M. Larivée, J. Turgeon, and J.-P. Lessard. (n.d.). Environmental filtering along a broad-scale acidity gradient shapes the structure of odonate communities. Ecology.

Blomberg, S. P., and T. Garland. 2002. Tempo and mode in evolution: Phylogenetic inertia, adaptation and comparative methods. Journal of Evolutionary Biology 15:899–910.

Blomberg, S. P., T. G. Jr, and A. R. Ives. 2003. Testing for Phylogenetic Signal in Comparative Data: Behavioral Traits Are More Labile. Evolution 57:717–745.

Bowler, K., and J. S. Terblanche. 2008. Insect thermal tolerance: What is the role of ontogeny, ageing and senescence? Biological Reviews 83:339–355.

Bozinovic, F., P. Calosi, and J. I. Spicer. 2011. Physiological correlates of geographic range in animals. Annual Review Of Ecology, Evolution, And Systematics 42:155–179.

Braune, E., O. Richter, D. Söndgerath, and F. Suhling. 2008. Voltinism flexibility of a riverine dragonfly along thermal gradients. Global Change Biology 14:470–482.

Burgmer, T., H. Hillebrand, and M. Pfenninger. 2007. Effects of climate-driven temperature changes on the diversity of freshwater macroinvertebrates. Oecologia 151:93–103.

Calosi, P., D. T. Bilton, J. I. Spicer, and A. Atfield. 2008. Thermal tolerance and geographical range size in the Agabus brunneus group of European diving beetles (Coleoptera: Dytiscidae). Journal of Biogeography 35:295–305.

Calosi, P., D. T. Bilton, J. I. Spicer, S. C. Votier, and A. Atfield. 2010. What determines a species' geographical range? Thermal biology and latitudinal range size relationships in European diving beetles (Coleoptera: Dytiscidae). Journal of Animal Ecology 79:194–204.

Castañeda, L. E., M. A. Lardies, and F. Bozinovic. 2004. Adaptive latitudinal shifts in the thermal physiology of a terrestrial isopod. Evolutionary Ecology Research 6:579–593.

Chen, I. 2012. Rapid Range Shifts of Species Associated with High Levels of Climatic Warming.

Science 1024:17–20.
Chiu, M. C., J. J. Kuo, and M. H. Kuo. 2015. Life stage-dependent effects of experimental heat waves on an insect herbivore. Ecological Entomology 40:175–181.
Chown, S. L., G. A. Duffy, and J. G. Sørensen. 2015. Upper thermal tolerance in aquatic insects. Current Opinion in Insect Science 11:78–83.
Chown, S. L., and J. S. Terblanche. 2006. Physiological Diversity in Insects: Ecological and Evolutionary Contexts. Advances in Insect Physiology 33:50–152.
Clusella-Trullas, S., and S. L. Chown. 2014. Lizard thermal trait variation at multiple scales: A review. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 184:5–21.
Corbet, P. 1980. Biology of Odonata. Annual Review of Entomology 25:89–217.
Corbet, P. 2004. Dragonflies, Behaviour and Ecology of Odonata. Page Harley Books, Martins.
Coyne, J. A., J. Bundgaard, and T. Prout. 1983. Geographic Variation of Tolerance to Environmental Stress in Drosophila pseudoobscura. The American Naturalist 122:474–488.
Dallas, H. F., and N. A. Rivers-Moore. 2012. Critical Thermal Maxima of aquatic macroinvertebrates: Towards identifying bioindicators of thermal alteration. Hydrobiologia 679:61–76.
Duarte, H., M. Tejedo, M. Katzenberger, F. Marangoni, D. Baldo, J. F. Beltrán, D. A. Martí, A. Richter-Boix, and A. Gonzalez-Voyer. 2012. Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities. Global Change Biology 18:412–421.
Duncan, R. P., P. Cassey, and T. M. Blackburn. 2009. Do climate envelope models transfer? A manipulative test using dung beetle introductions. Proceedings of the Royal Society B 276:1449–1457.
Environment Canada. 2017. Canadian Climate Normals. http://climate.weather.gc.ca/climate_normals.
Ernst, M. R., T. L. Beitinger, and K. W. Stewart. 1984. Critical Thermal Maxima of Nymphs of Three Plecoptera Species from an Ozark Foothill Stream Author (s): Mark R . Ernst , Thomas L . Beitinger and Kenneth W . Stewart Published by : The University of Chicago Press on behalf of the Society for Freshwater 3:80–85.
Eversham, B. C., and J. M. Cooper. 1998. Dragonfly species-richness and temperature: national patterns and latitude trends in Britain. Odonatologica 27:307–316.
Flenner, I., and G. Sahlén. 2008. Dragonfly community re-organisation in boreal forest lakes: rapid species turnover driven by climate change? Insect Conservation and Diversity 1:169–179.
Freckleton, R. P., and W. Jetz. 2009. Space versus phylogeny: disentangling phylogenetic and spatial signals in comparative data. Proceedings of the Royal Society B: Biological Sciences 276:21–30.
Frisbie, M. P., and R. E. Lee. 1997. Inoculative Freezing and the Problem of Winter Survival for Freshwater Macroinvertebrates. Journal of the North American Benthological Society 16:635–650.
Gaston, K. J., and J. I. Spicer. 2001. The relationship between range size and niche breadth: a test using five species of Gammarus (Amphipoda). Global Ecology & Biogeography 10:179–188.
Gouveia, S. F., J. Hortal, M. Tejedo, H. Duarte, F. A. S. Cassemiro, C. A. Navas, and J. A. F. Diniz-Filho. 2014. Climatic niche at physiological and macroecological scales: The thermal

tolerance-geographical range interface and niche dimensionality. Global Ecology and Biogeography 23:446–456.
Grafer, A. 1989. The phylogenetic regression. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 326:119–157.
Grewe, Y., C. Hof, D. M. Dehling, R. Brandl, and M. Brändle. 2013. Recent range shifts of European dragonflies provide support for an inverse relationship between habitat predictability and dispersal. Global Ecology and Biogeography 22:403–409.
Grigg, J. W., and L. B. Buckley. 2013. Conservatism of lizard thermal tolerances and body temperatures across evolutionary history and geography. Biology Letters 9:20121056–20121056.
Guisan, A., and N. E. Zimmermann. 2000. Predictive habitat distribution models in ecology. Ecological Modelling 135:147–186.
Gutiérrez-Pesquera, L. M., M. Tejedo, M. A. Olalla-Tárraga, H. Duarte, A. Nicieza, and M. Solé. 2016. Testing the climate variability hypothesis in thermal tolerance limits of tropical and temperate tadpoles. Journal of Biogeography 43:1166–1178.
Hansen, T. F. 1997. Stabilizing selection and the comparative analysis of adaptation. Evolution; international journal of organic evolution 51:1341–1351.
Harvey, P. H., and M. D. Pagel. 1991. The Comparative Method in Evolutionary Biology. Oxford University Press, Oxford.
Hickling, R., D. B. Roy, J. K. Hill, and C. D. Thomas. 2005. A northward shift of range margins in British Odonata. Global Change Biology 11:502–506.
Hijmans, R. J. 2017. raster: Geographic Data Analysis and Modeling. https://cran.r-project.org/package=raster.
Hijmans, R. J., S. E. Cameron, P. J.L., J. P.G., and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25:1965–1978.
Hof, C., M. Brändle, and R. Brandl. 2006. Lentic odonates have larger and more northern ranges than lotic species. Journal of Biogeography 33:63–70.
Hofmann, G. E., and A. E. Todgham. 2010. Living in the Now: Physiological Mechanisms to Tolerate a Rapidly Changing Environment. Annual Review of Physiology 72:127–145.
Huey, R. B., C. A. Deutsch, J. J. Tewksbury, L. J. Vitt, P. E. Hertz, H. J. Á. Pérez, and T. Garland. 2009. Why tropical forest lizards are vulnerable to climate warming. Proceedings of the Royal Society B: Biological Sciences 276:1939–1948.
Johnson, J. B., and K. S. Omland. 2004. Model selection in ecology and evolution. Trends in Ecology and Evolution 19:101–108.
Kellermann, V., B. Van Heerwaarden, C. M. Sgro, and A. A. Hoffmann. 2009. Fundamental Evolutionary Limits in Species Distributions. Science 1244:2–5.
Kellermann, V., V. Loeschcke, A. A. Hoffmann, T. N. Kristensen, C. Fløjgaard, J. R. David, J. C. Svenning, and J. Overgaard. 2012a. Phylogenetic Constraints In Key Functional Traits Behind Species' Climate Niches: Patterns Of Desiccation And Cold Resistance Across 95 Drosophila Species. Evolution 66:3377–3389.
Kellermann, V., J. Overgaard, A. A. Hoffmann, C. Flojgaard, J.-C. Svenning, and V. Loeschcke. 2012b. Upper thermal limits of *Drosophila* are linked to species distributions and strongly constrained phylogenetically. Proceedings of the National Academy of Sciences of the United States of America 109:16228–16233.
Kerr, J. T., A. Pindar, P. Galpern, L. Packer, S. G. Potts, S. M. Roberts, P. Rasmont, O.

Schweiger, S. R. Colla, L. L. Richardson, D. L. Wagner, and L. F. Gall. 2015. Climate change impacts on bumblebees converge across continents. Science 349:177–180.
Kimura, M. T. 2004. Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. Oecologia 140:442–449.
Kingsolver, J. G., H. Arthur Woods, L. B. Buckley, K. A. Potter, H. J. MacLean, and J. K. Higgins. 2011. Complex life cycles and the responses of insects to climate change. Integrative and Comparative Biology 51:719–732.
Klockmann, M., F. Günter, and K. Fischer. 2017. Heat resistance throughout ontogeny: body size constrains thermal tolerance. Global Change Biology 23:686–696.
Labra, A., J. Pienaar, and T. F. Hansen. 2009. Evolution of Thermal Physiology in *Liolaemus* Lizards: Adaptation, Phylogenetic Inertia, and Niche Tracking. The American Naturalist 174:204–220.
Leggott, M. A., and G. Pritchard. 1985. The life cycle of Argia vivida Hagen: Developmental types, growth ratios and instar identification (Zygoptera: Coenagrionidae). Odonalologica:201–210.
Lenoir, J., and J. C. Svenning. 2015. Climate-related range shifts - a global multidimensional synthesis and new research directions. Ecography 38:15–28.
Levy, O., L. B. Buckley, T. H. Keitt, C. D. Smith, K. O. Boateng, D. S. Kumar, and M. J. Angilletta. 2015. Resolving the life cycle alters expected impacts of climate change. Proceedings of the Royal Society B 282:20150837.
Lutterschmidt, W. I., and V. H. Hutchison. 1997. The critical thermal maximum: data to support the onset of spasms as the definitive end point. Canadian Journal of Zoology 75:1553–1560.
MacLean, H. J., J. K. Higgins, L. B. Buckley, and J. G. Kingsolver. 2016. Geographic divergence in upper thermal limits across insect life stages: does behavior matter? Oecologia 181:107–114.
Marais, E., J. S. Terblanche, and S. L. Chown. 2009. Life stage-related differences in hardening and acclimation of thermal tolerance traits in the kelp fly, Paractora dreuxi (Diptera, Helcomyzidae). Journal of Insect Physiology 55:336–343.
Marden, J. H. 1995. Large-scale changes in thermal sensitivity of flight performance during adult maturation in a dragonfly. Journal of Experimental Biology 198:2095–2102.
Martens, A. 1993. Influence of conspecifics and plant structures on oviposition site selection in Pyrrhosoma nymphula (Sulzer) (Zygoptera: Coenagrionidae). Odonatologica 22:487–494.
May, M. L. 1976. Thermoregulation and adaptation to temperature in dragonflies (Odonata : Anisoptera). Ecological Monographs 46:1–32.
May, M. L. 1981. Wingstroke frequency of dragonflies (Odonata: Anisoptera) in relation of temperature and body size. Journal of Comparative Physiology 144:229–240.
McCauley, S. J. 2006. The effects of dispersal and recruitment limitation on community structure of odonates in a artifical ponds. Ecography 29:585–595.
McCauley, S. J., J. I. Hammond, D. N. Frances, and K. E. Mabry. 2015. Effects of experimental warming on survival, phenology, and morphology of an aquatic insect (Odonata). Ecological Entomology 40:211–220.
McPeek, M. A. 2008. Ecological factors influencing the distribution and abundances of Odonata. Page 51–62. Dragonflies and damselflies: model organisms for ecological and evolutionary research.
Mcvey, M. E. 1984. Egg release rates with temperature and body size in libellulid dragonflies (Anisoptera). Odonalologica 13:377–385.

Misof, B., and et al. 2014. Phylogenomics resolves the timing and pattern of insect evolution. Science 346:763–767.
Munguía, M., C. Rahbek, T. F. Rangel, J. A. F. Diniz-Filho, and M. B. Araújo. 2012. Equilibrium of global amphibian species distributions with climate. PLoS ONE 7:1–9.
Munkemuller, T., S. Lavergne, B. Bzeznik, S. Dray, T. Jombart, K. Schiffers, and W. Thuiller. 2012. How to measure and test phylogenetic signal. Methods in Ecology and Evolution 3:743–756.
Ott, J. 2010. Dragonflies and climatic change - recent trends in Germany and Europe. BioRisk 5:253–286.
Paradis, E., J. Claude, and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–90.
Parmesan, C., N. Ryrholm, C. Stefanescu, J. K. Hill, C. D. Thomas, H. Descimon, B. Huntley, L. Kaila, J. Kullberg, T. Tammaru, W. J. Tennent, J. a Thomas, and M. Warren. 1999. Poleward shifts in geographical ranges of butterfly species associated with regional warming. Nature 399:579–583.
Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42.
Paulson, D. 2011. Dragonflies and Damselflies of the East. Princeton University Press, Princeton, New Jersey.
Pinkert, S., R. Brandl, and D. Zeuss. 2017a. Colour lightness of dragonfly assemblages across North America and Europe. Ecography 40:1110–1117.
Pinkert, S., K. D. B. Dijkstra, D. Zeuss, C. Reudenbach, R. Brandl, and C. Hof. 2017b. Evolutionary processes, dispersal limitation and climatic history shape current diversity patterns of European dragonflies. Ecography:795–804.
Pither, J. 2003. Climate Tolerance and Interspecific Variation in Geographic Range Size. Proceedings of the Royal Society B: Biological Sciences 270:475–481.
Pörtner, H. 2001. Climate change and temperature-dependent biogeography: Oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88:137–146.
Pritchard, G., L. D. Harder, and R. A. Mutch. 1996. Development of aquatic insect eggs in relation to te mperature and strategies for dealing with different thermal environments. Biological Journal of the Linnean Society 58:221–244.
Pritchard, G., and M. A. Leggott. 1987. Temperature, Incubation Rates and Origins of Dragonflies. Advances in odonatology 1:121–126.
R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing.
Radchuk, V., C. Turlure, and N. Schtickzelle. 2013. Each life stage matters: The importance of assessing the response to climate change over the complete life cycle in butterflies. Journal of Animal Ecology 82:275–285.
Rehn, A. C. 2003. Phylogenetic analysis of higher-level relationships of Odonata. Systematic Entomology 28:181–239.
Revell, L. J. 2010. Phylogenetic signal and linear regression on species data. Methods in Ecology and Evolution 1:319–329.
Richter, O., F. Suhling, O. Müller, and D. Kern. 2008. A model for predicting the emergence of dragonflies in a changing climate. Freshwater Biology 53:1868–1880.
Rinehart, J. P., S. a L. Hayward, M. a Elnitsky, L. H. Sandro, R. E. Lee, and D. L. Denlinger. 2006. Continuous up-regulation of heat shock proteins in larvae, but not adults, of a polar

insect. Proceedings of the National Academy of Sciences of the United States of America 103:14223–14227.
Savard, M. 2011. Atlas préliminaire des libellules du Québec (Odonata).
Sesterhenn, T. M., E. E. Reardon, and L. J. Chapman. 2013. Hypoxia and lost gills: Respiratory ecology of a temperate larval damselfly. Journal of Insect Physiology 59:19–25.
Sinclair, B. J. 1999. Insect cold tolerance: How many kinds of frozen?
Sinclair, B. J., L. E. Coello Alvarado, and L. V. Ferguson. 2015. An invitation to measure insect cold tolerance: Methods, approaches, and workflow. Journal of Thermal Biology 53:180–197.
Slatyer, R. A., M. Hirst, and J. P. Sexton. 2013. Niche breadth predicts geographical range size: A general ecological pattern. Ecology Letters 16:1104–1114.
Spicer, J. I., and K. J. Gaston. 1999. Physiological diversity and its ecological implications. Blackwell Science, Oxford.
Stevens, M. M., S. Jackson, S. A. Bester, J. S. Terblanche, and S. L. Chown. 2010. Oxygen limitation and thermal tolerance in two terrestrial arthropod species. Journal of Experimental Biology 213:2209–2218.
Stoks, R., A. Ordoba-Aguilar, and A. Córdoba-Aguilar. 2012. Evolutionary Ecology of Odonata: A Complex Life Cycle Perspective. Annual Review of Entomology 57:249–65.
Suhling, F., I. Suhling, and O. Richter. 2015. Temperature response of growth of larval dragonflies – an overview. International Journal of Odonatology 18:15–30.
Sunday, J. M., A. E. Bates, and N. K. Dulvy. 2011. Global analysis of thermal tolerance and latitude in ectotherms. Proceedings of the Royal Society B: Biological Sciences 278:1823–1830.
Sunday, J. M., A. E. Bates, and N. K. Dulvy. 2012. Thermal tolerance and the global redistribution of animals. Nature Climate Change 2:686–690.
Sunday, J. M., A. E. Bates, M. R. Kearney, R. K. Colwell, N. K. Dulvy, J. T. Longino, and R. B. Huey. 2014. Thermal-safety margins and the necessity of thermoregulatory behavior across latitude and elevation. Proceedings of the National Academy of Sciences of the United States of America 111:5610–5.
Sunday, J. M., G. T. Pecl, S. Frusher, A. J. Hobday, N. Hill, N. J. Holbrook, G. J. Edgar, R. Stuart-Smith, N. Barrett, T. Wernberg, R. A. Watson, D. A. Smale, E. A. Fulton, D. Slawinski, M. Feng, B. T. Radford, P. A. Thompson, and A. E. Bates. 2015. Species traits and climate velocity explain geographic range shifts in an ocean-warming hotspot. Ecology Letters 18:944–953.
Symonds, M. R., and S. P. Blomberg. 2014. A primer on phylogenetic generalised least squares. In Modern phylogenetic comparative methods and their application in evolutionary biology. Springer Berlin Heidelberg.
Terblanche, J. S., J. A. Deere, S. Clusella-Trullas, C. Janion, and S. L. Chown. 2007. Critical thermal limits depend on methodological context. Proceedings of the Royal Society B: Biological Sciences 274:2935–42.
Terblanche, J. S., C. J. Klok, and S. L. Chown. 2005. Temperature-dependence of metabolic rate in Glossina morsitans morsitans (Diptera, Glossinidae) does not vary with gender, age, feeding, pregnancy or acclimation. Journal of Insect Physiology 51:861–870.
Terblanche, J. S., C. J. Klok, E. S. Krafsur, and S. L. Chown. 2006. Phenotypic plasticity and geographic variation in thermal tolerance and water loss of the tsetse Glossina pallidipes (Diptera : Glossinidae): Implications for distribution modelling (vol 74, pg 786, 2006).

American Journal of Tropical Medicine and Hygiene 74:786–794.
Thomas, C. D., C. D. Thomas, A. Cameron, A. Cameron, R. E. Green, R. E. Green, M. Bakkenes, M. Bakkenes, L. J. Beaumont, L. J. Beaumont, Y. C. Collingham, Y. C. Collingham, B. F. N. Erasmus, B. F. N. Erasmus, M. F. De Siqueira, M. F. De Siqueira, A. Grainger, A. Grainger, L. Hannah, L. Hannah, L. Hughes, L. Hughes, B. Huntley, B. Huntley, A. S. Van Jaarsveld, A. S. Van Jaarsveld, G. F. Midgley, G. F. Midgley, L. Miles, L. Miles, M. a Ortega-Huerta, M. a Ortega-Huerta, a T. Peterson, a T. Peterson, O. L. Phillips, O. L. Phillips, S. E. Williams, and S. E. Williams. 2004. Extinction risk from climate change. Nature in press:145–8.
Tingley, M. W., W. B. Monahan, S. R. Beissinger, and C. Moritz. 2009. Birds track their Grinnellian niche through a century of climate change. Proceedings of the National Academy of Sciences 106:19637–19643.
Turgeon, J., R. Stoks, R. A. Thum, J. M. Brown, M. A. Mcpeek, J. Jurgeon, R. Stoks, R. A. Thum, J. M. Brown, and M. A. Mcpeek. 2005. Simultaneous quaternary radiations of three damselfly clades across the Holarctic. The American Naturalist 165:E78–E107.
Verberk, W. C. E. P., and P. Calosi. 2012. Oxygen limits heat tolerance and drives heat hardening in the aquatic nymphs of the gill breathing damselfly Calopteryx virgo (Linnaeus, 1758). Journal of Thermal Biology 37:224–229.
Vernon, P., and G. Vannier. 1996. Developmental patterns of supercooling capacity in a subantarctic wingless fly. Experientia 52:155–158.
Wiens, J. J., D. D. Ackerly, A. P. Allen, B. L. Anacker, L. B. Buckley, H. V. Cornell, E. I. Damschen, T. Jonathan Davies, J. A. Grytnes, S. P. Harrison, B. A. Hawkins, R. D. Holt, C. M. McCain, and P. R. Stephens. 2010. Niche conservatism as an emerging principle in ecology and conservation biology. Ecology Letters 13:1310–1324.
Wiens, J. J., and M. J. Donoghue. 2004. Historical biogeography, ecology and species richness. Trends in Ecology and Evolution 19:639–644.
Wiens, J. J., and C. H. Graham. 2005. Niche Conservatism: Integrating Evolution, Ecology, and Conservation Biology. Annual Review of Ecology, Evolution, and Systematics 36:519–539.
Wissinger, S. A. 1989. Seasonal Variation in the Intensity of Competition and Predation Among Dragonfly Larvae 70:1017–1027.
Wisz, M. S., J. Pottier, W. D. Kissling, L. Pellissier, J. Lenoir, C. F. Damgaard, C. F. Dormann, M. C. Forchhammer, J.-A. Grytnes, A. Guisan, R. K. Heikkinen, T. T. Høye, I. Kühn, M. Luoto, L. Maiorano, M.-C. Nilsson, S. Normand, E. Öckinger, N. M. Schmidt, M. Termansen, A. Timmermann, D. A. Wardle, P. Aastrup, and J.-C. Svenning. 2013. The role of biotic interactions in shaping distributions and realised assemblages of species: implications for species distribution modelling. Biological Reviews 88:15–30.

TABLES AND FIGURES

Table 1. Akaike's Information Criterion (AIC) based model selection used to estimate North American odonate species' climatic heat and cold limit based on their critical upper thermal maxima (CT_{max}) and supercooling points (SCP) in their adult (ad) and larval (lv) life stages.

Model	GLS				PGLS			
	AIC	Δ AIC	AIC weight	p	AIC	Δ AIC	AIC weight	λ
Heat Limit								
CT_{max}_ad + CT_{max}_lv + int.	61.474	3.649	0.063	0.890	63.324	3.549	0.065	0.248
CT_{max}_ad + CT_{max}_lv	59.547	1.722	0.166	0.738	61.390	1.615	0.171	0.231
CT_{max}_ad	57.825	0.000	0.392	0.519	59.775	0.000	0.383	0.106
CT_{max}_lv	57.887	0.062	0.380	0.546	59.787	0.012	0.381	-0.111
Cold Limit								
SCP_ad + SCP_lv + int.	69.676	3.866	0.073	0.595	64.982	2.333	0.159	-0.422
SCP_ad + SCP_lv	67.705	1.895	0.195	0.370	64.774	2.125	0.177	-0.347
SCP_ad	67.384	1.574	0.229	0.370	65.065	2.416	0.153	-0.474
SCP_lv	65.810	0.000	0.503	0.155	62.649	0.000	0.511	-0.470

Models with AIC values which differ by less than 2 are considered statistically equivalent. Estimates of λ indicate the phylogenetic signal present in the residuals of the regression model. AIC weights give the likelihood of the model given the data. Values of p indicates the significance of the model. OLS = ordinary least squares; PGLS = phylogenetic generalized least squares, int = interaction term; CT_{max} = critical upper thermal maximum; SCP = supercooling point; ad = adult; lv = larva.

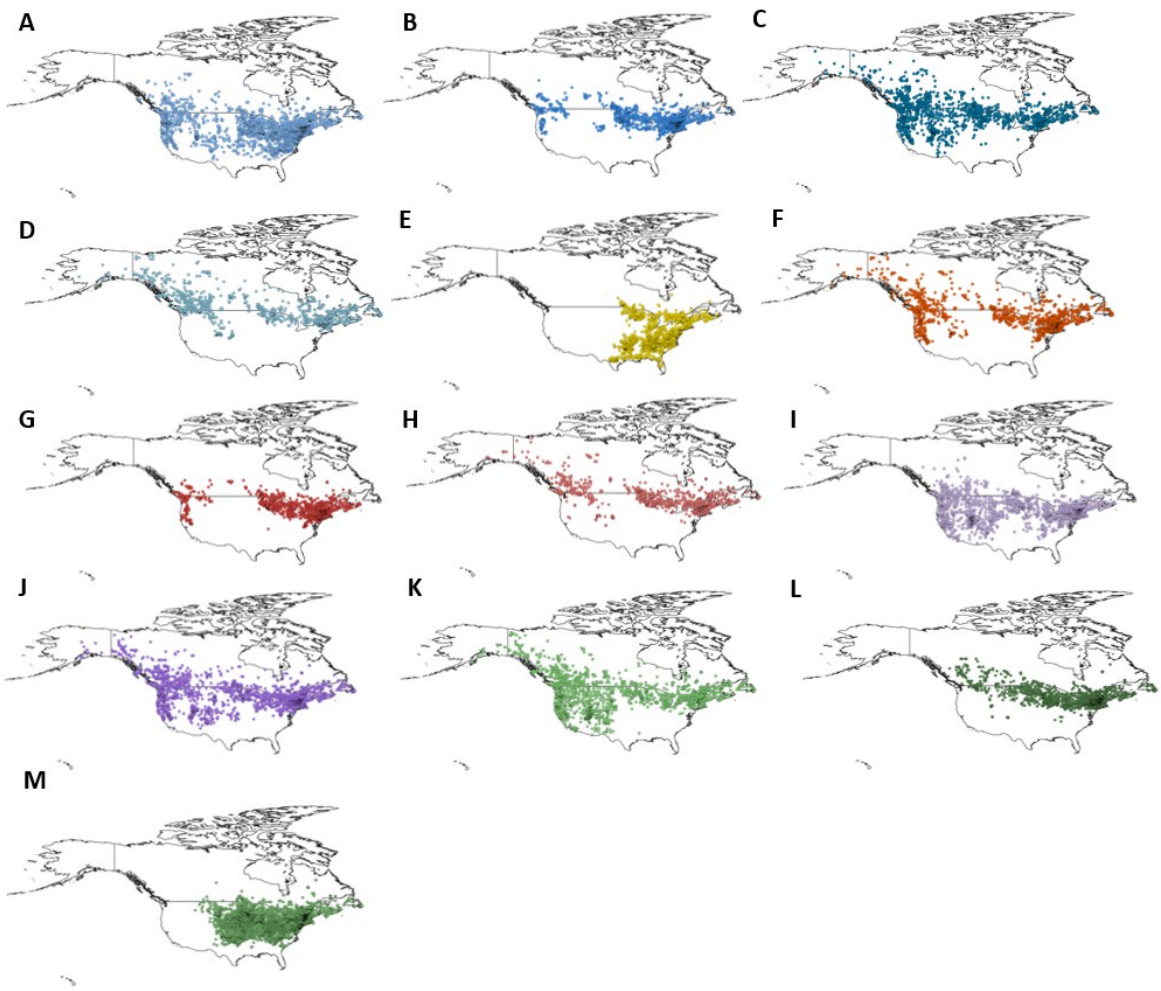

Figure 1. Geographic distributions of the 13 North American odonate species included in this study. Observations were used to estimate the heat and cold limits of species' climatic envelopes. (A) *Aeshna umbrosa*; (B) *Aeshna canadensis*; (C) *Aeshna interrupta* (D) *Aeshna eremita*; (E) *Hagenius brevistylus*; (F) *Cordulia shurtleffii*; (G) *Ladona julia*; (H) *Leucorrhinia proxima*; (I) *Lestes congener*; (J) *Lestes disjunctus*; (K) *Enallagma boreale*; (L) *Enallagma ebrium*; (M) *Ischnura verticalis*. Species from the same taxonomic family are shown in the same color; (blue) *Aeshnidae* (yellow) *Gomphidae*; (orange) *Corduliidae*; (red) *Libellulidae*; (purple) *Lestidae*; (green) *Coenagrionidae*.

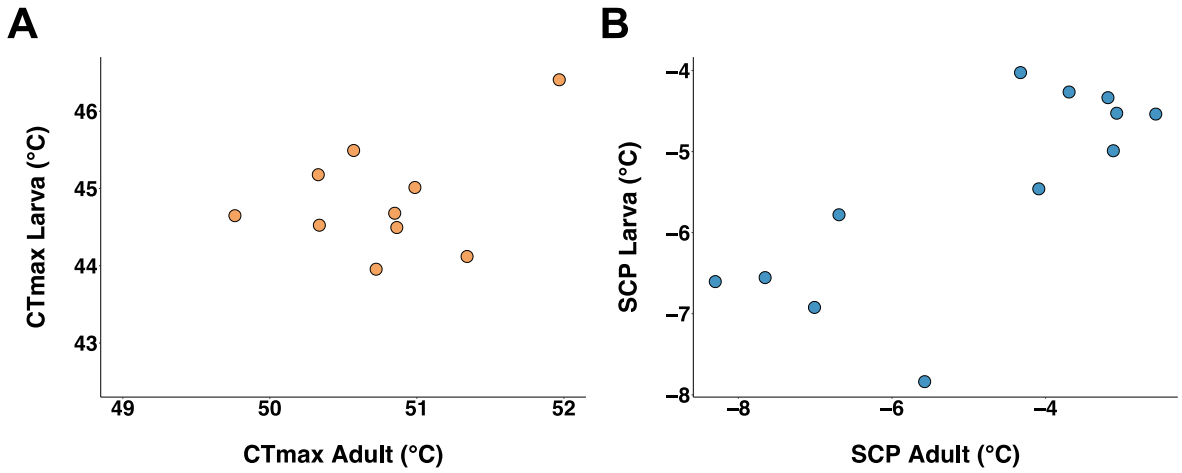

Figure 2. Correlation between the thermal limits of odonates in their adult and larval life stages. (A) correlation between the critical thermal maxima (CT_{max}; °C) of adults following exposure to 25 °C for 2 to 6 h and larvae following exposure to 20 °C for 7 to 10 d; (B) correlation between the supercooling points (SCP; °C) of adults after exposure to 15 °C for 2 to 6 h and larvae following exposure to 10 °C for 7 to 10 d. Each point represents individual odonate species.

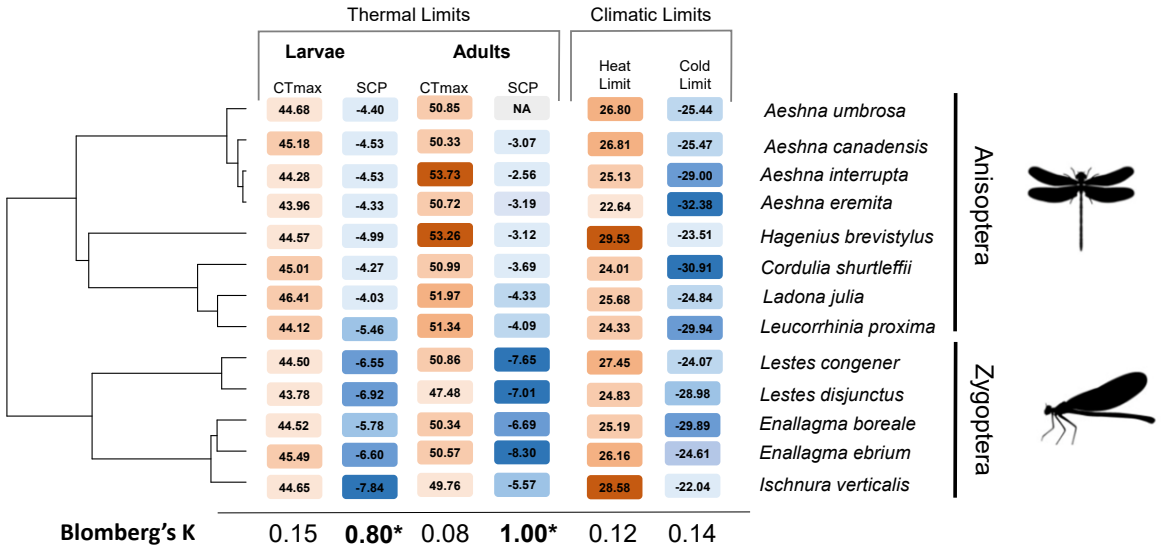

Figure 3. Phylogenetic signal in North American odonate species' thermal and climatic limits across their life cycle. The average values of species' critical thermal maxima (CT_{max}) and supercooling points (SCP) in the adult and larval stage, as well as their average climatic heat and cold limits are shown along with the associated values for Blomberg's K.

* $p < 0.001$

APPENDIX

Table A1. Summary of the taxonomic classification of the odonate species included in this study.

Species	Common name	Authority	Suborder	Family	Genus
A. umbrosa	Shadow darner	Walker, 1908	*Anisoptera*	*Aeshnidae*	*Aeshna*
A. canadensis	Canada darner	Walker, 1908	*Anisoptera*	*Aeshnidae*	*Aeshna*
A. interrupta	Variable darner	Walker 1908	*Anisoptera*	*Aeshnidae*	*Aeshna*
A. eremita	Lake darner	Scudder, 1866	*Anisoptera*	*Aeshnidae*	*Aeshna*
H. brevistylus	Dragonhunter	Selys, 1854	*Anisoptera*	*Gomphidae*	*Hagenius*
C. shurtleffii	American emerald	Scudder, 1866	*Anisoptera*	*Corduliidae*	*Cordulia*
L. julia	Chalk-fronted corporal	Uhler, 1857	*Anisoptera*	*Libellulidae*	*Ladona*
L. proxima	Belted whiteface	Calvert, 1890	*Anisoptera*	*Libellulidae*	*Leucorrhinia*
L. congener	Spotted spreadwing	Hagen, 1861	*Zygoptera*	*Lestidae*	*Lestes*
L. disjunctus	Common spreadwing	Selys, 1862	*Zygoptera*	*Lestidae*	*Lestes*
E. boreale	Boreale bluet	Selys, 1875	*Zygoptera*	*Coenagrionidae*	*Enallagma*
E. ebrium	Marsh bluet	Hagen, 1861	*Zygoptera*	*Coenagrionidae*	*Enallagma*
I. verticalis	Eastern forktail	Say, 1839	*Zygoptera*	*Coenagrionidae*	*Enallagma*

Table A2. Sampling sites located within la *Réserve Faunique Duchénier* where odonate adults and larvae were collected for this study.

Sampling Site	Latitude	Longitude	Substrate
Lac Bébé	48.205	-68.549	Some vegetation, cobble, gravel
Lac Blanc	48.178	-68.651	No vegetation, cobble, gravel
Lac Croisé	48.131	-68.639	Ample vegetation, mud, silt
Lac Cyprien	48.093	-68.740	Ample vegetation, mud, silt
Lac Dugas	48.214	-68.626	Ample vegetation, mud, silt
Lac France	48.198	-68.583	No vegetation, cobble, gravel
Lac Portage	48.186	-68.674	Ample vegetation, mud
Lac Quatres Martres	48.144	-68.684	Ample vegetation, mud, silt
Lac Rond	48.146	-68.710	Ample vegetation, mud, silt
Lac Touradi	48.139	-68.672	Some vegetation, cobble, gravel

Table A3. Summary of species' physiological thermal limits (°C; ± SE) in their larval and adult stage. CT_{max} = critical thermal maxima; SCP = supercooling point; n = sample size.

Species	Larvae				Adults			
	CT_{max}		SCP		CT_{max}		SCP	
	Mean (SE)	n	Mean (SE)	n	Mean (SE)	n	Mean (SE)	n
A. umbrosa	44.68 (0.31)	15	-4.40 (0.20)	19	50.85 (0.75)	4	NA	0
A. canadensis	45.18 (0.43)	14	-4.53 (0.71)	5	50.33 (0.44)	20	-3.07 (0.51)	15
A. interrupta	44.28 (0.77)	6	-4.53 (0.29)	14	53.73 (2.45)	4	-2.56 (0.04)	4
A. eremita	43.96 (0.40)	36	-4.33 (0.42)	14	50.72 (0.64)	17	-3.19 (0.39)	19
H. brevistylus	44.57 (0.53)	14	-4.99 (0.30)	16	53.26 (0.69)	15	-3.12 (0.35)	15
C. shurtleffii	45.01 (0.57)	23	-4.27 (0.25)	19	50.99 (0.60)	16	-3.69 (0.51)	16
L. julia	46.41 (0.33)	27	-4.03 (0.39)	16	51.97 (0.41)	26	-4.33 (0.47)	17
L. proxima	44.12 (0.55)	10	-5.46 (0.65)	8	51.34 (0.63)	19	-4.09 (0.51)	17
L. congener	44.50 (0.49)	22	-6.55 (0.23)	12	50.86 (1.36)	14	-7.65 (0.92)	7
L. disjunctus	43.78 (1.05)	4	-6.92 (0.33)	8	47.48 (0.78)	24	-7.01 (0.41)	19
E. boreale	44.52 (0.38)	4	-5.78 (1.05)	3	50.34 (1.00)	16	-6.69 (0.54)	15
E. ebrium	45.49 (0.83)	28	-6.60 (0.51)	6	50.57 (1.30)	24	-8.30 (0.50)	22
I. verticalis	44.65 (0.46)	34	-7.84 (0.64)	13	49.76 (0.69)	19	-5.57 (0.40)	18

Table A4. Species' climatic heat and cold limits (°C; ± SE) respectively calculated based on the 95th percentile of the hottest temperatures of the warmest month (BIO5) and 5th percentile of the coldest temperatures of the coolest month (BIO6) and the total number of occurrence records used to extract these values.

Species	95th percentile BIO5 (SE)	5th percentile BIO6 (SE)	Total Occurrence Records
Aeshna umbrosa	26.809 (0.336)	-25.442 (0.281)	2059
Aeshna canadensis	25.588 (0.311)	-25.471 (0.163)	1082
Aeshna interrupta	25.126 (0.443)	-29.002 (0.316)	1420
Aeshna eremita	22.637 (0.476)	-32.376 (0.175)	855
Hagenius brevistylus	29.525 (0.424)	-23.514 (0.168)	1253
Cordulia shurtleffii	24.008 (0.421)	-30.908 (2.168)	1153
Ladona julia	25.680 (0.291)	-24.842 (0.715)	1061
Leucorrhinia proxima	24.331 (0.416)	-29.944 (0.356)	847
Lestes congener	27.447 (0.371)	-24.073 (0.225)	1698
Lestes disjunctus	24.832 (0.405)	-28.977 (0.276)	1626
Enallagma boreale	25.185 (0.484)	-29.888 (0.299)	1520
Enallagma ebrium	26.157 (0.291)	-24.611 (0.188)	1308
Ischnura verticalis	28.582 (0.203)	-22.038 (0.172)	3229

Table A5. Correlations between average thermal tolerance traits in the larval or adult life stages and the number of individuals measured to obtain averages for each species. CT_{max} = critical thermal maxima; SCP = supercooling point.

Physiological Trait	Sample Size Range	Pearson's correlation coefficient	p
CT_{max} Larvae	4 - 36	0.364	0.222
CT_{max} Adults	4 - 26	-0.477	0.100
SCP Larvae	3 - 19	0.439	0.133
SCP Adults	4 - 22	-0.240	0.452

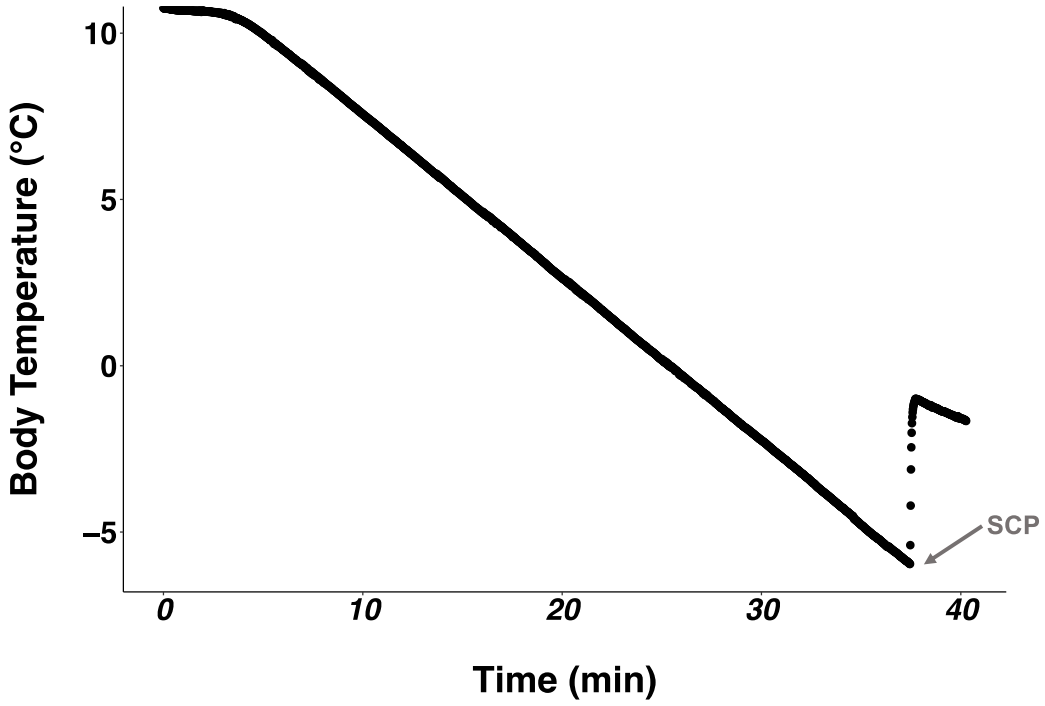

Figure A1. Body temperature of an odonate larva during supercooling point (SCP) experiment. The body temperature of the individual is gradually cooled until it's internal body fluids begin to freeze at which point we observe a spike in the individual's body temperature indicating the start of the phase change. The individual's lowest body temperature before the temperature increases is classified as its SCP, indicate by an arrow.

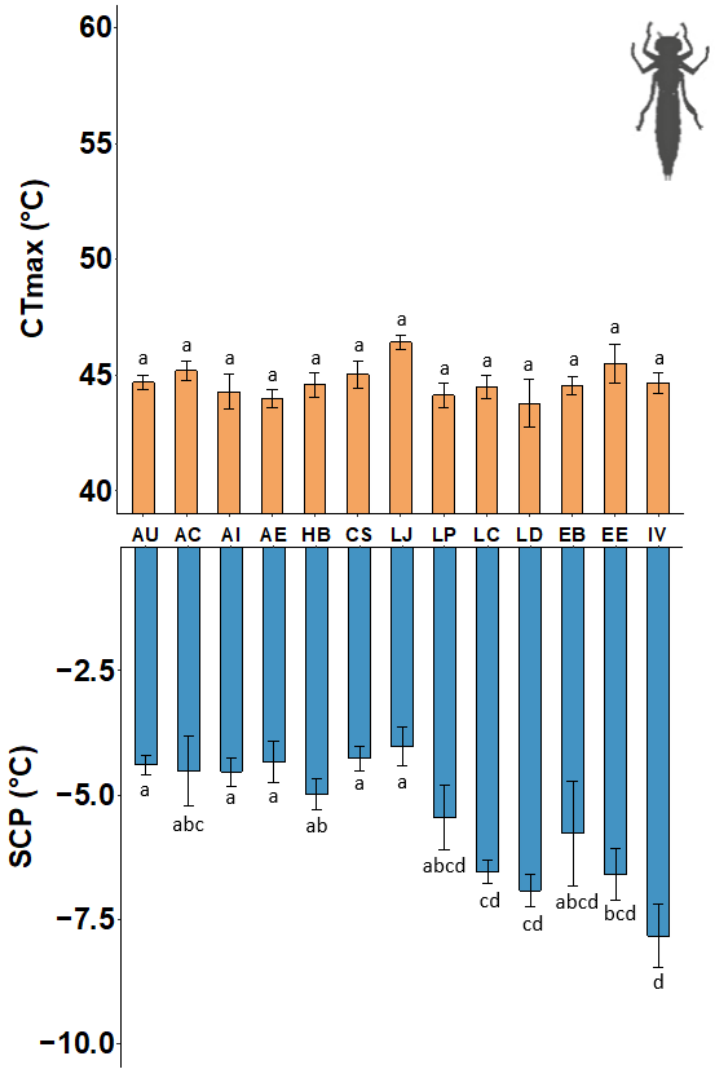

Figure 2A. Upper and lower thermal limits of North American odonate species in their larval stage. (A) Critical thermal maxima (CT_{max}) measured after exposure to 20 °C for 7 to 10 d. (B) Supercooling points (SCP) measured after exposure to 10 °C for 7 to 10 d. Bars represent mean values for each species (°C; ± SE). Letters indicate significant differences between species following false discovery rate (FDR) corrections ($p < 0.05$). AU - Aeshna umbrosa; AC – *Aeshna canadensis*; AI – *Aeshna interrupta*; AE – *Aeshna eremita*; HB – *Hagenius brevistylus*; CS – *Cordulia shurtleffii*; LJ – *Ladona julia*; LP – *Leucorrhinia proxima*; LC – *Lestes congener*; LD – *Lestes disjunctus*; EB – *Enallagma boreale*; EE – *Enallagma ebrium*; IV – *Ischnura verticalis*.

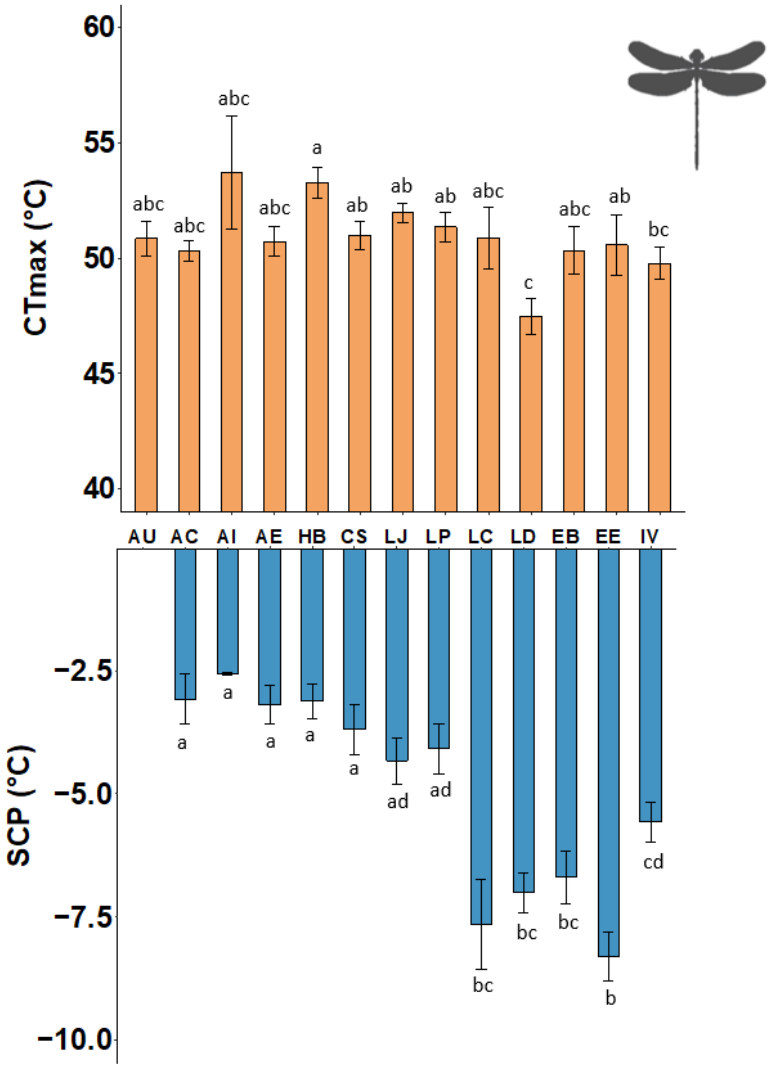

Figure 3A. Upper and lower thermal limits of North American odonate species in their adult stage. (A) Upper critical thermal maxima (CT_{max}) measured after exposure to 25 °C for 2 to 6 h. (B) Supercooling points (SCP) measured after exposure to 15 °C for 2 to 6 h. Bars represent mean values for each species (°C; ± SE). Letters indicate significant differences between species following false discovery rate (FDR) corrections ($p < 0.05$). AU - Aeshna umbrosa; AC – *Aeshna canadensis*; AI – *Aeshna interrupta*; AE – *Aeshna eremita*; HB – *Hagenius brevistylus*; CS – *Cordulia shurtleffii*; LJ – *Ladona julia*; LP – *Leucorrhinia proxima*; LC – *Lestes congener*; LD – *Lestes disjunctus*; EB – *Enallagma boreale*; EE – *Enallagma ebrium*; IV – *Ischnura verticalis*.