

# Temperature-body size responses in insects: a case study of British Odonata

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**Abstract.** 1. Body size is highly correlated with physiological traits, fitness, and trophic interactions. These traits are subject to change if there are widespread reductions of body size with warming temperatures, which is suggested as one of the ‘universal’ ecological responses to climate change. However, general patterns of body size response to temperature in insects have not yet emerged.

2. To address this knowledge gap, we paired the wing length (as a proxy for body size) of 5331 museum specimens of 14 species of British Odonata with historical temperature data. Three sets of analyses were performed: (i) a regression analysis to test for a relationship between wing length and mean seasonal temperature within species and subsequent comparisons across species and suborders; (ii) an investigation of whether the body size of species has an effect on sensitivity to warming temperature; and (iii) a linear-mixed effects model to investigate factors that potentially affect temperature–size response.

3. The regression analysis indicated that wing length is negatively correlated with mean seasonal temperatures for Zygoptera, whereas Anisoptera showed no significant correlation with temperature.

4. There is a significant decline in wing length of all Zygoptera (but not Anisoptera) with collection date, suggesting that individuals emerging later in the season are smaller.

5. Life-cycle type was not important for predicting wing length–temperature responses, whereas sex, species, and suborder were indicated as important factors affecting the magnitude of temperature–size responses in Odonata.

6. Overall, wing lengths of Zygoptera are more sensitive to temperature and collection date than Anisoptera.

**Key words.** Anisoptera, body size response, climate change, temperature–size rule (TSR), museum collections, Zygoptera.

## Introduction

Along with species distribution shifts and phenological changes, a decrease in body size is suggested to be a universal ecological response of organisms to warming temperature (Gardner *et al.*, 2011; Ohlberger, 2013). In ectotherms, body temperature depends on environmental temperature, which in turn, can affect body size through both developmental and metabolic rate

(Atkinson, 1994, Sheridan & Bickford, 2011). Previous studies have found that the developmental rate of ectotherms is faster at lower latitudes and warmer temperatures (Atkinson, 1994, Angilletta *et al.*, 2004) and is more sensitive to temperature than growth rate (Zuo *et al.*, 2012). Consequently, adult size should be smaller in warm environments compared with individuals growing in cooler temperatures and higher latitudes; this relationship is sometimes referred to as the temperature–size rule (TSR) (Atkinson, 1994).

As well as the direct effects of warming temperature on the developmental rate of ectotherms that can ultimately reduce

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adult body size, changing food quality and availability with changing temperature can have an indirect effect (Ozgul *et al.*, 2009; Ozgul *et al.*, 2010; Gardner *et al.*, 2011). Previous studies show that many ectothermic taxa do not follow the TSR, especially insects (Sota *et al.*, 2002; Blanckenhorn, 2004; de Queiroz & Ashton, 2004; Wilson *et al.*, 2019). This is assumed to be a result of season length effects (i.e. longer warm seasons correspond to a longer developmental period), voltinism type (the number of generations per year), sexual size-dimorphism (SSD), trophic level, habitat type, and food quality (Cressa *et al.*, 2008; Kingsolver & Huey, 2008; Schutze & Clarke, 2008; Gibert & DeLong, 2014; Horne *et al.*, 2015; Fenberg *et al.*, 2016).

General patterns of insect body size response to temperature have therefore not yet emerged. This may be partly because it can be challenging to isolate the overall effect of temperature on adult body size given the complex life cycles and ecology of many insects. For example, adult body size may be directly or indirectly affected by the temperature experienced during each larval stage or whether a species overwinters as an egg or larva (Puzzle, 2004). In addition, voltinism type, which can range from semivoltine (needing more than one year to complete the life cycle), univoltine (one generation per year), bivoltine (two generations per year) or multivoltine (more than two generations per year), is considered to affect temperature–body size response (Horne *et al.*, 2016).

To make matters more complicated, the body size of each sex may respond differently to temperature or have no response at all (Fenberg *et al.*, 2016). Furthermore, it is unknown whether different phylogenetic groups within an insect order (e.g. between suborders) exhibit similar temperature size responses.

A potentially powerful way to determine if there are general body size responses of insects to temperature is to study both sexes of multiple species with varying life-history strategies over long historical time scales, from the same geographical region. Natural history collections are valuable resources of such data. In the present study, we use museum collections of adult British Odonata as a model group for examining general patterns of insect body size responses to warming temperature because: (i) there is a large amount of historical data available for several Odonata species in museum collections; (ii) Odonata have seasonal regulation of key life-history traits triggered by temperature (e.g. timing of egg hatch and adult emergence (Has-sall & Thompson, 2008; Stoks & Córdoba-Aguilar, 2012)); (iii) Odonata exhibit a variety of life-history strategies, including differences in voltinism type (Corbet *et al.*, 2006), overwintering stage (Verberk *et al.*, 2008), and adult emergence period (Corbet *et al.*, 2006); (iv) monthly regional temperature records are available from the whole of the British Isles from 1910 (U.K. Meteorological Office); and (v) by measuring adult body size only, the same life stage across all years and seasons is consistent, and thus avoids issues of size variation in juvenile life stages and age structure.

In addition to the effects of temperature on adult body size through development rate, temperature could affect adult body size of Odonata by affecting their emergence time (Cothran & Thorp, 2006; Penn, 2015). For example, studies on the damselflies *Coenagrion puella* and *Coenagrion mercuriale*, as

well as on the dragonfly *Sympetrum danae*, found a negative correlation between adult body size and emergence date (Banks & Thompson, 1985; Michiels & Dhondt, 1989; Purse & Thompson, 2003).

Studies of temperature–size responses in insects should initially be examined at the intraspecific level because species specific traits such as seasonal life cycle will determine which temperature variable (e.g. seasonal or monthly) best predicts adult size. Then, comparisons across species can be made to assess whether there are any general temperature size responses. For example, previous studies have shown that the average size of a species can explain the strength of body size response to temperature change (Forster *et al.*, 2012; Tseng *et al.*, 2018). In aquatic organisms, large species exhibit greater reduction in body size in response to warming temperature than small species (Forster *et al.*, 2012). This could be explained by oxygen limitation because the reduced surface area to body mass ratio in large species leads to decreased respiratory surface area, resulting in greater reduction of adult body size in large species compared with small species (Forster *et al.*, 2012).

To investigate whether there are body size responses of Odonata to temperature, we analysed species specific responses by pairing mean seasonal temperature during the larval growth period for each species with wing length measurements for 14 British Odonata species, collected between 1910 and 1980. We then compared results across species and by suborder to determine if there are any general patterns of temperature size response across Odonata. In addition, we investigated: (i) whether the sexes responded differently to temperature; (ii) whether body size correlates with collection date; and (iii) potential drivers that could correlate with the magnitude of temperature–size responses of Odonata, including average adult size of species, life-history type, and suborder. To our knowledge, this is the first study that assesses body size response to temperature among different Odonata species across historic to modern temporal scales in the same region.

We hypothesized that given the classically assumed TSR, adult wing length (as a proxy for body size) will decrease during years with warmer temperatures. In addition, according to previous studies (Forster *et al.*, 2012; Tseng *et al.*, 2018), we also expected an increase in the magnitude of wing length reduction with increasing temperature in larger species. Thus, Anisoptera could be expected to have a greater reduction in wing length than Zygoptera. Furthermore, negative correlations are expected between temperature and body size for univoltine species as their development is time constrained. Previous studies, however, indicate a converse to the TSR in univoltine insects species (Horne *et al.*, 2015; Fenberg *et al.*, 2016; Wilson *et al.*, 2019). Positive or no correlation between temperature and wing length is predicted in semivoltine species as a result of their extended growing season in warmer years. Thus, we hypothesized that suborder and/or life cycle type could be predictors of the direction and magnitude of species responses to warming temperature. According to previous studies (Banks & Thompson, 1985; Michiels & Dhondt, 1989; Purse & Thompson, 2003), we also expected smaller wing lengths for specimens collected later in the year as a result of time-constraints on larval development.

**Table 1.** The 14 Odonata species used in this study and their voltinism type (Corbet & Brooks, 2008).

	Species	n	Corbet's type	Voltinism type	Environmental variables
Anisoptera	<i>Aeshna cyanea</i>	179	spring species	semivoltine	spring ( $y_{-1}$ ) to spring ( $y_0$ )
	<i>Aeshna grandis</i>	53	spring species	semivoltine	spring ( $y_{-1}$ ) to spring ( $y_0$ )
	<i>Aeshna mixta</i>	151	summer species	Univoltine	spring ( $y_0$ )
	<i>Anax imperator</i>	68	spring species	semivoltine	spring ( $y_{-2}$ ) to winter ( $y_0$ )
	<i>Brachytron pratense</i>	194	spring species	semivoltine	spring ( $y_{-2}$ ) to winter ( $y_0$ )
	<i>Libellula quadrimaculata</i>	235	spring species	semivoltine	spring ( $y_{-2}$ ) to winter ( $y_0$ )
	<i>Somatochlora metallica</i>	64	summer species	semivoltine	summer ( $y_{-2}$ ) to spring ( $y_0$ )
	<i>Sympetrum striolatum</i>	482	summer species	Univoltine	spring ( $y_0$ )
Zygoptera	<i>Calopteryx splendens</i>	684	summer species	semivoltine	summer ( $y_{-2}$ ) to spring ( $y_0$ )
	<i>Calopteryx virgo</i>	838	summer species	semivoltine	summer ( $y_{-2}$ ) to spring ( $y_0$ )
	<i>Coenagrion puella</i>	530	obligatorily univoltine	Univoltine	summer ( $y_{-1}$ ) to spring ( $y_0$ )
	<i>Ischnura elegans</i>	730	obligatorily univoltine	Univoltine	summer ( $y_{-1}$ ) to spring ( $y_0$ )
	<i>Lestes sponsa</i>	581	summer species	Univoltine	spring ( $y_0$ )
	<i>Pyrrhosoma nymphula</i>	542	spring species	semivoltine	spring ( $y_{-2}$ ) to winter ( $y_0$ )

Environmental variables for each characteristic type are calculated from the regional U.K. mean monthly temperature series. For each variable  $y_{-2}$  is 2 years before the collecting year,  $y_{-1}$  is a year before the collecting year, and  $y_0$  is the collecting year.

## Materials and methods

This study was based on analyses of 5331 specimens of 14 species of British Odonata, including eight Anisoptera species and six Zygoptera species (Table 1). These species were selected based on their range of life cycle types and the availability of specimens from U.K. museums (see Supporting information, Table S1, S2).

### Data acquisition

Among the morphological traits of insects, wing size and wing shape have been used to study size responses to environmental or climate change (Hassall, 2015; Bai *et al.*, 2016; Stewart & Vodopich, 2018). Here, wing length was used as a proxy for body size as justified by previous studies (Haas & Tolley, 1998; Corbet, 1999). Additionally, studies on body size in insects (Clapham & Karr, 2012) and Odonata (Worthen & Jones, 2006) suggest a strong correlation between body dimensions and wing length. Many studies on other insects suggested a reflection of body size in wing length (Dudley, 2000; Loh *et al.*, 2008; Beasley *et al.*, 2012; Bai *et al.*, 2016). Furthermore, wing length remains constant in museum specimens, whereas other insect body parts, including the thorax and abdomen, shrink over time (Krogmann & Holstein, 2010; Gilbert, 2011).

All specimens were imaged in a standardized template alongside specimen label(s), scale bar, and a colour checker (see Supporting information, Figure S1). Wing length was obtained by measuring the distance between the base of the leading edge of the wing and the distal end of the first radial anterior vein (R1) of the right-hind wings of each specimen (see Supporting information, Figure S1). Collection location and date were manually extracted from specimen labels.

The regional air monthly temperature time-series data are derived from the U.K. Meteorological Office ([www.metoffice.gov.uk/climate/uk/summaries/datasets](http://www.metoffice.gov.uk/climate/uk/summaries/datasets)) and were used to calculate climate variables for analyses. Although Odonata larvae are aquatic and not directly exposed to air temperatures

during development, previous studies indicate a linear relationship between air and stream water temperature (Erickson & Stefan, 2000) and air temperature and lake water temperature in the upper metre of the water column (Livingstone & Lotter, 1998). Most of the Odonata species in the present study breed in small ponds and lakes. We used mean regional temperature, which compensates for any small variations in water temperature during larval development.

Three sets of analysis were performed in R (R Core Team, 2014): (i) a stepwise regression analysis of each species to investigate body size responses to mean seasonal temperature during their larval growing periods; (ii) an investigation of whether body size of species has an effect on sensitivity to warming temperature (interspecific variation); and (iii) a linear-mixed effects model to investigate the influence of random factors, including suborder, life-history type, and sex on temperature size responses across species.

### Individual species analysis

Previous work found that species with different life histories (e.g. spring and summer species) or voltinism type can affect phenological responses to warming temperature (Hassall *et al.*, 2007). Thus, mean seasonal temperature of the larval period was calculated for each species based on their voltinism type and life cycle characteristics as defined by Corbet *et al.* (2006) and used as independent variables (Table 1). Prior to analysis of each species, the variance inflation factor (VIF) was estimated to test the multicollinearity among environmental variables. Variables having  $VIF_{Weight}$  higher than 3 were removed from the models. A multiple linear regression analysis was performed followed by stepwise linear regression in both directions (forward and backward selection) using the MASS package in R (Venables & Ripley, 2002) to select the best final model with important variables from all possible subset models.

Odonata body size may vary through the season (Cochran & Thorp, 2006; Penn, 2015); thus, it is important to consider emergence date as a factor that could affect adult body size.

Collection date, which can be considered as a proxy for emergence date (discussed below), was therefore also included as an independent variable in the individual species analyses (Bried, 2009).

#### Interspecific analysis

For each species, the percentage change in wing length per °C was calculated using the mean seasonal temperature variables identified in the multiple linear regression analyses described above. Percentage wing length change was then calculated using the formula  $[\exp(\text{slope}) - 1] \times 100$ ; slopes were calculated using the natural log of wing lengths to account for any scaling effects that may have resulted from the differences in size between species and sexes (Forster *et al.*, 2012). Then to investigate if species' size is related to sensitivity to temperature, a linear regression analysis was performed between the percentage change of wing length (per °C) and mean wing length of each species.

For the linear mixed effects model, four independent temperature variables including mean winter, mean spring, mean summer and mean autumn temperature of the larval period was calculated for each species. For example, for *Calopteryx splendens* and *Calopteryx virgo*, which are semivoltine species, mean winter temperature was calculated from two years of winter temperature data during their larval growing period (year before their emergence and year of emergence). The linear mixed-effects model was used to examine whether there were relationships between the dependent variable (log wing length), the four fixed independent variables (including mean winter, spring summer, and autumn temperature) and the random effect variables (species, sex, suborder, and life-history type) using the *lme4* package (Bates *et al.*, 2017).

## Results

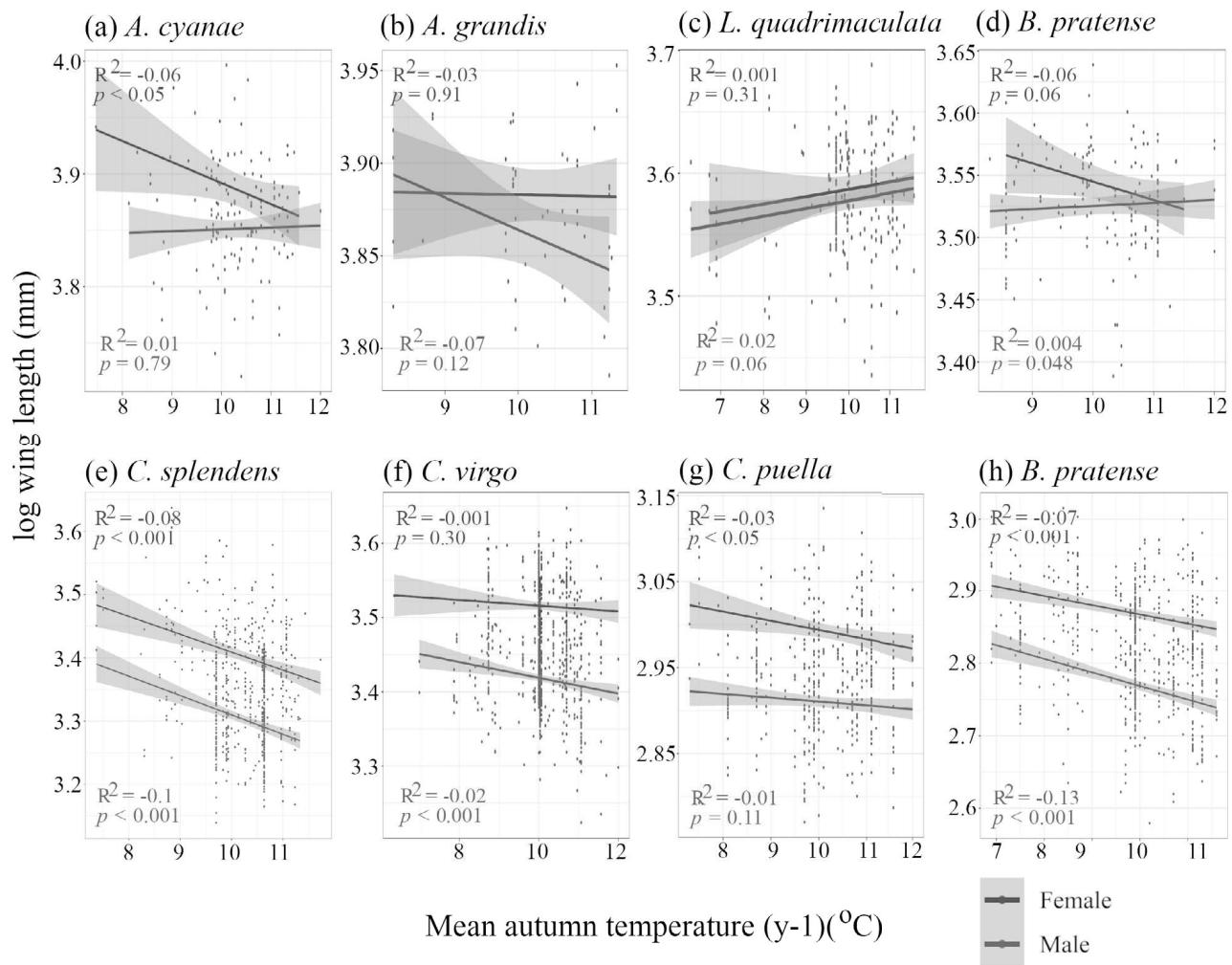
The stepwise regression analysis showed significant temperature-size relationships in all Zygoptera species and three Anisoptera species, *Aeshna cyanea*, *Anax imperator* and *Brachytron pratense* (Table 2). Mean autumn temperature of the year before emerging ( $y_{-1}$ ) and mean spring temperature of the emerging year ( $y_0$ ) were selected and retained as candidates of the fitted model to explain wing length variation of most Zygoptera species (Figs 1e-h and 2e-h). *Calopteryx splendens*, *C. virgo*, *C. puella* and *Ischnura elegans* showed a significantly smaller wing size with increasing mean autumn temperature of  $y_{-1}$  in both sexes (Fig. 1e-h). Most Zygoptera species, including *C. splendens*, *C. virgo*, *C. puella* and *Lestes sponsa*, also showed significantly reduced wing length with warming spring temperature of  $y_0$  (Fig. 2e-h). By contrast, most Anisoptera showed nonsignificant wing length change with mean spring temperature of  $y_0$  (Fig. 2a-d).

The percentage change of wing length per degree °C was calculated for each species to examine whether there are interspecific differences in response and whether mean body size is related to the strength and direction of temperature size responses. We found a significant positive correlation between

**Table 2.** Coefficients of the stepwise regression model for examining the effects of mean seasonal temperature, sex and day (collection date) on dragonfly wing length across studied species to investigate the effects of each variable on each species wing length.

	Anisoptera										Zygoptera										
	<i>Aeshna cyanea</i>	<i>Aeshna grandis</i>	<i>Aeshna mixta</i>	<i>Anax imperator</i>	<i>Brachytron pratense</i>	<i>Libellula quadrimaculata</i>	<i>Somatochlora metallica</i>	<i>Sympetrum striolatum</i>	<i>Calopteryx splendens</i>	<i>Calopteryx virgo</i>	<i>Coenagrion puella</i>	<i>Ischnura elegans</i>	<i>Lestes sponsa</i>	<i>Ptychosoma nymphula</i>							
Intercept	<b>52.48***</b>	57.68***	NS	27.08***	<b>33.94***</b>	33.98***	46.64***	27.39***	<b>46.43***</b>	<b>41.87***</b>	<b>24.81***</b>	<b>20.52***</b>	<b>25.07***</b>	<b>23.76***</b>							
Day	NS	NS	NS	NS	NS	NS	NS	NS	-0.01***	-0.02***	-0.01***	-0.01***	-0.01***	-0.01***							
Y-2	Spring				0.56**	NS	NS	NS							NS	NS	NS	NS	NS	NS	
	Summer				NS	NS	NS	NS							NS	NS	NS	NS	NS	NS	
Y-1	Autumn				NS	NS	NS	0.23*	NS	0.47*	NS	NS	NS	NS	NS	0.39***				-0.15**	
	Winter				NS	NS	NS	NS							NS	-0.39***				NS	
	Spring				<b>0.53*</b>	NS	NS	-0.36*	NS	NS	NS	NS	NS	NS	NS	NS	0.40***				
	Summer				NS	NS	0.83*	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.29***	NS	0.19***		
	Autumn				NS	NS	NS	0.22*	NS	NS	NS	NS	NS	NS	NS	NS	-0.86***	-0.19*	-0.14*		
	Winter				NS	NS	0.21*	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.18**	NS	NS	-0.31***	
Y0	Spring				NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.68***	-0.17**	-0.29***	NS	-0.21***
	Sex				NS	-1.14*	NS	1.44*	-0.69*	NS	NS	NS	NS	NS	NS	NS	-2.96***	-3.11***	-1.51***	-1.63***	-1.30***
	Adjusted $r^2$				<b>0.18***</b>	NS	0.08*	NS	0.08*	NS	NS	NS	NS	NS	NS	0.47***	0.60***	0.45***	0.53***	0.30***	
																				0.33***	

A negative symbol shows a negative relationship between wing length and the variables. Significant levels are indicated: \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ , respectively. Blank cells are variables that were not included in a species analysis. NS, nonsignificant variable. Significant models are indicated in bold.



**Fig. 1.** Linear regression of log wing length of Anisoptera (a) to (d) and Zygoptera (e) to (h) versus mean autumn ( $y_{-1}$ ) temperature. Red dots and lines represent females and blue dots and lines represent males. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

percentage change of wing length change per  $^{\circ}\text{C}$  with mean wing length across species ( $r = 0.60$ ,  $P < 0.05$ ) (Fig. 3a).

Although males are significantly smaller than females in all Zygoptera species, the magnitude and direction of wing length response to temperature is similar in both sexes (Figs 1e–h and 2e–h). Three Anisoptera species (*Aeshna grandis*, *A. imperator* and *B. pratense*) also exhibit a significant effect of sex on wing length responses to temperature (Table 2), which indicates that wing length responses are different in magnitude or direction between the sexes. It appears that females have more sensitivity to temperature, although wing length responses in both sexes are nonsignificant (Fig. 1a–d and Fig. 2a–d).

The stepwise regression analysis shows significantly smaller wing length with later collection date in all six Zygoptera ( $P < 0.01$ ) (Fig. 4a), whereas Anisoptera species exhibit a non-significant wing length difference across the emergence season ( $P > 0.05$ ) (Fig. 4b).

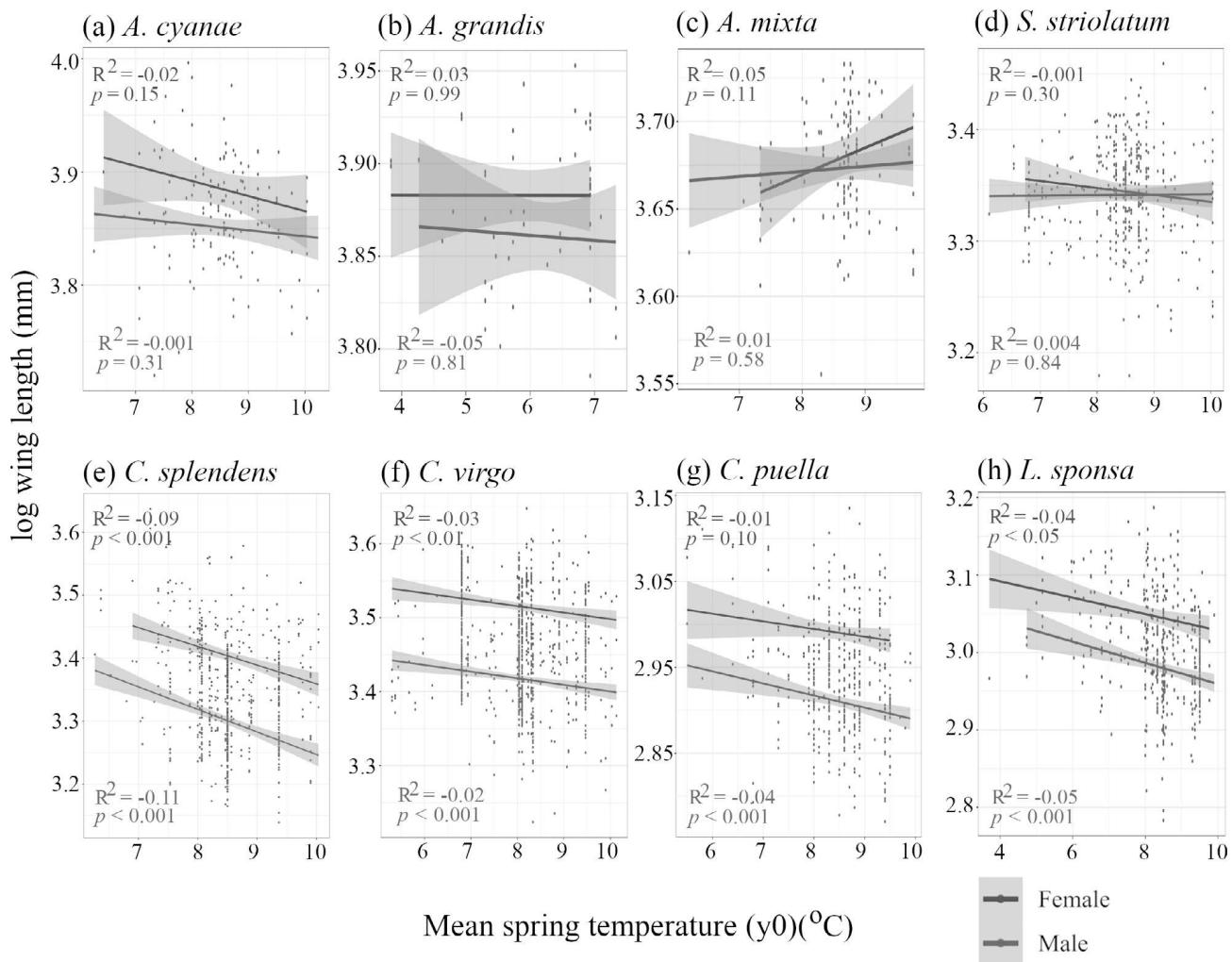
A significantly different wing length response is noted between Anisoptera and Zygoptera (Fig. 3b). Thus, when comparing across all species, using the linear-mixed effects model,

we incorporated suborder as a random effect variable *in lieu* of correcting for phylogeny.

The linear-mixed effects model indicated that the inclusion of species, sex and particularly suborder, was the best predictive model for wing length variation of Odonata (Table 3; see also Supporting information, Table S3). Given the importance of suborder in the linear-mixed effects model, an independent samples *t*-test was run between percentage wing length change per degree temperature change between Anisoptera and Zygoptera, which indicated a significant difference of magnitude of wing length change ( $P < 0.01$ ) (Fig. 3b). On average, the wing length in Zygoptera decreases by 1.10 mm (SE = 0.81) per  $^{\circ}\text{C}$ , whereas the wing lengths of Anisoptera as a whole, do not change with temperature (0.39 mm; SE = 0.49).

## Discussion

There are many different factors that need to be considered when investigating body size response to temperature in insects,



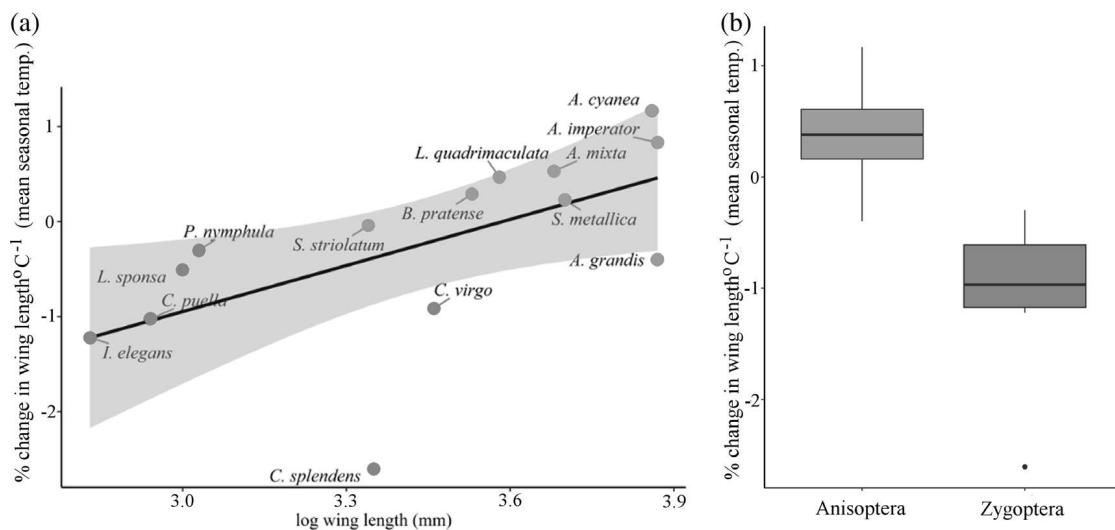
**Fig. 2.** Linear regression of log wing length of Anisoptera (a) to (d) and Zygoptera (e) to (h) versus mean spring ( $y_0$ ) temperature. Red dots and lines represent females and blue dots and lines represent males. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

including voltinism type, overwintering stage, phylogenetic relationships, and sex. These factors also affect which temperature variables should be used for examining temperature–size responses. Although we find no universal response to temperature and its proxies, we find that Zygoptera tend to have stronger and more consistent responses to seasonal temperature and collecting date than Anisoptera. Also, suborder is indicated as the most important factor affecting the direction and magnitude of wing length responses to temperature. Below, we discuss the likely causes of these results and their implications for future trajectories of temperature–size responses in Odonata.

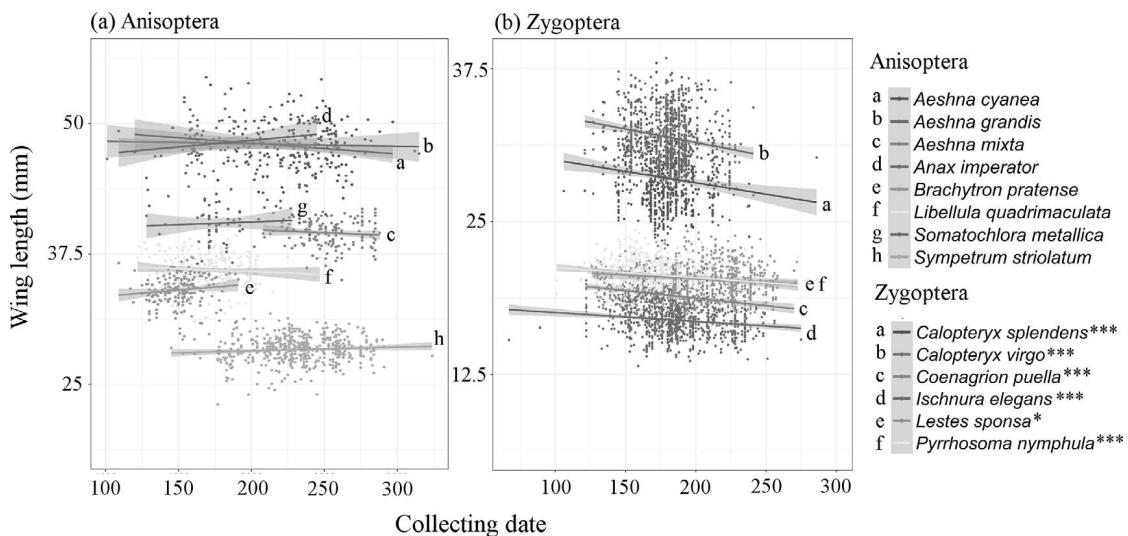
There were significant negative correlations between wing length of Zygoptera species and mean seasonal temperature, in particular mean temperature of autumn  $y_{-1}$  and mean temperature of spring  $y_0$  (Figs 1 and 2). This response corresponds with the temperature–size rule (Atkinson, 1994) because smaller body sizes are found in warmer temperatures. Higher temperatures disproportionately increase developmental rate, resulting in smaller adults (Atkinson, 1994, Angilletta *et al.*, 2004). In

terms of life-history characters, some species are recorded as facultative univoltine species that can switch to a semivoltine lifecycle in parts of their distribution range. *Coenagrion puella* and *I. elegans* (Zygoptera) have been recorded as facultative univoltine species in the U.K. (Corbet *et al.*, 2006). Thus, years with relatively cool mean seasonal temperature might result in larvae switching to a semivoltine life cycle, thereby extending their development for an additional year, and resulting in a larger body size on emergence.

In terms of sexual size dimorphism, if the sexes respond in the same direction and rate to temperature, then the magnitude of SSD would not be predicted to change with increasing temperature. Conversely, if the size of one sex responds to temperature and the other does not, or they respond in opposite directions, then the magnitude of SSD may be predicted to change with warming temperatures (Fenberg *et al.*, 2016). Because most Zygoptera in the present study exhibit SSD (see Supporting information, Figure S2) but the males and females have equally negative size responses to temperature



**Fig. 3.** (a) Percentage change in mean wing length per  $^{\circ}\text{C}$  of each species plotted against natural log wing length for mean seasonal temperature of the season that is indicated as the best variable for explaining wing length variation of each species (stepwise regression analysis) (Table 2). A correlation analysis indicated a significant positive correlation between percentage change in wing length per degree temperature change and average wing length (as a proxy of body size) ( $r = 0.60, P < 0.05$ ). (b) Percentage change in mean wing length per  $^{\circ}\text{C}$  change of Anisoptera and Zygoptera. A t-test indicated significant difference of percentage change in wing length between Anisoptera (mean = 0.39) and Zygoptera (mean = -1.10) ( $P < 0.01$ ). [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].



**Fig. 4.** Linear regressions of hindwing lengths and collecting date (as a proxy of emergence date) of (a) Anisoptera and (b) Zygoptera. Significant results indicated by asterisks: \*\*\* $P < 0.001$  and \* $P < 0.05$ . [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

(Figs 1 and 2), we can be fairly confident that the magnitude of SSD will generally be stable with warming temperatures for this taxon. Anisoptera, on the other hand (excluding *A. cyanea*) do not exhibit SSD (see Supporting information, Figure S2).

In females, bigger body sizes potentially have greater fecundity, so large females may be selected for. For Anisoptera, correlations between mean seasonal temperature and female wing length were non- or weakly significant (Figs 1a–d and 2a–d). However, similar to males, wing length decreased in female Zygoptera, thus the relative size difference between males and females does not change with respect to temperature.

The linear-mixed effects model indicates that suborder is the most important driver affecting the magnitude of species wing length responses to warming temperature. By contrast, life-history type is excluded from the best fit model, which indicates less selective pressure for life-history type on the wing length responses to temperature. Previous studies have suggested that larger species are likely to have a greater reduction in size than smaller species as temperature increases (Forster *et al.*, 2012; Horne *et al.*, 2015; Tseng *et al.*, 2018). However, the results obtained in the present study show the opposite, where small species have a greater reduction in size than large species

**Table 3.** Coefficients of the linear mixed effects model for examining the significant effects of mean seasonal temperature on dragonfly wing length across studied species.

Variables	Random effects			Intercept	Mean seasonal temperature			
	Suborder	Species	Sex		Summer	Autumn	Winter	Spring
Coefficients	0.099 ± 0.31 <sup>†</sup>	0.048 ± 0.220 <sup>†</sup>	0.003 ± 0.054 <sup>†</sup>	3.487	0.004***	-0.009***	-0.004***	0.002

A negative symbol shows a negative relationship between wing length and the variables.

Significant levels are indicated: \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ , respectively.

<sup>†</sup>Variance (mean ± SD) of random variables.

as temperature increases. Interestingly, Anisoptera species are not likely to show any significant wing length changes with temperature. This result may be because those anisopteran species are strongly territorial and large male body size is linked to mating success (Sokolovska *et al.*, 2000; Córdoba-Aguilar, 2008).

Within the Zygoptera, both *C. splendens* and *C. virgo* also exhibit territoriality and therefore, based on the results above, could be expected to show no body size change or get bigger with warmer temperatures, especially because they are semi-voltine, and so have less time-constraints on development than univoltine species. However, our results indicate that, in both species, the adult males (and females) are smaller when they grow at higher temperatures. A possible explanation is that territoriality in *Calopteryx* is weaker than in many Anisoptera. Previous studies on *Calopteryx* indicate that male wing pigmentation is a more important mating cue than territory holding (Córdoba-Aguilar, 2002; Córdoba-Aguilar & Cordero-Rivera, 2005). Moreover, Koskimaki *et al.* (2009) showed that *C. virgo* had three mating tactics: (i) territorial; (ii) nonterritorial (sneaking); and (iii) nonterritorial (wandering). They found that the body size of wandering *C. virgo* males was smaller than territorial individuals. This may partly account for our results, although it could be that other factors that differ between Anisoptera and Zygoptera have also affected their size responses to temperature. However, further study is required to fully untangle the mechanisms behind the differential wing size responses between Anisoptera and Zygoptera.

#### Seasonal cline in wing length

A significant negative response between wing length and collection date in all the studied Zygoptera species implies that individuals flying late in the season tend to be smaller than those of the same species flying early in the season. This finding corresponds well with previous field studies on U.K. populations of the zygoteran *Coenagrion mercuriale* (Purse & Thompson, 2003; Lowe *et al.*, 2009), which found larval exuviae emerging late in the season were smaller than those emerging early in the season, as well as studies in other Zygoptera species (Mahdjoub *et al.*, 2015). This is likely because larval development is time-constrained later in the season and therefore larvae accelerate their developmental rate, which comes at the cost of a smaller adult body size (Johansson & Rowe, 1999).

By contrast to Zygoptera, the wing lengths of the Anisoptera that we studied do not significantly change with collection date.

A previous study on an anisopteran species *Sympetrum danae*, revealed a decline in body size with emergence date (Michiels & Dhondt, 1989), whereas a study on *Sympetrum rubicundulum*, found no significant body size change across the emergence period (Van Buskirk, 1987). Interestingly, most Zygoptera and some Anisoptera, such as *S. danae*, are nonterritorial. Most Anisoptera, including *S. rubicundulum*, are territorial where body size is relatively important for mating success in males (Sokolovska *et al.*, 2000; Córdoba-Aguilar, 2008). Thus, small larvae of territorial species could delay emergence, enabling more time to grow and emerge as larger adults later in the year, thereby increasing their territorial competitiveness (Rowe & Ludwig, 1991; Fuselier *et al.*, 2007).

Adult Anisoptera generally have a slightly greater longevity than adult Zygoptera, which may mean that collection date is not as good a proxy for emergence date in Anisoptera compared with Zygoptera. Thus, this may account for the nonsignificant correlation of wing length with collection date in Anisoptera. To assess the influence of earlier emergence dates relative to collecting dates we have randomly assigned earlier emergence dates between 0 and 30 days before collection across the dataset. These simulated datasets have resulted in the same conclusions (see Supporting information, Table S4) highlighting that the potential disparity between emergence and collecting date does not influence the overall conclusions. Furthermore, an analysis of collecting date in response to temperature (see Supporting information, Figure S3) highlights that warmer years result in earlier collecting dates, indicating the collecting date is likely a good proxy for the date of emergence.

#### Conclusions

In conclusion, we find that: Zygoptera do show a decrease in wing length as temperature increases and as the season advances, which corresponds well with previous studies on other insects (Ghosh *et al.*, 2013; Horne *et al.*, 2015; Tseng *et al.*, 2018). By contrast, Anisoptera showed either a nonsignificant response or become larger (one species) or smaller (two species) with increasing temperature, possibly as a result of selection for larger individuals in strongly territorial species. In contrast to our hypothesis, Zygoptera, which have a smaller body size than Anisoptera, showed greater sensitivity to temperature than Anisoptera. Suborder is the main factor that affects Odonata temperature-size responses. We also hypothesized that univoltine species would show decreasing size with increasing temperature, whereas semivoltine species would show less

sensitivity. This was not the case, however, and suborder was a better predictor of size response irrespective of life cycle type. Other studies show that life cycle type is important for predicting temperature–size responses. For example, the adult body sizes of some insects get larger with increasing temperatures, most notably univoltine and the first generation of bivoltine butterflies (Fenberg *et al.*, 2016; Wilson *et al.*, 2019). Individuals of Zygoptera flying late in the season tend to be smaller than those of the same species flying early in the season. Taken together, our results and these recent studies support the idea that there is no universal temperature–size response for insects and that the results will depend upon multiple factors.

Although we find no universal body size response to warming seasonal temperature in Odonata, adult Zygoptera tend to be smaller in years that have warm seasonal temperatures during larval development. We presume that this is because warmer temperature accelerates the developmental rate of damselfly larvae at the cost of smaller adult body size. Future studies should further examine whether temperature size responses in insects vary between various taxonomic categories (i.e. at the order and suborder levels) and whether other morphological features, such as wing shape are also affected by temperature. Additionally, further studies investigating whether territoriality affects wing length response in both Anisoptera and Zygoptera are needed.

## Acknowledgements

The authors thank to Amoret Spooner (Oxford University Museum of Natural History), Ashleigh Whiffin (National Museums Scotland), and Michael R. Wilson (National Museum of Wales) for providing access to the collections in their care. This project is funded by the Royal Thai Government.

RW collected and analysed the data and led the writing of the paper. PBF, PGL, SJB, and BWP helped conceive the ideas for the research and analyses and provided direction for the writing.

The authors declare that they have no conflicts of interest.

## Data availability

The R scripts used for data analyses and the raw specimen data, including unique identifier, location (latitude, longitude), collecting date, wing length, and corresponding temperature data, are available at: <https://doi.org/10.5519/0095579>.

## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** (a) Image of a pinned specimen with labels indicating collector, location and collecting date, specimen barcode, a colour checker, and a scale bar. (b) Wing length measurement of Odonata wing between the base of the leading edge of the wing and the distal end of the first radial anterior vein (R1).

**Figure S2.** Box plots of females and males hindwing length for (a) eight species in suborder Anisoptera and (b) six species in suborder Zygoptera. Significant results indicated: \*\*\* $P < 0.05$ .

**Figure S3.** Regression analysis between the average temperature of larval period and collecting date, which is a proxy of emergence date.

**Table S1.** Number of specimens of 14 British Odonata species from museum collections in the U.K. and number of specimens.

**Table S2.** Number of specimens of 14 British Odonata species from each decade.

**Table S3.** Akaike information criterion approach to compare linear-mixed effects models contained different sets of random effects variables.

**Table S4.** Correlation coefficients and significant values ( $P$ -value) between collection date (original collection date and simulation data) and wing length of each Anisoptera species.

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Accepted 30 January 2020

First published online 26 February 2020

Associate Editor: Christopher Hassall