

# Can thermoregulation explain differences in habitat selection and distribution range in *Calopteryx* damselflies?

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## ABSTRACT

Body temperature is important for the behavioural and ecological performance of winged insects whose body temperature must exceed ambient temperature to fly. Although thermoregulation may affect geographical distribution and habitat selection of closely related species. The few studies that have been done on this subject have shown mixed results. Our aim was to study whether thermoregulation affects the coexistence of *Calopteryx* damselflies. We studied the body temperature of *C. virgo* and *C. splendens* under both field and laboratory conditions using an insect thermometer. These damselflies are ideal for studying thermoregulation, because they are closely related, but vary in colouration and size – factors that heavily influence insect thermoregulation. Results from our field and laboratory studies were consistent. Individual temperatures followed ambient temperatures linearly on a level ~3 °C above it. *C. virgo* could maintain higher body temperatures than *C. splendens*. Individual body temperature at the activation, or the time before activation was not affected by species, sex or wing size. However, *C. virgo* warmed up faster than *C. splendens* in laboratory conditions. Thermoregulation may be a key factor explaining differences in geographical distribution, habitat preferences and behavioral differences between these species. The more melanized species *C. virgo* has a northern distribution range than less melanized *C. splendens*. Additionally, *C. virgo* inhabits shadier brooks and rivers whereas *C. splendens* prefers open and sunny habitats. *C. virgo* also wakes up earlier and is a more effective territorial contestant than *C. splendens*. These differences may partly be explained by their thermoeological differences.

## 1. Introduction

Most of the insects, such as dragonflies and damselflies, are either thermoregulating or thermoconforming ectotherms, relying primarily on external heat sources like solar radiation and ambient temperature to elevate their body temperatures to levels sufficient for optimal behavioral and ecological performance (Castillo-Pérez et al., 2022; May, 1976). Some insect species are able to heat their thorax several degrees above the ambient temperature (Castillo-Pérez et al., 2022; Krogh and Zeuthen, 1941; May, 1976; Newport, 1837). However, insect thermoregulation is largely influenced by the species' colouration (Ellers and Boggs, 2003; Laakso et al., 2021; Lacey et al., 2010; Watt, 1968), body size (Heinrich, 1996; Laakso et al., 2021; May, 1976) and reflectivity (Willmer and Unwin, 1981). Also, body shape and posture (e.g. the obelisk posture where the odonate raises its abdomen until the tip points at the sun thus minimizing the solar radiation and preventing overheating) are known to influence thermoregulation in some odonate

species (Castillo-Pérez et al., 2022; Corbet, 1999; May, 1976).

Melanin is a common dark pigment known to influence insect body temperatures through absorption of solar radiation (Goulson, 1994; Gunn, 1998; Kingsolver and Wiernasz, 1991; Svensson and Waller, 2013; Watt, 1968; Watt and Hutchinson, 1969): more melanized individuals i.e. those with darker body colour heat up and attain higher steady-state body temperatures than less melanized individuals i.e. those with lighter body colour (Heinrich, 1996). Especially useful darker colors can be in colder environments: darker wing colors have been hypothesized to be linked with higher latitudes (Outomuro and Ocharan, 2011; Svensson and Waller, 2013; Valle, 1937; Watt, 1968; Watt and Hutchinson, 1969) and altitudes (Loayza-Muro et al., 2013). Indeed, direct evidence shows that in the Northern Hemisphere, species are darker in northern regions and lighter in southern ones (Dreisig, 1995; Hassall, 2014; Hegna et al., 2013; Svensson and Waller, 2013; Valle, 1937).

Although the benefits of increased solar absorption using

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physiological means seem straightforward, previous studies show mixed results of thermoregulation and colouration or body size in different odonate species (Bots et al., 2008; Laakso et al., 2021; Moore et al., 2019; Outomuro and Ocharan, 2011; Rivas et al., 2016; Schreiner et al., 2020). Wing pigmentation, wing spot size or body colouration did not affect body temperature or thermoregulation in different damselfly species (Bots et al., 2008; Outomuro and Ocharan, 2011; Rivas et al., 2016). However, some studies found a link between wing pigmentation or sex and thermoregulation or body temperature in certain dragonfly or damselfly species (Laakso et al., 2021; Moore et al., 2019; Schreiner et al., 2020). In addition, body size seems to influence thermoregulation in certain dragonfly or damselfly species (May, 1976), for example in *Calopteryx* (Laakso et al., 2021; Outomuro and Ocharan, 2011). Moreover, a recent study by Moore and Khan (2023) found evidence that odonate species living in higher and colder elevations tend to have larger wings, which might suggest that larger wings enable improved heat accumulation.

Solar radiation or ambient temperature are not the only means by which some odonate species can increase their body temperature. Additional heating may be required in northern regions, where ambient temperatures are below sufficient levels, especially if flight is required (Heinrich, 1996). Many species are able to heat their thorax several degrees above the ambient temperature (Castillo-Pérez et al., 2022; Krogh and Zeuthen, 1941; May, 1976; Newport, 1837) using methods, such as wing whirring, basking, adjusting their position or moving to a warmer place (Castillo-Pérez et al., 2022; Borkenstein and Jödicke, 2022; May, 1976).

Given the role of thermoregulation on insect species, it is likely that it plays a significant part in larger ecological contexts, such as species habitat selection and geographical distribution, but this area has received little attention. To understand this deeper, we decided to explore the role of thermoregulation in differences in habitat selection and geographical distribution in a well-studied odonate system using field collected data together with a laboratory experiment. *Calopteryx splendens* and *Calopteryx virgo* are two closely related European damselfly species that have overlapping ecological niches (Wellenreuther et al., 2012). They usually occur in flowing water habitats in which they often live in sympatry, and since males of both species are territorial, they often engage in interspecific fights (Karjalainen and Hämäläinen, 2013; Suhonen et al., 2008; Tynkkynen et al., 2004, 2005, 2006). Both species are also very similar in body size and general appearance: males of both species are metallic blue or green, and females are typically metallic green or brown, *C. splendens* females being usually greener and *C. virgo* females being usually browner (Karjalainen and Hämäläinen, 2013; Valle, 1937). Males have a striking difference: whereas *C. virgo* males have completely pigmented dark wings, *C. splendens* males only have dark wing spots that usually cover less than half of the wing area (Karjalainen and Hämäläinen, 2013; Valle, 1937). Wings of the females in both species are clear with a little pigmentation making them typically translucently brownish in *C. virgo* females and translucently greenish in *C. splendens* females (Karjalainen and Hämäläinen, 2013; Valle, 1937). There are some differences in their habitat selection and distribution range: *C. splendens* prefers more open habitats with less vegetation and canopy and more sunlight whereas *C. virgo* occurs also in shadier brooks and river with a lot of abundant vegetation and canopy (Karjalainen and Hämäläinen, 2013; Valle, 1937). *C. virgo* has also more northern distribution range than *C. splendens* in Finland and northern Europe (Karjalainen and Hämäläinen, 2013; Schütte and Schrimpf, 2002; Svensson and Waller, 2013; Valle, 1937). These attributes and differences between the two species make them excellent for evaluating the role of thermoregulation in habitat selection, distribution range and species coexistence.

The main objective of this study was to research the thermal biology of *C. splendens* and *C. virgo* damselflies, with a focus on identifying the intrinsic and extrinsic factors driving interspecific variation. Based on previous studies, we predict melanism, i.e. the darkness of the wings and

body, to be a significant factor in the thermoregulation of the *Calopteryx* species. Therefore, we expect *C. virgo* as the darker species to be more influenced by solar radiation than *C. splendens* and males to be more influenced than females since they have darker wings. Because *C. virgo* tolerates shadier habitats and slightly colder, northern regions than *C. splendens* (Karjalainen and Hämäläinen, 2013; Schütte and Schrimpf, 2002; Svensson and Waller, 2013), it may be able to activate and function properly in lower body temperatures compared to *C. splendens*. Enhanced thermoregulation due to darker appearance could be visible as lower activation temperatures and faster activation times. It also seems likely that *C. virgo* has higher body temperatures than *C. splendens* in the same ambient temperature.

## 2. Material and methods

### 2.1. Field data

Data for the field study were collected in 2000, during mid-June to end of July, in a single flight season of these damselflies (see also Karjalainen and Hämäläinen, 2013). Damselflies were captured from two sympatric locations (Pitkäjoki, Toivakka, 62° 7' N, 26° 2' E and Mustajoki, Jyväskylä, 62° 16' N, 25° 31' E), and one allopatric population with only *C. virgo* (Vasarajoki, Jyväskylä, 62° 20' N, 25° 37' E) (Supplementary Table 1). All three populations were situated in Central Finland. The populations were on small, shallow, slowly flowing rivers with clear water and abundant aquatic and semi-aquatic vegetation. Only mature individuals of both species and sexes were chosen for this study. The damselflies were captured during daytime (between ~ 10:00–16:00). This is the warmest and sunniest part of the day in the summer. Damselflies could fly later in the evening as well, because the sun sets late in the summertime (Fält, 2011). However, we wanted to measure them during day when they are most active and when the temperature is the highest to prevent error (Fält, 2011). The field data were collected by JS.

Damselflies were captured only in sunny weather conditions with no rain or wind. They were captured during daytime when they are the most active and when the temperature is typically highest (10:00–16:00). A standard butterfly net was used to capture the specimens. Individuals were observed for 2–3 min prior capturing by the same observer (JS) to prevent error in the observing accuracy differences between the different observers. Only resting specimens were selected, to negate any activity-caused effects that might increase endothermic heat generation (e.g. flying or fighting) (Heinrich, 1996). In total we captured 290 damselfly individuals, of which 136 were *C. virgo* males and 36 females. Corresponding numbers for *C. splendens* were 87 males and 31 females (Supplementary Table 1).

Body temperatures of the damselflies were measured by setting the tip of the thermometer under the thorax of a damselfly immediately after each damselfly was captured. The flight activity of the insect depends on the temperature of the flight muscles, which are located on the thorax (e.g., Dudley, 2003) and for this reason we chose this position for the temperature probe (see also Bots et al., 2008). For the measurements we used an insect temperature probe (a copper-constant thermocouple Type MT-29/1B with time constant 0.015 s mounted in a 29-gauge hypothermic needle, accuracy  $\pm 0.1$  °C, Physitemp Instruments, New Jersey) (Bots et al., 2008). After this, each damselfly individual was marked with a silver marker (Edding® 780) to prevent the same individuals from being captured multiple times. Then each individual was freed back to the same locations where they were originally captured. Immediately after that the ambient temperature was measured 1 m above ground or water from a shared environment with the same temperature probe that was used to measure the damselfly's body temperatures. Also, the same person (JS) measured all the individuals to prevent measurement errors in measuring accuracy between the measurers and to keep the data consistent.

## 2.2. Laboratory data

Data for the laboratory study were collected in two summers (2011–2012), during the flight season of both species between early June and mid-August. In this part of the study, damselflies were captured from two sympatric populations in Southwestern area of Finland: Mietoinen (60° 37' N, 21° 55' E) and Tarvasjoki (60° 34' N, 22° 43' E) (Supplementary Table 2). Totally, 138 individuals of damselflies were collected of which 49 were *C. virgo* (20 females, 29 males) and 87 were *C. splendens* (39 females, 50 males) (Supplementary Table 2).

Damselflies were captured using a standard butterfly net only in sunny weather conditions with no wind or rain. Ten individuals were captured each day, and they were placed individually in 0.5-L plastic jars, in which they were transported to the laboratory. Only one individual was placed in one jar to avoid stress between individuals. Tiny holes were made into the plastic jars to let the air flow to the jar to prevent damselflies from suffocating. To maintain a suitable moisture level, a moist piece of paper tissue was placed into each jar. Styrofoam cooler with two ice bricks was used to maintain body temperature of the damselflies relatively low (approximately 5–15 °C) to prevent heat shock. The individuals were placed in a cooling room with a temperature of 10 °C (see more details in Laakso et al., 2021). Damselflies were measured in the laboratory on the same day as they were captured. The time elapsed between capture and the test was approximately 1–2 h.

In the laboratory, we used the same insect temperature probe as in the field measurements. First, the temperature probe was placed below the thorax of the damselfly. A natural roosting posture of the damselflies was mimicked by placing a test tube below the damselfly. Damselfly was bound to the test tube with a narrow thread to prevent it from escaping. The entire set-up was placed in a white Styrofoam box (Bots et al., 2008; Laakso et al., 2021).

The damselfly body temperature was cooled down to 3–4 °C temperature by placing ice bricks in the box. The white box was selected for the experiment to prevent radiation absorbing to the walls and to direct most of the radiation to the damselfly. Then the Styrofoam box was placed under a light source in a dark room. The sodium lamp (IDMAN, 681 20 SON-T-400, 1 x SON-T 400 W, 230 W, IP 23) was used as the light source in this experiment because it is radiating the same spectrum of light as the Sun, including UV and infra-red radiation (Bots et al., 2008; Laakso et al., 2021).

At the beginning of the experiment, the body temperature of the damselfly was allowed to rise to 5 °C by removing the ice bricks from the box. From that point we started measuring the time to the point when the damselfly started to activate by flapping its wings. At that point we measured the activation time and body temperature of the damselfly and then the damselfly was removed from the set-up (Bots et al., 2008; Laakso et al., 2021). Wing length of the damselfly and wing spot size of the males were measured by Mitutoyo digital caliper (to the nearest ±0.01 mm). Wing length was used to measure the body size of the individual (Aromaa et al., 2019; Koskimäki et al., 2004). After this, wings of the damselflies were marked with tiny numbers by using the same silver marker as in the field study section (Edding® 780) to prevent capturing and measuring the same individuals twice. After the laboratory experiment, all the damselflies were returned to their original populations on the same day (Laakso et al., 2021). This process was repeated for several days until the sample was collected (n = 136 individuals). The temperature measurements in the laboratory were done by only one person (LL) to prevent errors in measuring accuracy between different persons.

## 2.3. Statistics

### 2.3.1. Field data statistics

Before statistical tests we explored assumption of statistic tests such as normality and heteroscedasticity, which are prerequisites for the application of the statistical tests used (Ranta et al., 1989).

Heteroscedacity of this dataset was tested with the Levene's test. There was no heteroscedacity in our field data ( $F_{3, 286} = 1.12$ ;  $P = 0.34$ ).

The Levene's test was also used to compare equality of error variances in the data. This test is used in general to test if the null hypothesis that the error variance of the dependent variable is equal across the different groups (Levene, 1960). Desing was to test Intercept + Ambient temperature (°C) + Sex + Species + Sex x Species in comparison to dependent variable Body temperature. The variance of this test was not significant ( $F_{3, 286} = 1.121$ ;  $P = 0.341$ ). Paired *t*-test was used to see whether the difference between body temperature and ambient temperature was significant. We used regression analysis (Ranta et al., 1989) to evaluate how damselflies' body temperature was related to the ambient temperature. Slope value of the regression line was used to indicate whether the body temperate changed faster or slower than ambient temperature: if the slope was higher than 1, body temperature rose faster than ambient temperature, and if it was lower than 1, the temperature rose slower than the ambient temperature. Difference between the observed regression slope value and expected value 1 was tested by using the following formula (Ranta et al., 1989):

$$t = \frac{b - \beta_0}{sb}$$

In this formula  $\beta_0 = 1$ , *b* is the slope of regression line and *sb* is standard error of the slope (Ranta et al., 1989). In this calculation, the *P*-value that corresponds to value that was calculated with degrees of freedom *n* - 2. Low *P*-value (lower than 0.05) means that slope (*b*) is different than 1.

The data were analyzed with General linear models (GLM) (Supplementary Table 3). The dependent variable in this model was body temperature and it was measured in relation to the ambient temperature (covariate) (Supplementary Table 3). Independent fixed variables were species (*C. virgo* and *C. splendens*), sex (males and females) and the interaction between sex and species (Supplementary Table 3). Continuous variables were normally distributed. The link function employed in the model was identity. The fixed factors were species and sex (Supplementary Table 3).

### 2.3.2. Laboratory data statistics

In laboratory data, we also wanted to explore assumption of statistic tests such as normality and heteroscedasticity before the statistical tests. Heteroscedacity of this dataset was tested with the Levene's test. Levene's test was used to compare equality of error variances in the laboratory data. This test is used in general to test if the null hypothesis is that the error variance of the dependent variable is equal across the different groups (Levene, 1960) (Supplementary Table 4). There was no statistically significant difference in variances across groups ( $F_{3, 134} = 1.583$ ;  $P = 0.196$ ). This means that we did not find any heteroscedacity in our laboratory data.

AICc (Akaike Information Criterion for small sample sizes) was used to compare the models to find the model that best explained the body temperature in these damselflies (Anderson et al., 2000; Burnham et al., 2011; Burnham & Andersson, 2004; Suhonen et al., 2008). This approach was used to detect the most fitted models for the dataset (Anderson et al., 2000; Burnham & Anderson, 2000; Burnham et al., 2011; Burnham & Andersson, 2004). The models with the lowest  $\Delta\text{AICc}$  (= AICc (Most fitted model) - AICc (alternative model)) are the most suitable for the dataset (Anderson et al., 2000; Burnham et al., 2011; Burnham & Andersson, 2004). The higher the  $\Delta\text{AICc}$  value, the more the model is lacking support but may still be worth considering (Anderson et al., 2000; Burnham et al., 2011; Burnham & Andersson, 2004; Suhonen et al., 2008) (Supplementary Table 5 a). As there were multiple suitable options for the most fitted models for the dataset, we also did model averaging for AICc (Bartoń, 2025; Dixon, 2022; Supplementary Table 5 b).

Effects of body size of and wing spot size on thermoregulation in

*Calopteryx* -species were analyzed by general linear model (GLM) (Supplementary Table 6 a, b, c). Dependent variable in the analysis was reaction time of the damselfly from the beginning of the experiment (s). In the analysis of warming up from 5 °C to the point of activity, explanatory variables were species, sex and their interactions and the covariate was the length of wing. The results were calculated with GLM where covariate was wing length (Supplementary Table 6 a, b, c).

Temperature accumulation rate (°C/s) was analyzed in relation to wing length, species and sex and interaction between species and sex and intercept. The dependent variable of the analysis was temperature accumulation rate. Temperature accumulation rate is the increase of temperature as a measure of time. It was calculated using the following formula:

$$\text{Temperature accumulation } (^\circ\text{C} / \text{s}) = \frac{(\text{Temperature at the time of activation} - 5^\circ\text{C})}{\text{Time for activation (s)}}$$

Heteroscedasticity problem was found from the activation time dataset ( $P = 0.002$ ). Therefore,  $\log_{10}$  transformation was used to stabilize the dataset. This procedure corrected the problem from the data ( $P = 0.63$ ). Most of the statistical analyses of both field data and laboratory data were conducted in IBM SPSS statistics 25. Model averaging for AICc was conducted with R (Bartoń, 2025; Dixon, 2022).

### 3. Results

#### 3.1. Field data

We found that damselfly average body temperatures were approximately 3 °C higher than ambient temperatures (Table 1). *C. virgo* males had lower temperatures than their female counterparts (Table 1), but this difference was not significant. Also, there was no significant difference in body temperatures in the *C. splendens* sexes, but the males had slightly lower temperatures than females even not significant (Table 1). Male temperatures between species did not differ significantly nor did it differ between females. However, when sexes were combined, *C. virgo* had significantly higher body temperatures than *C. splendens* (Tables 1 and 2, Figs. 1 and 2).

Damselflies' body temperatures rose linearly relative to ambient temperatures, and the slope value was approximately 1 (Table 3; Figs. 1 and 2). Males had a slightly steeper slope than females (Table 3) and these differences were particularly high in *C. virgo* between males and

**Table 1**

Field study results on the mean difference between body temperatures and ambient temperatures. The significance of the difference between body temperatures and ambient temperatures were calculated using a paired *t*-test. "N" stands for number of study individuals, "Mean °C" indicates the mean temperature difference between damselflies' body temperature (measured from the surface of the thorax) and the observed ambient temperature. "Range" indicates minimum and maximum values. Males are indicated with a ♂, females with a ♀.

	N	Mean °C	Range	SD	t	df	P
<i>C. virgo</i> ♂	136	3.08	0.00–8.50	1.40	25.68	135	<0.001
<i>C. virgo</i> ♀	36	3.49	0.20–9.30	1.76	11.91	35	<0.001
<i>C. v.</i> all	172	3.17	0.00–9.30	1.49	27.96	171	<0.001
<i>C. splendens</i> ♂	87	2.69	0.00–6.50	1.48	17.01	86	<0.001
<i>C. splendens</i> ♀	31	2.70	0.00–7.70	1.78	8.48	30	<0.001
<i>C. s.</i> all	118	2.70	0.00–7.70	1.55	18.86	117	<0.001
Pooled data	290	2.98	0.00–9.30	1.53	33.15	289	<0.001

females (Table 3; Fig. 1). Coefficient of determination ( $r^2$ ) was twice as high in males than in females in both species, indicating that males' body temperatures followed more closely the ambient temperatures than females' body temperatures did (Table 3; Fig. 1).

#### 3.2. Laboratory data

In the AICc test, we found the most fitted models (models with  $\Delta\text{AICc} < 2$ ) for our dataset explaining body temperature were species ( $\Delta\text{AICc} = 0.00$ ), wing + species ( $\Delta\text{AICc} = -0.57$ ) and species + sex ( $\Delta\text{AICc} = -1.62$ ) (Supplementary Table 5 a). Since three models have  $\Delta\text{AICc} < 2$ , we did model averaging approach to our dataset (Bartoń, 2025; Dixon, 2022; Supplementary Table 5 b).

Remarkably, no differences were observed in the temperature at the time of activation between both species and sexes (Table 4). It did not differ between *C. virgo* males or females (Table 4), nor between *S. splendens* males (Table 4) or females (Table 4), and neither did body size affect it (Table 4).

Time elapsed for the damselfly to become active was also similar between species, sexes, and body sizes. There were no differences between *C. virgo* males or females (Table 4) and *C. splendens* males or females (Table 4). Body size was a continuous variable in the test, but there were no differences in activation time between the different body sizes either (Fig. 3, Tables 4 and 5).

However, heating rate (or temperature increase over time) (°C/s), was significantly higher in *C. virgo* compared to *C. splendens* (Tables 4 and 5). Heating rate did not differ between *C. virgo* males and females (Tables 4 and 5), nor between *S. splendens* males and females (Tables 4 and 5). Wing length did not affect the heating rate either (Table 4).

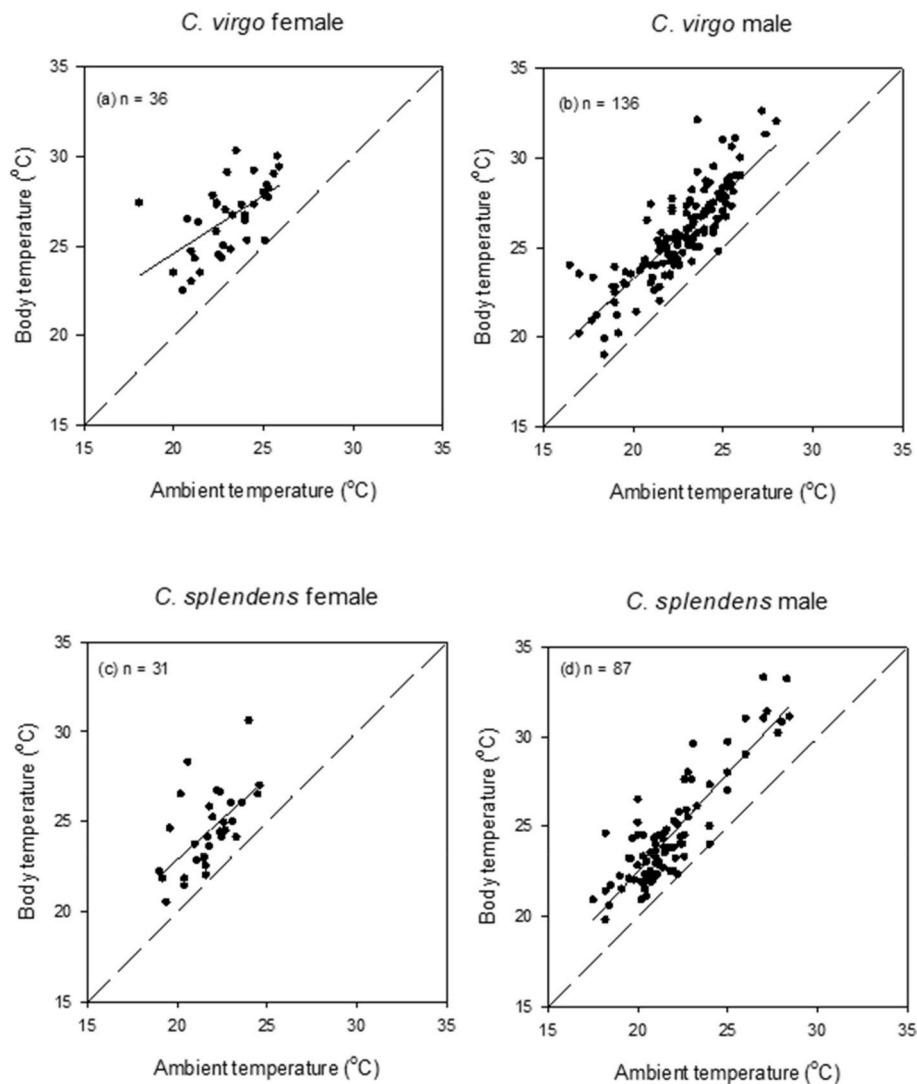
### 4. Discussion

Our findings confirm that the body temperatures of *Calopteryx virgo* and *C. splendens* closely follow ambient temperatures but consistently remain approximately 3 °C higher (Table 1, Fig. 1). This pattern aligns with previous studies (Schreiner et al., 2020; Svensson and Waller, 2013), reinforcing the notion that thermoregulation in these species

**Table 2**

The field study results of the GLM analysis on the damselflies' body temperature. The body temperature (dependent variable) was measured in relation to the ambient temperature (covariate), species (*C. virgo* and *C. splendens*), sex (males and females) and the interaction between species and sex. The GLM model was significant ( $\chi^2 = 343.36$ ;  $df = 4$ ;  $P < 0.001$ ). Statistically significant results are in bold.

Variable	df <sub>1</sub> , df <sub>2</sub>	$\chi^2$	P	SE	95 % Confidence Intervals Upper bound, lower bound
Intercept	1, 285	16.81	<0.001	0.900	1.62, 5.17
Species	1, 285	8.41	<0.005	0.211	0.03, 0.84
Sex	1, 285	1.04	0.309	0.317	-0.62, 0.63
Species x Sex	1, 284	0.95	0.329	0.425	-0.43, 1.25
Ambient temperature	1, 285	580.58	<0.001	0.041	0.89, 1.05



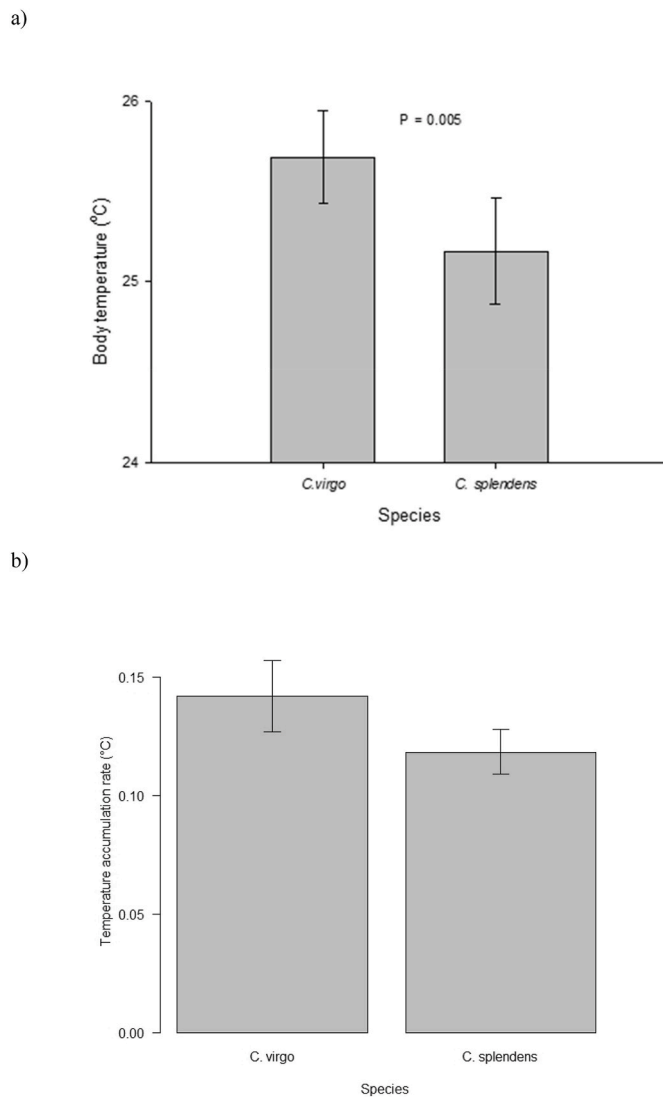
**Fig. 1.** Relationship between ambient temperatures and damselflies' body temperatures. (a) *C. virgo* females, (b) *C. virgo* males, (c) *C. splendens* females and (d) *C. splendens* males. Dashed line that cuts the square is where ambient temperatures and body temperatures have an equal value. If a dot is above the dashed line, body temperature is higher than ambient temperature, and *vice versa*. Solid lines represent the regression line between ambient and body temperature.

operates within a predictable margin above environmental temperature. Notably, *C. virgo* exhibited significantly higher body temperatures than *C. splendens*, although sex-based differences in body temperature were absent in both species (Table 2). This is in line with other *Calopteryx* studies (Laakso et al., 2021; Outomuro and Ocharan, 2011). Our findings expand the previous research by highlighting the more pronounced temperature dependence in males, whose body temperatures followed ambient temperatures at a steeper slope than females, suggesting stronger environmental coupling in male thermoregulation (Table 2).

Furthermore, our laboratory experiment revealed no effect of species, sex, or wing length on body temperature at the time of activation or at the time elapsed before activation (Table 5), contrasting with earlier studies that have suggested morphological or behavioral thermoregulatory adaptations (Laakso et al., 2021; Outomuro and Ocharan, 2011; Schreiner et al., 2020; Svensson and Waller, 2013). However, we observed that *C. virgo* accumulated heat at a significantly higher rate ( $^{\circ}\text{C}/\text{s}$ ) than *C. splendens*, indicating faster thermal responsiveness. This result underscores potential ecological or physiological differences between the species, which may be critical in behavioural thermoregulation and activity patterns. Our findings provide further nuance to the ongoing discussion of odonate thermoregulation and suggest paths for future research into the physiological mechanisms

underlying these thermal differences.

We found that *C. virgo* has higher body temperatures than *C. splendens*, which seem to explain differences in habitat selection between these *Calopteryx* species. *C. virgo* is found in shadier and colder habitats than *C. splendens* (Wellenreuther et al., 2012). *C. virgo* also wakes up earlier (Fält, 2011), so it stands to reason that it has evolved coping mechanisms for lower temperatures that are more efficient than in *C. splendens*. This may result in higher body temperatures in similar environments with additional benefits to behavior. In interspecies territorial fights, *C. virgo* males are more likely to win fights than their *C. splendens* counterparts (Tynkkynen et al., 2004). Higher body temperatures may be the reason for this due to increased metabolism and better fighting capability. Hotter males also seem to be more efficient in attracting females (Tsubaki et al., 2010), suggesting an advantage that females prefer. Indeed, if "hotter" males are more likely to win fights and copulate at a higher rate, over time this can lead to more efficient thermoregulation and higher body temperatures. Of course, becoming hotter is advantageous only in specific areas and habitats, and when the increase in body temperatures does not negatively interfere with other physiological or behavioral aspects. Indeed, the fact that past studies show a wide range of different responses to ambient temperatures in odonates (Bots et al., 2008; Marden et al., 1996; Samejima and Tsubaki,



**Fig. 2.** a) Estimated marginal means of body temperatures in the field study. 95 % confidence intervals in the two damselfly species *C. virgo* and *C. splendens*. *C. virgo* has higher body temperatures than *C. splendens* ( $P < 0.05$ ). b) Estimated Marginal Means of variable Species in laboratory experiment. Dependent variable of the test was Temperature accumulation rate ( $^{\circ}\text{C}/\text{s}$  for the activation time). *C. virgo* has a higher temperature accumulation rate than *C. splendens* ( $P < 0.05$ ).

2010; Schreiner et al., 2020; Svensson and Waller, 2013; Watanabe and Taguchi, 1993) may be the result of local adaptation to the specific climate in the specific species. The faster warming rate of *C. virgo* may provide a competitive advantage in more northern regions and shadier habitats or during early morning activity periods, allowing them to

activate faster than *C. splendens*. These thermoregulatory differences seem to explain differences in habitat selection, foraging efficiency, reproductive success, and geographical distribution between these species.

We found that body temperatures of the study species were approximately  $3^{\circ}\text{C}$  higher than ambient temperature. In previous *Calopteryx* studies the relationship between body temperature and ambient temperature varied between 1 or slightly over 1 (Schreiner et al., 2020) to much lower than 1 in *C. splendens* (Svensson and Waller, 2013) or even negative ( $-0.57$ ) in *C. virgo* (Svensson and Waller, 2013). These differences between the studies may be due to ambient temperature variations between study areas in northern America ( $16\text{--}41^{\circ}\text{C}$ ) (Schreiner et al., 2020), in southern Sweden ( $20\text{--}38^{\circ}\text{C}$ ) (Svensson and Waller, 2013) and in Finland in this study ( $16\text{--}28^{\circ}\text{C}$ ). It may be that *Calopteryx* species have developed adaptations for specific local temperature regimes, partly explaining the differences between these studies. For example, *Calopteryx* species in Finland may require more efficient heat absorption and higher body temperatures relative to ambient air than North American species, which may require more efficient cooling and lower body temperatures relative to ambient temperatures. It is also possible that methodological differences, especially in measuring body temperatures, may explain some of the differences between these studies. Some of the studies have been made with thermometer in laboratory conditions (e. g. Bots et al., 2008; Laakso et al., 2021; Outomuro and Ocharan, 2011) and some have been made with thermal camera in field conditions (e. g. Schreiner et al., 2020; Svensson and Waller, 2013). However, further studies are definitely needed to understand better the differences in thermoregulation between *Calopteryx* species and how the study methods affect the results of the studies.

According to our results, *C. virgo* was able to heat up faster than *C. splendens*, and it would be logical to assume that melanism i.e. their darker wings were the key factor in explaining this difference (Laakso et al., 2021). However, if wing melanization had a strong effect on heating ability, we should see significant differences between males and females, since wing melanization is considerably different between them. On the other hand, there is a difference in female wing melanization between these two species, and the heating rate was higher in *C. virgo* females ( $0.13^{\circ}\text{C}/\text{s}$ ) than in *C. splendens* females ( $0.12^{\circ}\text{C}/\text{s}$ ). Thus, wing pigmentation may have a distinct effect on heating rate even though sex differences were non-significant in our data. Previous studies imply that melanism (i.e. species darkness) influences insect thermoregulation, with increasing melanin level in higher altitude and northern latitude (Acquah-Lampsey et al., 2020; Pinkert et al., 2017; Zeuss et al., 2014; Hegna et al., 2013; Svensson and Waller, 2013). It has to be noted that there was a geographical and temporal difference between our field study and laboratory experiments. Given the nature of adaptive plasticity, especially in insects, it is possible that the  $2^{\circ}$  difference in latitude and/or 15-year time gap causes differences between the study sites and their individuals. It is possible that 15 years is enough for our study species to e.g. adapt to rising global temperatures, therefore creating a response difference between our field and laboratory studies. However, both datasets were analyzed separately, and they provided a congruent

**Table 3**

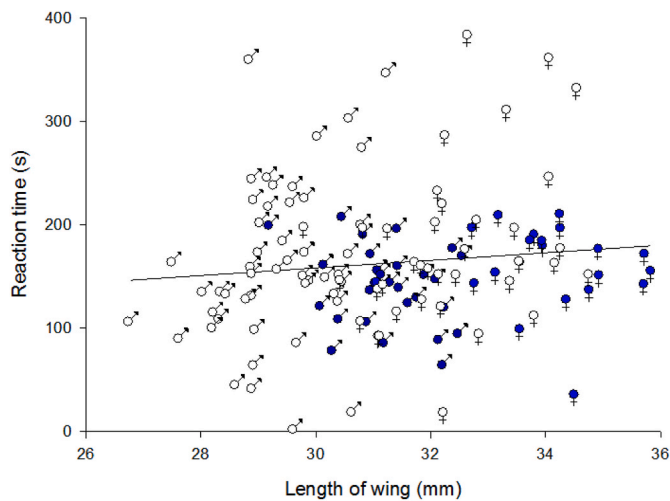
Field study results of the regression analysis between damselflies' body temperatures and ambient temperatures. The  $r^2$  is coefficient of determination. P (1) indicates the statistical significance of the differences between the observed slope value and the expected slope value of 1. P(R) is the P-value of the regression model. Statistically significant results are in bold.

	N	$r^2$	intercept (SE)	slope (SE)	P(1)	F	$df_1, df_2$	P(R)
<i>C. virgo</i> ♂	136	0.70	4.40 (1.21)	0.94 (0.05)	0.12	320.02	1, 134	<0.001
<i>C. virgo</i> ♀	36	0.34	11.63 (3.48)	0.65 (0.15)	<0.01	18.66	1, 34	<0.001
<i>C. virgo</i> all	172	0.64	5.37 (1.18)	0.90 (0.05)	<0.05	311.57	1, 170	<0.001
<i>C. splendens</i> ♂	87	0.76	0.72 (1.44)	1.09 (0.07)	0.90	277.11	1, 85	<0.001
<i>C. splendens</i> ♀	31	0.34	4.83 (4.84)	0.90 (0.22)	0.33	16.59	1, 29	<0.005
<i>C. splendens</i> all	118	0.70	1.20 (1.43)	1.07 (0.07)	0.84	269.74	1, 116	<0.001
Pooled data	290	0.68	3.08 (0.89)	0.99 (0.03)	0.37	622.63	1, 288	<0.001

**Table 4**

Laboratory experiment results. “N” indicates number of studied individuals, “Wing” indicates mean hind wing length (mm), “°C Activ.” indicates mean temperature at the moment of activation, “t Activ.” indicates the mean elapsed time to activation, and “°C/s” indicates the mean heat accumulation over time, i.e. heating rate. “SD” indicates standard deviation. Numbers in parentheses show the range of value (minimum-maximum) (see also [Supplementary Table 7](#)).

	N	Wing	SD	°C Activ.	SD	t Activ.	SD	°C/s	SD
<i>C. vir</i> ♂	29	31.30 (29.22–32.58)	0.81	24.14 (19.40–31.33)	3.12	143.36 (67.50–210.62)	37.30	0.14	0.03
<i>C. vir</i> ♀	20	34.04 (31.05–35.82)	1.16	24.49 (14.70–27.70)	2.95	157.41 (33.63–208.17)	41.25	0.13	0.04
<i>C. vir</i>	49	32.42 (29.22–35.82)		24.28 (14.70–31.33)		149.10 (33.63–210.62)		0.14	
<i>C. spl</i> ♂	50	29.34 (26.78–31.26)	0.94	22.68 (5.10–33.70)	4.42	166.68 (5.12–362.83)	76.04	0.12	0.05
<i>C. spl</i> ♀	39	32.37 (29.78–34.74)	1.24	24.14 (6.10–29.20)	4.33	176.66 (16.28–381.29)	75.37	0.12	
<i>C. spl</i>	89	30.67 (26.78–34.74)		23.32 (5.1–33.7)		171.05 (5.12–381.29)		0.12	
Total	139	31.29 (26.78–35.82)	1.97	23.66 (5.1–33.7)	3.99	163.25 (5.12–381.29)	65.64	0.13 (0.02–0.3)	0.04



**Fig. 3.** Reaction times of the damselflies in the laboratory experiment. Reaction time from 5 °C temperature in *C. virgo* (Dark) and *C. splendens* (Light) males (♂) and females (♀) in relation to the wing length in the heating experiment under sodium lamp as a light source radiating a spectrum which corresponds the spectrum of natural sunlight in the lab.

trend of the difference in thermoregulation between the species. We also wanted to see if the thermal responses of the study species are similar or if there has been a change in different time periods. Zygopterans may exhibit plasticity patterns in phenotypes and physiology for the response of climate change and therefore we measured their thermoregulation in two different time points. Even though there have been 15 years between the datasets, our results suggest that the thermal responses of these species have similar patterns in both time periods, which is in line with our expectations.

Interestingly, wing size did not affect any measured factor, suggesting that if wing pigmentation has an effect, it may not be related to wing size, or the effect of wing area has a very little effect. This idea may be worth studying in the future. Also, in future studies of *Calopteryx* thermoregulation, body pigmentation and body size are some attributes that should be taken into consideration. *C. virgo* is slightly larger in size than *C. splendens*, and there are small differences in body colouration between the species (Karjalainen and Hämäläinen, 2013; Laakso et al., 2021). Males of both species are very similar in body colouration: they are metallic blue or green in their thoracic and abdominal colouration whereas the females of both species are usually metallic green or brown in thoracic and abdominal colouration, *C. virgo* female being usually browner and *C. splendens* female usually greener (Karjalainen and Hämäläinen, 2013). Body pigmentation has not been studied in dragonflies and damselflies a lot as many studies are focused on wing pigmentation, but the few previous studies have not found a connection (Bots et al., 2008). Also, previous studies have mixed results in body size: some of them have not found correlation between body size and thermoregulation (Outomuro and Ocharan, 2011; Bots et al., 2008),

**Table 5**

Statistical tests of the laboratory study. We used General Linear Models (GLM) to analyze individual body temperature at the time of activation (°C Activ.), time elapsed to activation (t Activ.), and temperature accumulation over time, i.e. heating rate (°C/s). The time elapsed to activation (t Activ.) has been log<sub>10</sub> transformed. Independent variables of this test were Species, Sex, Wing, and Species x Sex interact. Adjusted R<sup>2</sup> for the “°C Activ.” model was 0.026, 0.001 for the “t Activ.” model, and 0.023 for the “°C/s” model. The latest model of the test was (F<sub>4, 133</sub> = 1.82, P = 0.129). Estimator values were (Intercept = 0.251, Wing = -0.0041, Species = 0.022, Sex = -0.0091; Species x Sex = 0.004) and their associated standard errors were (Intercept = 0.111, Wing = 0.003, Species = 0.013, Sex = -0.0014; Species x Sex = 0.015). Statistically significant results are bolded in the table.

Dependent variable	Source	Parameter	SE	F	df <sub>1</sub> , df <sub>2</sub>	P
°C Activ.	Intercept	8.12	10.55	0.65	1, 133	0.42
	Species	0.10	0.92	0.01	1, 133	0.92
	Sex	0.37	1.17	0.10	1, 133	0.76
	Wing	0.49	0.33	2.25	1, 133	0.14
t Activ.	Intercept	1.38	0.63	4.82	1, 134	<b>&lt;0.05</b>
	Species	-0.10	0.06	1.45	1, 134	0.23
	Sex	0.03	0.07	0.20	1, 134	0.66
	Wing	0.03	0.02	1.70	1, 134	0.19
°C/s	Intercept	0.25	0.11	5.61	1, 133	<b>&lt;0.05</b>
	Wing	-0.00	0.00	1.43	1, 133	0.24
	Species	0.02	0.01	6.09	1, 133	<b>&lt;0.05</b>
	Sex	-0.01	0.01	0.26	1, 133	0.61
	Species x Sex	0.00	0.02	0.09	1, 133	0.78

whereas other studies have found (Laakso et al., 2021; May, 1976). Our previous study found that fastest individuals to warm up are darkest in color and smallest in body size (Laakso et al., 2021). This idea needs to be studied more in the future.

Our measurements revealed great variability in body temperatures, time elapsed prior activation and heating rate (Tables 1 and 4). We also found that female body temperatures were much less affected by ambient temperatures, than male temperatures: coefficient of determination (r<sup>2</sup>) was half of what males had (Table 3). Our data therefore suggests that there are several factors beyond ambient temperature and solar radiation that affect *Calopteryx* thermoregulation. Females and males have different physiological needs, e.g. females need to produce a huge number of eggs (Corbet, 1999) and males need to win territorial fights (Suhonen et al., 2008; Tynkkynen et al., 2004), and these drive

different optimization schemes. Egg production for examples, may require a somewhat steadier body temperature level more independent from ambient temperature (e.g. Berger et al., 2008; Geister et al., 2008; Jones et al., 1982), which may cause the difference we see between the two sexes. We know from previous studies that female egg production is dependent on temperature (e.g. Berger et al., 2008; Geister et al., 2008; Jones et al., 1982), but how well this is visible in odonate systems remains to be studied. Successfully winning territorial fights on the other hand, may require much higher body temperatures than ambient temperatures would allow (e.g. Castillo-Pérez et al., 2022; Samejima and Tsubaki, 2010; Schreiner et al., 2020). Moreover, we do not yet know what temperature difference (in ambient or body temperatures) has biological relevance, regardless of whether it is statistically significant or not. Non-significant temperature variations may in fact be biologically and ecologically significant when observed for a long enough time period. However, studies of this nature would require temporally very long and robust datasets to verify the significance of small-scale differences. That being said, rising global temperatures should give ample reason to study this phenomenon.

In conclusion, our data suggests that both *Calopteryx* species can maintain their body heat temperatures 3 °C higher than ambient temperatures. We also noted that *C. virgo* can maintain higher body temperatures and it can warm up faster than *C. splendens*. Interestingly, we did not find any statistically significant effects that wing size, or sex might have on *Calopteryx* thermoregulation. According to our results, wing melanism did not show any significant trends in relation to thermoregulation. There is great variance in our data, suggesting that other factors might play an important role in thermoregulation as well. For example, thoracic pigmentation and behavioral mechanisms could be influential and require further studying. Future research should focus on studying melanism and wing pigmentation in relation to thermoregulation, as well as exploring the impact of other factors such as thoracic pigmentation and behavioral mechanisms. Our observations that *C. virgo* and *C. splendens* are different in their thermoregulation shed new light in their geographical range, habitat preference, coexistence, and physiological behavior. Thermoregulation appears to be a significant factor in e.g. species niche partitioning, and it should be considered more thoroughly in future studies; for example, the correlation between body temperature and ambient temperature could be used to model body temperatures and possible changes in behaviour and distribution range in these species as well as in many other different species of odonates and other insects.

### Glossary

- Calopterygidae is a family of damselflies in the suborder of Zygoptera (Karjalainen and Hämäläinen, 2013)
- Coexistence is a situation when different species share the same environment (Darwin, 1859)
- Ectotherm is a creature whose body temperatures are regulated by their environment (Heinrich, 1996)
- Melanism is an increased amount of pigmentation, for example in the skin or wings of a creature (Heinrich, 1996)
- Niche partitioning is a process where natural selection drives competing species in different directions and different niches (Darwin, 1859)
- Odonata is an order of insects containing dragonflies and damselflies (Corbet, 1999)
- Thermoecology is a study of the interactions between temperature and different organisms (Heinrich, 1996)
- Thermoregulation is the maintenance and regulation of a particular temperature in a living body (Heinrich, 1996)

### CRedit authorship contribution statement

**Linda Laakso:** Writing – original draft, Visualization, Resources,

Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Jaakko J. Ilvonen:** Writing – original draft, Validation, Investigation, Formal analysis. **Jukka Suhonen:** Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2025.104255>.

### Data availability

The data underlying this research work is available in Zenodo data repository. The URL for the data is <https://zenodo.org/records/16893971> and the DOI for the data is <https://doi.org/10.5281/zenodo.16893971>.

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