Time stress and temperature explain continental variation in damselfly body size[1]

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ABSTRACT
Body size is among the most important biological variables but despite much measurement of this trait, the factors driving its variation are not fully understood. Here, I describe variation in body size in the damselfly *Calopteryx maculata* to establish whether variations in growth and development observed in response to experimental manipulation of temperature and time stress in the laboratory can be scaled-up to variation among natural populations. 907 specimens of *C. maculata* males were collected from 34 sites across the species' entire range in North America during the summer of 2010. A general measure of body size was derived from a series of wing and leg measurements. I compare the fit of models based on latitude (Bergmann's rule), temperature (the temperature-size rule) and seasonal effects (a combination of temperature and time stress) using Akaike's Information Criterion (AIC). The U-shaped relationship between size and latitude was best explained by a seasonality model containing both photoperiod and temperature. The presence of both these terms suggests that time stress dominates in the southern part of the range, reducing body size by accelerating development. However, the temperature-size rule dominates in the northern part of the range, increasing body size closer to the northern range margin. The best-fit model of geographic variation in size is in accordance with previous laboratory studies of temperature and photoperiod in damselflies and theoretical work, indicating that the findings from such studies can be applied to natural populations. These findings are likely applicable to any species with complex life histories inhabiting seasonal environments.

INTRODUCTION
Body size is one of the most important biological variables, influencing or trading-off against a huge array of ecological parameters, including dispersal (Jenkins, et al. 2007), developmental rates (Nijhout, et al. 2010), trophic interactions (Brose, et al. 2006), fecundity (Roff 2002), and abundance (White, et al. 2007). While larger body size is generally considered to be selected for by both ecological and sexual components of natural selection (Clutton-Brock 1988), there are also a range of selection pressures which favour smaller sizes (Blanckenhorn 2000). Thus the benefits of large size at maturity in terms of fecundity and competition are traded off against the increased risk of mortality that arises while growing to that size (Abrams, et al. 1996, Roff 2002). Widely-accepted correlates of inter- and intraspecific variation in body size include latitude (reviewed by Meiri and Dayan 2003) and temperature (Atkinson 1994), although the mechanism behind these patterns still proves elusive (Angilletta Jnr. and Dunham 2003).

Bergmann's rule (Bergmann 1847), the observation that individuals and species at higher latitudes tend to be larger at maturity, is among the few rules in biology. It has been estimated that this pattern holds for the majority (between 62% and 83%) of vertebrate species (reviewed by Millien, et al. 2006). The first mechanisms proposed to explain Bergmann's rule involved the decreased surface area:volume ratio (SAVR) of larger animals and the benefits that this would have in reducing heat loss in colder climates. However, evidence against the simple thermal explanation includes (i) that temperature is not the only environmental variable that varies with latitude that appears to influence size (Yom-Tov and Nix 1986), (ii) that modification of SAVR is not as efficient a thermoregulatory strategy as alternatives such as fur and

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vascular control (Scholander 1955), and (iii) Bergmann's rule appears to apply to some ectotherms, where regulation of heat loss is unlikely to be significant (Atkinson 1994, but cf Mousseau 1997).

Chown and Gaston (2010) provide a comprehensive overview of body size variation in insects through space and review 58 studies describing intraspecific variation in size. Of these 58 studies, 28 exhibited positive relationships (known as "Bergmann clines", after Bergmann 1847), 19 negative relationships (known as "converse Bergmann clines", Blanckenhorn, et al. 2006), 2 curvilinear patterns (e.g. Johansson 2003), and 2 sawtooth patterns (as described by Roff 1980) between size and latitude (see also Blanckenhorn and Demont 2004 for further reviews). However, experimental attempts to generate sawtooth clines have met with mixed results (Kivelä, et al. 2011). Similar variation occurs in studies of altitudinal variation in body size – out of 29 studies reviewed by Chown and Gaston, 15 showed positive relationships, 11 showed negative relationships, and 3 showed no relationships between size and altitude. Common garden experiments have shown, at least in some taxa, that size variation has a genetic component in addition to being influenced by environmental variables such as temperature or nutrition (e.g. De Block, et al. 2008, Weeks, et al. 2002). However, while numerous descriptions of geographical variation in body size have been made, there has been little attempt to make formal connections to the proximate mechanisms underlying that variation (Chown and Gaston 2010).

The "temperature-size rule", often used interchangeably with Bergmann's rule due to the strong correlation between latitude and temperature, typically states that individuals reared at colder temperatures mature at larger sizes than individuals reared at warmer temperatures (Atkinson 1994, Atkinson and Sibly 1997). It has been suggested previously to differentiate the two rules as follows: Bergmann's rule is the description of an association between temperature and size in natural populations while the temperature-size rule is a description of the thermal reaction norms relating temperature to size in laboratory experiments (Angilletta Jnr. and Dunham 2003), although this ignores additional factors involved in latitude-size relationships described above. It has been demonstrated that many ectotherms actually mature at larger sizes at higher temperatures (Mousseau 1997), which is the more intuitive pattern based on availability of energy for metabolic processes. The current leading explanation for why some species follow the temperature-size rule while others do not involves differences in the temperature dependence of development (i.e. the reaching of ontogenetic milestones) and growth (i.e. the accumulation of biomass) (Forster, et al. 2011, Walters and Hassall 2006, Zuo, et al. 2012).

The Odonata (dragonflies and damselflies) are considered model organisms for the study of numerous ecological and evolutionary phenomena (Cordoba-Aguilar 2008) and are particularly well-understood in terms of growth and development. Previous laboratory studies have demonstrated that temperature (Pickup and Thompson 1990) and time stress (Stoks, et al. 2008) influence the rates of development and growth. Body size measurements taken as part of mark-recapture studies have demonstrated a decline in size over the season (Banks and Thompson 1985). However, field studies of variation in body size between populations of Odonata are relatively rare. Qualitative assessments seem to indicate that some species increase in size at higher latitudes or altitudes while others decline in size (see Table A.6.3 in Corbet 1999 for a review). In the only large-scale study, Johansson (2003) found a curvilinear (U-shaped) relationship between latitude and body size in *Enallagma cyathigerum*. Unsurprisingly this was also related to temperature, due to the correlation between temperature and latitude. Johansson went on to predict that obligately univoltine species would exhibit negative relationships between latitude and body size, in accordance with other ectotherms (Mousseau 1997). Measurement of water temperature in the field is even rarer, although a study conducted at a reservoir receiving thermal effluent found that of nine species, eight exhibited negative relationships between size and temperature (four of which were statistically significant) and one a non-significant positive relationship (Cothran and Thorp 1982). Body

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size in the damselfly *Calopteryx splendens* has also been shown to increase with increasing pH within a catchment (Chaput-Bardy, et al. 2007).

While there has been considerable work done on the relationship between odonate size and environmental conditions in the laboratory, there have been relatively few large-scale studies attempting to test whether those patterns occur in wild populations (cf. Johansson 2003). Here, I evaluate the fit of three competing models that could explain geographical variation in body size of *Calopteryx maculata*. The extensive distribution of this species (essential for studying geographical variation in size, Chown and Gaston 2010) and univoltinism across its range (discussed below) makes *C. maculata* an ideal model species to evaluate hypotheses relating to geographical variation in body size.

Bergmann's rule – If Bergmann clines were the primary drivers of body size, I would expect to see a latitudinal cline that better explains variation in size than a simple environmental temperature measure. This might manifest as an increase or decrease in size towards higher latitudes, the direction being difficult to predict due to interspecific variation in the response to temperature of development and growth (see Atkinson 1995 for an example of Ephemeroptera that exhibit contrasting temperature-size relationships, and Chown and Gaston 2010 for a broader taxonomic survey). The fit to latitude would incorporate other latent variables, such as productivity or precipitation, that lend additional explanatory power to a latitude-based model beyond a pure temperature model.

Temperature-size rule – The response of particular species to temperature is difficult to predict, as mentioned above. The superiority of the temperature-size rule over Bergmann's rule would manifest as a closer fit to the data for temperature compared to latitude, if temperature itself is the proximate factor driving variation in size. A sampling strategy covering a range of longitudes with varying distances from the coast across latitudes should reduce the correlation between latitude and temperature to make this effect clearer.

Seasonality – Both temperature and latitudinal effects may act on odonate development to produce variation in body size at maturity. In odonates, development is contingent not only on thermal reaction norms, where increasing temperatures reduce size at emergence, but also on perceptions of time stress in the form of photoperiod. Photoperiodic fluctuations, which influence the rate of growth and the rate of development (De Block and Stoks 2003), are intrinsically tied to latitude. In some species, the net effect of photoperiod on growth and development time is a reduction in size at emergence (De Block and Stoks 2003), while in other species growth rate compensates for reduced development time resulting in no net change in size (Strobbe and Stoks 2004). Individuals inhabiting different latitudes (and, hence, experiencing different photoperiods) will experience different temperatures as a result of the sampling strategy. As a result, the seasonality model will include temperature and latitude-specific photoperiod as well as a potential interaction between the two variables.

METHODS
Study species
Calopteryx maculata is one of the most common and best-studied riverine damselflies in North America. As such, it is capable of sustaining extensive sampling from populations without risk of demographic collapse. The three published studies that recorded the voltinism of *C. maculata* have found the species to be univoltine across its range, including at around 45.5°N in Algonquin Park, Ontario (Martin 1939), around 37.2°N in Virginia (Burcher and Smock 2002), and around 35.5°N in North Carolina, (Paulson and Jenner 1971), which avoids complications due to varying numbers of generations per year (as was the case with a previous study of *Enallagma cyathigerum*, Johansson 2003).

Field sampling

A total of 907 male specimens of *C. maculata* were collected from 34 sites across the range by 25 collectors (Figure 1, see Table 1 for details of sites and sample sizes). Females were not collected to limit damage to populations that exhibited high variability in the numbers of animals between years, and because males were easier than females for volunteer collectors to discriminate from the congener *C. aequabilis*. Collections took place between 13 May and 7 August 2010 and sample sizes from each site varied between 4 and 84 individuals (mean=26.7 ±2.9 SE). Specimens were sent to the author's lab for measurement. Wings were dissected from the body as close to the thorax as possible and the right hind leg was removed. Where the right hind leg was missing the left hind leg was taken.

Measurements

The four wings and hind leg were mounted on transparent, adhesive tape. Wings were scanned using the slide scanner on an Epson V500 PHOTO flatbed scanner with fixed exposure at 1200dpi. Due to differences between individuals in the accuracy of dissections, all wing images were modified to omit the arculus and all regions before the first cross-veins (Figure S1). Wing length (the length from the costal end of the vein separating the arculus from the discoidal cell to the tip of the wing) was calculated for each of the four wings, along with the length of the hind tibia. All measurements were carried out in ImageJ (Rasband 1997-2007). Twenty specimens were randomly selected for a repeatability analysis. The mean error for fore wing length was 0.17%, for hind wing length was 0.15% and for hind tibia length was 0.56%. During measurement, any damage to wings was noted and those measurements (length or area) which could not be accurately quantified were excluded. This resulted in the exclusion of seven fore wing and nine hind wing lengths. Specimens missing hind legs were also excluded, leaving a total of 879 specimens for the analysis.

Due to high collinearity between size measurements, a composite measure of body size was defined as the first principal component (PC1) of fore wing and hind wing lengths and the length of the hind tibia. While wing areas were also measured, they were not included as they are not independent of wing lengths. PC1 explained 93.4% of the variance in the three size measures and was significantly and negatively correlated (p<0.001 in all cases) with all measurements (fore wing length, r=-0.992, hind wing length, r=-0.991, tibia length, r=-0.694). Raw data are included in Table S1.

Temperature data

Temperature data were extracted from the US National Oceanic and Atmospheric Administration (NOAA) National Climate Data Center (http://www.ncdc.noaa.gov/cdo-web/) for US sampling sites and Environment Canada's National Climate Data and Information Archive (http://climate.weatheroffice.gc.ca/climateData/canada_e.html). Weather stations were chosen for their proximity to sampling sites: mean distance between each site and its weather station was 22.9km ±4.2 (SE), with a maximum distance of 121km (see Table S2 for a list of weather stations, locations and identification codes). Twenty-nine of the weather stations were within 30km of the sampling sites. Mean daily temperature at each site was calculated from the minimum and maximum daily temperatures ((min+max)/2) for a one year period between 1 July 2009 and 30 June 2010. Where daily data were missing from the record, values were interpolated based on 4th order polynomial models which fitted very well to the data (R^2 between 0.744 and 0.882, see Table S2 for parameter estimates). Studies have demonstrated that thermal thresholds exist for the development of odonate eggs (11.25°C in *Argia vivida*, Leggott and Pritchard 1985, 12°C in *Coenagrion puella*, Waringer and Humpesch 1984) and larvae (8°C in Ischnura elegans, Thompson 1978). Since calculations of developmental thresholds have not been made for *C. maculata*, I used a base of 10°C to calculate degree day accumulation (DD_{10}) over the year

at each site. Stream temperatures have been shown to correlate well with atmospheric temperatures, supporting the use of atmospheric temperature as a proxy for water temperature (Pilgrim, et al. 1998).

Data analysis
Variables were transformed for normality where appropriate after assessment using Shapiro-Wilk tests. Mixed effects models were constructed in the lme4 library (Bates, et al. 2011) in R (R Development Core Team 2012), with site as a random effect and body size (PC1) as the response variable. Fixed effect predictors from which models were constructed were (i) latitude and latitude^2 (the "Bergmann model"), (ii) DD_{10} and DD_{10}^2 (the "temperature-size model") or (iii) photoperiod (on 1st May), photoperiod^2, DD_{10}, DD_{10}^2 and the photoperiod×DD_{10} interaction (the "seasonality model").

Akaike's Information Criterion (AIC, a measure of goodness-of-fit taking into account the number of parameters) was used to evaluate (i) the improvement in model fit due to the addition of quadratic terms in the geographic, temperature, and seasonality models, (ii) the improvement in model fit due to the addition of ordinal date (days since 1st January, averaged if sampling occurred over multiple days) of the collection, and (iii) the relative performance of the three candidate models. Model comparison was carried out using the aictab function in the AICcmodavg package (Mazerolle 2012) in R. This procedure calculated the AICc (AIC accounting for small sample sizes), ΔAICc (the difference between the AICc of the focal model and that of the best model), and w_i (the "Akaike weight", which gives the ratio of ΔAICc values for each model relative to the entire set of candidate models). Models for which ΔAIC<2 have substantial support compared to the top model, 3<ΔAIC<7 indicates considerably less support and ΔAIC>10 indicates essentially no support (Burnham and Anderson 2002).

Two nearby populations may exhibit similar morphological traits simply by virtue of their proximity rather than as a result of some shared causal process. Hence, spatial proximity will reduce the independence of data within the study as well as clouding potential causal relationships. This spatial autocorrelation (SAC) must be controlled for in analyses if model residuals exhibit spatial autocorrelation (Diniz-Filho, et al. 2003). All model residuals were tested for spatial autocorrelation in R. A final methodological point to consider is that of multicollinearity, which is introduced in this analysis as a consequence of including temperature and photoperiod across a wide geographical area. However, while multicollinearity interferes with the interpretation of significance of individual terms within the models, the statistical significance of goodness of fit of the final model is not affected (Graham 2003).

Additional supporting data
In an earlier analysis of variation in body size in *C. maculata*, Huggins (1926) describes variation in 106 males and 65 females from 21 sites across North America. Collection dates are not available for these smaller samples of specimens, though it is stated that specimens were caught from particular sites in different years, and Huggins provides only average local temperature measurements so a rigorous statistical analysis such as that described above is not possible. Nevertheless, I include Huggins' data as a comparison with my own and test for non-linearity in the relationships between latitude and body size, and temperature and body size using AIC to compare general linear models (GLMs) weighted by the sample size. I extract comparable mean annual temperature data to match Huggins' temperature data from the WorldClim dataset (Hijmans, et al. 2005). Huggins' measurement of both males and females permits a test of Rensch's rule, which states that sexual size dimorphism (SSD) increases with trait size when SSD is male-biased and decreases with trait size when SSD is female-biased. I test for SSD in Huggins' measurements using Wilcoxon matched-pairs tests on site means, and for the relationship between SSD and trait size using major axis regression in the lmodel2 package (Legendre 2011) in R to take into account the error in both measurements.

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RESULTS

All aspects of body size showed considerable variation across the range (Table 1). There is no evidence of a sudden change in mean or variance of body size with latitude (see error bars in Figure 2A), which has been suggested as a hallmark of changing voltinism (Hassall and Thompson 2008, Roff 1980). For these reasons, I conclude that *C. maculata* is univoltine throughout its range. The composite measure of body size (-PC1, since PC1 was negatively correlated with all size measures) showed significant negative linear and U-shaped quadratic relationships with latitude as well as temperature (Table 2, Figure 2, see Figure S2 for plots of data for individuals).

The temperature-size model produced AICc values of 9718.7 and 9704.7 (ΔAIC=13.9) for the linear and quadratic fits, respectively. The Bergmann model produced AIC values of 9721.5 and 9717.1 (ΔAIC=4.5) for the linear and quadratic fits, respectively. The seasonality model produced the most parsimonious fit (lowest AIC) with quadratic terms for both temperature and photoperiod, when compared against two linear terms (ΔAIC=26.6), quadratic temperature plus linear photoperiod (ΔAIC=13.5), and quadratic photoperiod plus linear temperature (ΔAIC=9.6). An interaction between the two linear terms also produced a substantially less parsimonious fit (ΔAIC=10.1). From these ΔAIC values, there is support for the inclusion of quadratic terms in all three models. The addition of ordinal date produced models with only slightly higher AIC values for the Bergmann (ΔAIC=2.7), temperature-size (ΔAIC=2.7), and seasonality models (ΔAIC=2.8) and so date was not included. None of the three models produced spatially-autocorrelated residuals so no control for spatial autocorrelation was employed.

Based on the ΔAICc values for the three main models, it can be concluded that the temperature-size model and the Bergmann model have lower overall explanatory power compared to the seasonality model (Table 2A). Of greatest interest here is the interpretation of the model containing photoperiod and temperature, shown in Figure 2B. The pattern is consistent with two complementary processes: increases in time stress reduce growth, accelerate development, and reduce size at emergence between the southern range margin and the centre (as observed in laboratory experiments, Stoks, et al. 2008). Following that, the temperature-size rule drives an increase in body size between the centre of the range and the northern margin (also consistent with laboratory experiments on a range of species, Pickup and Thompson 1990).

When compared with the data from this study, the data collected by Huggins are remarkably similar (Figure 3). Females have larger fore wing lengths (Mann-Whitney U=46.0, p=0.001) and hind wing lengths (U=44.5, p=0.001) compared to males, though there was no difference between sexes in hind tibia lengths (U=85.5, p=0.103). A quadratic term in the general linear models provided an improved fit to the body size data compared to linear models for both the latitude-size relationship (ΔAICc=10.8) and the temperature-size relationship (ΔAICc=6.6) in males. The quadratic GLMs explained 65.9% of the variation between temperature and size, and 77.6% of the variation between latitude and size. This was also true for Huggins' smaller sample of female *C. maculata*, where quadratic GLMs improved the fit to the latitude-size (ΔAICc=7.9) and temperature-size (ΔAICc=6.9) relationships. Female GLMs explained 74.8% of the variation in the latitude-size relationship and 67.6% of the variation in the temperature-size relationship. For the 13 sites at which both males and females were collected, there was significant female-biased SSD in all three traits (fore wing length: V=91, p=0.002; hind wing length: V=91, p=0.002; hind tibia length: V=55, p=0.006). Major axis regression of female size on male size yielded a significant slope that did not differ from one in fore wing length (slope=1.056 (95% CI: 0.863-1.296), p=0.001), hind wing length (slope=1.048 (95% CI: 0.873-1.259), p=0.001), and hind tibia length (slope=0.955 (95% CI:

0.467-1.907), p=0.003, see Figure 4). Based on these limited data, I suggest that Rensch's rule does not hold in this system.

DISCUSSION

I provide the first analysis of whole-range variation in body size in a damselfly to test the leading hypotheses for variations in this important biological trait. I demonstrate that a model of variation involving not only temperature but time stress (related to photoperiod) out-performs models corresponding to the Bergmann and temperature-size rules. It is likely that this best fit model combining latitudinal effects in the form of time stress with temperature can explain variation in body size in many animals with complex life cycles inhabiting seasonal environments.

The negative relationship between latitude and body size in this study was predicted by Johansson (2003) for univoltine species and is in accordance with other ectotherms (Mousseau 1997). However, the relationship between temperature and photoperiod displayed in Figure 2B suggests that the pattern of body size across latitudes represents "two ends of a continuum" (Blanckenhorn and Demont 2004). Previous studies have shown that photoperiodic increases in growth rates can either (i) undercompensate for increases in development rate leading to smaller size at emergence (De Block and Stoks 2003), or (ii) fully compensate for increases in development rate through countergradient variation leading to equal size at emergence under different photoperiods (Strobbe and Stoks 2004). The latitudinal decline in body size in the results presented here suggests that there may be under compensation in growth rates due to photoperiod in *C. maculata* that dominates the latitude-size patterns at lower latitudes. In the middle of the range, this decline slows and stops, then body size increases again towards the northern range margin. This is consistent with the temperature-size rule predominating at higher latitudes where temperatures are lowest.

The pattern found in specimens collected for this study closely resemble the pattern found by previous studies, including that by Huggins (1926). In other studies of variation in *C. maculata* morphology, Taylor and Merriam (1995) found variation in mean fore wing length between sites of 27.1mm to 28.5mm (ca. 5%), and Pither and Taylor (2000) found that fore wing length varied across three sites between 32.258 and 33.380mm (ca. 3%), while hind wing length varied between 30.968 and 32.089mm (ca. 4%), both studies carried out in southern Canada. In a more detailed study of geographic variation, Waage (1979) also found a slight decline in female fore wing length from 32.5mm at 36°N to 30mm at ca. 42.5°N, followed by an increase in size to 34.3mm at 47°N. Waage's results closely mirror those of Huggins and the present study, suggesting that the U-shaped latitude-size pattern is genuine.

Interactions between photoperiod and temperature have been studied in a number of odonates.   Most studies of time stress in damselflies have been conducted on *Lestes* sp. (12 out of 14 studies reviewed by Stoks, et al. 2008) due to its strong response to time stress. However, this response is almost certainly not universal in odonates, given the high proportion of species inhabiting tropical latitudes without significant photoperiodic variation. The response may not even be consistent within genera, as low temperatures inhibited growth rate responses to time stress in *Enallagma aspersum*, but not *Enallagma hageni* (Ingram and Jenner 1976). That said, the finding that *C. maculata* varies strongly in size with latitude is not surprising. Substantial variation in the response of growth rates to thermal conditions must be possible to permit full development in a single year at widely-varying temperature regimes and the sensitivity of calopterygid damselflies to changing photoperiod has already been demonstrated (Plaistow and Siva-jothy 1999). Evolutionary and plastic responses in growth rate to different temperatures have likely played a key role in the successful colonisation by insects of temperate, seasonal latitudes (see Danks 2007 for a review).

Since the data are field observations of body size without common garden rearing, I cannot discount the presence of adaptive countergradient variation in growth rates or other genetic effects. Such countergradient variation may be present in *Enallagma cyathigerum*, which showed variation in mass at emergence (De Block, et al. 2008). This result has been proposed as an explanation for the U-shaped relationship between latitude and body size found in this same species by Johansson (2003). However, the three samples on which the study by De Block et al. (2008) was based exhibit different voltinism in the field (two are univoltine while one is semivoltine), and this may complicate results if there are adaptations associated with voltinism. Other studies in Odonata have demonstrated adaptations to local photoperiodic patterns in terms of diapause induction (Norling 1984). The effects on body size of cohort-splitting (where individuals within the same population exhibit different generation times) and changes in voltinism due to thresholds of development are unclear. Johansson (2003) proposed that the patterns seen in body size across latitudes in his study were indicative of changes in voltinism but the fact that *E. cyathigerum* is either bivoltine, split bi- and univoltine, univoltine, split uni- and semivoltine or semivoltine between 47.48°N and 56.53°N (see the Appendix in Corbet, et al. 2006) across that study area complicates the interpretation of those results. As outlined above, *C. maculata* is likely to be univoltine across its range, reducing any error associated with voltinism. Cabanita and Atkinson (2006) have suggested that cohort splitting may negate the effects of seasonal time constraints in altering size at emergence in some aquatic insects, although it is unclear how often this occurs.

The correlational nature of my study leaves open the possibility for other drivers of body size in Odonata. Previous studies have shown that prey availability influences growth rates and size in damselflies (Lawton, et al. 1980, Pickup and Thompson 1990). Prey community structure will certainly vary geographically due to abiotic and biotic requirements of the prey species. However, there is no reason to suspect that prey *availability* will vary systematically for a generalist predator like an odonate larva. Fragmented habitats have been shown to contain *C. maculata* individuals that have larger wings (Taylor and Merriam 1995), although it is unclear whether this is a result of spatial segregation of phenotypic variation (i.e. ecological processes) or selection for particular phenotypes (i.e. evolutionary processes). Predation by fish has also been implicated in driving variation in macroinvertebrate body sizes (Blumenshine, et al. 2000), but this acts by selecting among, rather than within, species. A final possibility as a driver of body size is larval competition. High larval densities are associated with smaller body size which may be due to food limitation (Banks and Thompson 1987), although high larval densities may also result in increased mean population body size through preferential cannibalism of smaller larvae (Van Buskirk 1989).

The difficulties of extending the results of this study to other taxa stem from the lack of rigorous investigation of the interaction between temperature and photoperiod. While few groups have been studied in such detail as the Odonata, there are other groups for which data exist. Lepidoptera have been shown to exhibit greater increases in growth rate in response to increasing temperature when under time stress (Gotthard, et al. 2000) – exactly as Figure 2B suggests is the case for *C. maculata*. However, studies in anurans have produced equivocal results (Laurila, et al. 2001). The results of these studies may be complicated by the presence of additional seasonal cues such as pond drying, which is known to affect development in anurans (Altwegg 2002) and odonates (Sawchyn and Gillott 1974).

The results of this study constitute the most thorough investigation of damselfly size variation in the field and the first demonstration that patterns detected by laboratory manipulations of temperature and photoperiod are present in natural populations. The best-fit model explaining body size variation is consistent with theory in describing a situation where time stress reduces body size at low latitudes and decreasing temperatures increase body sizes at higher latitudes. The additive effect of these two linear

patterns results in the U-shaped relationship between size and latitude. While sexual selection is thought to be a primary driver of body size evolution (Blanckenhorn 2000), the lack of evidence for Rensch's rule in this species (in contrast with results from mammal taxa, Fairbairn 1997) suggests that this may not be an important factor in this system. By carefully selecting a study organism that is univoltine across its range, the unknown effects of changing voltinism are avoided. Due to the degree of variation detected and the non-linearity of the relationship between size and latitude, it is imperative that researchers wishing to study body size variation in the field make use of the full range of the species that they are investigating. Indeed, a reanalysis of previous studies of latitudinal variation in body size (e.g. those listed in Chown and Gaston 2010) is warranted to look at the patterns observed in the context of the entire range. I predict that the variation between studies will be at least partially explained by the choice of sampling region within the range. For organisms with clearly defined pre-adult stages, the temperature-size rule cannot be considered in isolation from the effects of time stress caused by variations in the length of the growing season. Further work is required on (i) the interactions between multiple cues beyond temperature and time stress in order to fully characterise variation in body size across a species' range and (ii) the effects of voltinism shifts on body size in ecologically important taxa such as the Odonata.

Supplementary information
Figure S1 – Dorsal drawing of *C. maculata* showing regions of the wing omitted from measurements redrawn from Hassall and Thompson (2009)).
Figure S2 – Raw plot of body size measurements (length of the right fore wing in mm) of male *Calopteryx maculata* at 34 sites across the USA and Canada.
Table S1 - Individual measurements for specimens of male *Calopteryx maculata*, and date of collection (days since 1 January).
Table S2 – Details of weather stations used in the calculation of degree day data.

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TABLES

Table 1 – Summary of measurements and variation in 906 specimens of male *Calopteryx maculata*. DD_{10}= degree day accumulation above 10°C between 1 July 2009 and 30 June 2010, Date=ordinal date (days since 1 January), $Temp_{Ann}$=mean annual temperature.

Region	Site	Site code	Date	DD_{10} (°C)	$Temp_{Ann}$ (°C)	Photoperiod (hrs, 1 May)	Latitude (°N)	Longitude (°E)	Fore wing length (mm, ±SE)	Hind wing length (mm, ±SE)	Hind tibia length (mm, ±SE)	N
Canada, ON	Blakeney Falls	ON_A	151	1143.1	5.5	13.574	45.268	-76.250	28.55 (±0.17)	27.37 (±0.16)	8.044 (±0.06)	23
Canada, ON	Dorset	ON_B	212	1037.3	4.5	13.574	45.271	-78.960	27.05 (±0.31)	25.95 (±0.31)	7.772 (±0.10)	7
Canada, ON	Heber Down	ON_C	159	1238.3	7.7	13.502	43.941	-78.988	27.87 (±0.14)	26.66 (±0.14)	8.458 (±0.08)	20
Canada, ON	Lucknow	ON_D	209	1237.5	6.8	13.502	43.954	-81.497	25.92 (±0.17)	25.00 (±0.16)	7.279 (±0.06)	20
Canada, ON	North Bay	ON_E	171.3	940.7	5.2	13.556	44.947	-21.876	28.16 (±0.09)	27.05 (±0.09)	8.855 (±0.04)	84
Canada, ON	Peterborough	ON_F	166	1068.5	6.0	13.522	44.315	-78.343	27.22 (±0.20)	26.16 (±0.19)	7.820 (±0.07)	20
Canada, ON	Ridgetown	ON_G	192	1262.0	8.8	13.424	42.439	-81.831	26.82 (±0.16)	25.93 (±0.16)	7.472 (±0.05)	18
Canada, ON	Sault Ste Marie	ON_H	175.7	849.6	3.7	13.649	46.582	-84.300	27.31 (±0.10)	26.24 (±0.10)	8.254 (±0.05)	60
Canada, ON	Serena Gundy Park	ON_I	196	1508.8	8.4	13.490	43.716	-79.353	27.39 (±0.13)	26.46 (±0.13)	7.917 (±0.06)	25
Canada, QC	Dunany	QC_A	176	1194.0	4.7	13.601	45.758	-74.304	26.40 (±0.35)	25.36 (±0.31)	7.764 (±0.08)	15
Canada, QC	Shawinigan	QC_B	178	975.1	4.4	13.645	46.514	-72.679	27.25 (±0.19)	26.22 (±0.18)	8.042 (±0.09)	33
USA, AR	Smithville	AR	199.5	2769.3	14.1	13.139	36.235	-91.470	27.63 (±0.16)	26.67 (±0.16)	8.191 (±0.06)	35
USA, FL	8 Mile Creek	FL	177	3593.6	19.8	12.914	30.483	-87.326	28.16 (±0.32)	27.20 (±0.29)	7.860 (±0.09)	20
USA, GA	Conyers Monastery	GA_A	216	2746.1	16.2	13.031	33.584	-84.073	27.36 (±0.27)	26.39 (±0.27)	7.811 (±0.14)	11
USA, GA	Rome	GA_B	173.5	2956.8	15.1	13.065	34.443	-85.150	28.22 (±0.16)	27.24 (±0.12)	8.015 (±0.06)	20
USA, IA	Gateway Hills Park	IA_A	172	1476.6	8.6	13.402	42.008	-93.647	26.85 (±0.14)	25.86 (±0.14)	7.522 (±0.05)	20
USA, IA	Odebolt	IA_B	196	1344.8	8.2	13.416	42.274	-95.129	26.94 (±0.09)	25.96 (±0.09)	7.788 (±0.04)	73
USA, IL	Rockford	IL_A	175	1529.5	9.3	13.412	42.211	-88.976	26.25 (±0.15)	25.36 (±0.15)	7.503 (±0.06)	20
USA, KY	Fossil Creek	KY	158	2116.0	13.1	13.205	37.773	-84.561	28.50 (±0.13)	27.36 (±0.13)	8.500 (±0.07)	25
USA, MD	Folly Quarter Creek	MD	194	2672.2	12.1	13.271	39.255	-76.927	26.01 (±0.14)	25.15 (±0.14)	7.438 (±0.06)	33
USA, MI	Johnson Creek	MI	173.5	1599.1	8.8	13.422	42.399	-83.528	26.96 (±0.12)	26.05 (±0.10)	7.573 (±0.05)	24
USA, MO	Eleven Point River	MO_A	156	2047.6	13.1	13.162	36.793	-91.331	27.50 (±0.18)	26.58 (±0.19)	8.197 (±0.14)	12
USA, MO	White River	MO_B	156	2177.7	13.7	13.156	36.654	-92.230	27.11 (±0.13)	26.03 (±0.11)	8.041 (±0.08)	25
USA, MS	Starkville	MS	186	2996.5	16.8	13.030	33.567	-89.041	29.02 (±0.18)	27.97 (±0.18)	8.126 (±0.11)	26
USA, NE	Chappell	NE_A	167	1526.9	8.1	13.357	41.083	-102.467	27.08 (±0.17)	26.15 (±0.23)	7.622 (±0.09)	6
USA, NE	Kimball	NE_B	181	1098.4	9.6	13.364	41.232	-103.843	26.27 (±0.14)	25.45 (±0.13)	7.460 (±0.06)	32
USA, NE	Leigh	NE_C	182	1457.8	8.7	13.387	41.701	-97.247	26.07 (±0.12)	25.07 (±0.12)	7.266 (±0.06)	25
USA, OH	Mt Vernon	OH	198	1305.2	9.3	13.325	40.405	-82.487	27.71 (±0.14)	26.58 (±0.13)	8.195 (±0.06)	40
USA, SC	Four Holes Swamp	SC_A	195	3015.3	17.8	13.017	33.212	-80.348	31.21 (±0.32)	30.35 (±0.32)	9.308 (±0.10)	21
USA, SC	Little Creek	SC_B	196	2872.2	15.4	13.081	34.842	-82.402	27.73 (±0.18)	27.11 (±0.21)	7.847 (±0.06)	29
USA, TX	Powderly	TX	133	3087.8	17.1	13.038	33.753	-95.605	28.70 (±0.15)	27.55 (±0.14)	8.055 (±0.05)	22
USA, VT	Lamoille River	VT_A	169	1317.4	6.4	13.542	44.681	-73.068	27.17 (±0.33)	26.05 (±0.28)	7.775 (±0.16)	4
USA, VT	West Haven	VT_B	205	1274.2	7.4	13.485	43.624	-73.362	26.77 (±0.14)	25.84 (±0.15)	7.873 (±0.14)	17
USA, VT	Winooski River	VT_C	190.3	1290.4	4.9	13.636	46.352	-72.571	26.52 (±0.14)	25.59 (±0.13)	7.829 (±0.06)	42

Table 2 – Evaluation of three competing mixed effects models to explain variation in body size (-PC1, as PC1 is negatively related to size measurements; see text for details) in *Calopteryx maculata*. N=879 specimens in all cases, and site (N=34) is a random effect in all models. Information theory statistics (AICc, ΔAICc and w_i) allow comparison between models (see text for details).

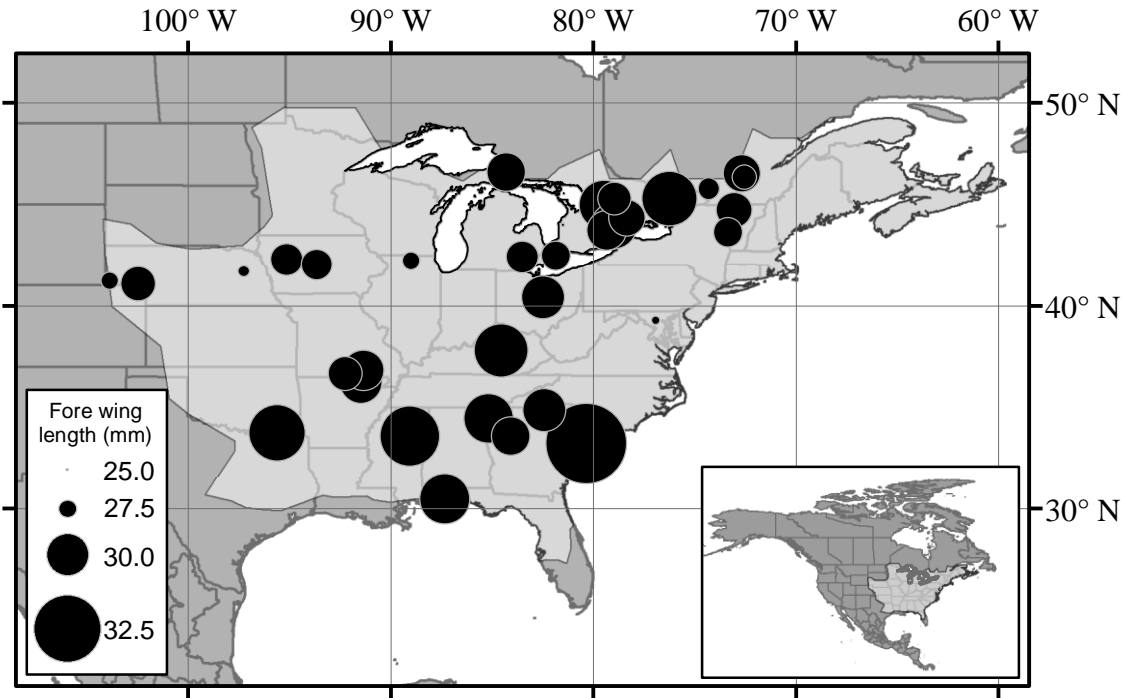|	t	p	AICc	ΔAICc	w_i
(A) Seasonality model					
Intercept	1.356	0.147	9680.3	0.0	1.000
$\log(DD_{10})$	-0.313	0.736			
$\log(DD_{10})^2$	0.283	0.761			
Photoperiod	-1.225	0.189			
Photoperiod^2	1.213	0.194			
(B) Temperature-size model					
Intercept	2.259	0.023	9704.7	24.4	<0.001
$\log(DD_{10})$	-2.330	0.019			
$\log(DD_{10})^2$	2.401	0.016			
(C) Bergmann model					
Intercept	2.859	0.005	9717.0	36.7	<0.001
Latitude	-2.677	0.008			
Latitude^2	2.500	0.013			

Figure 1 – Geographic distribution of *Calopteryx maculata* (light area) within North America (see inset). Filled circles show right fore wing length in mm at 34 locations throughout the range, with the size of the symbol being proportion to the length of the wing (see scale in the lower left).
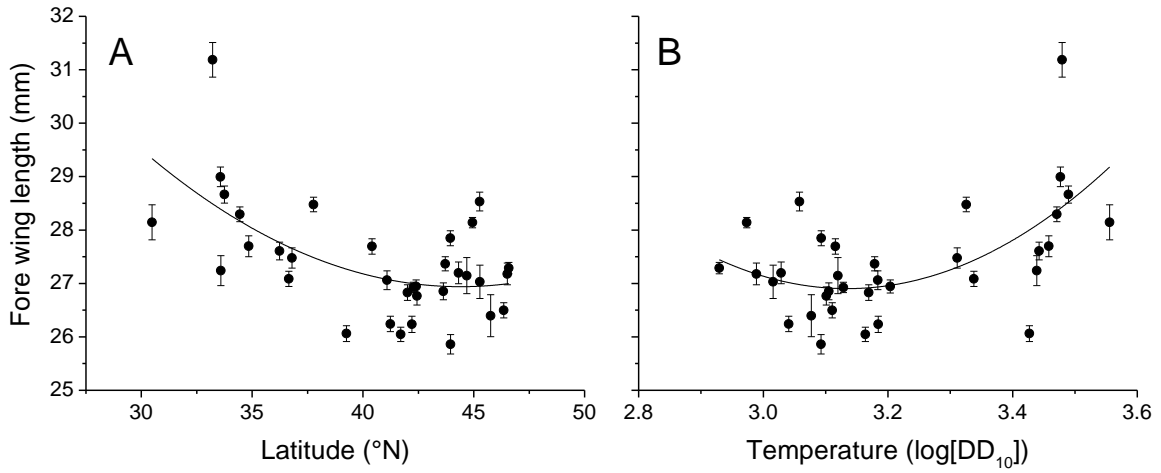
Figure 2 – Quadratic relationships between body size (PC1) and (A) latitude and (B) temperature (degree days above 10°C between 1st July 2009 and 30th June 2010, "DD_{10}") in male specimens of the damselfly *Calopteryx maculata*. Error bars are 1SE. These two plots correspond to (A) the Bergmann model, and (B) the temperature-size model.
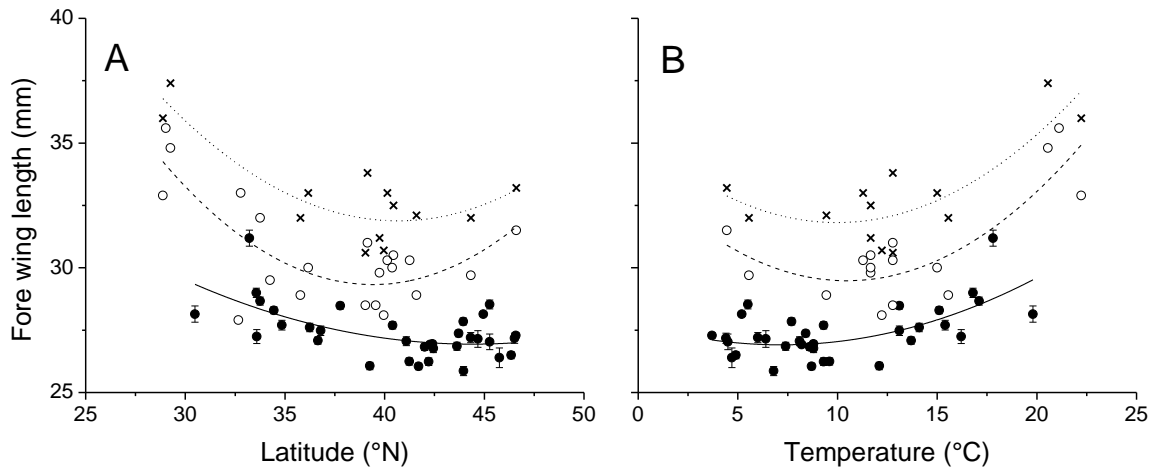
Figure 3 – A comparison latitude- and temperature-size relationships in *Calopteryx maculata* from this study (filled symbols with solid line) with those of Huggins (1926): open symbols with dashed line represents Huggins' male specimens, crosses with dotted line represent Huggins' female specimens (see text for statistics). Measurements vary in magnitude between studies as a result of different measurement methods.
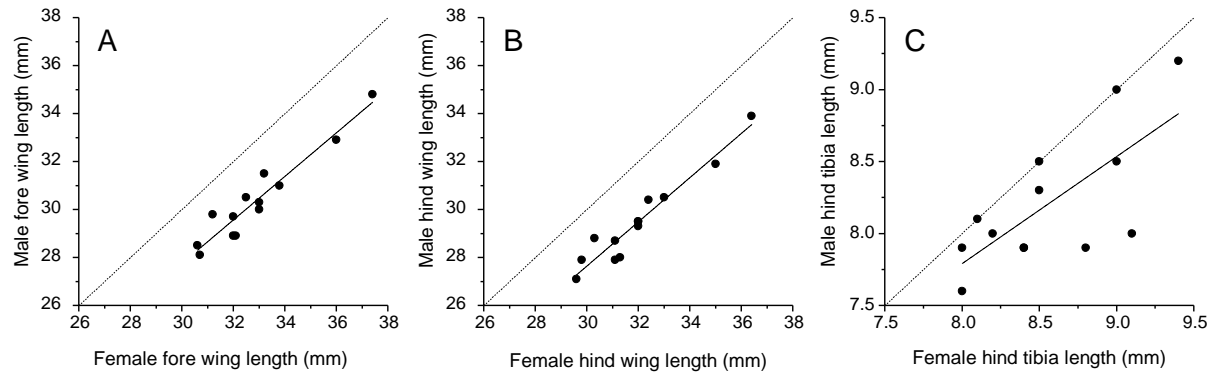
Figure 4 – A comparison of variation in male and female body size measurements (fore wing length (A), hind wing length (B), and hind tibia length (C)) in *Calopteryx maculata* as a test of Rench's rule. Dotted line indicates 1:1 relationship. Measurements are taken from Huggins (1926).