University of Southampton

School of Ocean and Earth Sciences

Insect size and shape responses to temperature: a case study of British Odonata and Chironomidae (Diptera)

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Abstract

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This PhD project investigated the response of wing length (as a proxy of body size) of Odonata and Chironomidae, and wing shape of Odonata to temperature and latitude. Three complementary data sources were used: natural history collections, field data, and mesocosm experiments. While natural history collections are valuable resources of specimens collected over long historical time scales, field data provides a modern perspective and mesocosm experiments provide a window on the future, under a predicted climate warming scenario.

This PhD project used 5,331 museum specimens of 14 British Odonata species representing different life cycle types to examine the potential drivers of body size and wing shape responses to latitude and temperature (Chapter II and V). To control for latitude a field survey was performed during the summer of 2018 at Edington, Somerset to compare modern wing length of three species with historical data based on museum specimens of the same species collected at the same locality (Chapter III). Furthermore, to investigate body size responses of chironomids under a future temperature scenario, 1,976 adult specimens of six chironomid species were collected from mesocosm experiments which comprised ponds at ambient temperature and ponds maintained at 4°C higher than ambient (Chapter IV).

The results of Chapters II, III and IV showed that species and suborder (within Odonata) were significant factors affecting the magnitude of the temperature-size responses in Odonata and Chironomidae. Wing lengths of Zygoptera (Odonata) and Chironomidae are more sensitive to temperature and collection date than Anisoptera (Odonata). Zygoptera and Chironomidae tend to get smaller with increasing temperature, likely due to higher temperatures disproportionately increasing developmental rate, resulting in smaller adults. Anisoptera showed no significant correlation with temperature, possibly due to selection for larger individuals in Anisoptera which are strongly territorial species. Adults of Zygoptera and Chironomidae emerging towards the end of the summer tend to be smaller than those emerging earlier in the season, likely due to larval development being time-constrained later in the season and as a result, larvae accelerate their developmental rate which comes at the cost of a smaller adult body size.

The results of Chapter V indicated non-significant correlations between environment and wing shape in Anisoptera, while there were significant influences of latitude and mean seasonal temperature on wing shape in Zygoptera species, with broader and shorter wings found at lower latitudes with warmer temperatures. This finding corresponds well with a result of Chapter II which found shorter wing length with increasing temperature in all zygopteran species in the study.

Overall, the results of this PhD project show that there are different factors influencing the temperature-size responses of insects, including phylogenetic relationships, sex, behaviour and life cycle types. Although this study found no universal temperature-size responses in the focal taxa, Zygoptera and Chironomidae tend to have stronger negative body size responses to warming temperature and emergence date than Anisoptera. In addition, the study shows that wing shape variation in Zygoptera is more sensitive and adaptive to latitude and temperature than in Anisoptera.

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I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

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Chapter I

Introduction:

This chapter reviews the current literature for temperature-size responses and wing shape responses of insects, introduces the general aims and summarizes the study design of this PhD.

1.1. Introduction

According to IPCC assessment (IPCC, 2018), climate change, as a consequence of anthropogenic activities, has become an important issue in ecology, biodiversity and conservation research. The main characteristics of predicted global climate change are an increase in the average global temperature, and changes in precipitation patterns and intensity (Hansen et al., 2006, IPCC, 2018). In the past 30 years, global mean temperature has increased at the rate of 0.2°C per decade (Hansen et al., 2006), and there is a predicted increase of 3-4℃ in the average global temperature by 2100 (Forster & Artaxo, 2005, World Bank, 2012, IPCC, 2018, Kjellstrom & McMichael, 2013). The resulting change in mean regional temperature affects many aspects of the ecology and life history of species, including their abundance and distribution, as well as changes in phenology and body size, leading to ecosystem alteration (e.g., Halpin, 1997, Walther et al., 2002, Thomas et al., 2004).

Freshwater ecosystems are particularly vulnerable to global climate change because water temperature is directly dependent on local and regional climate, and freshwater ecosystems can be more isolated and fragmented (e.g. lakes, ponds) compared to terrestrial and marine habitats (Woodward et al., 2010). Warmer temperature can lead to higher metabolism and productivity in freshwater ecosystems. Temperature changes affect many aspects of aquatic insects, including changes in their distribution range (Sundar, 2017), abundance (Sweeney & Schnack, 1977), life cycle (voltinism type) (Jonsson et al., 2015), phenology (Jonsson et al., 2015), behaviour and phenotype (Sweeney et al., 1992), for example, changing colour and body size (Ward & Stanford, 1982) which relate to population and community dynamics.

Body size is a fundamental character which influences multiple aspects of an organism as it is often correlated with certain physiological traits, fitness, behaviour, life history, resource use and trophic interactions (Cressa et al., 2008, Kingsolver & Huey, 2008, Gibert & DeLong, 2014). Among insects, larger females typically have higher potential fecundity and larger males have higher mating rate and success (Elgar & Pierce, 1988, Berrigan, 1991, Berger et al., 2008).

Environmental temperature is an important abiotic factor affecting the body size of insects, with a reduction in body size proposed as a third universal response to warming, after distributional and phenological change (Gardner et al., 2011). This chapter will provide background theory and relevant previous studies on insect body size responses to increasing temperature, particularly in Odonata and Chironomidae. Wing shape is also recognised as an important trait correlated with size, and related to fitness and flight performance in insects (Powell, 1997, Iriarte & Hasson, 2000, Van Heerwaarden & Sgrò, 2011), thus changes in wing shape in response to temperature will be reviewed. Finally, at the end of this chapter, the main questions and aims of this study will be considered.

1.1.1. General ecogeographical rules of body size and shape responses to temperature

In terms of ecogeographical-size relationships, three rules are typically cited. **Bergmann's rule** states there will be a latitudinal cline in the body size of endothermic homeotherms, proposing that homeotherms at high latitudes tend to have a larger body size than those at lower latitudes (Bergmann, 1847) (Figure 1.1). This rule can be explained by thermo-regulation mechanisms: larger organisms have a lower surface area to volume ratio; thus they radiate less body heat than smaller organisms which is an advantage at higher latitudes. However, this rule was originally proposed to account for interspecific latitudinal body size clines in endothermic species. In contrast, **James' rule** accounts for intraspecific latitudinal body size clines in endothermic species in relation to temperature (James, 1970). This rule states that, within a species, larger individuals are generally found at higher latitudes with lower temperatures (Figure 1.1).

When looking directly at temperature, rather than latitude, **the temperature-size rule (TSR)** states that for ectotherms their developmental rate is faster in warmer temperatures, consequently, the final size of organisms becomes smaller than those growing in lower temperatures (Atkinson, 1994, Angilletta et al., 2004). Apart from the effect of temperature on developmental rate, and therefore body size, TSR could influence aerodynamics in flying organisms. In particular, wing loading is calculated as body weight divided by wing area and previous studies show that in colder climates there is a decline in wing loading (Stalker, 1980, Pétavy et al., 1997), an outcome of decreased body weight or increased wing area (David et al., 2011). Hence, at higher latitudes with a colder climate, a greater wing area relative to body weight is predicted. However, previous studies have shown that some species do not appear to follow these rules (Sota et al., 2002, Blanckenhorn, 2004, de Queiroz & Ashton, 2004). A previous study on butterflies found larger body size with increasing temperature during the late larval stages, due to more available time for development (Wilson et al., 2019).

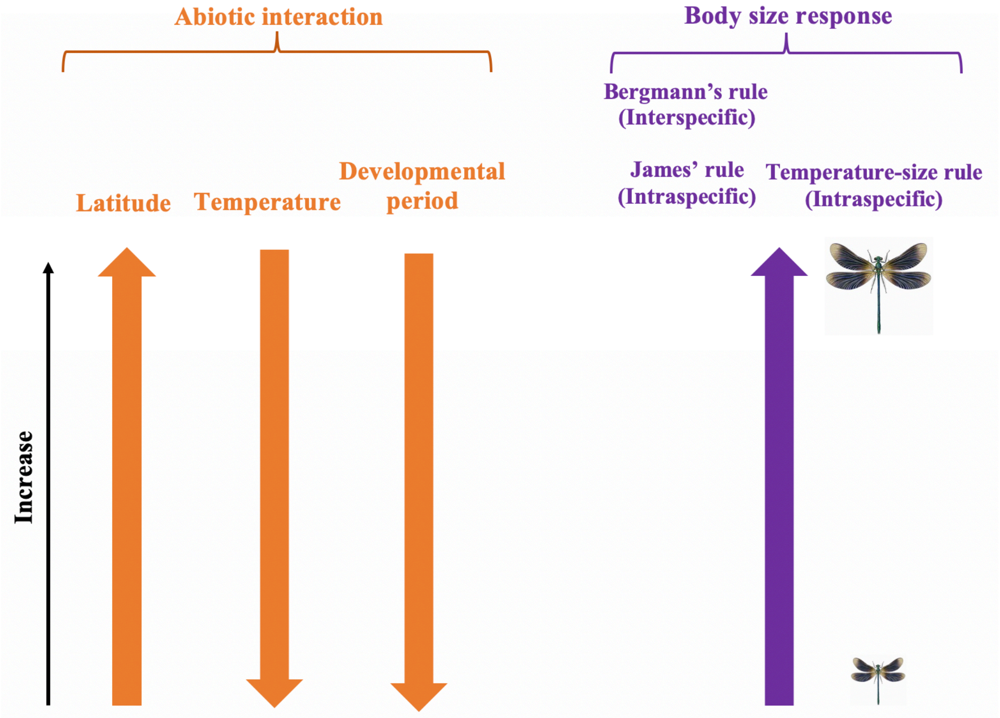

Fig 1.1: A summary of the abiotic variables (orange) and their influence on body size (purple) as predicted by various ecogeographical rules.

In addition to the general rules about wing size in relation to temperature mentioned above, the sensitivity of temperature-body size responses can potentially be influenced by mean body size of a species. A meta-analysis across terrestrial, freshwater and marine taxa (Forster, et al., 2012) and a study on beetles (Tseng et al., 2018) suggested that the average body size of a species can explain the magnitude of body size response to temperature change. In aquatic organisms, larger species exhibit greater reduction in body size in response to warming temperature than smaller species (Forster et al., 2012). One possible explanation is the oxygen limitation hypothesis: warmer water has less capacity to hold oxygen, which can exacerbate the increased energetic cost of respiration in larger aquatic invertebrates (Woods, 1999, Forster et al., 2012, Aguilar-Alberola & Mesquita-Joanes, 2014).

In addition to size, wing shape is heritable and considered to be an important trait related to fitness and flight performance in insects (Powell, 1997, Iriarte & Hasson, 2000, Van Heerwaarden & Sgrò, 2011). Wing performance can influence a species' dispersal ability (Bouget & Oger, 2015) and reflect adaptation to local environmental factors at the population level (Haas & Tolley, 1998).

Allen's rule (Allen, 1877) states that the appendages such as limbs, ears and tails in endothermic organisms are shorter, more rounded and more compact in cold climates and at high latitudes. Hence, on the basis of thermodynamics, a latitudinal cline in the proportion of surface area to volume is expected in endotherms. In ectothermic flying organisms, wing shape is likely to relate more to aerodynamics than thermodynamics (Norberg, 1995). In cold regions, increased wing area can reduce wing loading and compensate for reduced flight performance (Lehmann, 1999, Dudley, 2000). In previous studies of a butterfly and a fruit fly, shorter and more rounded wings correlated with larger wing area, and ultimately reduced wing loading, were found at higher latitudes (Azevedo et al., 1998, Vandewoestijne & van Dyck, 2011).

1.1.2. The body size of Odonata and Chironomidae in response to warming climate

Several groups, including dragonflies (Insecta: Odonata) and non-biting midges (Insecta: Diptera: Chironomidae), have been used to investigate the consequences of climate change on freshwater ecosystems (Stoks et al., 2014, Choudhary & Ahi, 2015). These two taxonomic groups are highly diverse with global distributions, and are sensitive to warming and other aspects of climate change, such as changes in salinity and hydroperiod (Stoks et al., 2014). However, they have different life cycles and play different roles in freshwater ecosystems: Odonata are hemimetabolous and top predators in fishless habitats (McPeek, 1998), while Chironomidae, which are holometabolous, are an important link in food webs between producers and secondary consumers, and are an important food resource for fish and other aquatic predators (Walshe, 1951). Additionally, Odonata are often univoltine or semivoltine, while chironomids are univoltine or multivoltine. Thus, it is interesting to compare their responses to warming temperature.

Odonata is a hemimetabolous order of aquatic insects including the dragonflies (suborder Anisoptera) and the damselflies (suborder Zygoptera), with complex life-cycles. They have three life-stages, with the eggs and larvae inhabiting aquatic systems, followed by a terrestrial adult stage. Most of the life-cycle of Odonata species is spent in the larval stage, where they develop until day length and temperature triggers the final moult when they emerge as adults (Stoks & Córdoba-Aguilar, 2012).

Odonata have many strong behavioral and life cycle connections to temperature (Hassall et al., 2007), and so are suitable as a model taxon for examining phenotypic responses to warming temperature (Hassall, 2015a). In particular Odonata 1) have seasonal regulation of key life history traits triggered by temperature, such as the timing of egg hatch and adult emergence (Hassall & Thompson, 2008); 2) they have life history (voltinism) and behavioral (territoriality) differences between species, and 3) they have a large historical data record available in museum collections.

Odonata species exhibit differences in voltinism, the number of generations per year (Corbet et al., 2006), and overwintering stage, being either larvae or eggs (Verberk et al., 2008). Furthermore, Odonata can be categorized into spring or summer species according to synchronization in their emergence period and the presence or absence of larval diapause in the final larval stage (Corbet et al., 2006). This is useful for understanding temperature-size responses for the reasons discussed below.

In general, given the classically assumed TSR, adult wing length (as a proxy for body size) is expected to decrease during years with warmer temperatures. However, previous studies have shown the converse in univoltine insect species (one generation per year), owing to more time being available for development (Horne et al., 2015, Fenberg et al., 2016, Wilson et al., 2019). Within Odonata, the obligate univoltine damselfly *Lestes viridis*, has been shown to accelerate its development rate as day length shortens to ensure adults emerge in that year (Stoks et al., 2006). This results in adults being smaller towards the end of the flight season than those emerging earlier in the flight season. In response to shorter development times at higher latitudes, Odonata species with a facultative univoltine life-cycle can shift to a semivoltine life-cycle (one generation in two years or more). As a result, an increase in body size is likely to be found in populations with a semivoltine life-cycle due to their longer development period (Johansson, 2003). By the same token, in a year with warmer seasonal temperatures, particularly a warmer winter or autumn, there would be a lengthening of the developmental period, resulting in a larger adult body size (Roff, 1980).

The Chironomidae (Diptera) are a family of non-biting midges. They are holometabolous insects that are ubiquitous in freshwater ecosystems and considered keystone species as they play an important role in nutrient cycling (Kelly and Roger, 2004). Furthermore, chironomids are excellent indicators of environmental change, due to their high local abundance and sensitivity to a range of environmental perturbations (Francis, 2004).

Many studies on Chironomidae in relation to climate change have focused on the influences of warming temperature on their distribution and abundance (Francis, 2004, Nyman et al., 2005, Brooks & Langdon, 2014, Matthews-Bird et al., 2016). However, only a few studies have investigated the effects of environmental temperature on their body size. While some studies have found an increased body size with increasing temperature, contrary to the TSR (Roff, 1980, Reynolds & Benke, 2005, Hannesdóttir et al., 2010), others have found a decline in body size with increasing temperature, following the TSR (Frouz et al., 2009, McKie & Cranston, 2005). However previous studies have been limited in the number of focal taxa examined and have been restricted to laboratory studies, limiting the broader conclusions that can be made about chironomid responses to temperature in natural environments.

1.1.3. Wing shape in response to latitude and temperature

Although wing shape has been widely studied in relation to aerodynamics (Hedenström, 2002, Sudo et al., 2005), research on wing shape as a function of environment and latitude in insects has rarely been studied. A recent study on the butterfly *Pieris rapae* (Bai et al., 2015) and another on the fly *Polietina orbitalis* (Alves et al., 2016) found latitudinal variation in wing shape, and suggested that these variations are an adaptation to environmental conditions or as a result of isolation from other populations. A rounded forewing in the butterfly *Pieris rapae* is considered an adaptive trait to more precipitation and higher temperatures at lower latitudes (Bai et al., 2015). In addition, individuals with larger wings are likely found in mountain areas, while those with smaller wings are found on the plains.

Within Odonata a previous study on *Calopteryx virgo meridionalis* showed strong correlation of wing shape with latitude, and that wing shape might be a result of the selection pressure within each region to maintain their flight performance (Outomuro & Johansson, 2011). This study also suggested that sexual selection in wing sexual ornaments can be a selective factor influencing wing shape variations in this damselfly species.

1.1.4. Sexual size and shape dimorphism

Sexual size dimorphism (SSD) is the difference in body size and associated morphological traits between the sexes (Fairbairn, 1997). Sexual size dimorphism is often reported for Odonata (Gribbin & Thompson, 1991, Corbet & Hoess, 1998). Odonata exhibit both male-biased SSD (males larger than females) and conversely female-biased SSD (Serrano-Meneses et al., 2008). Female-biased SSD could be advantageous for females to maximize their potential fecundity. However, male-biased SSD is expected in species that exhibit territorial mating strategies (Crowley & Johansson, 2002) as larger males could be at an advantage for male-male competition and territorial defence (Andersson, 1994, Sokolovska et al., 2000). Furthermore a previous study on Odonata showed that larger species tend to show more male biased SSD (Johansson et al., 2005).

For chironomids, a study on *Chironomus crassicaudatus* found that the body size of males is more sensitive to temperature and declines faster with increasing temperature than females (Frouz et al., 2009). Several studies of Chironomidae indicated that males have a faster development rate than females (Armitage, 1995, Stevens, 1998), and that the females of several species have slower development during the last larval instar (Danks, 1978, Stevens, 1998). A study on *Chironomus plumosus* found stabilizing selection on male body size, where bigger males tend to live longer but have a lower mating success, while small males could have an advantage in mating swarms (Neems et al., 1998). This study suggested that the probability of mating is greatest in small individuals because smaller males are faster (McLachlan, 1986, Neems et al., 1990). Thus, it does not seem to be important for male chironomids to maintain their body size at higher temperatures.

Within Odonata sexual shape dimorphism (SShD) has been studied in the damselfly family Calopterygidae (Anders & Rüppell, 1997). This study suggested that wing beat frequency is an important aspect of courtship in males, thus sexual displays might potentially be a crucial driver of sexual wing shape dimorphism in some Odonata taxa (Anders & Rüppell, 1997, Outomuro & Johansson, 2011). Additionally, a study of *Calopteryx virgo* found sexual wing shape dimorphism was influenced by both natural and sexual selection pressure, with female wing shape explained by latitude, but male wing shape variation was mostly explained by wing pigmentation (Outomuro & Johansson, 2011). This study indicated that narrow hind wings correlated with less pigment on the wings (Outomuro & Johansson, 2011).

Even though sexual shape dimorphism (SShD) is well studied in *Calopteryx* damselflies due to their well-known mating behaviour and ecology, there are no studies in other Odonata taxa, limiting our understanding.

This study uses wings to investigate the body size and shape responses of Odonata and body size of Chironomidae to latitude and temperature. In insects wing length has a significant correlation with body size (Haas & Tolley, 1998), therefore, changes in wing length can be ultimately used to indicate changes in body size. Surprisingly, even though body size is tightly correlated to temperature, there is very little research on the body size and wing shape pattern responses in Odonata (Hassall & Thompson, 2008), and only few a recent studies focus on Chironomidae body size in relation to environmental temperature (Jonsson et al., 2015, Schütz & Füreder, 2018). Furthermore, despite many studies on sexual size dimorphism in insects (Fairbairn & Preziosi, 1994, Johansson et al., 2005, Serrano-Meneses et al., 2008), if and how the sexes differentially respond to temperature is unknown for Odonata and Chironomidae. This is the first study of temperature-size responses across many species of Odonata and chironomids, which have different life-history strategies. Moreover, this study uses museum specimens, collected across the UK over the last century. The museum collections are useful in providing historical data for research.

1.2. The aims of PhD

Understanding how different taxa respond to warming temperature is crucial for determining which taxa are vulnerable to the predicted regional temperature changes. The overall aim of this PhD project is to explore the responses of wing length and shape of Odonata and Chironomidae to temperature. Furthermore, it is interesting to compare the responses of these taxa due to the difference in their body size, trophic level in the food web, and the complex life cycles of Odonata compared to Chironomidae.

This project specifically aims to:

1) Explore intra- and interspecific responses of wing length, a proxy for body size, to increasing temperature **(Chapter II, III, IV)**.

2) Explore potential drivers that could influence the direction and strength of temperature-size responses, for example, sex, suborder, voltinism type and the average body size of species **(Chapter II, IV)**.

3) Investigate any correlation between collecting date, which is a proxy for emergence date, and body size **(Chapter II, IV)**.

4) Investigate the influence of latitude and environmental temperature on wing shape **(Chapter V)**.

1.3. Study design

This study used different sources of data to explore the effects of environmental temperature on wing shape of Odonata and wing length of Odonata and Chironomidae at various spatial and temporal scales.

The following chapter (**Chapter II)** uses historic data from museum collections of Odonata, dating back 110 years. While museum collections are valuable in providing long time-series data for research, the collections can sometimes be biased towards specific localities or species.

The next chapter (**Chapter III)** We collected modern data of selected Odonata species from a field survey at Edington Somerset during 2018. The modern data was compared with historic data from the same locality to remove any effects of latitude.

The following chapter **(Chapter IV)** explores the responses to predicted future temperatures, examined in field mesocosm experiments. Mesocosms, run at current and predicted future temperature scenarios, provided data on body size responses in chironomids to warming climate. These mesocosms can provide more realistic conditions than indoor laboratory experiments, but are less complicated than natural ecosystems (Kangas & Adey, 2008).

Then, **Chapter V** uses museum collections of Odonata to examine the influences of latitude and environmental temperature on wing shape. A geometric morphometric approach was used to analyse wing shape variation in selected Odonata species.

Finally, key findings, conclusions and future directions of research are summarized in **Chapter VI**.

Chapter II

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

This chapter used museum collections of 14 British Odonata species to investigate temperature-body size responses and potential drivers that could correlate with the magnitude of temperature-size responses of Odonata.

Author contributions: 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.

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

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Abstract

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. To address this knowledge gap, we paired the wing length (as a proxy for body size) of 5,331 museum specimens of 14 species of British Odonata with historical temperature data. Three sets of analyses were performed 1) a regression analysis to test for a relationship between wing length and mean seasonal temperature within species and subsequent comparisons across species and suborders, 2) an investigation of whether the body size of species has an effect on sensitivity to warming temperature and, 3) a linear-mixed effects model to investigate factors that potentially affect temperature-size response. The regression analysis indicated that wing length is negatively correlated with mean seasonal temperatures for Zygoptera, while, Anisoptera showed no significant correlation with temperature. 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. 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. 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*

2.1. 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 to 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 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 thought to be due to 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 instance, 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 thought 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 sub-orders) 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 geographic region. Natural history collections are valuable resources of such data. In this 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 1) there is a large amount of historical data available for several Odonata species in museum collections; 2) Odonata have seasonal regulation of key life history traits triggered by temperature (e.g. timing of egg hatch and adult emergence (Hassall & Thompson, 2008, Stoks & Córdoba-Aguilar, 2012)); 3) 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); 4) monthly regional temperature records are available from the whole of the British Isles from 1910 (UK Meteorological Office); and 5) 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 *C. mercuriale*, and 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, as 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 to small species (Forster et al., 2012).

To investigate if 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 1) tested whether the sexes responded differently to temperature; 2) investigated if body size correlates with collection date; and 3) investigated 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 1) given the classically assumed TSR, adult wing length (as a proxy for body size) will decrease during years with warmer temperatures. 2) 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 are predicted in semivoltine species due to their extended growing season in warmer years. Thus, 3) we hypothesized that suborder and/or life cycle type could be predictors of the direction and magnitude of species responses to warming temperature. 4) 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 due to time-constraints on larval development.

2.2. Materials and methods

This study was based on analyses of 5,331 specimens of 14 species of British Odonata, including eight Anisoptera species and six Zygoptera species (Table 2.1). These species were selected based on their range of life cycle types and the availability of specimens from UK museums (Table S2.1 in appendix).

2.2.1. 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, 2015a, 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 (i.e. Dudley, 2000, Loh et al., 2008, Beasley et al., 2012, Bai et al., 2016). Furthermore, wing length remains constant in museum specimens, while 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 (Fig S2.1 in appendix). 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 (Fig S2.1 in appendix). Collection location and date were manually extracted from specimen labels.

Table 2.1: The 14 Odonata species used in this study and their voltinism type (Corbet & Brooks, 2008). Environmental variables for each characteristic type are calculated from the regional UK mean monthly temperature series. For each variable y-2 is two years before the collecting year, y-1 is a year before the collecting year, and y0 is the collecting year.

	Species	**N**	**Corbet's type**	**Voltinism type**	**Environmental variables**
Anisoptera	*Aeshna cyanea*	179	spring species	semivoltine	spring (y-1) to spring (y0)
	Aeshna grandis	53	spring species	semivoltine	spring (y-1) to spring (y0)
	Aeshna mixta	151	summer species	Univoltine	spring (y0)
	Anax imperator	68	spring species	semivoltine	spring (y-2) to winter (y0)
	Brachytron pratense	194	spring species	semivoltine	spring (y-2) to winter (y0)
	Libellula quadrimaculata	235	spring species	semivoltine	spring (y-2) to winter (y0)
	Somatochlora metallica	64	summer species	semivoltine	summer (y-2) to spring (y0)
	Sympetrum striolatum	482	summer species	Univoltine	spring (y0)
Zygoptera	*Calopteryx splendens*	684	summer species	semivoltine	summer (y-2) to spring (y0)
	Calopteryx virgo	838	summer species	semivoltine	summer (y-2) to spring (y0)
	Coenagrion puella	530	obligatorily univoltine	Univoltine	summer (y-1) to spring (y0)
	Ischnura elegans	730	obligatorily univoltine	Univoltine	summer (y-1) to spring (y0)
	Lestes sponsa	581	summer species	Univoltine	spring (y0)
	Pyrrhosoma nymphula	542	spring species	semivoltine	spring (y-2) to winter (y0)

The regional air monthly temperature time-series data are derived from the UK Meteorological Office (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 our 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): 1) a stepwise regression analysis of each species to investigate body size responses to mean seasonal temperature during their larval growing periods; 2) an investigation of whether body size of species has an effect on sensitivity to warming temperature (interspecific variation); and 3) 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.

2.2.2. Individual species analyses

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 2.1). Prior to analysis of each species, the Variance Inflation Factor (VIF) was estimated to test the multicollinearity among environmental variables. Variables having $\mathrm{VIF}_{\mathrm{Weight}}$ 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 (Cothran & 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).

2.2.3. Interspecific analyses

For each species, the percentage change in wing length per ºC 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(slope)−1) × 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 percent change of wing length (per ºC) 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 *C. 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 if 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., 2015).

2.3. Results

The stepwise regression analysis showed significant temperature-size relationships in all Zygoptera species and three Anisoptera species, *Aeshna cyanea*, *Anax imperator* and *B. pratense* (bold text Table 2.2). Mean autumn temperature of the year before emerging (y-1) and mean spring temperature of the emerging year (y0) were selected and retained as candidates of the fitted model to explain wing length variation of most Zygoptera species (Fig 2.1e-2.1h, 2.2e-2.2h). *Calopteryx splendens*, *C. virgo*, *Coenagrion puella* and *Ischnura elegans* showed a significantly smaller wing size with increasing mean autumn temperature of y-1 in both sexes (Fig 2.1e-2.1h). Most Zygoptera species, including *Calopteryx splendens*, *C. virgo*, *Coenagrion puella* and *Lestes sponsa*, also showed significantly reduced wing length with warming spring temperature of y0 (Fig 2.2e-2.2h). In contrast, most Anisoptera showed non-significant wing length change with mean spring temperature of y0 (Fig 2.2a-2.2d).

The percent change of wing length per degree ºC was calculated for each species to examine if 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 percent change of wing length change per ºC with mean wing length across species (r=0.60, p<0.05; Fig 2.3a).

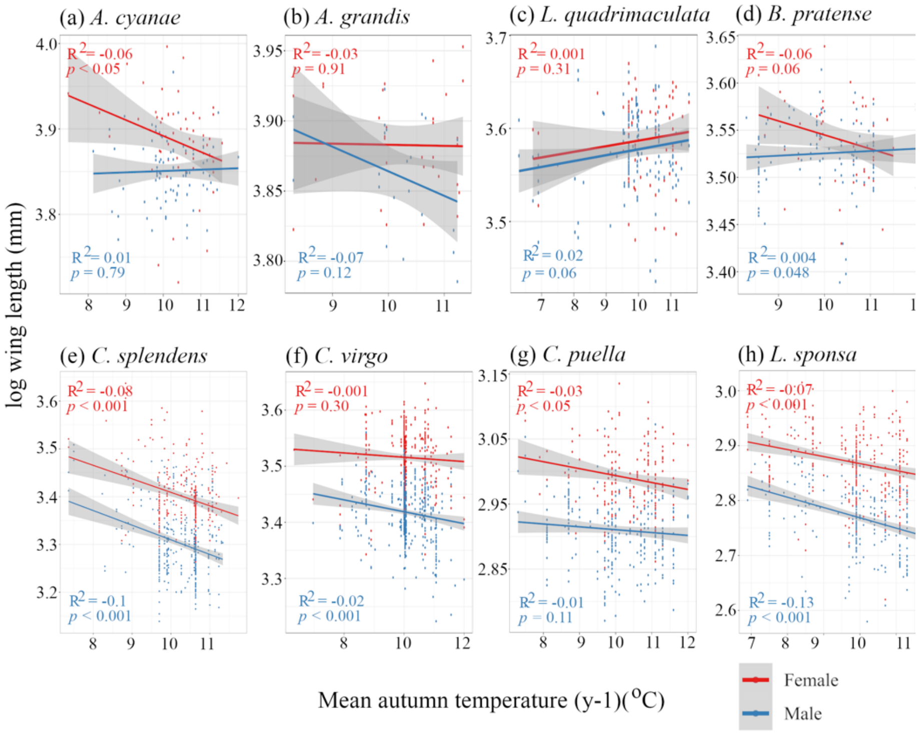
Although males are significantly smaller than females in all Zygoptera species, the magnitude and direction of wing length response to temperature is similar in both sexes (Fig 2.1e-2.1h and 2.2e-2.2h). Three Anisoptera species (*Aeshna grandis*, *Anax imperator* and *B. pratense*) also exhibit a significant effect of sex on wing length responses to temperature (Table 2.2), which indicates that wing length responses are different in magnitude or direction between the sexes. It seems females have more sensitivity to temperature, but wing length responses in both sexes are non-significant (Fig 2.1a-2.1d and Fig 2.2a-2.2d).

The stepwise regression analysis shows significantly smaller wing length with later collection date in all six Zygoptera (p<0.01; Fig 2.4a). Whereas Anisoptera species exhibit non-significant wing length difference across the emergence season (p>0.05; Fig 2.4b).

A significantly different wing length response is noted between Anisoptera and Zygoptera (Fig 2.3b). 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 S2.3 in appendix; Table 2.3). Given the importance of suborder in the linear-mixed effects model, an independent samples *t*-test was run between percent 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 2.3b). On average, the wing length in Zygoptera decreases by 1.10mm (SE=0.81) per ºC, whereas the wing lengths of Anisoptera as a whole, do not change with temperature (0.39mm, SE=0.49).



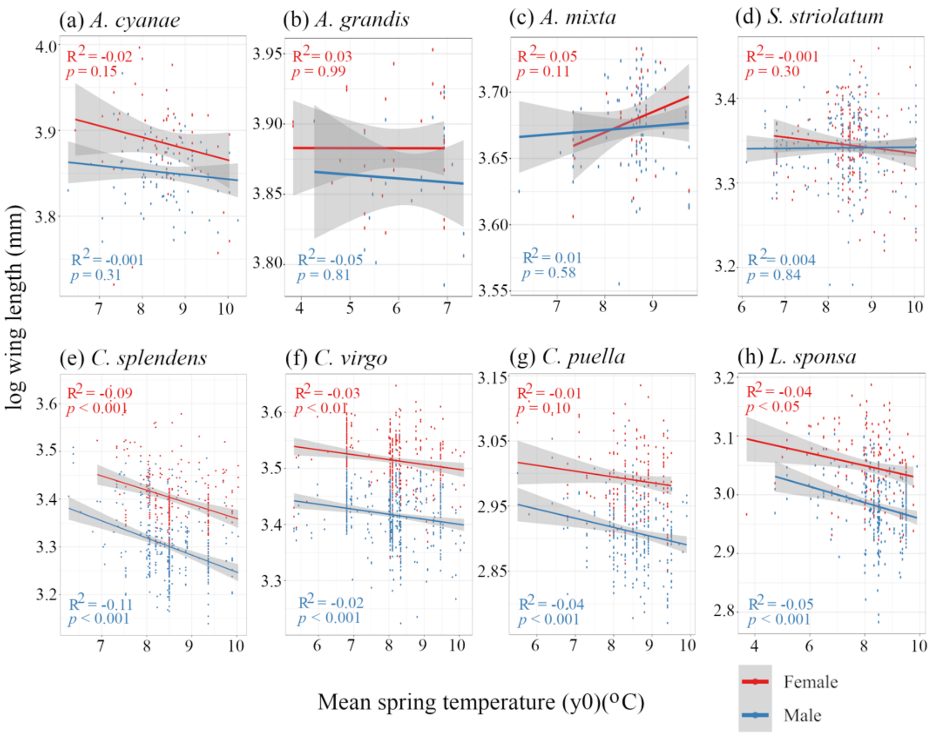


Fig 2.1: Linear regression of log wing length of Anisoptera (a)-(d) and Zygoptera (e)-(h) vs. mean autumn (y-1) temperature. Red dots and line represent females and blue dots and lines represent males.

Fig 2.2: Linear regression of log wing length of Anisoptera (a)-(d) and Zygoptera (e)-(h) vs. mean spring (y0) temperature. Red dots and line represent females and blue dots and lines represent males.

Table 2.2: 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					
		Aeshna cyanea	*Aeshna grandis*	*Aeshna mixta*	*Anax imperator*	***Brachytron pratense***	*Libellula quadrimaculata*	*Somatochlora metallica*	*Sympetrum striolatum*	***Calopteryx splendens***	***Calopteryx virgo***	***Coenagrion puella***	***Ischnura elegans***	***Lestes sponsa***	***Pyrrhosoma nymphula***
	Intercept	**52.48*****	57.68***	NS	27.08***	**33.94*****	33.98***	46.64***	27.39***	**46.43*****	**41.87*****	**24.81*****	**20.52*****	**25.07*****	**23.76*****
	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**
	Summer				NS	**NS**	NS	NS		**NS**	**NS**				**NS**
	Autumn				NS	**NS**	NS	-0.13***		**NS**	**0.39*****				**-0.15****
Y-1	Winter				NS	**0.23***	NS	0.47*		**NS**	**-0.39*****				**NS**
	Spring	**0.53***	NS		NS	**-0.36***	NS	NS		**0.40*****	**NS**				**NS**
	Summer	**NS**	NS		0.83*	**NS**	NS	NS		**NS**	**-0.29*****	**NS**	**0.19*****		**NS**
	Autumn	**NS**	NS		NS	**NS**	0.22*	NS		**-0.86*****	**-0.19***	**-0.14****	**-0.31*****		**-0.21*****
Y0	Winter	**NS**	NS		NS	**0.21***	NS	NS		**-0.18****	**NS**	**NS**	**NS**		**NS**
	Spring	**NS**	NS	NS				NS	NS	**-0.68*****	**-0.17****	**-0.29*****	**NS**	**-0.29*****	
	Sex	**NS**	-1.14*	NS	1.44*	**-0.69****	NS	NS	NS	**-2.96*****	**-3.11*****	**-1.51*****	**-1.63*****	**-1.30*****	**-1.30*****
	Adjusted R^2	**0.18*****	NS	NS	NS	**0.08***	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 as *, **, *** for p<0.05, 0.01 and 0.001, respectively. Blank cells are variables that were not included in a species analysis. NS is non-significant variable.

Table 2.3: Coefficients of the linear mixed effects model for examining the significant effects of mean seasonal temperature on dragonfly wing length across studied species. A negative symbol shows a negative relationship between wing length and the variables. Significant levels are indicated as *, **, *** for $p<0.05$, 0.01 and 0.001, respectively. ǂ is variance ± standard deviation of random variables.

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

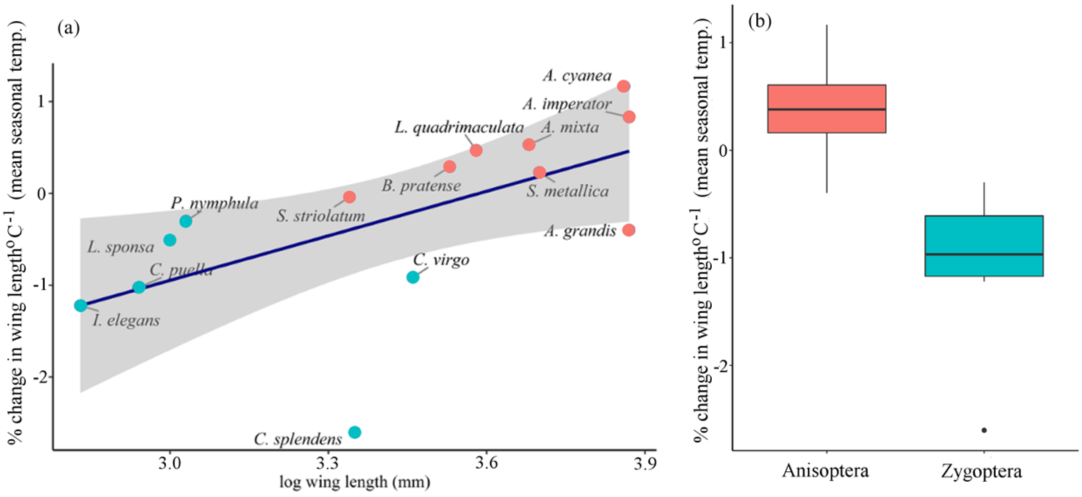


Fig 2.3: (a) Percentage change in mean wing length per °C of each species plotted against natural log wing length for mean seasonal temperature of the season that is indicated as the best variable for explaining wing length variation of each species (a stepwise regression analysis; Table 2.2). A correlation analysis indicated significant positive correlation between percent 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 °C change of Anisoptera and Zygoptera. A *t-test* indicated significant difference of percent change in wing length between Anisoptera ($\bar{x} = 0.39$) and Zygoptera ($\bar{x} = -1.10$) ($p<0.01$).

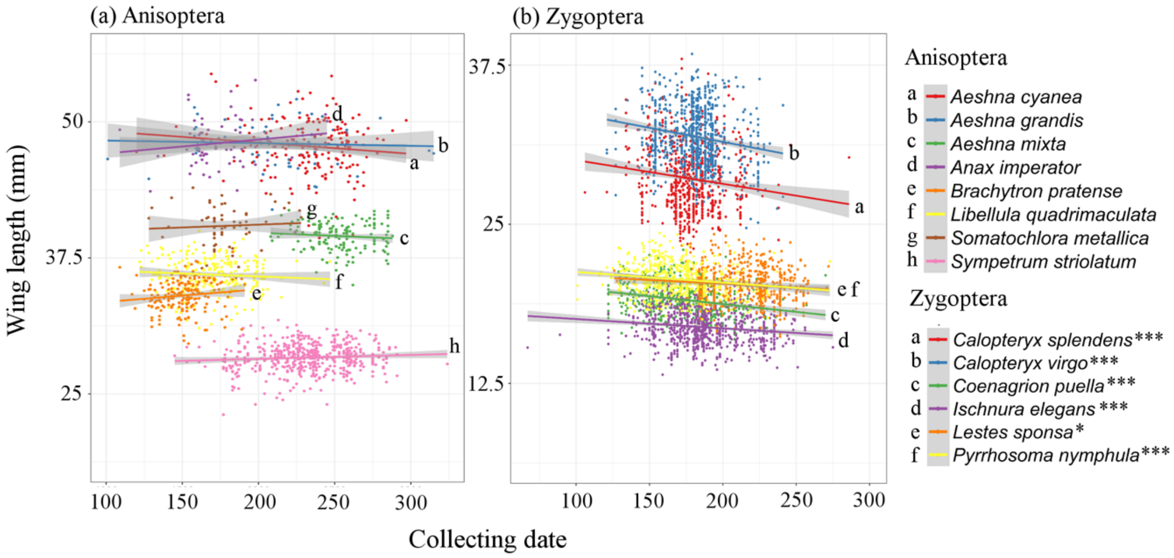


Fig 2.4: Linear regressions of hindwing lengths and collecting date (as a proxy of emergence date) of (a) Anisoptera and (b) Zygoptera. Significant results indicated by *** (p<0.001) and * (p<0.05).

2.4. Discussion

There are many different factors that need to be considered when investigating body size response to temperature in insects, 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 will 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 y0 (Figures 1 and 2). This response corresponds with the temperature-size rule (Atkinson, 1994), as smaller body sizes are found in warmer temperatures. Higher temperatures disproportionately increase developmental rate, resulting in smaller adults (Atkinson, 1994, Angilletta, 2004). In terms of life-history characters, some species are recorded as facultative univoltine species which can switch to a semivoltine lifecycle in parts of their distribution range. *Coenagrion puella* and *Ischnura elegans* (Zygoptera) have been recorded as facultative univoltine species in the UK (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 this study exhibit SSD (Fig S2.2 in appendix), but the males and females have equally negative size responses to temperature (Fig 2.1 and 2.2), 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 (Fig S2.2 in appendix).

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 (Fig 2.1a-2.1d, 2.2a-2.2d). 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. In 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, our results show the opposite, whereby small species have a greater reduction in size than large species as temperature increases. Interestingly, Anisoptera species are likely not 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 *Calopteryx 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 as they are semivoltine, 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; 1) territorial 2) non-territorial (sneaking), and 3) non-territorial (wandering). They found that the body size of wandering *C. virgo* males was smaller than territorial individuals. This may partly account for our results, but 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 UK populations of the zygopteran, *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, and studies in other Zygoptera species (Mahdjoub et al., 2015). This is likely due to the fact that 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).

In contrast to Zygoptera, the wing lengths of the Anisoptera 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), while 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 non-territorial. Most Anisoptera, including *S. rubicundulum,* are territorial where body size is relatively important for mating success in males (Sokolovska et al., 2000, Córdoba-Aguilar et al., 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 to Zygoptera. Thus, this may account for the non-significant correlation of wing length with collection date in Anisoptera. In order 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 Table S2.4 in appendix) 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 (Figure 2.3S in appendix) highlights that warmer years result in earlier collecting dates, indicating the collecting date is likely a good proxy for the date of emergence.

2.5. Conclusions

In conclusion, we find that 1) 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). In contrast, Anisoptera showed either a non-significant response or become larger (one species) or smaller (two species) with increasing temperature, possibly due to selection for larger individuals in strongly territorial species. 2) Contrary to our hypothesis, Zygoptera, which have a smaller body size than Anisoptera, showed greater sensitivity to temperature than Anisoptera. 3) Suborder is the main factor that affects Odonata temperature-size responses. We also hypothesized that univoltine species would show decreasing size with increasing temperature but that semivoltine species would show less sensitivity. This, however, proved not to be the case and suborder was a better predictor of size response irrespective of life cycle type. Other studies, however, 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). 4) 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 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 sub-order levels) and whether other morphological features, such as wing shape are also affected by temperature. Additionally, further study to investigate whether territoriality affects wing length response in both Anisoptera and Zygoptera is needed.

Appendix

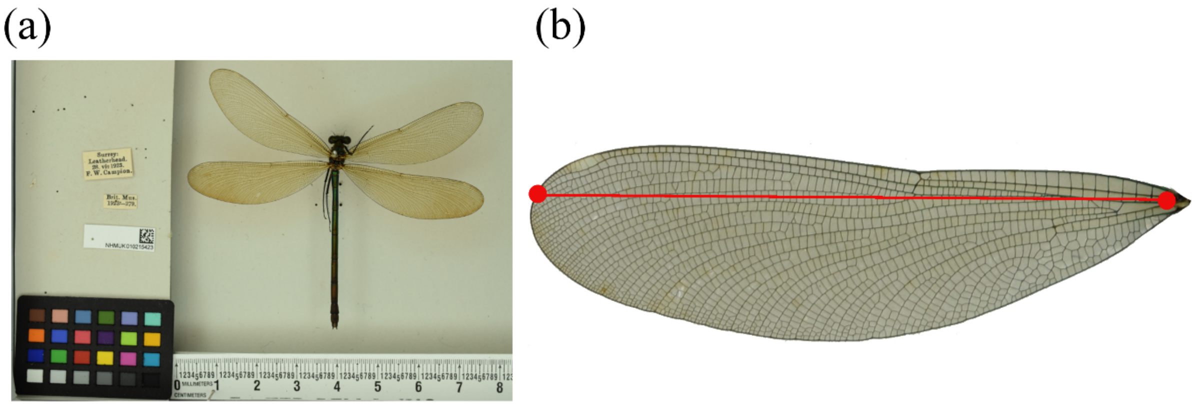


Fig S2.1: (a) Image of a pinned specimen with labels indicating collector, location and collecting date, specimen barcode, a color checker and a scale bar. (b) Wing length measurement of Odonata wing between the base of the leading edge of the wing and the distal end of the first radial anterior vein (R1).

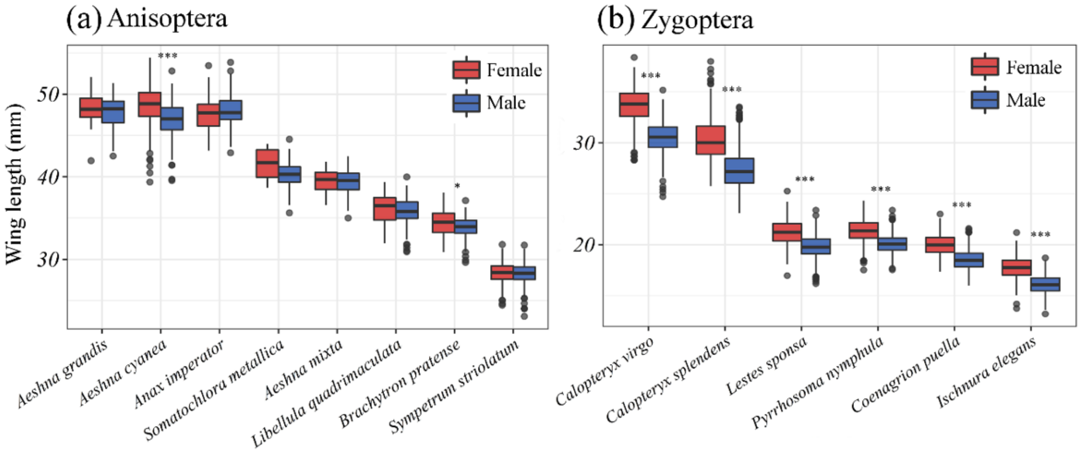


Fig S2.2: Box plots of females and males hindwing length for (a) eight species in suborder Anisoptera and (b) six species in suborder Zygoptera. Significant results indicated by *** ($p<0.001$) and * ($p<0.05$).

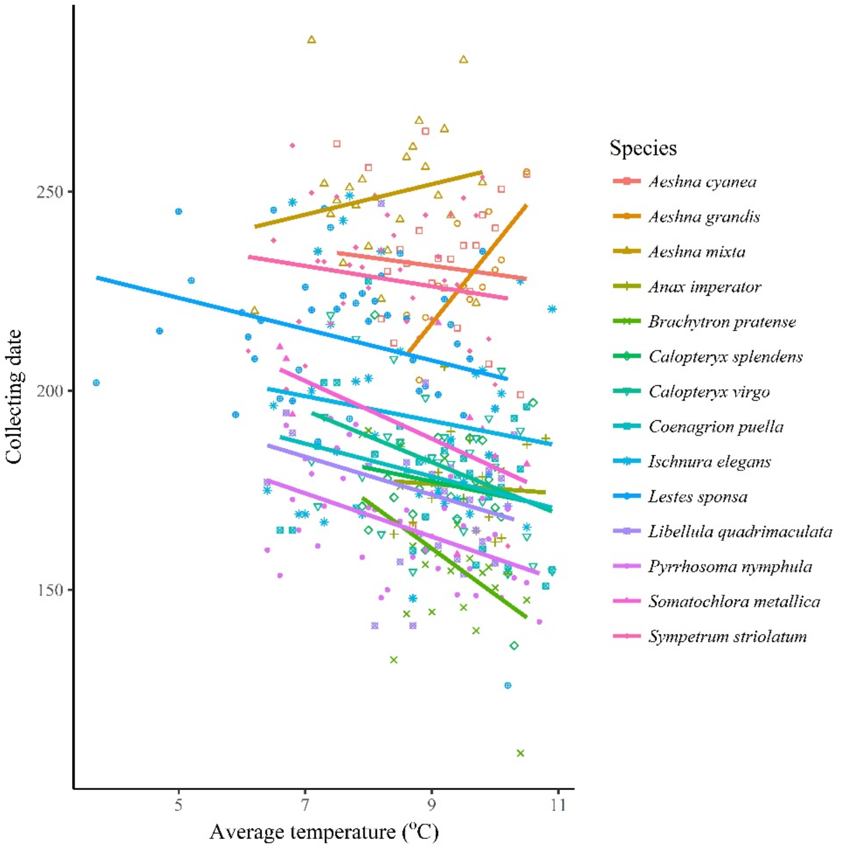


Fig S2.3: Regression analysis between the average temperature of larval period and collecting date which is a proxy of emergence date.

Table S2.1: Number of specimens of 14 British Odonatan species from museum collections in the UK and number of specimens.

Species	NHM (London)	NMS (Scotland)	OUMNH (Oxford)	NMW (Cardiff)	Liverpool	Manchester	Total
Anisoptera							
Aeshna cyanea	166	43	66	36	-	-	**311**
Aeshna grandis	158	-	-	-	-	-	**158**
Aeshna mixta	143	10	57	18	-	-	**228**
Anax imperator	105	5	44	9	-	-	**163**
Brachytron pratense	149	37	49	18	-	-	**253**
Libellula quadrimaculata	367	-	-	-	-	-	**367**
Somatochlora metallica	171	-	-	-	-	-	**171**
Sympetrum striolatum	452	59	151	62	-	-	**724**
Zygoptera							
Calopteryx splendens	877	35	57	-	47	26	**1042**
Calopteryx virgo	822	85	138	126	-	-	**1171**
Coenagrion puella	526	29	129	41	-	-	**725**
Ischnura elegans	682	184	114	30	-	-	**1010**
Lestes sponsa	461	75	112	64	-	-	**712**
Pyrrhosoma nymphula	441	186	84	53	-	-	**764**
Total	**5520**	**748**	**1001**	**457**	**47**	**26**	**7799**

Table S2.2: Number of specimens of 14 British Odonatan species from each decade.

Species	**1910s**	**1920s**	**1930s**	**1940s**	**1950s**	**1960s**	**1970s**	**1980s**	**1990s**	**Total**
Anisoptera										
Aeshna cyanea	26	21	28	53	16	1	5	5	2	**179**
Aeshna grandis	0	3	5	15	22	8	0	0	0	**53**
Aeshna mixta	25	42	17	38	13	3	1	2	0	**151**
Anax imperator	20	18	8	11	8	0	1	1	1	**68**
Brachytron pratense	52	33	25	58	19	5	0	2	0	**194**
Libellula quadrimaculata	18	49	23	119	17	8	0	1	0	**235**
Somatochlora metallica	5	7	24	16	11	2	0	0	0	**64**
Sympetrum striolatum	121	46	63	170	56	16	7	2	1	**482**
Zygoptera										
Calopteryx splendens	67	70	148	315	48	10	3	12	11	**684**
Calopteryx virgo	42	72	106	307	221	47	2	0	41	**838**
Coenagrion puella	125	76	112	150	45	2	16	4	0	**530**
Ischnura elegans	158	72	257	148	51	11	27	5	1	**730**
Lestes sponsa	179	68	48	205	40	23	9	5	4	**581**
Pyrrhosoma nymphula	116	43	122	145	48	34	23	7	4	**542**
Total	954	620	986	1750	615	170	94	46	65	**5331**

Table S2.3: AIC approach to compare linear-mixed effects models contained different sets of random effects variables.

Model	Random effects variables	AIC	Chi-square	Df (Chi-squre)	*p*-value
Model1	species + sex + suborder + life history	-11865.6	1530.751	1	<2e-16***
Model2	sex	1331.7	0.000	0	<2e-16***
Model3	life history	1312.1	19.605	0	<2e-16***
Model4	suborder	-91.5	1403.604	0	<2e-16***
Model5	species	-10334.0	10242.478	1	<2e-16***
Model6	suborder + sex + life history	-387.2	256.053	1	<2e-16***
Model7	**suborder + sex + species**	**-11867.6**	**11480.398**	**0**	**<2e-16*** **
Model 8	suborder + life history + species	-10336.9	0.000	0	1
Model 9	sex + life history + species	-11860.4	0.000	0	1
Model 10	species + sex	-11862.4	1530.429	1	<2e-16***
Model 10	species + suborder	-336.8	0.000	0	1
Model 11	species + life history	-10338.9	10002.068	0	<2e-16***
Model 12	sex + suborder	1312.4	0.000	0	1
Model 13	sex + life history	-10332.0	11644.455	0	<2e-16***
Model 14	suborder + life history	-133.2	0.000	0	1

Table S2.4: Correlation coefficients and significant values (*p*-value) between collection date (original collection date and simulation data) and wing length of each Anisoptera species.

Species	***r* (*p*-value)**	
	Original collecting date	**30days-period simulation**
Aeshna cyanea	0.005 (*p*=0.95)	0.003 (*p*=0.98)
Aeshna grandis	-0.234 (*p*=0.09)	-0.181 (*p*=0.19)
Aeshna mixta	-0.082 (*p*=0.36)	-0.078 (*p*=0.38)
Anax imperator	0.139 (*p*=0.31)	0.064 (*p*=0.64)
Brachytron pratense	0.087 (*p*=0.26)	0.079 (*p*=0.31)
Libellula quadrimacuata	-0.085 (*p*=0.21)	-0.067 (*p*=0.32)
Somatochlora metallica	-0.017 (*p*=0.90)	-0.027 (*p*=0.84)
Sympetrum striolatum	0.093 (*p*=0.07)	0.099 (*p*=0.06)

Chapter III

Temperature-size responses in three species of British Odonata over 100 years

This chapter investigates whether body size changes over historic to modern time scales in the three British odonate species (*Calopteryx splendens*, *Coenagrion puella* and *Sympetrum striolatum*) are related to temperature by excluding geographic and intra-annual (month) effects. Wing lengths of museum specimens collected in the same location between 1912 and 1992 are compared to specimens collected and measured in the field during the summer of 2018.

Author contributions: RW collected and analysed the data and led the writing of the chapter. PBF, PGL, SJB, and BWP helped collected data in field, conceive the ideas for the research and analyses and provided direction for the writing.

Abstract

Body size is a fundamental trait of insects and is correlated with individual fitness, trophic interactions and ecosystems functioning. Global warming over the last century may lead to a decrease in insect body size, in accordance with the Temperature-Size Rule. This study aims to investigate the influences of warming temperature on wing length, as a proxy for body size, in three species of British Odonata. Wing lengths of *Sympetrum striolatum*, *Calopteryx splendens* and *Coenagrion puella* from museum collections, collected in the same locality between 1912 -1992, were compared with wing length data from a field survey during the summer of 2018. The results indicate non-significant wing length changes in *S. striolatum* but significantly longer wing length, with increasing temperature in *Calopteryx splendens*. Warmer temperatures provide a longer growing season for larval development, ultimately resulting in larger adult body sizes, which is beneficial for territorial competitiveness in this species. In contrast, the non-territorial *Coenagrion puella* shows a negative wing length response to increasing temperature suggesting that warming accelerates larval developmental rate and leads to smaller adult body size. These findings support the suggestion that there is no universal size response to temperature in Odonata, and that other factors may ultimately drive changes in body size.

Key-words: *Anisoptera, body size response, climate change, temperature-size rule (TSR), warming, Zygoptera*

3.1. Introduction

In the 20th century, the global average temperature has increased by about 0.6℃ (IPCC, 2018). Many previous studies found that the developmental rate of organisms is faster at warmer temperatures, consequently, the final size of organisms becomes smaller than those growing in lower temperatures, a phenomenon known as the temperature-size rule (TSR) (Atkinson, 1994). The reason for this size response to temperature is that higher temperatures accelerate the developmental rate of organisms rather than their growth rate (Atkinson, 1994, Angilletta et al., 2004). For example, an experiment on *Lestes eurinus* reported faster larval development at higher temperature (Lutz, 1974) and a laboratory study on the development of *Sympetrum striolatum* found faster egg development in higher temperatures (Koch, 2015). Thus, with climate warming, ectothermic organisms are expected to become smaller over time, which can negatively affect fitness and life history (Cressa et al., 2008, Kingsolver & Huey, 2008, Gibert & DeLong, 2014). However, temperature is not the only factor that can affect the adult body size of insects - sexual selection, the availability of food resources, and behavioural factors (e.g. territoriality) can also be important (Wonglersak et al. 2020).

Apart from temperature, latitude can also have indirect effects on the adult body size of insects by influencing the length of the growth-season and food availability. The limited time available for development and growth at higher latitudes can lead to smaller adult body size (Blanckenhorn & Demont, 2004, Mousseau, 1997). Additionally, temperature could impact the adult body size of Odonata by affecting their date of adult emergence (Cothran & Thorp, 2006, Penn, 2015). On one hand larvae may be larger with later emergence as they have a longer time to develop, while conversely, late emerging larvae of univoltine species may be smaller as they have to accelerate their development rate to emerge in the correct season and this comes at the cost of a smaller adult body size (Johansson & Rowe, 1999).

Despite several studies investigating the effects of temperature on insect body size (Sota et al., 2002, Bidau & Martí, 2008, Horne et al., 2015), to our knowledge there is no study focused on temperature-body size responses that exclude ecogeographical effects and the influence of adult emergence date. Thus it is interesting to compare the adult body size of several odonate species, collected from the same location and during the same time of year, but in different years experiencing different seasonal temperatures.

Specifically, this study uses British Odonata to examine body size responses to temperature through time, excluding spatial effects. Three species were selected: *Sympetrum striolatum*, *Calopteryx splendens* and *Coenagrion puella*, as they fulfil several criteria: (1) they represent both suborders Anisoptera and Zygoptera; (2) include univoltine and semivoltine species; (3) include territorial and non-territorial species; and (4) they have sufficient historical samples collected from the same site. Wing length was measured using museum specimens collected from the same location between 1912 and 1992, and compared to specimens collected and measured in the field during the summer of 2018. The historical and modern data were analysed to investigate whether body size changes in the selected species were related to temperature change.

For *Coenagrion puella*, which is a univoltine, non-territorial, Zygoptera species, we expected a smaller adult body size in response to climate warming, as a result of a faster development rate of larvae in warmer temperatures (Atkinson, 1994, Angilletta, 2004).

For *Calopteryx splendens,* which is a semivoltine, territorial, Zygoptera species where body size may be important for territory defence, we expected no body size change or a bigger size with warmer temperatures, especially as they are semivoltine, and so have less time-constraints on development. However, the results presented in a previous study (Chapter 2) show that *C. splendens* tend to have a smaller body size with increasing temperature (Wonglersak et al., 2020). This may be because territoriality in *Calopteryx* is weaker than in many Anisoptera. By using specimens collected over a longer period of time, but from the same locality and during the same time of year we can better understand temperature-body size response in this species.

For *Sympetrum striolatum*, a univoltine, territorial, Anisoptera species, we predict no significant change in adult body size, following the previous results over a wider geographic scale but shorter timescale (Chapter 2, Wonglersak et al., 2020), with the assumption that this is because this species exhibits territorial behaviour, which is likely to select for larger body size to enhance territorial defence (Sokolovska et al., 2000, Córdoba-Aguilar, 2008).

3.2. Materials and Methods

3.2.1. Data acquisition

Three species of British Odonata (*Calopteryx splendens*, *Coenagrion puella* and *Sympetrum striolatum*) were selected for this analysis based on their life history, territorial behaviour, their known size responses to temperature (see Chapter 2 of this thesis) and the availability of sufficient museum specimens collected at a single locality during the 1900s (1912-1992). Wing length was obtained for museum specimens as outlined in Chapter 2, and in the field 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 using digital vernier callipers. These species were surveyed in the field during two time periods: 7th – 8th June 2018 for *Calopteryx splendens* and *Coenagrion puella*, and 15th -16th August 2018 for *Sympetrum striolatum*. To avoid measuring the same specimen multiple times, a hindwing was marked using orange nail polish before the specimen was released. As the identification of female *Coenagrion puella* is unreliable in the field, only male data are available for this species.

The regional monthly air temperature time-series data are derived from the UK Meteorological Office (www.metoffice.gov.uk/climate/uk/ summaries/datasets) and were used to calculate climate variables for analyses. 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) (Table 3.1).

Table 3.1: The three Odonata species used in this study and their voltinism type (Corbet & Brooks, 2008). Environmental variables for each type are calculated from the England South West mean monthly temperature series. For each variable y-2 is two years before the year of collection, y-1 is a year before the year of collection, and y0 is the year of collection. Number of specimens collected in each year is in table S1.

Species	**N**			**Corbet's type**	**Voltinism type**	**Environmental variables**
	Year of museum specimens	**No. of specimens**	**Field collection (2018)**			
Calopteryx splendens	1925	1	49	summer species	semivoltine	summer (y-2) autumn (y-2) winter (y-1) spring (y-1) summer (y-1) autumn (y-1) winter (y0) spring (y0)
	1932	1				
	1942	129				
	1943	72				
	1963	1				
	1982	1				
	1992	1				
	Total	206				
Coenagrion puella	1918	2	114	summer species	univoltine	summer (y-1) autumn (y-1) winter (y0) spring (y0)
	1924	1				
	1936	1				
	1941	6				
	1943	3				
	1944	1				
	1946	12				
	1947	2				
	1948	21				
	1950	9				
	Total	58				
Sympetrum striolatum	1912	1	25	obligatorily univoltine	univoltine	spring (y0)
	1924	1				
	1933	1				
	1934	1				
	1937	2				
	1938	2				
	1939	1				
	1941	15				
	1942	50				
	1943	3				
	1944	6				
	1945	1				
	1946	28				
	1948	4				
	1950	5				
	1953	1				
	1964	1				
	Total	123				

3.2.2. Data analysis

The modern wing length data was compared to the historical data from the museum collection, collected at the same time of year in all years, to investigate wing size difference over time from the same location, without the influence of seasonal effects. In addition to mean seasonal temperature, year was included in the data analysis to examine wing length changes over time. Prior to the analysis of each species, the Variance Inflation Factor (VIF) was estimated to test the multicollinearity among environmental variables. Variables with $\mathrm{VIF}_{\mathrm{Weight}}$ higher than three were removed from the models (Quinn & Keough, 2002, Zuur et al., 2010). Then 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. If sex is indicated as an important factor, then subsequent analysis for each sex was performed.

The Akaike Information Criterion (AIC) (Burnham & Anderson, 2002) was used in order to perform model selection using the *MuMIn* package (Barton, 2015) in R. On the basis of the $\mathrm{AIC}_{\mathrm{C}}$, the *dredge* function in the package was used to rank a set of models, which were generated based on all possible combinations of the explanatory variables (environmental variables). A set of candidate models was selected when ΔAIC of the models was equal to or less than 7 (Burnham et al., 2011). The model averaged coefficient of each variable was extracted. An importance score was determined and ranked from 0 to 1 that indicates the frequency of each variable in the set of candidate models. These two values were used to select the most important variables for explaining wing length. Furthermore, to ensure a selection of the most important variables, the nested function in *MuMIn* package (Barton, 2015) was performed to remove nested models. This can enhance model selection by reducing the chance of excessively complex model selection (Richards et al., 2011).

3.3. Results

The VIF indicated multicollinearity between year and other temperature variables, thus year was excluded from the multiple regression analysis. However, a two-way analysis of variance (ANOVA) was performed to determine whether there is significant difference between mean wing length of historical specimens and modern specimens.

For *Sympetrum striolatum*, a univoltine anisopteran species, a two-way ANOVA indicated non-significant differences between mean wing length of historical and modern data (p>0.05) (Table 3.2). Also, stepwise regression and AIC analysis found no significant correlation between wing length and any mean seasonal temperature for either sex (p>0.05)(Table 3.3, 3.4).

For *Calopteryx splendens,* which is a semivoltine zygopteran species, the stepwise regression analysis indicated that wing length positively correlates with mean temperature of summer (y-2) in both sexes (Fig 3.1a, Table 3.3). A significant positive correlation between wing length and mean autumn temperature of y-2 is found in females (p<0.05) (Fig 3.1b, Table 3.3). Whereas males showed positive wing length responses to mean winter and spring temperature of y-1 (p<0.01)(Fig 3.1c-d, Table 3.3). A two-way ANOVA showed a significant difference between mean wing length of historic and modern data and between sexes (p<0.01 and p<0.001, respectively) (Table 3.2).

For *Coenagrion puella*, which is a univoltine zygopteran species, the stepwise regression analysis showed significant wing length decrease with increasing mean autumn temperature of y-1 and mean spring temperature of y0 in males ($p<0.05$)(Fig 3.1e-f). Whereas wing length of females showed non-significant correlation with mean seasonal temperature (Table 3.3). A two-way ANOVA indicated a significant difference between mean wing length of historic and modern data and between sexes ($p<0.001$) (Table 3.2).

Table 3.2. A two-way ANOVA between wing length, temperature condition and sex. Significant levels are indicated as **, *** for $p<0.01$ and 0.001, respectively. NS is non-significant variable.

Species	Temperature condition	Sex	Temperature*sex
Sympetrum striolatum	NS	NS	NS
Calopteryx splendens	**	***	NS
Coenagrion puella	***	***	NS

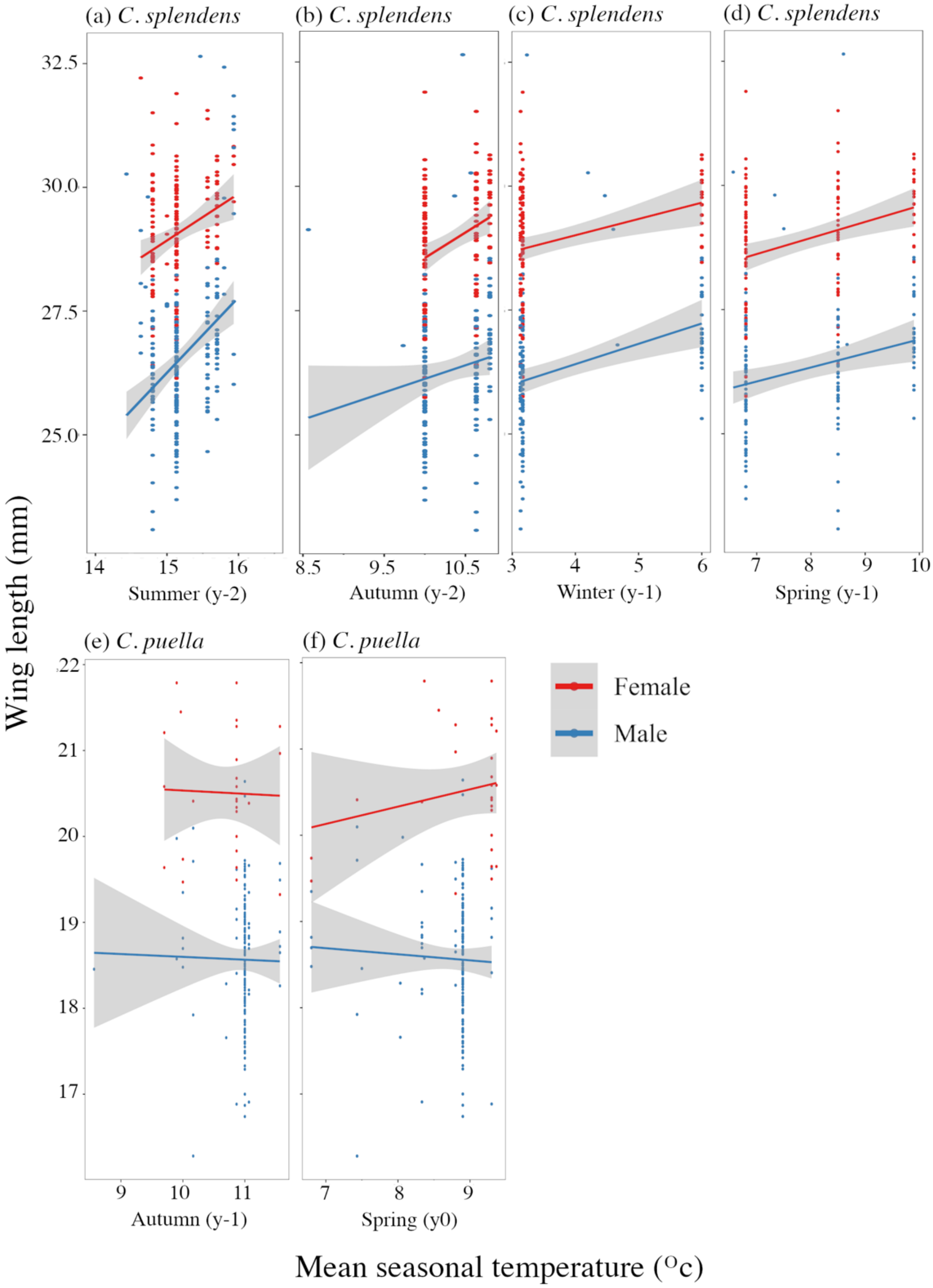


Fig 3.1: Linear regression of wing length of *Calopteryx splendens* (a)-(d) and *Coenagrion puella* (e)-(f) vs mean seasonal temperature. Red dots and line represent females and blue dots and lines represent males.

Table 3.3. Stepwise regression analysis between wing length and mean seasonal temperature and collecting date. A subsequent analysis for each sex was performed when factor (sex) was a significant variable. y-2 is two years before the collecting year, y-1 is a year before the collecting year, and y0 is a collecting year. Blank cells are variables that were not included in a species analysis. Significant levels are indicated as *, **, *** for $p<0.05$, 0.01 and 0.001, respectively. NS is non-significant variable and NA is variable that was excluded from the analysis because the VIF is higher than 3.

Species	**Adjusted R^2**	**Factor (sex)**	**Collecting year**	**y-2**		**y-1**				**y0**	
				Summer	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**
Sympetrum striolatum	NS	NS	NS								NS
Calopteryx splendens	0.64***	-2.71***	NA	3.31***	1.06**	0.92***	NA	-5.29***	NA	NS	NA
Female	0.11***		NA	0.60*	0.92**	NS	NS	NS	NA	NS	NA
Male	0.34***		NA	3.87***	NS	0.67**	0.45**	NS	NA	NA	NA
Coenagrion puella	NS	-1.73***	NA					NS	NS	NS	NS
Female	NS		NA					NS	NS	NS	NA
Male	0.11***		NA					NS	-0.30*	NS	-0.32*

Table 3.4. Parameters of the set of candidate models using The Akaike Information Criterion (AIC) for predicting wing length of all individuals of selected species. Values in bold correspond to variables retained after nested models were removed. Importance score and number of candidate models for each sex are indicated if factor (sex) is determined to be an important factor. Blank cells are variables that were not included in a species analysis. NA is variable that was excluded from the analysis because the VIF is higher than 3.

			Factor (sex)	Year	Summer (y-2)	Autumn (y-2)	Winter (y-1)	Spring (y-1)	Summer (y-1)	Autumn (y-1)	Winter (y0)	Spring (y0)
S. striolatum	All	Importance scores	0.42									0.74
		N containing models	3									4
		Coefficient estimate	-0.21									-0.27
		Std. Error	0.16									0.14
C. splendens	All	Importance scores	**1.00**	NA	**1.00**	**1.00**	**1.00**	NA	**1.00**	NA	0.26	NA
		N containing models	**2**	NA	**2**	**2**	**2**	NA	**2**	NA	1	NA
		Coefficient estimate	**-2.71**	NA	**3.29**	**1.08**	**0.92**	NA	**5.29**	NA	-0.02	NA
		Std. Error	**0.14**	NA	**0.80**	**0.37**	**0.15**	NA	**0.80**	NA	0.11	NA
	Female	Importance scores		NA	**0.30**	**0.35**	**0.36**	0.15	**0.30**	NA	**0.30**	NA
		N containing models		NA	**4**	**5**	**5**	1	**4**	NA	**4**	NA
	Male	Importance scores		NA	**1.00**	0.57	**1.00**	**0.63**	0.57	NA	NA	NA
		N containing models		NA	**3**	2	**3**	**2**	2	NA	NA	NA
C. puella	All	Importance scores	**1.00**	NA					0.19	0.19	0.40	0.25
		N containing models	**18**	NA					5	5	8	6
		Coefficient estimate	**-1.83**	NA					0.01	-0.04	0.13	0.06
		Std. Error	**0.20**	NA					0.10	0.18	0.11	0.19
	Female	Importance scores		NA					0.20	**0.76**	**0.77**	**NA**
		N containing models		NA					2	**6**	**5**	**NA**
	Male	Importance scores		NA					0.18	0.19	**0.35**	0.25
		N containing models		NA					5	5	**8**	6

3.4. Discussion

The results indicate that the three species showed different wing size responses to temperature over the 100-year timescale. The non-significant correlation between wing length and mean seasonal temperature and non-significant ANOVA between mean wing length over historical and modern timescales in *Sympetrum striolatum* from this single locality corresponds well with the study of this species using museum specimens from throughout Britain (Chapter 2, Wonglersak et al., 2020). This Anisoptera species is territorial and a large adult size is likely important for mating success (Sokolovska et al., 2000, Córdoba-Aguilar et al., 2008), thus they need to maximise their body size. Previous studies have suggested that small larvae of territorial dragonfly species can delay their emergence, allowing more time to grow and thus emerge as a larger adult (Rowe & Ludwig, 1991, Fuselier et al., 2007).

Calopteryx splendens showed significantly bigger size with warmer of summer of y-2 in both sexes but the correlation is notably stronger in males. A significantly bigger size with warmer of winter of y-1 and spring y-1 is found in males, whereas females showed bigger size with mean autumn temperature of y-2. Additionally, an ANOVA indicated significantly bigger size of both sexes of *C. splendens* in modern specimens compared with historical specimens. Interestingly, this finding of increasing size with temperature and over period of time in *C. splendens* is in agreement with the expectation but in contrast to the previous study (Chapter 2, Wonglersak et al., 2020). We assume that because *C. splendens* has territorial behaviour, wing size, which is a proxy for body size (Haas & Tolley, 1998, Corbet, 1999, Worthen & Jones, 2006), is an important factor for mating success in males (Sokolovska et al., 2000, Córdoba-Aguilar et al., 2008). Thus, males probably try to maximise their adult body size, and small larvae could get an advantage from warmer winter or autumn by delaying their emergence time and enabling more time for growing and developing (Rowe & Ludwig, 1991, Fuselier et al., 2007). This assumption requires further study by collecting more modern data throughout their emergence season to illuminate the influences of territoriality on body size responses to temperature in this species.

According to the previous study using museum collections (Wonglersak et al., 2020), females of *C. splendens* are expected to be smaller with increasing temperature. Interestingly, in the current study, the results of the regression analysis and ANOVA indicated significantly longer wings with increasing temperature and significant larger wing length in modern specimens than historic specimens. The negative response of wing length to warming temperature found in the previous study (Wonglersak et al., 2020) could be an indirect effect of latitude and emergence date of each individual. Extending the larval development period at high latitude has been recorded in some Zygoptera species (Corbet et al., 2006). Thus, years with relatively cool mean seasonal temperature might result in larvae switching to a three-year life cycle, thereby extending their development for an additional year, and resulting in a larger body size on emergence in *C. splendens*.

This study used specimens from same locality that were collected at the same time of year in all years, thus we aimed to exclude ecogeographical and emergence date effects. Our finding of longer wing lengths in female *C. splendens* with warming temperature is consistent with previous studies in butterflies (Fenberg et al., 2016, Wilson et al., 2019). This finding implies that in a year with warmer temperatures more time is available for larvae to develop and emerge as bigger adults, than for larvae from the same locality growing in a year with cooler temperature, which thus emerge as smaller adults (Sota et al., 2002, Blanckenhorn, 2004, de Queiroz & Ashton, 2004).

Coenagrion puella showed a negative correlation between temperature and wing length in males, indicating that warming temperature reduced male wing size. This finding corresponds well with the previous study using museum specimens from a wider range of localities (Wonglersak et al., 2020) and the temperature-size rule (Atkinson, 1994), where warming temperature disproportionately increases developmental rate, resulting in smaller adults (Atkinson, 1994, Angilletta, 2004). Since *C. puella* is not territorial, this species is not under such selection pressure to produce large males. However, museum specimens of the selected species in this study have a large record in a small number of years. Thus, this limitation could affect the analysis and result of this chapter.

3.5. Conclusions

In conclusion, We find that: 1) species with territorial behaviour showed either non-significant wing length changes (*S. striolatum*) or significantly longer wing length (*C. splendens*) with increasing temperature and over a 100-year timescale. As warmer temperature provides a longer growing season for larval development, these species ultimately benefit from bigger adult body size for their territorial competitiveness; 2) negative wing length response with increasing temperature in *C. puella* suggested that warming could accelerate larval developmental rate and lead to smaller adult size.

Although this study found that wing length responses of *Sympetrum striolatum* and *Coenagrion puella* correspond well with the previous results using museum specimens from a wider range of localities (Wonglersak et al., 2020), the results for *Calopteryx splendens* contradicted those from Wonglersak *et al*. (2020). The reasons for this require further study but are likely related to the effects on wing length of latitude and adult emergence date, which may counteract the direct effects of temperature on size in this species.

Chapter IV

Insect body size changes under future warming projections: a case study of Chironomidae (Insecta: Diptera)

This chapter collected six species of Chiromidae over a two-year period (2017-2018) from mesocosms experiments comprising ten artificial outdoor experimental ponds with five ponds at ambient temperature and five ponds at 4℃ higher than ambient temperature, to uncover if there are any general body size responses to temperature. Mesocosms were chosen as they provide more realistic conditions compared to indoor laboratory experiments, but are less complicated than natural ecosystems.

Author contributions: 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.

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PRIMARY RESEARCH PAPER

Insect body size changes under future warming projections: a case study of Chironomidae (Insecta: Diptera)

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Abstract

Chironomids are a useful group for investigating body size responses to increasing temperature due to their high local abundance and sensitivity to environmental change. We collected specimens of six species of chironomids every two weeks over a two-year period (2017-2018) from mesocosm experiments using five ponds at ambient temperature and five ponds at 4℃ higher than ambient temperature. We investigated 1) wing length responses to temperature within species and between sexes using a regression analysis, 2) interspecific body size responses to test whether the body size of species influences sensitivity to warming, and 3) the correlation between emergence date and wing length. We found a significantly shorter wing length with increasing temperature in both sexes of *Procladius crassinervis* and *Tanytarsus nemorosus*, in males of *Polypedilum sordens,* but no significant relationship in the other three species studied. The average body size of a species affects the magnitude of the temperature-size responses in both sexes, with larger species shrinking disproportionately more with increasing temperature. There is a significant decline in wing length with emergence date across most species studied (excluding *Polypedilum nubeculosum* and *P. sordens*), indicating that individuals emerging later in the season tend to be smaller.

Key-words: *body size response, climate change, mesocosm, temperature-size rule (TSR), warming*

4.1. Introduction

Since the 1990s, climate change as a consequence of anthropogenic activities has become an important issue in ecological, biodiversity and conservation research. The main characteristics of predicted global climate change are a change in precipitation patterns and intensity, stochastic temperature variability, and an increase in the average global mean temperature, that has increased at the rate of 0.08-0.14℃ per decade since 1951 (Hansen et al., 2006, IPCC, 2014). Furthermore, there is a likely increase of at least 1.5 ℃ in average global temperature by 2100 for most representative concentration pathways (RCPs) of greenhouse gas emissions (IPCC 2014).

Change in mean regional temperature affects several aspects of the ecology and life history of species, including their abundance, distribution and body size (e.g., Halpin, 1997, Walther et al., 2002, Thomas et al., 2004). It is generally known that the developmental rate of early life stages in ectotherms depends on environmental temperature, known as the temperature-size rule (TSR) (Atkinson, 1994), which ultimately affects the final adult body size. Adult body size subsequently correlates with individual fitness, population growth, and ecosystem functioning (Bonner & Peters, 1985). Within species, changes in the final adult body size can affect individual fitness. Particularly among insects, larger females have higher potential fecundity and larger males may have a higher mating success (Elgar & Pierce, 1988, Berrigan, 1991, Berger et al., 2008). In addition to individual fitness, increasing temperature can relay the effects of changes in body size to species interactions and community dynamics. For example, a reduction in body size in species that are major food resources within a community can indirectly change food web structure and community dynamics by altering feeding rates and predator-prey interactions (Boukal et al., 2019). The ecological effects of body size reductions are more apparent in aquatic ecosystems because food webs of aquatic ecosystems have stronger size-structure than terrestrial habitats (Sentis et al., 2017, Boukal et al., 2019). Thus, understanding how warming affects the body sizes of animals toward the base of the food chain (e.g. chironomids) will allow us to better predict its potential cascading effects on ecological functioning in the future.

Non-biting midges (Diptera: Chironomidae) are a highly diverse aquatic insect family with global distributions (Sæther, 2000, DeWalt et al., 2010). Chironomids are highly abundant, have short generational times, and play a crucial role in aquatic food webs as a resource for other invertebrates, fish and birds. They have different feeding strategies across sub-families, including herbivores, predators, detritivores, grazers and filter-feeders (DeWalt et al., 2010), and there is considerable variation and flexibility in voltinism (Tokeshi, 1995). Additionally, they are considered keystone species as they play an important role in nutrient cycling (Kelly and Roger, 2004). Changes in chironomid body size can ultimately affect predator-prey relationships. Thus, they are a useful insect-model to assess the influence of temperature on adult body size.

As found with other arthropods, ambient temperature significantly affects chironomid developmental and growth rate, and ultimately their final adult body size (Pinder, 1986, Sankarperumal & Pandian, 1991, Stevens, 1998, Mackey, 2006, Frouz et al., 2009), especially in temperate regions where there are large differences between winter and summer temperatures.

Previous laboratory-based studies on chironomids (e.g. McKie & Cranston, 2005, Frouz et al., 2009, Baek et al., 2012) and in nature (e.g. Oliver, 1971, Pinder, 1986, Kobayashi, 1998) have found a negative correlation between adult body size and temperature. However, other studies have found a bell-curved relationship between body size and developmental rate, with temperatures above 20℃ resulting in an increased body size due to a reduced developmental rate (Maier et al., 1990, Frouz et al., 2009, Baek et al., 2012).

In terms of Sexual Size Dimorphism (SSD), a study on *Chironomus crassicaudatus* found steeper declines in the body size of males with increasing temperature than in females (Frouz et al., 2009). Furthermore, studies of Chironomidae recorded faster development of males compared to females (Armitage, 1995, Stevens, 1998). Additionally, the females of many chironomid species have slower development during the last larval instar (Danks, 1978, Stevens, 1998). This is possibly because bigger females are correlated with higher fitness as they could have a bigger clutch size, whereas male fitness does not appear to correlate with body size (Xue & Ali, 1994). However, a previous study on *Chironomus plumosus* found stabilizing selection on male body size. Larger males tend to live longer but have lower mating success, whereas small males might be at an advantage in swarms and could be more aerobatic (Neems et al., 1998). Furthermore, smaller males are faster, which is beneficial for mating success (McLachlan, 1986, Neems et al., 1990), so it may not be important for males to maintain their body size at higher temperatures.

Study of morphological responses of organisms to increasing temperature is complicated because morphology, body size and body shape, could be influenced by factors other than temperature, for instance, sexual selection, mortality risk, precipitation or food availability (Tokeshi, 1995). According to studies on other aquatic insects, body size may also vary through the season (Cothran & Thorp, 2006, Penn, 2015, Wonglersak et al., 2020). A negative correlation between adult body size and emergence date is indicated in previous studies on a tropical stream mayfly (Ephemeroptera) (Sweeney et al., 1995) and many species of British dragonflies (Odonata) (Wonglersak et al., 2020). However, as far as We know, this relationship between adult body size and emergence date has not been investigated in chironomids. Thus, more experimental studies are needed in order to examine this relationship and to determine the direction and strength of temperature-size responses within and across chironomid species.

Even though there are some laboratory studies on the influence of temperature on chironomid body size (i.e. Oliver, 1971, Pinder, 1986, McKie & Cranston, 2005, Frouz et al., 2009, Baek et al., 2012), my study approach differs by using mesocosms. These were artificial, outdoor, experimental ponds, with five ponds kept at ambient temperature and five ponds at 4°C higher than ambient temperature. We used this approach to investigate body size responses of chironomids to a predicted future temperature scenario within and across species over their entire emergence period. As these mesocosms have daily temperature fluctuation they can provide more realistic conditions than indoor laboratory experiments, but are less complicated than natural ecosystems (Kangas & Adey, 2008).

My study uses mesocosms to investigate intraspecific adult body size responses of chironomids to increasing temperature under a future temperature scenario and the sensitivity of temperature-body size changes between sexes. Additionally, we also investigate interspecific body size responses to temperature by comparing body size change between ambient and heated conditions across species and sex, and explore the correlation between body size and the emergence date of chironomids.

We hypothesized that: 1) a smaller adult body size is expected in the heated treatments due to a faster developmental rate, in agreement with previous studies on chironomids (e.g. Oliver, 1971, Pinder, 1986, Kobayashi, 1998). 2) We expect the magnitude of body size decline with increasing temperature to be higher in large species than small species as suggested by several studies of other invertebrate taxa (Forster et al., 2012, Tseng et al., 2018). 3) The body sizes of adult Chironomidae are expected to be smaller at later emergence dates in both heated and ambient conditions, as larval development rate accelerates to allow emergence before the end of the warm season as found in Odonata (Banks & Thompson, 1985, Michiels & Dhondt, 1989, Wonglersak et al., 2020).

4.2. Materials and Methods

4.2.1. Sampling

The experiments were conducted over a two-year period (2017-2018) using ten mesocosm ponds at the Freshwater Biological Association River Laboratory, Dorset, United Kingdom (2°10`W, 50°13`N). The ten mesocosm ponds have been fully functioning since 2006 (Yvon-Durocher et al., 2010). Each pond has a 2.5m^2 surface area with 1.8m diameter and contains about 1m^3 of water that may vary seasonally. These ponds consist of five ponds at ambient temperature, and five artificially warmed to remain 4°C above ambient temperature at all times (see mean daily temperature in Fig S4.1). The mesocosms are warmed by a heating element connected to a thermal couple (Fig. 4.1), which monitors the temperature of both warmed and ambient ponds. The initial colonization of species started in 2005 with organisms from surrounding habitats, with the exception of fish, which are the main predators of freshwater invertebrates (Yvon-Durocher et al., 2015). All the ponds are unshaded and have similar environmental conditions, especially in terms of water temperature, trophic conditions, and aquatic vegetation.

A floating emergence trap, covering 0.25m^2, was put into each of the ten mesocosms (Fig. 4.1). All insects were collected from the traps every two weeks, preserved in absolute ethanol and kept in a refrigerator until processed. Specimens were collected through spring, summer and autumn from 4th May 2017 to 16th November 2017, and 28th February 2018 to 10th October 2018.

Fig 4.1: The mesocosms at Freshwater Biological Association (FBA), River Laboratory, East Stoke, Dorset, UK. Each pond has a 2.5m^2 surface area with 1.8m diameter and contains about 1m^3 of water that may vary seasonally. Heated ponds are warmed by a heating element connected to a thermocouple (yellow arrow). Emergence

traps (red arrow) were put into each of ten ponds (5 traps in ambient ponds, 5 traps in heated ponds).

4.2.2. Identification

Six chironomid species, including males and females of *Ablabesmyia monilis* (N=256), *Chironomus piger* (N=191), *Tanytarsus nemorosus* (N=905), *Procladius crassinervis* (N=488), and males of *Polypedilum nubeculosum* (N=87) and *Polypedilum sordens* (N=49), were identified and selected for the study based on the availability of specimens collected from the mesocosms (Table 4.1). Female *Polypedilum* spp. could not be separated by morphology, thus only male specimens were used.

All specimens were initially sorted by morphotype and only species with more than 50 specimens were selected for analysis. As only males can be reliably identified to species from morphology (Langton & Pinder, 2007), a male specimen from each morphotype was selected and used as a reference specimen for morphological identification. The wings were removed from the reference specimen and mounted on a microscope slide using HYDRO-MATRIX®, permanent mounting medium. Following this the rest of the specimen was heated at 70°C in 10% KOH for 5-10 minutes, washed in glacial acetic acid and then water, and finally mounted on the same slide as the wings. Species were identified following Langton & Pinder (2007).

4.2.3. DNA barcoding for species identification

DNA barcoding was used to confirm the morphological species identifications and to link females of each species to the identified males. However, the females of *Polypediulm nubeculosum* and *P. sordens* could not be distinguished morphologically to species and so females of these species were not included in this study. Representative specimens from each morphospecies were extracted using either the Qaigen DNEasy Blood and Tissue kit or a 5% Chelex solution (Walsh et al., 1991). DNA barcoding followed standard in-house protocols using LCO1490 and HCO2198 primers (Folmer et al., 1994). Each reaction consisted of 1mM total dNTPs, 3mM MgCl2, 1.25u Bio-Taq DNA polymerase (Bioline), 0.1µM each primer and 1x reaction buffer. Cycling conditions were: initial denaturation 94°C for 1min followed by 35 cycles of 94°C for 30s, 48°C for 30s and 72°C for 30s, with a final elongation of 10min at 72°C. PCR products were visualised using gel electrophoresis, purified using Agencourt AMPure XP beads and then sequenced bi-directionally using BigDye terminator reaction mix v3.1 in a 3730xl DNA analyser (Applied Biosystems) at the NHM sequencing facility. Sequences were deposited in GenBank (Accession numbers is MW448368-MW448379).

4.2.4. Data acquisition

The right wing of all specimens was removed and slide-mounted. All wings were imaged using either a Canon 5DsR camera and MPE-65 lens or ZEISS Axio Scan z1, both at 5x magnification.

Wing length was used as previous studies have shown wing length is a good proxy for body size (McLachlan, 1986, Ali, 1994, Xue & Ali, 1994 and Fyodorova & Azovsky, 2003). Images of wings with scale were measured using ImageJ (version 1.51). The scale was set in ImageJ using the scale bar to measure for a known distance (the image resolution was 1222 pixels per mm). Each right wing was measured from the humeral cross-vein (HC) to the end of costal extension (CE) (Fig. 4.2). To confirm the precision of wing length measurements, 50 specimens were remeasured, and a correlation analysis between the original wing lengths and remeasured wing lengths was performed. We found a very high correlation between original wing length measurements and remeasurements (r=0.99, p<0.001). Wing images used in this study are available on the NHM data portal (https://doi.org/10.5519/0003569). Specimens within each species collected from different mesocosms were pooled by temperature treatment for analysis to increase the sample size. Data analysed from 2011-2012 showed that an individual mesocosm has no effect on community structure, especially on the phytoplankton community (Yvon-Durocher et al., 2015) which is a dominant food resource for chironomids (Armitage, 1995). Nevertheless, the community structure and species in these ponds may have changed since this date and thus the mesocosms may not constitute exact replicates.

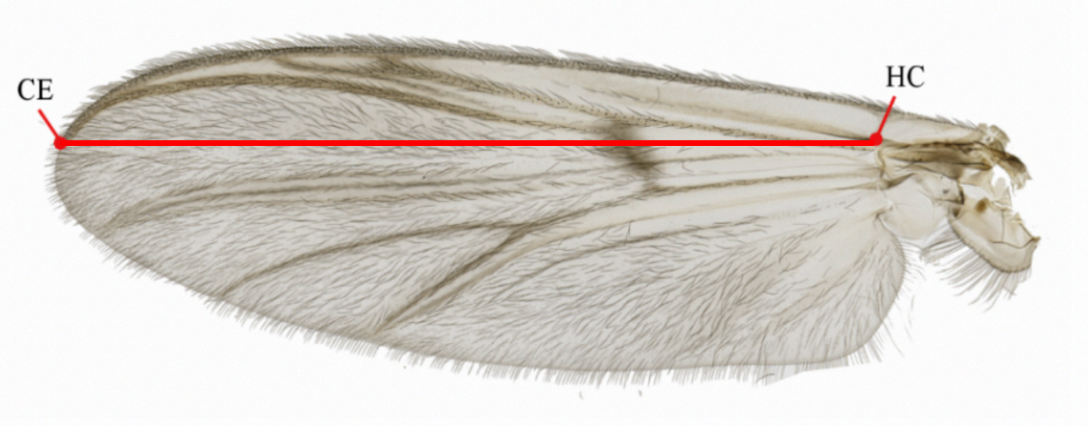


Fig 4.2: Wing morphology of *Polypedilum sordens* and wing length measurement from humeral cross-vein (HC) to the end of costal extension (CE).

4.2.5. Individual species analysis

All analyses were performed in R (version 1.2.5033) (R Core Team, 2014). Within each species, generalised linear models (GLMs) were performed twice to explore the influence of the independent variables including temperature condition, year and emergence date, on wing length which is a dependent variable. First, a full model was developed to explore the effects of temperature condition, emergence date, sex and year on wing length. A second, reduced model which excluded year from the set of independent variables was then run. The full and reduced models were compared using the Akaike Information Criterion (AIC) (Table S4.1) to select the most parsimonious model.

In order to examine the effect of warming on wing length, a one-way ANOVA and a Tukey's post-hoc test were used to test the difference in mean wing length between ambient and heated conditions. The relationship between emergence date and wing length was examined with a linear regression (Kenney & Keeping, 1962). A locally weighted regression was fitted to the model (Cleveland & Devlin, 1988) if species exhibited non-linear patterns.

4.2.6. Interspecific analysis

The percentage wing length change between treatments was calculated from mean wing length change between ambient and heated conditions for each sex of each species (Table S4.2) using the following formula; $\left(\frac{\overline{x}_{\text{heated}} - \overline{x}_{\text{ambient}}}{\overline{x}_{\text{ambient}}} \times 100\right)$. Then to investigate if species' size is related to sensitivity to temperature, a linear regression analysis was performed between the percent change of wing length between treatments and mean wing length of each species and sex.

4.3. Results

The reduced model GLM, which excluded year, of each species is shown in table 4.2 as the AIC approach indicated that a model without year explained the variation of wing length better than the full model including year. The regression indicated a strong significant negative (p<0.001) effect of temperature condition (ambient vs ambient +4°C) on wing length in three species: *Polypedilum sordens*, *Procladius crassinervis* and *Tanytarsus nemorosus* (Table 4.2). Also, a one-way ANOVA indicated significantly longer wing lengths in the ambient mesocosms than heated mesocosms in both sexes of *Procladius crassinervis* and *Tanytarsus nemorosus* (p<0.001) and in males of *Polypedilum sordens* (p<0.001) (Table 4,2; Fig. 4.3). Whereas, a non-significant difference in wing length between temperature conditions in both sexes of *Chironomus piger* and *Ablabesmyia monilis,* and male *Polypedilum nubeculosum* was found (Table 4.2; Fig. 4.3).

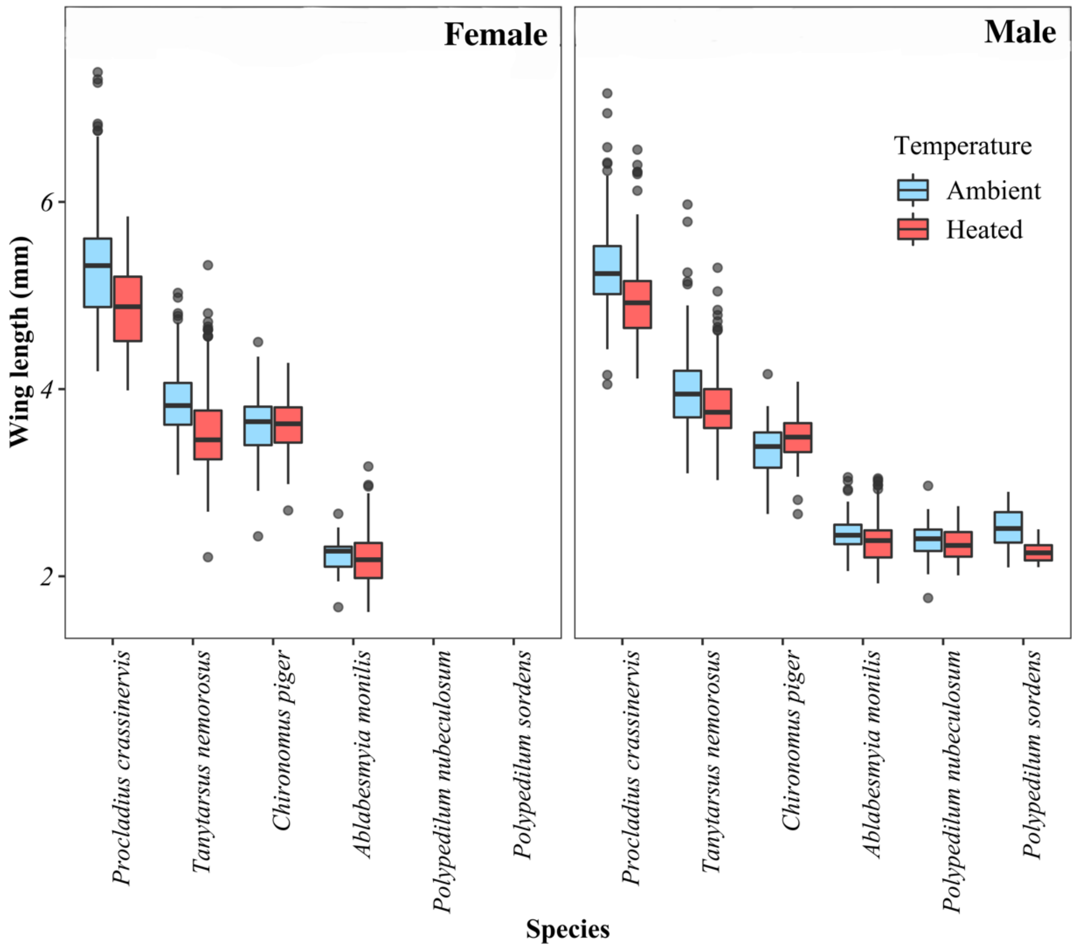


Fig 4.3: Box plots of the wing lengths of specimens between ambient and heated conditions for females (left) and males (right) of six chironomid species. For *P. nubeculosum* and *P. sordens*, the results of female wing length are not available because of the difficulty in identifying female specimens to species. Significant results indicated by *** (p<0.001).

Table 4.1: Species and numbers of chironomids used in this study. For *P. nubeculosum* and *P. sordens*, females are not available because of the difficulty in identifying female specimens to species.

Subfamily (tribe)	**Species**	**Female**		**Male**		**Number of specimens**
		Ambient	**Heated**	**Ambient**	**Heated**	
Chironominae (Chironomini)	*Chironomus piger*	45	44	56	46	191
	Polypedilum nubeculosum	-	-	53	34	87
	Polypedilum sordens	-	-	23	26	49
Chironominae (Tanytarsini)	*Tanytarsus nemorosus*	236	287	198	184	905
Tanypodinae (Pentaneurini)	*Ablabesmyia monilis*	45	97	36	78	256
Tanypodinae (Procladiini)	*Procladius crassinervis*	139	91	137	121	488
TOTAL						1,976

Table 4.2: The generalized linear model analysis of Chironomids species in this study. A negative symbol shows a negative relationship between wing length and the variables. Significant levels are indicated as *** for $p < 0.001$.

	Wing length					
	A. monilis	*C. piger*	*P. crassinervis*	*T. nemorosus*	*P. nubeculosum*	*P. sordens*
Emergence date	-0.003 (±0.001)***	-0.003 (±0.001)***	-0.005 (±0.001)***	-0.007 (±0.001)***	-0.001 (±0.001)	-0.001 (±0.001)
Temperature condition	-0.064 (±0.034)	-0.497 (±0.049)	-0.497 (±0.045)***	-0.276 (±0.023)***	-0.076 (±0.023)	-0.245(±0.051)***
Sex	0.201 (±0.032)***	-0.204 (±0.043)***	0.025 (±0.045)	0.107 (±0.024)***	NA	NA
Degrees of freedom	252	187	484	901	83	46

With respect to seasonal clines in wing length, significantly smaller wing lengths were found when adults emerged later in the season in the linear regressions of both sexes and both temperature conditions in *A. monilis*, *C. piger*, *P. crassinervis* and *T. nemorosus* (Fig. 4.4a-4.4d). While *Polypedilum nubeculosun* and *P. sordens* exhibited non-significant seasonal cline in wing length (Fig. 4.4e-4.4f). Additionally, a quadratic local regression exhibited obvious U-shaped curves between wing length and emergence date in *P. crassinervis* (Fig. 4.4c).

In terms of interspecific responses, all species, excluding *C. piger*, showed a negative percent wing length change between ambient and heated conditions in both sexes (Fig. 4.5). The range of wing length changes between treatments is from +0.84% to -9.68% in females and from +2.68% to -9.60% in males. However, correlations between percent change of wing length and mean wing length of each species are non-significant ($r = -0.69$ and -0.06, $p > 0.05$, in females and males, respectively), likely due to the limited number of species in this study.

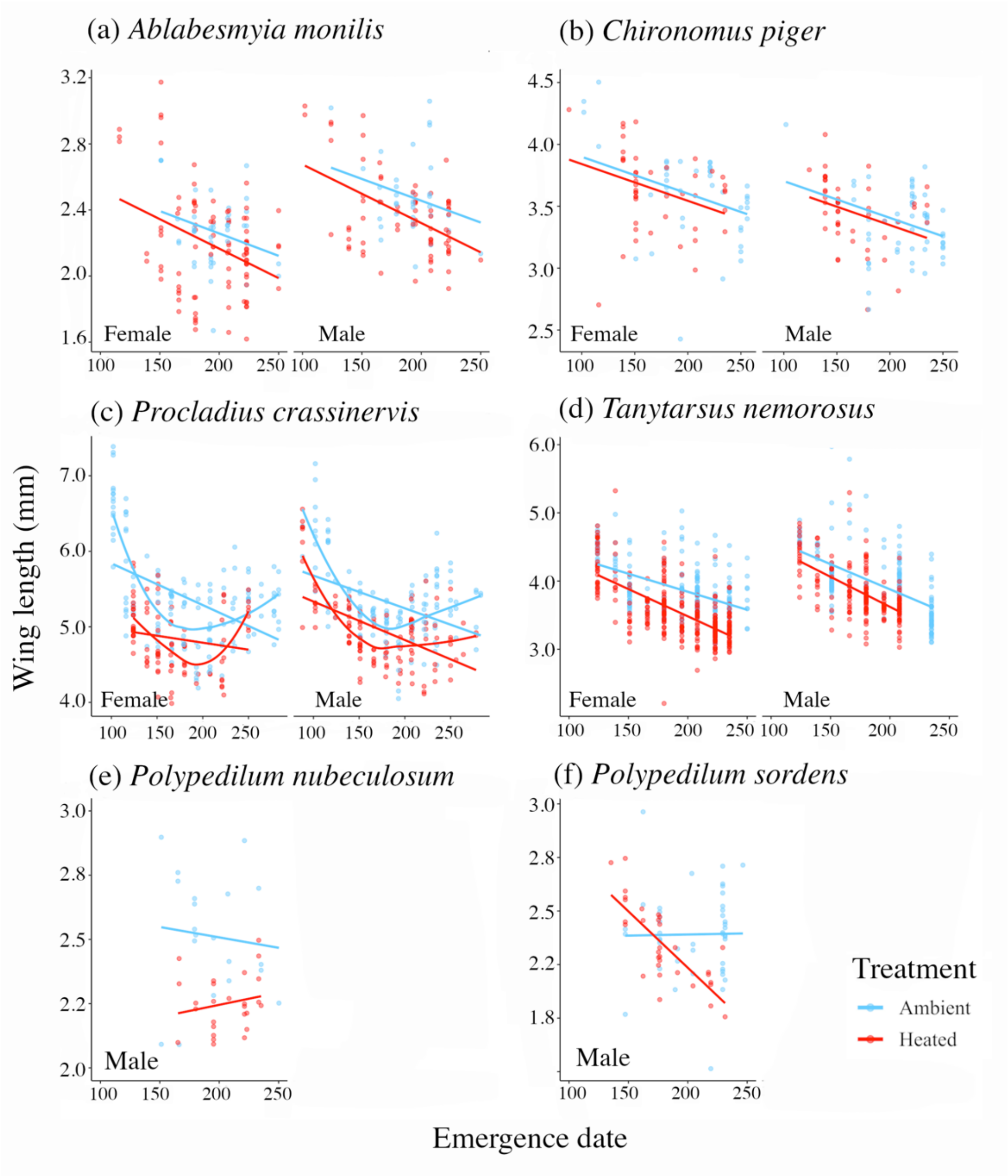


Fig 4.4: A regression plot of generalised linear models of wing length vs emergence date (number of the day) in females and males of six chironomid species. For *Procladius crassinervis*, local regression was applied because this species exhibits obvious U-shaped curves between wing length and emergence date. For *Polypedilum nubeculosum* and *P. sordens*, the results of female wing length are not available because of the difficulty in identifying female specimens to species. Red dots and lines represent heated (+4°C) conditions and blue dots and lines represent ambient condition.

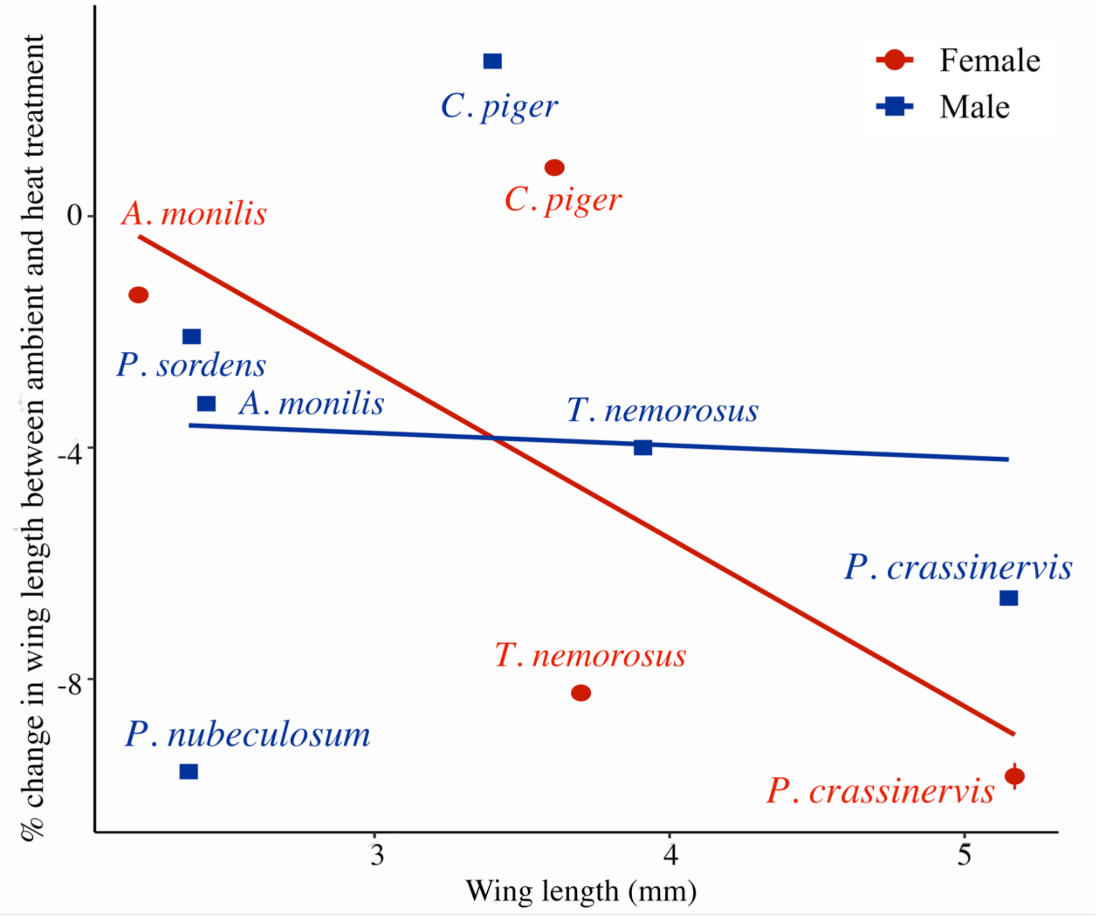


Fig 4.5: Percentage change in mean wing length between ambient and heated treatments for each species plotted against the mean wing length of each species. A correlation analysis indicated a non-significant negative correlation between percent change in wing length between treatments and average wing length per species in females (red) (r=-0.69, p>0.05) and males (blue) (r=-0.06, p>0.05).

4.4. Discussion

Although this study finds no universal pattern in the response of wing size to temperature among the chironomid species examined, we find that species either tend to get smaller under heated conditions or show non-significant body size changes (Fig.

4.3). Interestingly, a significant seasonal decline in wing length is exhibited across four species in this study: *A. monilis*, *C. piger*, *P. crassinervis* and *T. nemorosus*, in both heated and ambient conditions (Fig. 4.4). Below we discuss the likely cause of these responses and their consequences.

Although each species in my study tend to have shorter wings in heated conditions than ambient conditions across the emergence season (Fig. 4.4), this relationship is strongly significant in both sexes of *Procladius crassinervis*, *T. nemorous* and male *Polypedilum sordens*. This finding highlights that adult body size of these species gets smaller with increasing temperature (Fig. 4.3). This result corresponds well with the temperature-size rule (TSR) (Atkinson, 1994) which found that the developmental rate of ectotherms is faster at warmer temperatures, consequently, adult size should be smaller in warm environments compared to individuals growing in cooler temperatures (i.e. Pinder, 2002, Mackey, 2006, Frouz et al., 2009). However, the generalised linear models and one-way ANOVA indicated a non-significant wing length difference between ambient and heated conditions in *A. monilis*, *C. piger* and *P. nubeculosum* (Fig. 4.3).

These inconsistent body size-temperature responses between species may be influenced by differences in their life-history, particularly, the number of generations per year. A previous study on various arthropods suggested that voltinism is associated with temperature-size responses (Horne et al., 2015). Species with one generation per year (univoltine) tend to have non-significant or positive temperature-size responses, whereas species with multiple generations per year (multivoltine species) tend to support predications of the TSR (Atkinson, 1994). In my study, plots between number of specimens collected versus emergence date of each species (Fig S4.2) show that *A.*

monilis has a univoltine life cycle (one generation per year). *Chironmomus piger*, *P. nubeculosum* and *P. sordens* likely have a univoltine or bivoltine life cycle (one or two generations per year). While *P. crassinervis* and *T. nemorosus,* which exhibit a negative correlation between wing length and temperature, likely have a multivoltine life cycle (more than two generations per year). Thus, voltinism type could potentially explain these different responses between species. However, more work on the life-history of these species is essential for a more complete understanding of the underlying factors driving the different temperature-size responses we observed.

My results do not show different temperature-size responses between the sexes (Fig. 4.4). This result is inconsistent with previous studies (Xue & Ali, 1994, Frouz et al., 2009) and does not support my hypothesis that females will retain their body size to maximise fecundity, whereas small males will be at an advantage for swarming and mating success (McLachlan, 1986, Neems et al., 1990). However, sexual size dimorphism and the temperature-body size response in each sex of chironomids requires additional life-history data and further study to examine if the previously reported differential sensitivity between the sexes is applicable to the group more widely.

When comparing the response between species, there was a non-significant negative correlation between mean wing length and the percent change in wing length between treatments in both sexes (Fig. 4.5). This is probably because of the limited number of species in this study. This response contrasts with a recent study on Odonata which found larger species do not respond more to temperature than smaller species, however, this may have been due to the territorial nature of the larger Anisoptera (Wonglersak et al., 2020). Most anisopteran species are strongly territorial where large male body size is linked to mating success (Sokolovska et al., 2000, Córdoba-Aguilar, 2008). Thus, small larvae of Anisoptera may delay their emergence to emerge as larger adults later in the season, therefore increasing their territorial competitiveness. While chironomids have been reported to exhibit territorial feeding behaviour in the larval stage of some species, this does not correlate with body size (Imada, 2020, Brodin & Andersson, 2009). My results suggest that larger species tend to have a greater reduction in body size with increasing temperature than smaller species, consistent with previous aquatic and terrestrial studies (Forster et al., 2012, Horne et al., 2015, Tseng et al., 2018). The 'oxygen limitation hypothesis' might account for this pattern of interspecific responses (Woods, 1999) - warmer water holds less oxygen than cooler water which can exacerbate the increased energetic cost of respiration in larger aquatic invertebrates (Woods, 1999, Forster et al., 2012). A previous study of these mesocosms showed that the level of oxygen concentration is significantly lower in the warmed ponds than the ambient ones ($p<0.001$) (Zhu et al., 2020). Thus, larger chironomids would get disproportionately smaller than smaller species in warmer temperatures, however, this hypothesis requires more species to be studied.

With respect to a seasonal cline in wing length, the results indicate a significantly smaller wing length with a later emergence date in four species (in both heated and ambient conditions): *A. monilis*, *C. piger*, *P. crassinervis* and *T. nemorosus*. We hypothesise that larval development accelerates as the end of the warm season approaches, leading to smaller adults later in the season (Johansson & Rowe, 1999). The non-significant seasonal size-cline in *Polypedilum nubeculosum* and *P. sordens* may be an artifact resulting from the limited number of specimens available for our study.

Clear U-shaped curves between wing length and emergence date were observed in *P. crassinervis* (Fig. 4.4c). These U-shaped patterns could be a reflection of body size-temperature responses across the year. Individuals emerging early during spring and later in autumn when temperatures are cool tend to have a bigger size than those emerging when it is warmer during summer. Some chironomid species undergo larval diapause during unfavourable conditions (Oliver, 1971, Neumann & Krüger, 1985). We presumed that *P. crassinervis* probably adapts to winter temperature by arresting larval development during the winter months. Ultimately, bigger adult size early in the emergence season compensates for their larval diapause. The other species in this study probably avoid larval diapause and are forced to emerge before winter leading to smaller adults (McLachlan et al., 1995). However, this assumption must be evaluated further by investigating the life cycle and phenology of these species. Finally, while we have explored the influence of warming on the body size of some chironomid species, other biotic factors in natural ecosystems, such as predators, can also result in selection pressure which ultimately affects the body size of prey.

4.5. Conclusion

My results suggest that predicted future temperatures will likely lead to a reduction in the adult body size of some chironomids in both sexes. Larger-bodied species will likely be more negatively affected by increasing temperatures than smaller species. In particular, this reduction in body size has the potential to affect trophic interactions, especially predator-prey interactions. Chironomids are primary prey for other invertebrates, fish and birds, and smaller prey can affect the nutritional value per individual, attack rate, and handling time of predator-prey interactions (Thompson, 1975). However, we do not show a single consistent response to temperature across all species studied. Therefore, future studies should include additional species and seek a better understanding of the influence of life-cycles on body size response to temperature across the emergence period.

Appendix

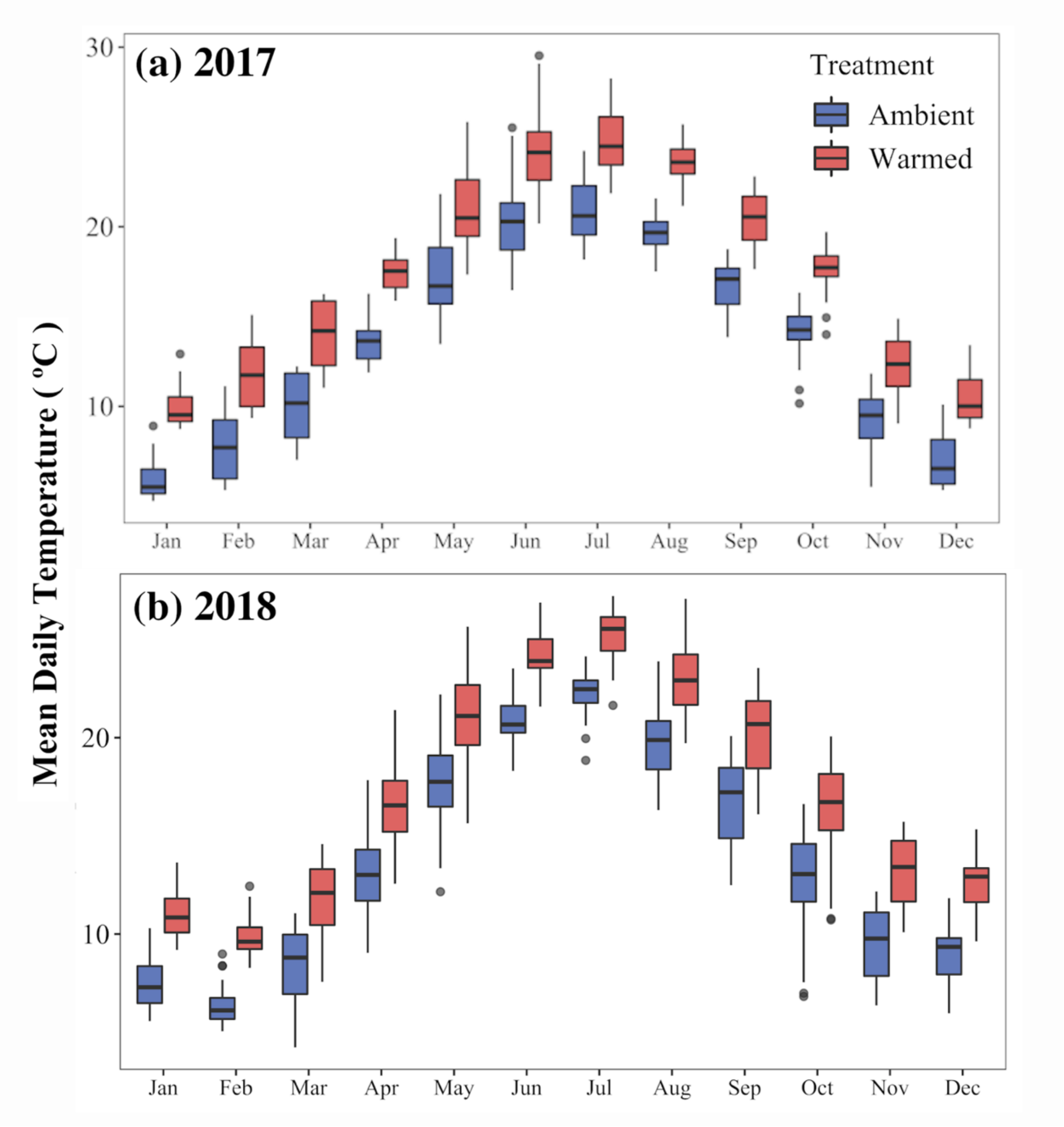
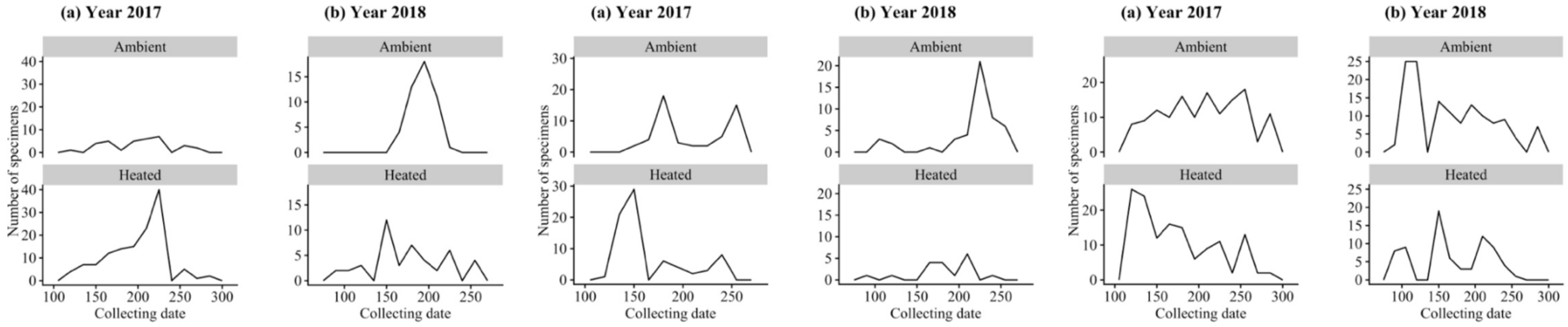


Fig S4.1: Temperature plot comparing mean daily temperature of ambient and heated (~4ºC higher) ponds between January 2017 to December 2018.

(A) *A. monilis* (B) *C. piger* (C) *P. crassinervis*



(D) *T. nemorosus* (E) *P. nubeculosum* (F) *P. sordens*

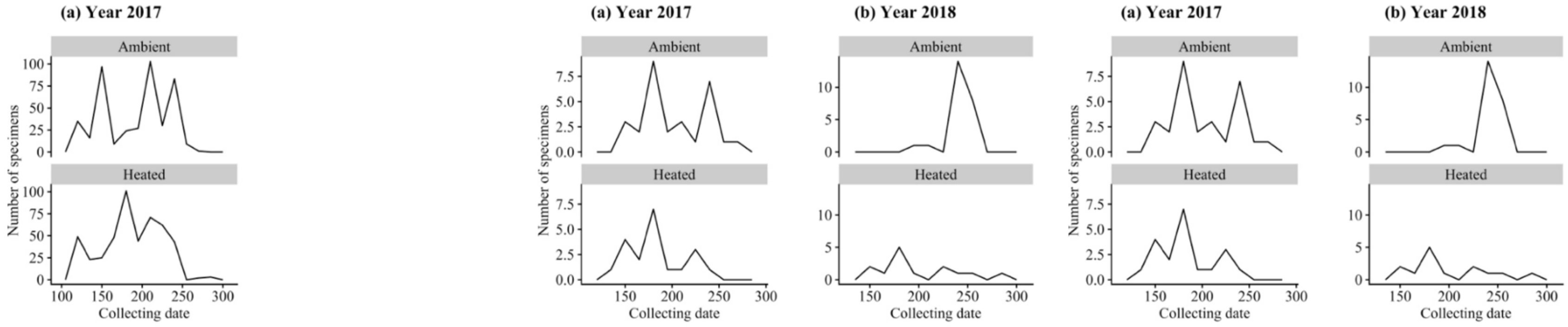


Fig S4.2: The emergence date of each species and number of specimens collected on each day.

Table S4.1: An AIC approach to compare generalised linear models contained different sets of variables. The most fitted model with lowest AIC value is indicated as bold text. A set of candidate models consists of 1) a full model, which includes year as an independent variable and 2) reduce model, which excludes year from independent variables.

	Wing length					
	A. monilis	*C. piger*	*P. crassinervis*	*T. nemorosus*	*P. nubeculosum*	*P. sordens*
D+T+S+Y	30.295	82.680	696.513	NA		
D+T+S	**8.503**	**81.173**	**694.513**	**665.87**		
D+T+Y					-39.282	-23.442
D+T					**-43.042**	**-33.745**

Table S4.2: Mean and standard deviation of wing length of each sex and each condition and significant level of sample *t*-test.

Species	**Mean ± SD**					
	Female			**Male**		
	Ambient	**Heated**	***p*-value**	**Ambient**	**Heated**	***p*-value**
Chironomus piger	3.60±0.38	3.63±0.32	0.76	3.36±0.28	3.45±0.27	0.08
Ablabesmyia monilis	2.22±0.18	2.19±0.31	0.39	2.48±0.24	2.40±0.27	0.10
Procladius crassinervis	5.37±0.66	4.85±0.43	**<0.001**	5.31±0.52	4.96±0.47	**<0.001**
Polypedilum nubeculosur	NA	NA	NA	2.40±0.19	2.35±0.18	0.25
Polypedilum sordens	NA	NA	NA	2.50±0.24	2.26±0.11	**<0.001**
Tanytarsus nemorosus	3.88±0.38	3.56±0.44	**<0.001**	3.99±0.47	3.83±0.39	**<0.001**

Chapter V

The influence of environmental variables on insect wing shape: a case study of British Odonata

This chapter used museum collections of 14 British Odonata species to investigate the influences of latitude and mean seasonal temperature on wing shape, sexual shape dimorphism and the correlation between body size and wing shape.

Author contributions: 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.

Abstract

Wing shape is highly correlated with flight performance and is suggested to be an adaptive trait to environmental conditions. In this study, the first of its kind, we evaluate the response of Odonata wing shape to latitude and specifically mean seasonal temperature, and how this is influenced by wing size and sex, using 5661 museum specimens of 14 species of British Odonata. Wing shape variation was analysed using a geometric morphometric approach. We tested for 1) sexual dimorphism in wing shape; 2) correlations between wing shape and wing size; and 3) correlations between wing shape and environmental variables, including latitude and mean seasonal temperature using a partial least square analysis. We found a significant difference in wing shape between sexes across twelve species in this study, most markedly in *Calopteryx splendens* and *C. virgo* which are known for their male display flights. This finding suggests that wing ornamentation and sexual selection are the main drivers of sexual wing shape dimorphism. A significant correlation between wing shape and length is found in all Zygoptera and two Anisoptera species, suggesting that variation in wing length across temperature conditions are also associated with wing shape alterations. A partial least square analysis indicated non-significant correlations between environment and wing shape in Anisoptera. Conversely a significant influence of latitude and mean seasonal temperature on wing shape is found in all Zygoptera species, with broader and shorter wings found at lower latitudes with warmer temperatures. Overall, the results suggest that wing shape of

Zygoptera species is more sensitive to local environmental conditions and adaptive to latitude and temperature than in Anisoptera.

Key words: *Anisoptera, geometric morphometric, museum collection, sexual shape dimorphism, temperature, wing shape response, Zygoptera*

5.1. Introduction

Wing shape is heritable and considered to be an important trait related to individual fitness and flight performance in insects (Powell, 1997, Iriarte & Hasson, 2000, Van Heerwaarden & Sgrò, 2011, Kovac et al., 2012). Flight performance can influence a species' dispersal ability (Bouget & Oger, 2015) and can reflect adaptation to local environmental factors, for example, temperature and precipitation at the population level (Haas & Tolley, 1998). In the past 30 years, global mean temperature has increased at the rate of 0.2°C per decade (Hansen et al., 2006, IPCC, 2018). Thus it is interesting to investigate how wing shape may be influenced by environmental change, particularly temperature.

In endotherms, Allen's rule (Allen, 1877) states that appendages, such as limbs, ears and tails, are shorter, rounder and more compact in cold climates and at high latitudes, a result of reducing the surface area to volume ratio to reduce heat loss. In ectothermic insects, wing shape is more likely related to aerodynamics and energetic costs than thermoregulation (Norberg, 1995). In cold regions, increased wing area can reduce wing loading and compensate for reduced flight performance (Lehmann, 1999, Dudley, 2000). Surprisingly, although wing shape has been widely studied in relation to aerodynamics (Hedenström, 2002, Sudo et al., 2005), there are few studies of wing shape as a function of environment and latitude in insects (Debat et al., 2003, García & Sarmiento, 2012).

Studies in both the butterfly *Pararge aegeria* (Vandewoestijne & van Dyck, 2011) and the fruit fly *Drosophila melanogaster* (Azevedo et al., 1998) found that shorter and more rounded wings increased wing area, thereby resulting in reduced wing loading at higher latitudes where lower temperatures reduce flight performance in insects (Lehmann, 1999). A study in butterflies found that individuals from higher latitudes show more rounded wings than individuals from low latitudes (Vandewoestijne & van Dyck, 2011).

Within Odonata, a study by Hassall (2015) suggested that geographical wing shape variation in *Calopteryx maculata* (Odonata: Zygoptera), is correlated with temperature. A separate study of *Calopteryx virgo* showed a strong variation of wing shape in relation to latitude in both males and females (Outomuro & Johansson, 2011). This study also indicated that wing shape variation might be a result of selection pressure within each region, with broader wing bases more common in damselflies living in open landscapes (Outomuro & Johansson, 2011). Additionally, a study in *Libellula quadrimaculata* found wing shape variation with altitude suggesting wing adaptation to different environmental conditions (Casanueva et al., 2017).

Regarding sexual dimorphism in wing shape, generally males of calopterygid damselflies have more rounded and compact wings to enhance their sexual display (Betts & Wootton, 1988, Outomuro & Johansson, 2013a). A study on *Calopteryx* damselflies suggested that wing beat frequency is an important aspect of courtship in males (Anders & Rüppell, 1997), thus sexual displays might potentially be a crucial driver of sexual dimorphism in wing shape in odonate taxa that exhibit courtship behaviour (Outomuro & Johansson, 2013a). Additionally, a study in *Calopteryx virgo* found sexual dimorphism in wing shape was due to both natural and sexual selection pressures (Outomuro & Johansson, 2013a). Variation in female wing shape was explained by latitude, but wing shape variation in males was mostly explained by the extent of wing pigmentation, which is important for mating success in this species (Outomuro & Johansson, 2013a). A study on *Nannophya pygmaea* (Anisoptera: Libellulidae) found that longer wings of males is usually correlated with high territorial success (Tsubaki & Ono, 1987). Even though sexual dimorphism in wing shape is well studied in calopterygid damselflies due to their well-known courtship behaviour, there is a gap in the knowledge of sexual dimorphism in wing shape in other Odonata (Gidaszewski et al., 2009).

Wing shape and body size are important factors affecting insect aerodynamics (Lehmann, 2002). As body size changes, wing shape might be altered to retain flight efficiency. A study in various holometabolous insects (Debat et al., 2003) and in *Drosophila* (Perrard et al., 2012) indicated a non-significant correlation between wing shape and body size. A study in the dragonfly family Calopterygidae suggested that the relationship between wing shape and body size is taxon dependent and can differ between closely related taxa (Outomuro et al., 2013b).

Wing shape variation in insects is crucial and has a direct effect on flight behaviour and performance (Donoughe et al., 2011). Flight efficiency can vary according to wing planform. A previous study in dragonflies pointed out that variation of wing veins is correlated with stroke-plane inclination (Wootton & Newman, 2008).

Additionally, a study in hawkmoth *Manduca sexta* indicated that wing deformation can affect overall aerodynamic forces (Mountcastle & Daniel, 2009).

This study is the first to provide an evaluation of wing shape across two distinct phylogenetic groups (Zygoptera and Anisoptera) within the insect order Odonata in relation to 1) sexual dimorphism, 2) wing length, and 3) latitude and temperature.

The following is predicted: 1) The difference in wing shape between males and females (sexual shape dimorphism) is expected to be greater in taxa in which males have wing ornament display (Serrano-Meneses et al., 2008); 2) In general, non-significant correlations between wing shape and wing length, which is a proxy for body size, are expected in Odonata species as reported on various holometabolous insects (Debat et al., 2003); 3) Wing shape is expected to correlate with latitude and temperature as reported in previous studies on other insect groups (Debat et al., 2003, García & Sarmiento, 2012). More rounded and broader wings, which correlate with bigger wing area, should be found at high latitudes with colder mean seasonal temperature to enhance flight performance and compensate for low wingbeat frequencies at lower temperature (Azevedo et al., 1998, Hassall, 2015b).

To test these hypotheses, museum specimens of 14 British Odonata species were used to explore intraspecific and interspecific variations in wing shape. A geometric morphometric approach was used to extract wing shape variables of these specimens. Then wing shape variables were used to test our predictions, as outlined above.

5.2. Materials and methods

This study was based on images of 5,661 specimens with entire hindwings, from 14 species of British Odonata, including eight Anisoptera species and six Zygoptera species (Table 5.1). These species were selected based on the availability of specimens from UK museums.

5.2.1. Data acquisition

All specimens were imaged using a standardized template with specimen label(s), scale bar, and a colour checker. Collection location and date was captured from specimen labels. Six landmarks were chosen based on the certainty of these landmarks being present on every wing of every species in the same suborder. In addition, 200 semi-landmarks were collected, starting from the basal section of the proximal end of the wing and proceeding in an anticlockwise direction around the wing periphery. Apart from traditional landmarks, semi-landmarks are intended to represent curves or outlines (Lele & Bookstein, 1999, Gunz & Mitteroecker, 2013, Dudzik, 2019). The semi-landmark data were processed to reduce their number to 15 equally spaced points that cover the wing outline (Figure 5.1). The first and last semi-landmarks were located at the junction of the first antenodal crossvein and the costa and at the tornus of the proximal end of the wing, respectively. These combined data, comprising six landmarks and 15 semi-landmarks, were used as the primary morphometric dataset. This dataset was digitized using tpsDig2 (Rohlf, 2008) available at https://life.bio.sunysb.edu/ee/rohlf/software.html.

The regional monthly air temperature time-series data were derived from the UK Meteorological Office (www.metoffice.gov.uk/climate/uk/summaries/datasets) and were used to calculate climate variables for analyses. Previous studies on the relationship between air temperature and water temperature indicate a linear relationship (Livingstone & Lotter, 1998, Erickson & Stefan, 2000), at least in standing water. Thus, although Odonata larvae are not directly exposed to air temperatures during larval development, mean regional air temperature can be used as a proxy for water temperature. 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) (Table 5.1).

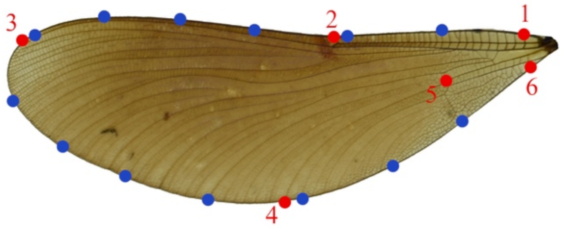


Fig 5.1: Six landmarks (red dots) and 15 semi-landmarks (blue dots) on hindwing periphery of *Calopteryx virgo*. The first and last semi-landmarks were on landmarks 1 and 6 and were removed from the dataset. Landmarks correspond to: 1- first antenodal cross vein (Ax1) at the basal of the proximal end of the wing, 2- nodus, 3- the first radial anterior (R1), 4- the first cubital vein (Cu1), 5- intersection between the second and third radial vein (R2+R3), and 6- anal crossing.

5.2.2. Data analyses

The workflow of data analysis is summarised in figure 5.2. For each species, a primary morphometric dataset was processed using procrustes superposition to minimize shape differences caused by position, scaling and rotation (Lele & Bookstein, 1999). Then procrustes superposition coordinates were processed in a principal component analysis

(PCA) (Hotelling, 1933, Smith, 2002). The PCA was used to reduce the dimensionality of the procrustes coordinate data to the minimum number of independent variables needed to retain 95% of the original wing shape variability.

Two-block partial least square regression analysis (2B-PLS) (Haenlein & Kaplan, 2004) was used to examine the influence of environmental variables on the wing shape of each species. The PLS tested the covariance between the following blocks of variables: 1) PCA scores (wing shape data) and 2) environmental variables, including latitude, mean seasonal temperature of the larval period and mean temperature of the larval period (Table 1). If PLS analysis indicated a significant correlation between two blocks of data, then a canonical variate analysis (CVA) (MacLeod, 2007) was carried out to maximize between-group separation defined with respect to the important variables influencing wing shape as indicated by the PLS regression analysis. Then a bootstrapped (1,000 iteration) log-likelihood ratio test (Woolf, 1957) was used to estimate the statistical significance of this group separation.

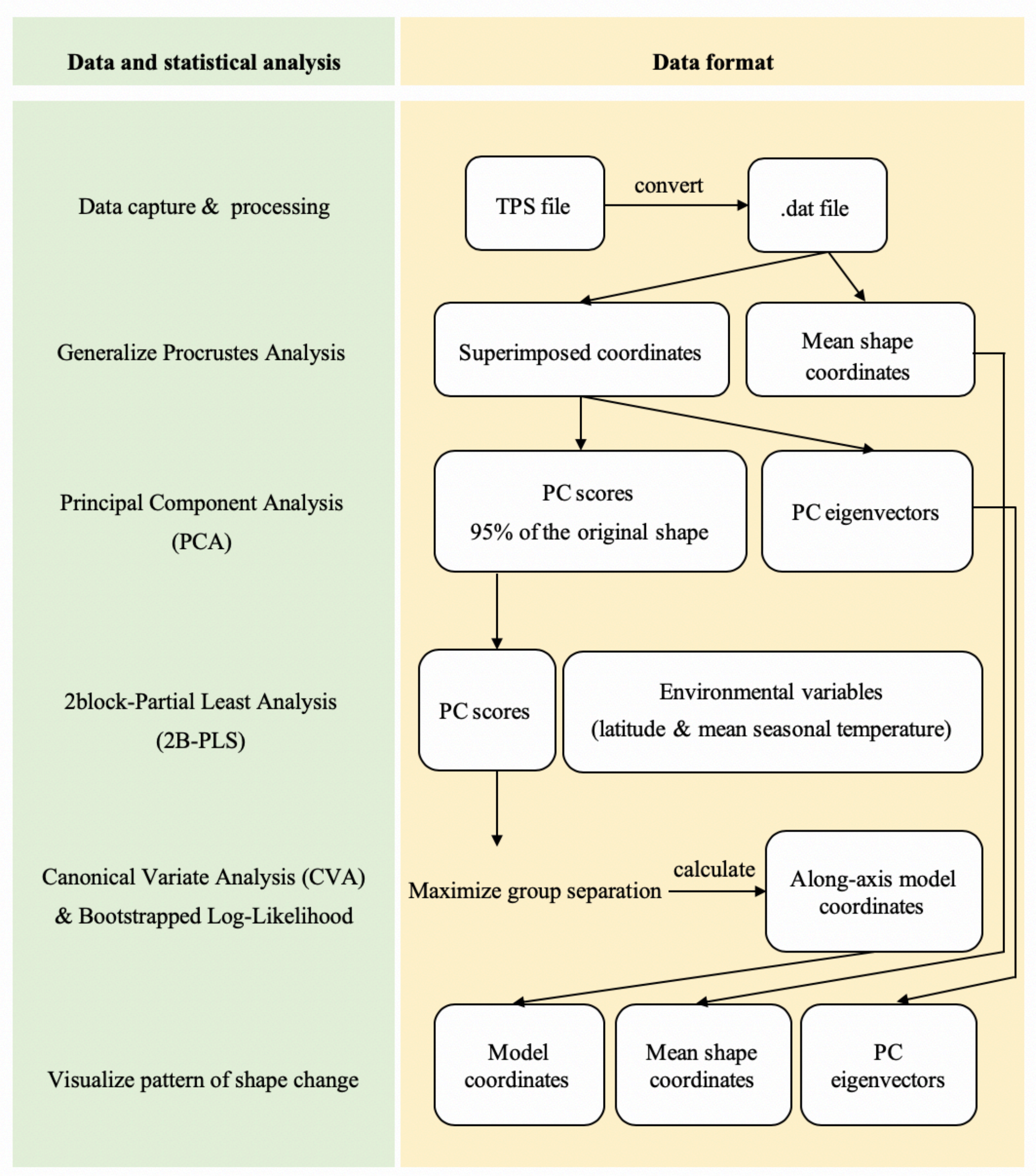


Fig 5.2: Workflow used for wing shape analysis.

For the variables that significantly affect wing shape, the pattern of shape change was visualized by calculating the direction of shape change for each of the first three PCA eigenvectors. Following this the variable values along the CVA discriminant axis were projected back into the PCA space.

In addition to environmental variables, wing shape difference between the sexes of each species were examined using a CVA to maximize the separation of each sex. A multivariate analysis of variance (MANOVA) was then performed to test for the differences in shape variables between males and females. If sex was determined as a significant factor of the shape variation, then a subsequent wing shape analysis for each sex was conducted against the environmental variables.

To assess the correlation between wing shape and wing length, which is used as a proxy of body size (Haas & Tolley, 1998, Corbet, 1999, Outomuro et al., 2013a), a linear regression analysis between the procrustes coordinates and wing length, was performed for each species. Additionally, a correlation analysis was performed to assess a correlation between wing length and centroid size which is a proxy for wing area. All wing shape analyses were performed using Mathematica version 12.1 (Wolfram Research, 2020). The linear regression analysis and MANOVA were performed in R (R Core Team, 2014) using *car* package (Fox et al., 2014).

5.3. Results

The number of retained PC axes required to summarize 95% of all wing shape variables of each species is indicated in Table 5.1. An analysis of each species found that only two species, *Anax imperator* and *Somatochlora metallica*, showed non-significant differences in shape variables between the sexes (MANOVA; $p>0.05$). While in all the other species, there was a significant discrimination of wing shape between the sexes (MANOVA; $p<0.01$) (Table 5.1). The mean wing shape of each sex in each species is visualized in Figure 5.3. Then, subsequent analyses for each sex were performed in the remaining 12 Odonata species (six Anisoptera and six Zygoptera).

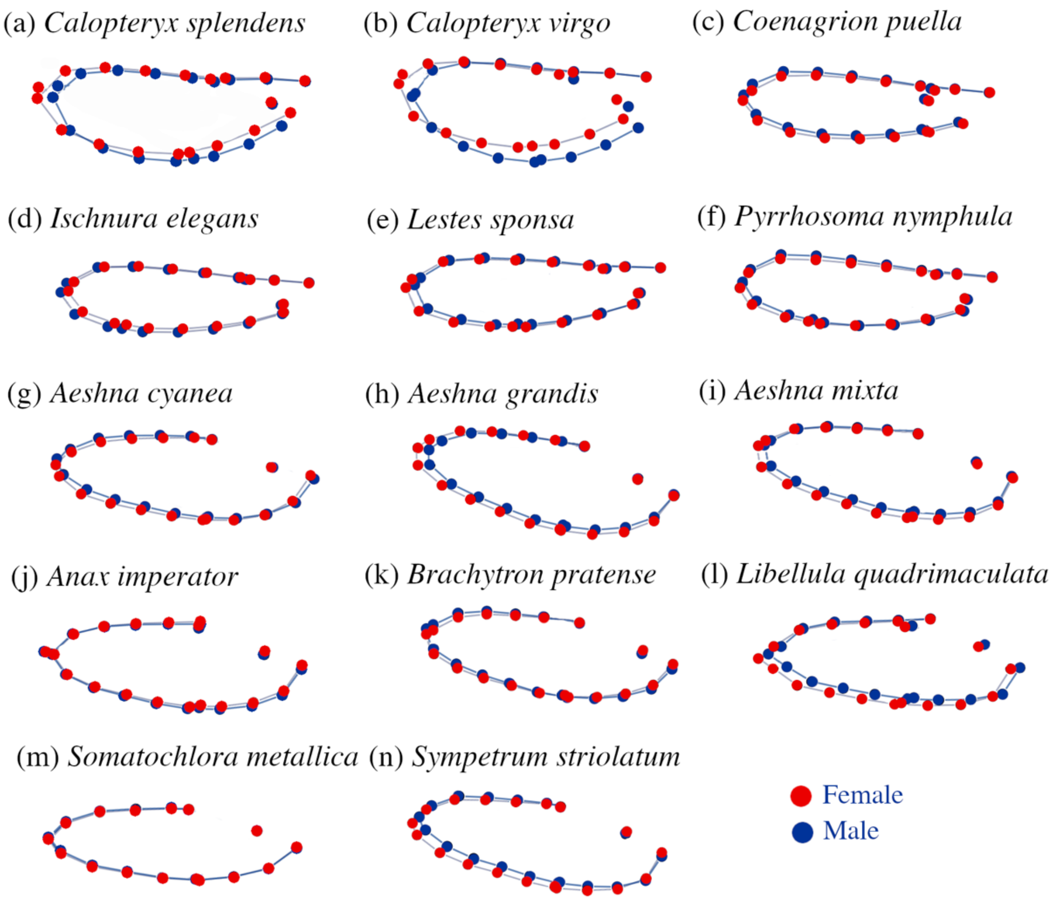

(a) *Calopteryx splendens* (b) *Calopteryx virgo* (c) *Coenagrion puella*
(d) *Ischnura elegans* (e) *Lestes sponsa* (f) *Pyrrhosoma nymphula*
(g) *Aeshna cyanea* (h) *Aeshna grandis* (i) *Aeshna mixta*
(j) *Anax imperator* (k) *Brachytron pratense* (l) *Libellula quadrimaculata*
(m) *Somatochlora metallica* (n) *Sympetrum striolatum*

Fig 5.3: Comparison of mean shape of the wing shape between female and male of Zygoptera species (a)-(f) and Anisoptera species (j)-(n). Percent correction of wing shape discrimination and significance level of MANOVA are indicated in Table 5.1.

5.3.1. Correlation of wing shape and wing length

The linear regression analysis of each species found that, in Anisoptera, only *Sympetrum striolatum* (both sexes) and *Somatochlora metallica* (males) have a significant correlation between wing shape and wing length (p<0.001, Table 5.1). Whereas both sexes of all Zygoptera species in this study showed a strongly significant correlation between shape variables and wing length (p<0.001, Table 5.1) indicating that as wings change length the shape changes.

5.3.2. The influences of latitude and temperature on wing shape

For Anisoptera, the discriminant function by CVA indicated non-significant wing shape discrimination across the environmental variables in all Anisoptera (bootstrapped; p>0.05), except *B. pratense*. The PLS analysis showed that mean spring temperature of two years before the collecting year (y-2) was positively correlated with the PLS1 in females of *B. pratense* (Table 5.2). The discriminant function by CVA indicated a significant wing shape difference across mean spring temperature of y-2 for female *B. pratense* (bootstrapped; p<0.001) (Figure 5.4). Thinner and longer wings were found in specimens experiencing cold spring temperature as larvae in y-2 (Figure 5.4).

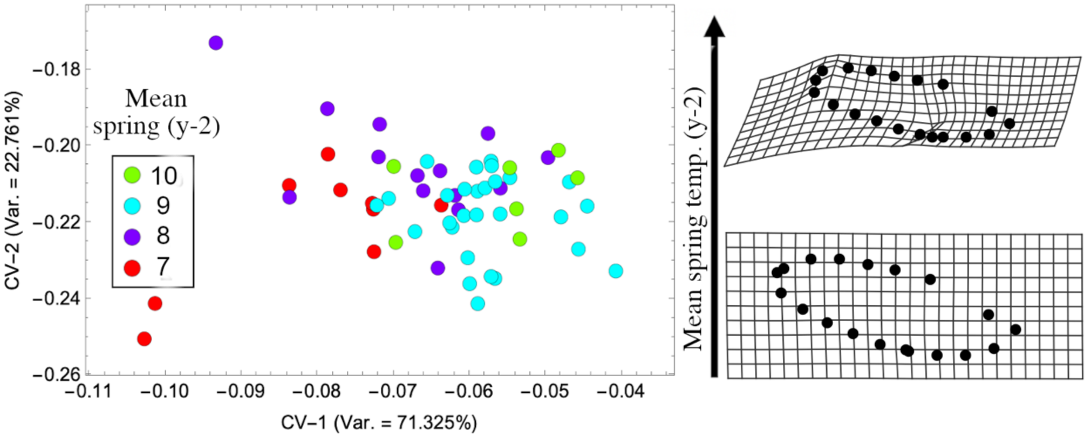


Fig 5.4: The scatter plot of wing shape of *B. pratense* on the first and second discriminant axes using a canonical variation analysis across mean spring temperature

of two years before the collecting year (y-2) and the model of mean shape changes of specimens from cold mean spring temperature to warmer temperature of y-2.

For all Zygoptera species, in both sexes, the PLS analysis showed strong significance in PLS1, which explained most of the variation in wing shape (Table 5.3-5.5). Each species is described below.

Calopteryx splendens: In females, PLS1 was negatively correlated with latitude and positively correlated with mean winter temperature of the collecting year (y0) (Table 5.3). While in males, PLS1 was positively correlated with latitude and negatively correlated with mean summer temperature of two year and one year before the collecting year (y-2 and y-1) and mean autumn temperature of y-1 (Table 5.3).

Calopteryx virgo: PLS1 was strongly positively correlated with latitude in both sexes (Table 5.3). The PLS1 showed negative correlation with mean spring and winter temperature of y0 in females and males, respectively. The discriminant function by CVA in both species also indicated a strong wing shape distinction across the environmental variables correlated with PLS1 (bootstrapped; $p<0.001$, Table 5.3). The model plots showed narrower and longer wings at higher latitude in both sexes of both species (Figure 5.5a-5.5d).

Coenagrion puella: showed a positive correlation between PLS1 and latitude in females and mean winter temperature of y0 in males (Table 5.4). Broader wings were found at lower latitude in females and in higher mean winter temperature of y0 in males (Figure 5.5e, 5.5f).

Ischnura elegans: PLS1 was negatively correlated with latitude in females, but positively correlated with mean spring temperature of y0 and mean temperature of larval period (bootstrapped; $p<0.05$; Table 5.4). Longer and narrower wings were found at higher latitude (Figure 5.6a). Males showed negative correlation between PLS1 and mean temperature of larval period (bootstrapped; $p<0.05$; Table 5.4). The model plot indicated rounded wings in higher mean temperature (Figure 5.6b).

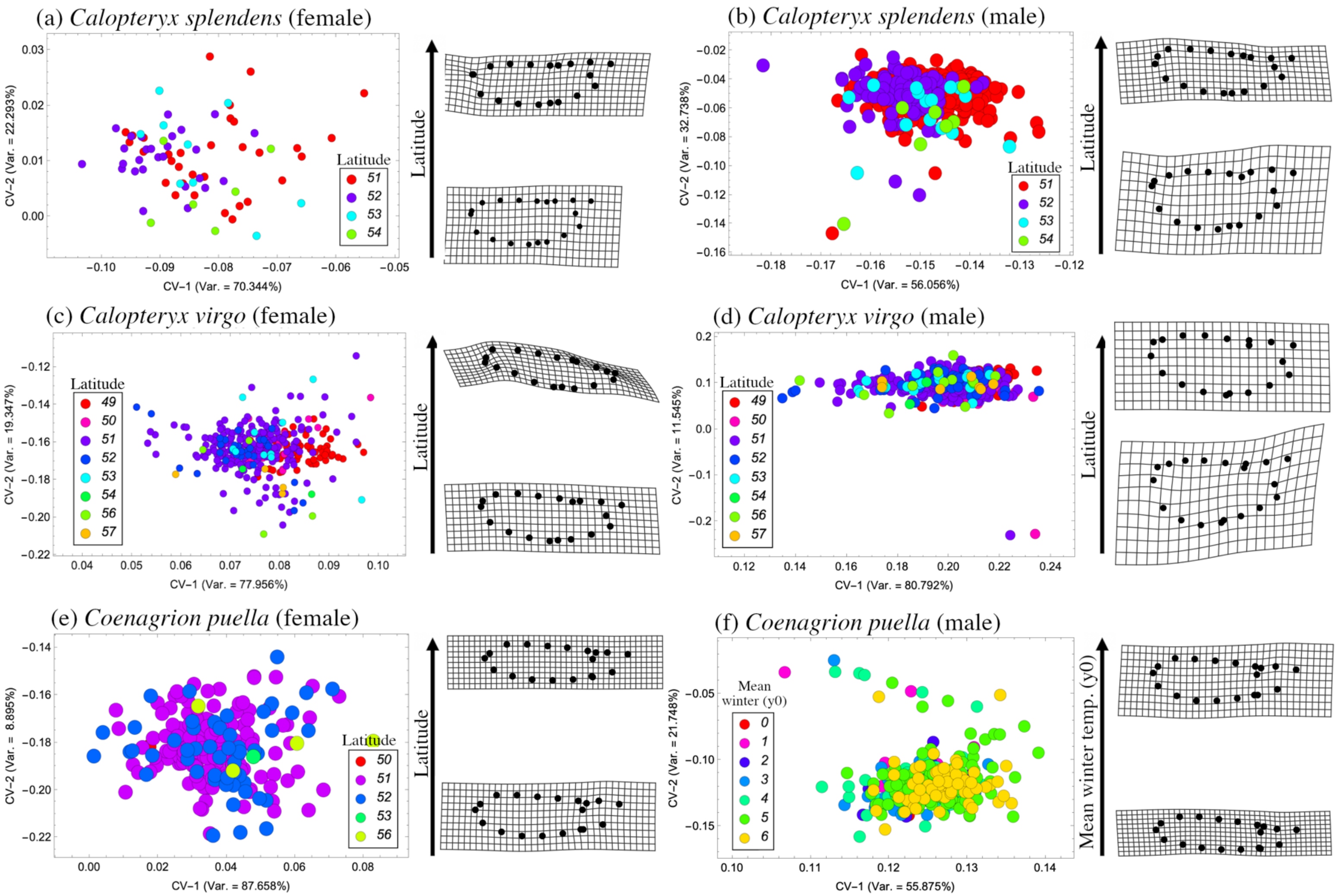
Lestes sponsa: showed a significant correlation between the PLS1 and mean spring temperature of y0 in both sexes (bootstrapped; $p<0.05$; Table 5.5). The model plots showed longer wings in cold mean spring temperature (y0) (Figure 5.6c, 5.6d).

Pyrrhosoma nymphula: PLS1 was negatively correlated with mean autumn temperature of y-1 in females (bootstrapped; $p<0.05$; Table 5.5). Higher mean temperature was associated with rounder wings (Figure 5.6e). While the PLS1 showed a positive correlation with mean summer temperature of y-1 in male *P. nymphula*, but showed a negative correlation with latitude (bootstrapped; $p<0.01$ in both variables; Table 5.5). Higher latitude correlated with narrower and longer wings (Figure 5.6f).

Table 5.1: The 14 Odonata species used in this study and their voltinism type (Corbet & Brooks, 2008). Environmental variables for each characteristic type are calculated from the regional UK mean monthly temperature series. For each variable y-2 is two years before the collecting year, y-1 is a year before the collecting year, and y0 is the collecting year. Columns include: the number of PC axes retaining 95 percent of wing shape variables; the significant level of correlation between wing shape variables and wing length; percent correctly discriminated by sex based on wing shape and the corresponding significance level of the MANOVA; and correlation between wing length and centroid size.

	Species	**N**		**Voltinism type**	**Environmental variables**	**PC axes**	**Correlation between wing shape and wing length**	**Sex discrimination**	**Correlation between wing length and centroid size**
		Female	**Male**						
Anisoptera	*Aeshna cyanea*	80	129	semivoltine	spring (y-1) to spring (y0)	6	NS	58.37**	NS
	Aeshna grandis	32	22	semivoltine	spring (y-1) to spring (y0)	6	NS	88.89***	NS
	Aeshna mixta	44	122	Univoltine	spring (y0)	8	NS	76.51***	NS
	Anax imperator	34	86	semivoltine	spring (y-2) to winter (y0)	4	NS	NS	NS
	Brachytron pratense	58	144	semivoltine	spring (y-2) to winter (y0)	6	0.27***	69.12***	0.87***
	Libellula quadrimaculata	79	162	semivoltine	spring (y-2) to winter (y0)	5	NS	69.87***	NS
	Somatochlora metallica	4	60	semivoltine	summer (y-2) to spring (y0)	7	NS	NS	NS
	Sympetrum striolatum	238	321	Univoltine	spring (y0)	7	0.04***	87.30***	0.87***
Zygoptera	*Calopteryx splendens*	255	417	semivoltine	summer (y-2) to spring (y0)	2	0.05***	85.43***	0.96***
	Calopteryx virgo	358	481	semivoltine	summer (y-2) to spring (y0)	5	0.38***	88.68***	0.81***
	Coenagrion puella	216	345	Univoltine	summer (y-1) to spring (y0)	5	0.21***	61.32***	0.10**
	Ischnura elegans	416	364	Univoltine	summer (y-1) to spring (y0)	8	0.09***	67.05***	0.78***
	Lestes sponsa	210	405	Univoltine	spring (y0)	6	0.14***	59.84***	0.82***
	Pyrrhosoma nymphula	268	311	semivoltine	spring (y-2) to winter (y0)	6	0.22***	75.65***	0.94***

Significant levels are indicated as *, **, *** for $p<0.05$, 0.01 and 0.001, respectively. NS is non-significant variable. NA is not applicable due to limited number of specimens.



(a) *Calopteryx splendens* (female)

(b) *Calopteryx splendens* (male)

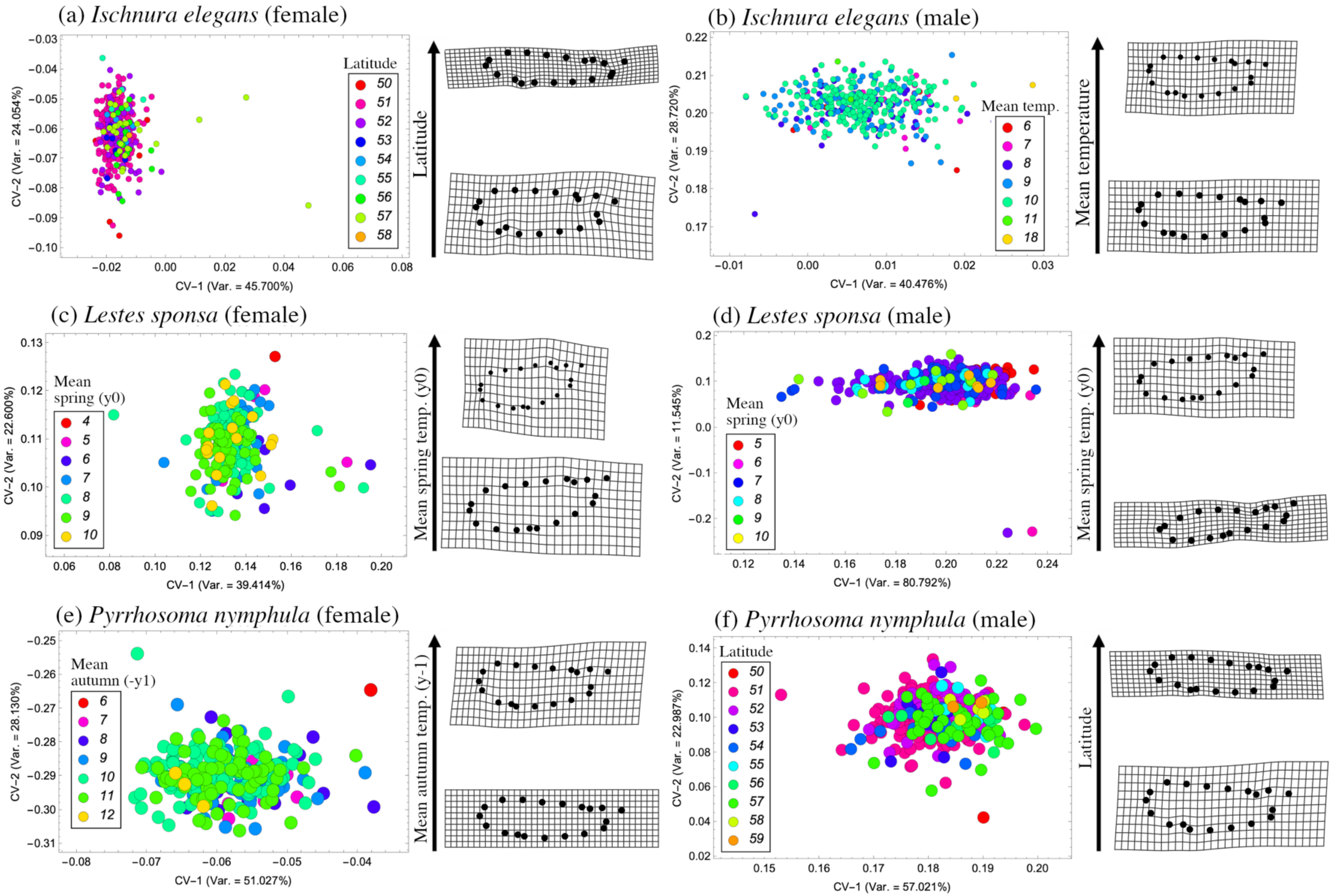
(c) *Calopteryx virgo* (female)

(d) *Calopteryx virgo* (male)

(e) *Coenagrion puella* (female)

(f) *Coenagrion puella* (male)

Fig 5.5: The CVA plot of wing shape of (a, b) *C. splendens,* (c, d) *C. virgo* and (e, f) *Coenagrion puella* on the first and second discriminant axes across the variables which showed the strongest influence on wing shape indicated by PLS-analysis and model of mean shape changes from lower to higher value of relevant variable.


(a) *Ischnura elegans* (female)

(b) *Ischnura elegans* (male)

(c) *Lestes sponsa* (female)

(d) *Lestes sponsa* (male)

(e) *Pyrrhosoma nymphula* (female)

(f) *Pyrrhosoma nymphula* (male)

Fig 5.6: The CVA plot of wing shape of (a, b) *I. elegans*, (c, d) *L. sponsa* and (e, f) *P. nymphula* on the first and second discriminant axes across the variables which showed the strongest influence on wing shape indicated by PLS-analysis and model of mean shape changes from lower to higher value of relevant variable.

Table 5.2: The PLS results between the wing shape of *Brachytron pratense* and environmental variables. Significant level indicated a significant of the CVA discriminant function using a bootstrapped log-likelihood ratio test. Significant levels are indicated as ***, **, * for $p<0.001$, $p<0.01$ and $p<0.05$, respectively.

	Female					Male					
	1***	2**	3*	4	5	1***	2**	3	4*	5	6
Brachytron pratense											
Latitude	-0.165	0.615	0.363	0.159	-0.227	0.469	0.006	-0.081	-0.119	-0.204	-0.359
Mean spring temperature (y-2)	**0.638*****	-0.245	0.345	-0.075	0.226	-0.294	0.102	-0.648	-0.193	-0.234	0.359
Mean summer temperature (y-2)	-0.154	0.339	0.086	-0.005	-0.199	0.023	0.623	-0.228	0.544	0.378	-0.035
Mean autumn temperature (y-2)	-0.063	0.057	-0.287	-0.730	-0.191	-0.127	0.560	0.073	-0.629	-0.023	-0.051
Mean winter temperature (y-1)	0.213	-0.009	-0.175	0.322	-0.499	-0.211	-0.259	-0.045	-0.402	0.678	-0.276
Mean spring temperature (y-1)	0.331	0.492	-0.435	0.290	0.467	-0.072	-0.204	-0.350	0.198	0.078	-0.517
Mean summer temperature (y-1)	0.183	0.379	0.372	-0.451	0.198	0.283	0.188	0.424	-0.024	0.275	0.225
Mean autumn temperature (y-1)	0.459	-0.011	0.091	0.006	-0.534	-0.358	-0.298	0.188	0.180	0.138	0.414
Mean winter temperature (y0)	0.073	0.085	-0.519	-0.173	-0.061	-0.554	0.153	0.419	0.150	-0.383	-0.403
Mean temperature	0.366	0.225	-0.153	-0.123	-0.161	-0.334	0.179	-0.021	0.028	0.231	-0.102
Singular values	1.105	0.674	0.296	0.132	0.021	0.631	0.405	0.192	0.101	0.077	0.034
Covariation (%)	49.60	30.27	13.28	5.93	0.92	43.84	28.11	13.32	7.02	5.37	2.34
Correlation	0.442	0.325	0.231	0.108	-0.008	0.286	0.233	0.123	0.157	0.094	0.015

Table 5.3: The PLS results between the wing shape of *Calopteryx splendens* and *C. virgo* and environmental variables. Significant level indicated a significant of the CVA discriminant function using a bootstrapped log-likelihood ratio test. Significant levels are indicated as ***, **, * for $p<0.001$, $p<0.01$ and $p<0.05$, respectively.

	Female		Male					
	1***	2**	1***	2**	3**	4*	5	6
Calopteryx splendens								
Latitude	**-0.524*****	-0.291	**0.221*****	-0.770	0.166	0.214	-0.116	-0.222
Mean summer temperature (y-2)	0.047	0.477	**-0.406****	0.018	-0.624	0.413	-0.337	-0.012
Mean autumn temperature (y-2)	0.226	-0.584	-0.224	-0.028	0.339	-0.436	-0.689	-0.056
Mean winter temperature (y-1)	-0.323	-0.005	0.224	0.148	-0.141	0.101	-0.008	0.348
Mean spring temperature (y-1)	-0.201	-0.149	-0.104	-0.250	-0.500	-0.587	0.043	-0.254
Mean summer temperature (y-1)	-0.213	0.378	**-0.459****	-0.456	0.092	-0.085	0.442	0.446
Mean autumn temperature (y-1)	0.317	0.237	**-0.489****	0.305	0.297	-0.020	0.330	-0.412
Mean winter temperature (y0)	**0.436*****	-0.309	-0.075	-0.090	0.142	0.439	-0.018	-0.459
Mean spring temperature (y0)	0.398	0.170	-0.259	-0.004	0.271	0.179	-0.294	0.427
Mean temperature	0.179	-0.011	-0.391	-0.113	-0.086	0.078	-0.071	-0.017
Singular values	0.617	0.178	0.702	0.232	0.145	0.091	0.054	0.035
Covariation (%)	77.60	22.40	55.81	18.43	11.49	7.25	4.26	2.76
Correlation	0.461	0.173	0.320	0.137	0.134	0.095	0.059	0.033

	Female					Male			
	1***	2***	3**	4*	5	1**	2*	3	4
Calopteryx virgo									
Latitude	**0.387*****	0.563	-0.192	0.008	-0.360	**0.619*****	0.462	-0.542	0.104
Mean summer temperature (y-2)	0.352	-0.136	-0.485	0.011	0.233	-0.033	0.327	0.290	0.419
Mean autumn temperature (y-2)	0.225	-0.409	0.229	-0.426	-0.114	-0.024	-0.471	-0.421	0.433
Mean winter temperature (y-1)	-0.354	-0.009	-0.103	-0.612	0.047	-0.060	0.129	0.192	0.563
Mean spring temperature (y-1)	**0.276*****	-0.172	-0.104	-0.248	-0.192	0.061	0.096	-0.242	0.077
Mean summer temperature (y-1)	-0.240	-0.230	0.144	-0.042	-0.690	-0.221	-0.267	-0.466	-0.075
Mean autumn temperature (y-1)	-0.021	-0.430	-0.087	0.209	0.358	-0.370	0.013	-0.094	-0.012
Mean winter temperature (y0)	-0.101	-0.312	-0.273	0.527	-0.375	**-0.573***	0.380	-0.337	-0.017
Mean spring temperature (y0)	**-0.387*****	0.178	-0.608	-0.183	-0.046	-0.124	0.383	-0.040	-0.427
Mean temperature	0.030	-0.318	-0.426	-0.158	-0.129	-0.280	0.263	-0.091	0.339
Singular values	0.346	0.284	0.262	0.108	0.092	0.282	0.134	0.110	0.085
Covariation (%)	31.68	25.99	23.99	9.91	8.42	46.18	21.92	18.01	12.90
Correlation	0.184	0.269	0.156	0.095	0.076	0.110	0.103	0.059	0.050

Table 5.4: The PLS results between the wing shape of *Coenagrion puella* and *Ischnura elegans* and environmental variables. Significant level indicated a significant of the CVA discriminant function using a bootstrapped log-likelihood ratio test. Significant levels are indicated as ***, **, * for $p<0.001$, $p<0.01$ and $p<0.05$, respectively.

	Female				Male					
	1**	2	3	4	1***	2**	3**	4*	5	6
Coenagrion puella										
Latitude	**-0.474***	0.228	0.496	-0.352	-0.447	-0.069	-0.162	0.691	-0.499	-0.208
Mean summer temperature (y-1)	0.367	0.239	-0.342	0.281	-0.023	0.416	-0.703	-0.426	-0.387	-0.030
Mean autumn temperature (y-1)	0.424	-0.717	0.455	0.090	0.259	-0.871	-0.273	-0.184	-0.246	-0.074
Mean winter temperature (y0)	0.179	0.351	0.026	0.339	**0.535***	0.215	0.447	-0.042	-0.534	-0.425
Mean spring temperature (y0)	0.574	0.202	-0.072	-0.781	0.470	0.099	-0.441	0.411	0.460	-0.440
Mean temperature	0.315	0.462	0.651	0.252	0.474	0.090	-0.104	0.370	-0.208	0.759
Singular values	0.544	0.171	0.137	0.074	0.385	0.207	0.141	0.046	0.035	0.017
Covariation (%)	58.74	18.49	14.77	8.00	46.35	24.92	17.02	5.49	4.15	2.08
Correlation	0.183	0.112	0.239	0.085	0.213	0.114	0.072	0.052	0.037	-0.007

	Female						Male					
	1***	2*	3	4	5	6	1***	2	3	4	5	6
Ischnura elegans												
Latitude	**-0.498***	0.434	0.250	-0.534	-0.464	0.000	**0.529***	-0.382	0.454	0.493	-0.353	0.000
Mean summer temperature (y-1)	0.381	-0.206	0.487	-0.608	0.360	-0.273	-0.347	0.318	0.317	0.652	0.455	-0.216
Mean autumn temperature (y-1)	0.410	0.718	0.320	0.375	-0.027	-0.270	-0.122	-0.584	-0.572	0.272	0.095	-0.484
Mean winter temperature (y0)	0.157	0.348	-0.763	-0.386	0.190	-0.296	-0.278	-0.496	0.594	-0.450	0.256	-0.236
Mean spring temperature (y0)	**0.469***	-0.304	-0.118	-0.057	-0.785	-0.236	-0.596	0.087	0.100	0.065	-0.767	-0.187
Mean temperature	**0.441***	0.201	-0.040	-0.228	-0.045	0.842	**-0.391***	-0.397	-0.063	0.224	0.076	0.793
Singular values	0.681	0.100	0.060	0.054	0.024	0.000	0.427	0.280	0.079	0.051	0.30	0.000
Covariation (%)	74.08	10.86	6.56	5.88	2.62	0.000	49.24	32.28	9.16	5.91	3.40	0.00
Correlation	0.243	0.104	0.070	0.038	0.015	0.014	0.162	0.133	0.078	0.050	0.012	0.022

Table 5.5: The PLS results between the wing shape of *Lestes sponsa* and *Pyrrhosoma nymphula* and environmental variables. Significant level indicated a significant of the CVA discriminant function using a bootstrapped log-likelihood ratio test. Significant levels are indicated as ***, **, * for p<0.001, p<0.01 and p<0.05, respectively.

	Female		Male	
	1***	2**	1***	2**
Lestes sponsa				
Latitude	-0.686	0.728	**-0.466*****	-0.885
Mean spring temperature (y0)	**0.728***	0.686	**0.855****	-0.466
Singular values	0.465	0.055	0.405	0.122
Covariation (%)	89.59	10.50	76.84	23.16
Correlation	0.274	0.158	0.258	0.075

	Female							Male				
	1***	2***	3	4	5	6	7	1***	2	3	4	5
Pyrrhosoma nymphula												
Latitude	0.473	-0.211	0.096	0.183	-0.485	-0.206	0.624	**-0.445****	-0.288	0.083	0.138	-0.518
Mean spring temperature (y-2)	-0.393	-0.234	-0.308	0.530	0.010	0.418	0.313	0.375	0.543	-0.152	0.579	-0.222
Mean summer temperature (y-2)	-0.068	-0.353	-0.275	-0.105	0.219	-0.564	0.071	0.054	-0.378	-0.394	-0.132	-0.184
Mean autumn temperature (y-2)	-0.224	0.600	-0.467	-0.029	-0.489	-0.294	-0.024	0.380	-0.380	-0.239	0.346	0.191
Mean winter temperature (y-1)	0.169	-0.456	-0.166	-0.458	-0.406	0.241	-0.323	0.064	-0.209	0.190	0.345	-0.454
Mean spring temperature (y-1)	-0.341	-0.070	-0.132	-0.538	0.192	0.105	0.474	0.388	-0.374	0.506	0.068	0.024
Mean summer temperature (y-1)	-0.363	-0.253	0.061	0.141	-0.461	0.121	-0.254	**0.383****	-0.100	-0.110	-0.315	0.156
Mean autumn temperature (y-1)	**-0.463***	-0.076	0.676	0.023	-0.150	-0.339	0.004	0.377	0.264	0.148	-0.528	-0.555
Mean winter temperature (y0)	0.140	-0.199	-0.198	0.390	0.198	-0.271	-0.336	0.034	0.011	0.641	0.070	0.203
Mean temperature	-0.237	-0.306	-0.239	-0.037	-0.034	-0.327	-0.000	0.264	-0.267	-0.144	-0.041	-0.179
Singular values	0.653	0.291	0.190	0.162	0.112	0.061	0.008	0.765	0.161	0.098	0.069	0.028
Covariation (%)	44.21	19.71	12.88	10.94	7.61	4.11	0.56	68.21	14.37	8.76	6.12	2.53
Correlation	0.278	0.224	0.085	0.177	0.094	0.048	0.017	0.263	0.059	0.055	0.053	0.022

5.4. Discussion

This study is the first using a geometric morphometric approach to assess intraspecific wing shape variation of various Odonata species under the influences of latitude and seasonal temperature. We found little influence of latitude and mean seasonal temperature of the larval period on hindwing shape in Anisoptera species, while the hindwing shape of Zygoptera species was significantly correlated with both latitude and seasonal temperature.

5.4.1. Sexual shape dimorphism

Our results show that hindwing shape in all the examined Zygoptera and Anisoptera species, excluding *A. imperator* and *S. metallica*, was sexually dimorphic. For Zygoptera, wing shape sexual dimorphism was strongly significant in all species, but especially so in *Calopteryx splendens* and *C. virgo* where sex discrimination was 85.43% and 88.68%, respectively. A previous study across many species of Calopterygidae found a strong correlation between male wing shape, especially in hind wings, and wing pigmentation, which plays an important role in male sexual displays (Córdoba-Aguilar, 2002, Outomuro & Johansson, 2011, Outomuro et al., 2013a). Thus, it could be implied that sexual display is a strong selective pressure for wing shape in male *Calopteryx* spp. and ultimately leads to wing shape differentiation between males and females. In *C. splendens* and *C. virgo*, we found that females generally have narrower and more elongated wings than males (Figure 5.2a, 5.2b). More rounded and compact wings aids wing beating (Anders & Rüppell, 1997) which is used in male courtship behaviour (Betts & Wootton, 1988, Outomuro & Johansson, 2011). While the other species examined in this study also show significant sex discrimination in wing shape, the variation is not as obvious (Figure 5.2c-5.2l).

5.4.2. Correlation of wing shape and wing length

We hypothesised that intraspecific wing shape variation in Odonata is not significantly correlated with body size as suggested on previous studies on various insect species (Debat et al., 2003). For Anisoptera species, only *Sympetrum striolatum* and *Somatochlora metallica* showed a significant correlation between wing length and shape. While the other Anisoptera species in this study indicated no relationship between shape and length in these species.

A previous study suggested that a correlation between wing shape and body size is taxon-specific and can be different even in closely-related species (Outomuro et al., 2013a). However, the different correlation patterns between species are complex and presumably are influenced by different sexual selection traits (e.g. wing coloration or territorial behaviour) or natural selection traits (e.g. flight performance) between species.

A significant correlation between wing shape and wing length in all Zygoptera species suggests that changes in wing length result in changes in shape in these species. This finding corresponds well with a study in *Calopteryx* damselflies (Outomuro et al., 2013a) and extends this to additional families within Zygoptera, including Coenagrionidae and Lestidae.

5.4.3. The influences of latitude and temperature on wing shape

The results show a non-significant correlation between the PLS axes and environmental variables in Anisoptera. This is in contrast with the predictions and indicates that wing shape in Anisoptera almost have no variation with the environmental parameters measured here. It might be that other factors, such as behavioural variables, are more important in influencing the wing shape of Anisoptera. For example, previous studies on Anisoptera wing shape highlight the effects of migratory behaviour and flight strategies on interspecific wing shape variation (Rajabi et al., 2018, Huang et al., 2020). Geographical range and vicariance may also influence wing shape variations (Hassall et al., 2009). Previous studies on some damselfly (Travis & Dytham, 1999, Hassall et al., 2009) and butterfly species (Hill et al., 1999) suggested wing shape variation at the expanding edge of their distribution range. Also, habitat fragmentation is indicated as a factor influencing wing shape variation in *Calopteryx maculata* (Taylor & Merriam, 1995).

The PLS analysis indicated that latitude is significantly correlated with wing shape variation in all Zygoptera species in this study. The model plots for each species suggested that hindwings of Zygoptera at higher latitude are longer and thinner than wings of the same species from lower latitude (Figure 5.5a-e, 5.6a, 5.6f). In terms of mean seasonal temperature of larval period, the wing shape of individuals collected from warmer years are rounder and broader than individuals collected from colder years (Figure 5.5b-5.5e). This finding is supported by a previous study on British Odonata which indicated that a reduction in wing length is associated with warmer temperature (Wonglersak et al., 2020). Wing lengths were also positively correlated with centroid size, and thus wing area, in all Zygoptera species and two Anisoptera species: *B. pratense* and *S. striolatum* (Table 5.1). Thus in these species, longer, thinner wings with a greater wing area are found at higher latitudes and in lower temperatures, whereas shorter, rounder wings with less wing area are found at lower latitudes and higher temperatures.

These findings are similar to a study across the entire North American distribution range of *Calopteryx maculata* (Hassall, 2015). Lower temperatures can reduce wing beat frequency and ultimately affect the flight performance of insects (Dudley, 2000), thus, individuals with longer and narrower wings, which positively correlate with wing area in the present study and imply reduction of wing loading, are generally expected at higher latitudes or in cooler temperatures (Azevedo et al., 1998, Hassall, 2015). The results of this study extend these findings across several species and two additional families in Zygoptera.

In the present study, specimens were collected only from Britain, thus expanding the sampled latitudinal range would extend the temperature range of the dataset, which might amplify the wing shape correlation with seasonal temperature. A study involving phylogenetic correlation could enlighten our understanding on the wing shape variation changes with temperature. Apart from wing variation between individuals, a study in dragonfly wings indicated that dragonflies can change wing shape during flight. Thus, it would be interesting for future research to incorporate wing deformation during flight for the wing shape analyses.

5.5. Conclusion

In conclusion, the first hypothesis expected that sexual dimorphism in wing shape would be more explicit in species with wing display courtship behaviour. My results indicated that sexual dimorphism in wing shape occurred in 12 of the 14 species, and was most obvious in *Calopteryx splendens* and *C. virgo,* in which the males exhibit wing display courtship behaviour.

Secondly, my results on the correlation between wing shape and body size are partially in agreement with my second hypothesis, suggesting that wing length is related to wing shape in Zygoptera, while this is not the case for most Anisoptera species, except for *Sympetrum striolatum* and *Somatochlora metallica*.

The third hypothesis, on the influence of latitude and temperature on wing shape was partially upheld. The Zygoptera species in this study do exhibit wing shape variation across latitude and seasonal temperature. Elongated wings were found at higher latitudes and colder temperatures, whereas rounded and shorter wings occurred at lower latitude and warmer temperature. In contrast there was almost no wing shape variations with latitude and seasonal temperature in Anisoptera, indicating that wing shape in Anisoptera is likely to be a non-adaptive trait to regional temperature and latitude, but may be more influenced by behavioural traits. However, future studies with more samples from a wider latitudinal range are needed to clarify any relationship between environment and wing shape in Anisoptera species.

Chapter VI

Conclusions:

This chapter presents the key findings and conclusions of this PhD project.

Investigating temperature-body size responses requires consideration of many different factors, including life history traits, phylogenetic relationship and sexual selection (Fig 6.1). This study examined the influence of environmental temperature on two disparate freshwater insect groups that have different life-history strategies and trophic positions, specifically the wing length of Odonata and Chironomidae, and the wing-shape of Odonata, at various spatial and temporal scales. The findings of this study suggest some general patterns in wing length shape response to temperature which are summarised below.

6.1. Intraspecific temperature-size responses

In **Chapter II and IV**, all Zygoptera and most chironomid species in the study showed a significant decline in wing length with warmer temperature. This finding implies that higher temperatures disproportionately increase developmental rate above growth rate, resulting in smaller adults, as predicted by the temperature-size rule (Atkinson, 1994).

However, contrary to general body size rules, Anisoptera species tend to have non-significant responses to environmental temperature. This is hypothesised to be due to the strong territorial behaviour of most Anisoptera species, with a large body size being linked to mating success. Thus, sexual selective pressure could override the increased developmental rate. Additionally, *Calopteryx splendens* collected in the field showed a positive wing length response with increasing temperature **(Chapter III)**. This contrary finding implies that within the same locality larvae emerge as bigger adults in a year with warmer autumn, winter and spring which has more available time for larvae to develop.

6.2. Interspecific temperature-size responses

The study on Chironomidae in **Chapter IV** showed that larger chironomid species are likely to have a greater decline in size than smaller species as temperature increases. This finding could be explained by the oxygen limitation hypothesis (Woods, 1999), in which warmer water holds less oxygen than cooler water, exacerbating the increased energetic costs of respiration in larger aquatic invertebrates compared with small ones (Forster et al., 2012). However other factors such as voltinism, feeding strategy and phylogenetic grouping should be considered in future studies with more species to further understand chironomid responses. For example, a study on various arthropods suggested that voltinism is associated with temperature-size responses (Horne et al., 2015). Univoltine species (one generation per year) tend to have non-significant or positive temperature-size responses, whereas multivoltine species (multiple generations per year) tend to support the TSR (Atkinson, 1994).

Interestingly, **Chapter II** on British Odonata indicated that suborder is an important factor affecting the direction of temperature-size responses. Anisoptera which are generally larger than Zygoptera showed non-significant size changes with warming temperature, whereas Zygoptera were smaller at higher temperatures. This contrast is likely due to territoriality in anisopteran species, as mentioned in section 6.1 (above). Within Zygoptera, small zygopteran species tend to have more sensitivity to temperature changes than larger ones. Taken together, these studies **(Chapters II and IV)** suggested that other factors, including taxonomic relationships, natural selection, sexual display and sexual behaviour can affect the temperature-size responses in insects.

6.3. Seasonal cline in wing length

Individuals of both Zygoptera (Odonata) and Chironomidae (Diptera) flying late in the season tend to be smaller than those of the same species flying early in the season **(Chapter II and IV)**. These results are consistent with those provided by Michiels & Dhondt, 1989 and Johansson & Rowe, 1999 and in their study of the dragonfly *Sympetrum danae* and damselfly *Lestes congener*, respectively, but extend these findings to several more families within Odonata and the fly family Chironomidae. These findings suggest that when larval development is time-constrained, larvae accelerate development towards the end of the emergence season, leading to a smaller adult body size later in the season.

However, within the Odonata this study found non-significant body size changes across the emergence season in most Anisoptera species **(Chapter II)**. This finding is probably because larvae of non-obligate univoltine species which are too small to emerge can delay their emergence until the following year and thus allow more time to develop (Rowe & Ludwig, 1991, Fuselier et al., 2007). Ultimately, these individuals will emerge as larger adults the following year, which will likely increase their territorial competitiveness.

Interestingly, while all Zygoptera and most chironomid species in the study exhibited wing length decline with warming, some chironomid species exhibited larger adult sizes during spring and autumn, with smaller adults found during summer **(Chapter IV)**. This is probably because Chironomids have much faster lifecycles and individuals emerging early during spring and later in autumn when temperatures are cool tend to have a bigger size than those emerging when it is warmer during summer, as a result of accelerated development rates. Thus, temperature variation during the year could affect the adult body size and lead to different sizes across the emergence season and more work is needed to understand the lifecycles of chrionomids.

6.4. Wing shape and ecogeographical responses

The study of wing shape in response to latitude and temperature **(Chapter V)** found that, in agreement with wing length, there was no significant influence in Anisoptera, indicating that wing shape in Anisoptera is likely to be a non-adaptive trait to regional temperature and latitude, but may be more influenced by behavioural traits.

Interestingly, the Zygoptera species in this study exhibited wing shape variation across latitude and temperature. Elongated wings were found at higher latitude and colder temperature, excluding *C. splendens* in Chapter III which exhibited shorter wing lengths with colder temperature. In **Chapter II**, on the influence of temperature on wing length, a significant correlation of temperature on wing length of these Zygoptera species was found (Wonglersak et al., 2020). The results suggested that wing shape variation in Zygoptera is more sensitive to local environment and more adaptive to latitude and temperature than in Anisoptera.

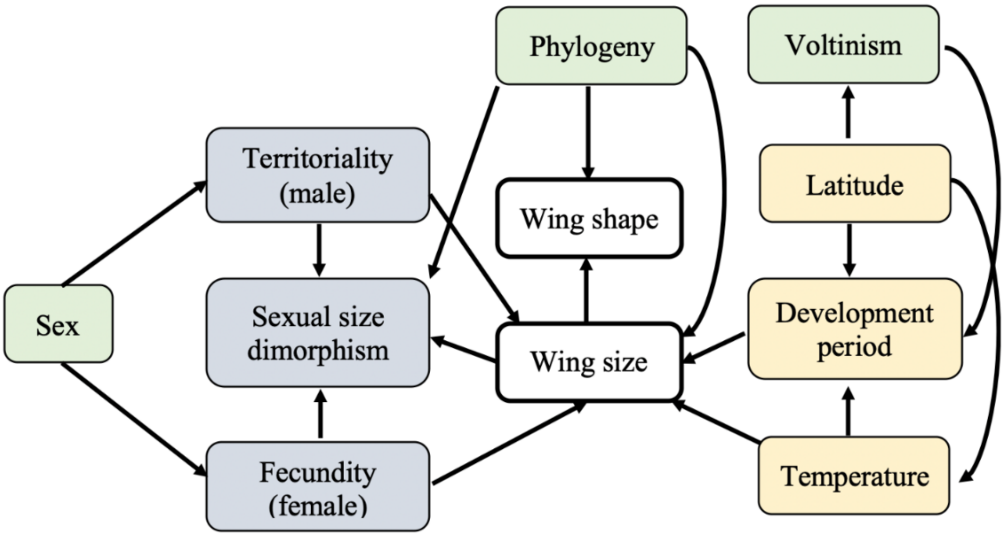


Fig 6.1: The links between abiotic (yellow) and biotic (green) factors that potentially influence wing size, shape and sexual dimorphism of Odonata and Chironomidae investigated in this study.

Overall, this study found that species of Odonata and Chironomidae could get smaller or have no significant body size changes with increasing temperature. The magnitude and direction of temperature-size responses is influenced by several factors, including sex, and suborder (phylogeny), which is linked to territoriality and life-history strategies. Additionally, individual body size of Odonata and Chironomidae can vary throughout their flight period, with declines towards the end of the flight period, or in the case of multivoltine chironomid species, declines in the warmer parts of the year.

The sensitivity of wing shape responses to temperature and latitude is greater in Zygoptera: longer and narrower wings are generally found in cooler temperatures, with a strong correlation between wing length and wing centroid size suggesting that elongated wings have a larger wing area than shorter rounded wings, resulting in less wing loading. Ultimately, this elongated wing can compensate for lower wing beat frequency and improve the overall flight efficiency for individuals in colder climates.

6.5. Future directions of research

The body size- and wing shape- responses of insects to warming temperature is complex and relates to many abiotic and biotic factors (Fig 6.1). This study examined the influences of some factors, including sex, suborder, territoriality and life-history strategies, on temperature-size responses in two disparate groups of insect. However, there are some aspects of wing shape and size responses to temperature that need to be further untangled in future studies for a better understanding of this topic and are discussed below.

Chapter II found significantly different body size-temperature responses between Zygoptera and Anisoptera, and hypothesized that territoriality in Anisoptera species could affect their body size responses to temperature. To test this hypothesis, a further study including more Anisoptera species which do not show territorial behaviour is needed. Furthermore, a study involved phylogenetic analysis would be interesting to test how different phylogenetic groups respond to temperature.

Chapter II and **III** found contradictory results of temperature-size responses in *Calopteryx splendens*. While **Chapter II** using museum collections, found a reduction in size of this species with increasing temperature, **Chapter III** which excluded the effect of latitude and emergence date, found an increase in size with increasing temperature. We hypothesize that this contradictory result is probably as an indirect effect of latitude and emergence date on individuals in **Chapter II**. To untangle this effect a field survey is required to collect wing length data at the same locality throughout the emergence season of this species. Furthermore, latitude and emergence date can influence the temperature-size responses on the other Odonata species, thus further field surveys on the other species is interesting.

The study on temperature-size response in chironomids **(Chapter IV)** found that three of the six species from this study showed a significant decline in size with increasing temperature, while the other species showed non-significant size changes with temperature. Thus, rearing these species for studying their life cycle and phenology in the UK range is beneficial for understanding their inconsistent responses to temperature.

More generally in this study, specimens were collected only from the UK which has a limited latitudinal range and the weakly- or non-significant species may in fact become more significant over a wider latitudinal range. In order to further untangle the effects of temperature on insects more studies are needed, focussing on different phylogenetic groups across the very diverse insect class and comparing different life cycle types and trophic positions. Furthermore, the major differences in climate conditions and developmental period between the temperate and tropical regions likely affects the sensitivity and adaptation of organisms to climate change, thus comparisons within and between taxon groups in these disparate regions are required.

Finally, a future study using museum collection of different insect groups, for example, Coleoptera (beetle and weevils) is essential to generate broader understanding on the effects of temperature on insects body size. Coleoptera is an interesting insect order for the next step of study they constitute the largest insect order, consisting of terrestrial and aquatic groups. Additionally, there are a large number of specimens available in museum collections. Furthermore, they have hard front wings which are ideal for size measurement. In addition, studies of insect body size involving machine learning for automatic body size measuring could be advantageous when working with a large number of specimens.

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