



# Cool Bands: Wing bands decrease rate of heating, but not equilibrium temperature in *Anartia fatima*



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## ABSTRACT

Butterflies regulate their internal thoracic temperature in order to optimize performance activities (e.g. flight, foraging). Previous research has shown that butterfly wings, particularly the innermost portions, play a role in thermoregulation. We investigated to see whether a lightly colored wing band would alter the thermal properties of the banded peacock butterfly (*Anartia fatima*) with two within subject experiments in a laboratory setting: (1) band color manipulation in which euthanized individuals were heated to thermal equilibrium with the band unaltered and then again with the wing darkened; (2) wing ablation in which individuals already run through experiment 1 were heated to equilibrium two more times; once with the outer portion of the wing including the band removed and then with the entire wing removed. Individuals were spread so that the dorsal surface of the wing was exposed to illumination from a lamp suspended above. Twelve *Anartia fatima* males were collected in Panama and were run through experiment one. Four individuals were run through experiment two. We found no effect of darkening the band on the internal thoracic equilibrium temperature, but the darkened band did increase the rate of heating. The wing ablation experiment revealed that wing removal lowered the internal thoracic equilibrium temperature but did not affect the heating rate. Therefore we show that butterfly bands may be important in butterfly thermoregulation and we discuss the importance of the wing band on thermoregulatory abilities in *Anartia fatima* with respect to the butterfly's natural history. We conclude that the wing band may allow butterflies to reduce heat stress induced by their warm environments.

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## 1. Introduction

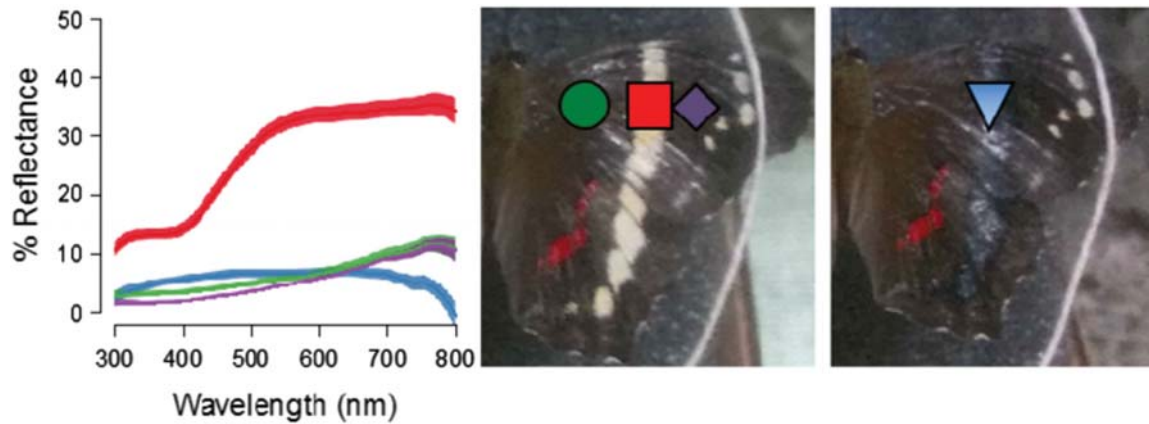
Thermoregulation is an important component of butterfly biology. Although the physical structures, such as wing size and thorax, ultimately limit thermoregulatory capacity, behavioral changes (e.g., basking, body postures) can greatly affect heat gain and loss and allow butterflies to keep their body temperature close to the optima for flight, foraging, etc. (Heinrich, 1972; Kingsolver and Moffat, 1982; Kingsolver and Wiernasz, 1991; Kleckova et al., 2014; Rawlins, 1980; Rutowski et al., 1994). Through basking and adjusting body posture, butterflies increase internal temperature by absorbing solar radiation or by conducting heat through body parts placed onto warmer substrates (Heinrich, 1993; Huey et al., 2003; Kingsolver, 1987). Wing flapping or increased exposure to wind decreases body temperatures through convective cooling.

Because butterflies are not able to change their coloration, wing color is thermally limiting in that darker coloration absorbs more solar radiation than lighter coloration (Berwaerts et al., 2001; Watt, 1968). Here, we revisit the question of how wing coloration affects thermoregulatory properties in a butterfly with a wing band.

Seminal research has demonstrated the effects of wing coloration on both the rate of heating and the internal thoracic temperature (Kammer and Bracchi, 1973; Schmitz, 1994; Wasserthal, 1975). Larger and darker wings (i.e., wings with more melanin) absorb more energy, thus increasing heat gain (Kammer and Bracchi 1973; Wasserthal 1975; Kingsolver 1987; Schmitz 1994). Although wing coloration is important, Wasserthal (1975) demonstrated that only the 15% of the wing surface area closest to the thorax (that is, coloration within 2–3 mm of the thorax) affects thoracic temperature, the main target for butterfly thermoregulation (Kingsolver, 1985; May 1979). However, these trends may not be applicable to all butterflies. Recent studies have shown a more complicated relationship between wing coloration and thoracic temperature.

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**Fig. 1.** Experimental set up and reflectance spectra of the un-manipulated and manipulated wing patterns. Left, the reflectance spectra are mean (lines) and standard error (shading). Center and right, the photographs depict the natural coloration of *A. fatima* (left) and the sharpie darkened band of the manipulated wing (right). The colored shapes in each photograph match the reflectance spectra: green circle=proximal brown; red square=natural band; purple diamond=distal brown; blue triangle=sharpie darkened band.

Wing coloration can be produced by pigments, structurally (e.g. photonic crystals), or by a combination of both (Nijhout, 1991). Iridescent structural coloration absorbs more solar radiation and has a greater effect on internal temperatures than pigmentary coloration (Bosi et al., 2008). Furthermore, De Keyser et al. (2015) found that color had less of an effect on thoracic temperature in small Lycaenid butterflies. Therefore, although there is a basic understanding of the role of coloration in butterfly thermoregulation, our understanding is based on a few species and further research is warranted. No research has explicitly tested the thermoregulatory function of wing bands, even though many banded butterflies occur in thermally challenging climates. Many butterflies have bands or stripes that are much darker or lighter than the wing background, and this difference in brightness could enhance or reduce the solar radiation absorbed by the wing and thorax.

*Anartia fatima* (Nymphalidae), the banded peacock butterfly, has a conspicuous yellowish band that fades to white with age and is set within a field of dark brown on both the dorsal and ventral wing surfaces of the fore and hind wings (Fig. 1). The continuous band of 3–4 mm in width runs from the anterior to posterior wing edges about halfway between the wing base and distal wing margin. The continuous band is set approximately halfway on the wing, 1.25 cm from the thorax and 1.25 cm from the wing margin. *Anartia fatima* is found in disturbed habitats in Central and South America where the environment can be very hot and dry (Silberglied et al., 1979). Furthermore, *A. fatima* perches on vegetation in open areas and may not be able to seek shade for thermal relief (Silberglied et al., 1979). Hence, *A. fatima* may be under strong thermoregulatory pressures to reduce overheating in its habitat and the wing band may function to reduce the absorption of heat by the thorax. We tested the effect of the wing band in *A. fatima* on both heating rate and thermal equilibrium in a laboratory setting. We predicted that the band would have a minimal effect on the two thermal metrics due to the band being further than .5 cm from the thorax, as is predicted from Wasserthal (1975). These experiments are the first to explicitly test the thermoregulatory role of wing bands in butterflies, which will further our understanding of evolutionary and ecological significance of wing bands and coloration of butterflies.

## 2. Methods

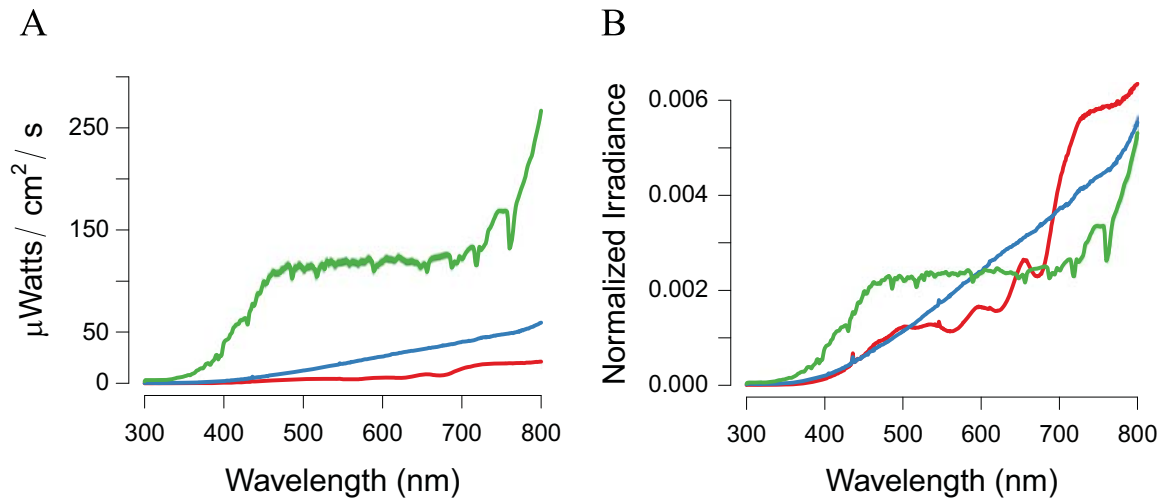
### 2.1. Butterfly collection

We collected 12 *Anartia fatima* males from gardens and fields in Gamboa, Panama (9.1167N, 79.7000W) during May of 2015; all individuals had no evident wing wear or scale loss (see Kemp (2006)). All specimens were euthanized by freezing and then stored individually in a freezer until use. For each trial, two butterflies were removed, weighed, and mounted on a .33 mm diameter type T thermocouple connected to a digital recorder (Dual J-T-E-K Thermocouple Thermometer, Digi-sense, Vernon Hills, IL). The thermocouple was inserted into the thorax, between the mesothoracic segments, to a depth of ~2 mm at an angle perpendicular to the frontal plane. The wings were then spread to an angle of approximately 180° relative to each other to achieve maximum light exposure.

### 2.2. Band manipulation experiment

We then measured reflectance at three locations on three parts of the wings: the brown wing surface outside of the band, within the band (both unaltered and darkened), and the brown inner wing surface (Fig. 1). We recorded reflectance perpendicular to the wing surface and relative to a white spectralon standard (Ocean Optics, Dunedin, FL). All reflectance spectra were recorded with SpectraSuite software (Ocean Optics, Dunedin, FL) from a bifurcated reflectance probe (Ocean Optics, Dunedin, FL) connected to a USB2000 spectrometer (Ocean Optics, Dunedin, FL). We recorded reflectance from 300 nm to 800 nm (UV to infrared). The spectra were then processed in R (cran) with the Pavo package (Maia et al., 2013). We extracted total reflectance (i.e. the total number of photons, calculated as the integral of the spectrum from 300 to 800 nm) for each patch (i.e. distal brown, unaltered band, proximal brown, and darkened band) for each individual.

Mounted butterflies were positioned and allowed to thermally equilibrate to room conditions of illumination and temperature, which was maintained within a half degree of 27 °C. Room illumination consisted of a single fluorescent light and had no detectable thermal effect, which we confirmed before each trial by measuring the surface temperature using a non-contact thermal gun (AR550, Smart Sensor, Intel Instruments) at four locations: external thorax, proximal side of band, band, and distal side of band. These temperatures were statistically no different from the



**Fig. 2.** Irradiance spectra of the experimental conditions and natural lighting conditions of *A. fatima*. A) Absolute irradiance in units of energy for the natural lighting of *A. fatima* habitat (green), unfiltered lamp (blue), and filtered lamp (red). B) Normalized irradiance for each condition. Normalized irradiance was calculated by dividing each energy value (binned by one nanometer) by the sum of all the energy values for each spectrum. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

air temperature and internal thoracic equilibrium temperature (repeated measures ANOVA;  $F_{4, 50} = 1.29$ ,  $p = 0.286$ ).

The trials consisted of heating each butterfly two times, once not-manipulated and then again with the darkened band, under a lamp (60 watt halogen, Satco S2248, Brentwood, NY) placed 28 cm above the butterflies. The light source was directly overhead of the two specimens, and light was passed through two band pass filters, one short wavelength transmissive and one long wavelength transmissive (L176R and L503R, Rosco Laboratories Inc, Stamford, CT), to achieve a spectrum closely resembling natural light (see Fig. 2 for irradiance spectra). Butterflies were heated for 15 min, which was sufficient for all samples to attain thermal equilibrium. Internal thoracic temperature was recorded each minute during the trial. Before ending the trial, surface temperatures were again recorded with the non-contact thermal gun. After 15 min, the lamp was turned off and butterflies were allowed to reequilibrate to room temperature (27 °C). We then painted each band black with a permanent marker (Sharpie, fine point, Downers Grove, IL). No obvious damage to wings occurred during the marking manipulation. Three spectral readings were taken of each artificially darkened band. The procedure was then repeated with the wings darkened: surface temperatures were recorded; butterflies were heated again for 15 min and internal thoracic temperatures were recorded at intervals of a minute: surface temperatures were recorded after 15 min. To control for body size, water loss, and order effects, post-trial weight and femur length were recorded at the end of each trial.

### 2.3. Wing ablation experiment

We selected four butterflies, in good condition, from the previous band manipulation experiment for the ablation experiment. They were weighed, then both wings were cut carefully along the proximal edge of the band, so each butterfly had only the portions of the wings proximal to the band intact. They were weighed, and then two butterflies were mounted on the thermocouples, on the same side of the lamp as they were in the previous experiment. After equilibration to room temperature (27 °C), surface temperatures were recorded from the thorax and the proximal wing, and the lamp was turned on. Internal thoracic temperature was

recorded every minute for 15 min, and after 15 min the surface temperatures were again recorded. Once all four had completed this trial, the butterflies were weighed, and their proximal wings were removed to leave only the thorax. These were weighed and mounted again for a final heating trial. The heating procedure was repeated exactly as before, but surface temperature was recorded only from the thorax. A final individual mass, without wings, was collected.

### 2.4. Calculations and statistics

The internal thoracic equilibrium temperature was calculated as the mean of the final three recorded values. Following Kammer and Bracchi (1973), we calculated the rate of heating as the slope of the internal thoracic temperature after the temperature had climbed higher than 3.2 °C above ambient. To ensure that this measurement correctly measured warming rate, we also calculated the rate of heating as the slope during the first two minutes of the trial and as a function of the time required to reach within one standard deviation of final equilibration temperature. As all metrics were statistically the same, we present only the first. The total reflectance was calculated as the mean of the proximal and distal integrals of each spectra. All data analyses were performed with the open software R (R Core Team, 2014) and the lme4 package.

We used generalized linear mixed models to compare the effects of wing darkening on the final equilibrium temperature and the rate of heating. We included pre-trial mass, femur length, and total reflectance as independent variables. Two random variables were also included: individual to control for the repeated measures design, and position on the right or left side of the lamp to control for possible unequal heating. To compare external temperatures recorded at the thorax, proximal wing, band, and distal wing, we used the same model but included a comparison of pre- and post-trial temperatures. Significance of fixed factors were calculated using Satterthwaite's method of denominator synthesis to generate the correct error terms. Likelihood ratios were used to test the significance of random factors (Satterthwaite, 1946). The effects of ablation on the equilibrium temperature and rate of heating of the internal thorax were analyzed using a repeated

**Table 1**

Generalized linear mixed model analysis of the effects darkening the wing band on the internal equilibrium temperature, heating rate, and external temperatures taken at the thorax, proximal wing, band, and distal wing of *A. fatima* butterflies (n=12). Initial mass, femur length, and wing brightness were included as fixed factors, and the individual and position (right or left of center of lamp) were included as random factors. The significance of random factors was tested using likelihood ratio tests. [Satterthwaite's \(1946\)](#) method of denominator synthesis was used to calculate the appropriate error terms.

	Type of factor	df effect	MS effect	Error df <sup>a</sup>	Error MS <sup>a</sup>	F/x <sup>2</sup>	p
<b>Equilibrium temperature</b>							
Band darkening	Fixed	1	0.02	11.00	0.32	0.07	0.79
Initial mass	Fixed	1	0.19	7.01	0.32	0.62	0.45
Femur length	Fixed	1	0.02	7.00	0.32	0.08	0.77
Brightness	Fixed	1	0.16	7.00	0.32	0.50	0.49
Individual	random	1	–	–	–	0.71	0.40
Position	random	1	–	–	–	9.87	<b>0.002</b>
<b>Heating rate</b>							
Band darkening	Fixed	1	0.46	11.00	0.035	13.40	<b>0.004</b>
Initial mass	Fixed	1	0.05	7.07	0.033	1.51	0.25
Femur length	Fixed	1	0.01	7.00	0.033	0.03	0.87
Brightness	Fixed	1	0.001	7.00	0.005	0.20	0.66
Individual	random	1	–	–	–	4.81	<b>0.03</b>
Position	random	1	–	–	–	2.18	0.14
<b>External thorax</b>							
Band darkening	Fixed	1	2.13	34	0.39	5.4	<b>0.02</b>
Initial mass	Fixed	1	0.40	8	0.40	1.0	0.34
Femur length	Fixed	1	0.02	8	0.40	0.05	0.84
Brightness	Fixed	1	1.21	8	0.39	3.1	0.11
Pre/post comp.	Fixed	1	1524	1	0.39	3881	< <b>0.001</b>
Individual	random	1	–	–	–	1.89	0.2
Position	random	1	–	–	–	< 0.001	1.0
<b>Proximal wing</b>							
Band darkening	Fixed	1	1.27	34.00	0.96	1.32	0.25
Initial mass	Fixed	1	0.12	7.08	1.00	0.12	0.73
Femur length	Fixed	1	0.04	7.00	1.00	0.04	0.84
Brightness	Fixed	1	2.43	7.00	0.96	2.53	0.15
Pre/post comp.	Fixed	1	2165	34.00	0.96	2249	< <b>0.001</b>
Individual	Random	1	–	–	–	0.98	0.3
Position	Random	1	–	–	–	1.54	0.2
<b>Band</b>							
Band darkening	Fixed	1	0.3	64	1.67	0.18	0.67
Initial mass	Fixed	1	1.1	8	1.48	0.74	0.41
Femur length	Fixed	1	0.1	8	1.11	0.09	0.77
Brightness	Fixed	1	1.7	8	1.53	1.11	0.32
Pre/post comp.	Fixed	1	3770	34	1.53	2459	< <b>0.001</b>
Individual	Random	1	–	–	–	1.23	0.3
Position	Random	1	–	–	–	0.00	1.0
<b>Distal wing</b>							
Band darkening	Fixed	1	0.3	41.00	2.98	0.1	0.74
Initial mass	Fixed	1	1.9	41.12	2.37	0.8	0.37
Femur length	Fixed	1	1.1	41.00	2.21	0.5	0.49
Brightness	Fixed	1	4.4	41.00	2.31	1.9	0.17
Pre/post comp.	Fixed	1	7397	41.00	2.32	3186	< <b>0.001</b>
Individual	Random	1	–	–	–	0.00	1.00
Position	Random	1	–	–	–	8.89	<b>0.003</b>

measures analysis of variance followed by a paired t-test with alpha error correction if significance was found.

### 3. Results

#### 3.1. Wing reflectance

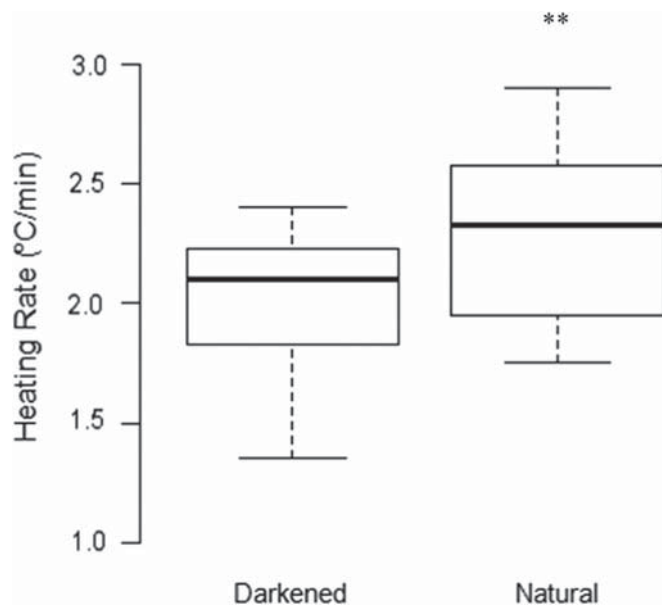
The total reflectance was significantly different between the four patches ( $F_{3,11} = 116.2$ ,  $p < 0.001$ ; [Fig. 1](#)), and post-hoc analyses revealed that the total reflectance of only the non-manipulated band was significantly different from other patches (i.e. distal, darkened band, and proximal). The non-manipulated band was

much brighter than the darkened band, with mean total reflectance of 143.5 and standard deviation of 28.51, while the darkened band had a mean total reflectance of 32.47 and standard deviation of 21.15.

#### 3.2. Band manipulation experiment

The initial masses of the butterflies were  $0.74 \pm 0.03$  g. Femur length was  $4.38 \pm 0.31$  mm. The internal thorax heating rate was faster in the darkened wing band treatment than the non-manipulated wing ([Table 1](#); [Fig. 3](#)) and was significantly different across individuals ([Table 1](#)). Each butterfly reached an internal thoracic equilibrium temperature that was 12 °C warmer than





**Fig. 3.** Heating rates for the two treatment groups in experiment one. Asterisks indicate a significant difference ( $p < 0.01$ ) between natural and manipulated conditions (Table 1).

ambient temperature, Fig. 4B and C. However, internal thoracic equilibrium temperature did not differ between the non-manipulated band and darkened wing manipulation (Table 1; Fig. 4A), although the position under the lamp did have a significant effect (Table 1). All parts of the wing increased in temperature during the trial (pre/post comparison in Table 1); however, there were no temperature differences between the two treatments for the wing surface, whether distal (Table 1; Fig. 4A), on the band (Table 1; Fig. 4A), or the proximal wing (Table 1; Fig. 4A). However, darkening the wing did increase the equilibrium temperature attained by the external thorax (Table 1; Fig. 4A). Femur length and pre-trial mass had no significant effect on any dependent variables. Average mass lost during heating was the same, approximately 6%, for both natural and darkened heating trials.

### 3.3. Wing ablation experiment

Full wing ablation significantly decreased the equilibrium temperature attained by the internal thorax ( $F_{3, 9} = 14.8$ ,  $p < 0.001$ , Figs. 5A and 6), but partial wing ablation had no significant effect on equilibrium temperature ( $F_{3, 8} = 0.18$ ,  $p = 0.91$ , Figs. 5A and 6). When the entire wing was removed, the internal equilibrium temperature of the thorax was about two and a half degrees less than the natural unaltered butterflies (a decrease of 6% from unmanipulated equilibrium temperature), while partial wing ablation was only half a degree less (a decrease of 1% from unmanipulated equilibrium temperature). Paired t-tests showed a significant difference between butterflies having darkened wings and completely ablated wings (Table 2; Figs. 5A and 6). There was no significant effect of the ablation experiment on the rate of heating (Figs. 5B and 6).

## 4. Discussion

We predicted that darkening the wing band would have minimal effects on the internal thoracic equilibrium temperature and the rate of heating in *Anartia fatima*. We did find that the band slows the rate of heating in *A. fatima*, but we did not find any

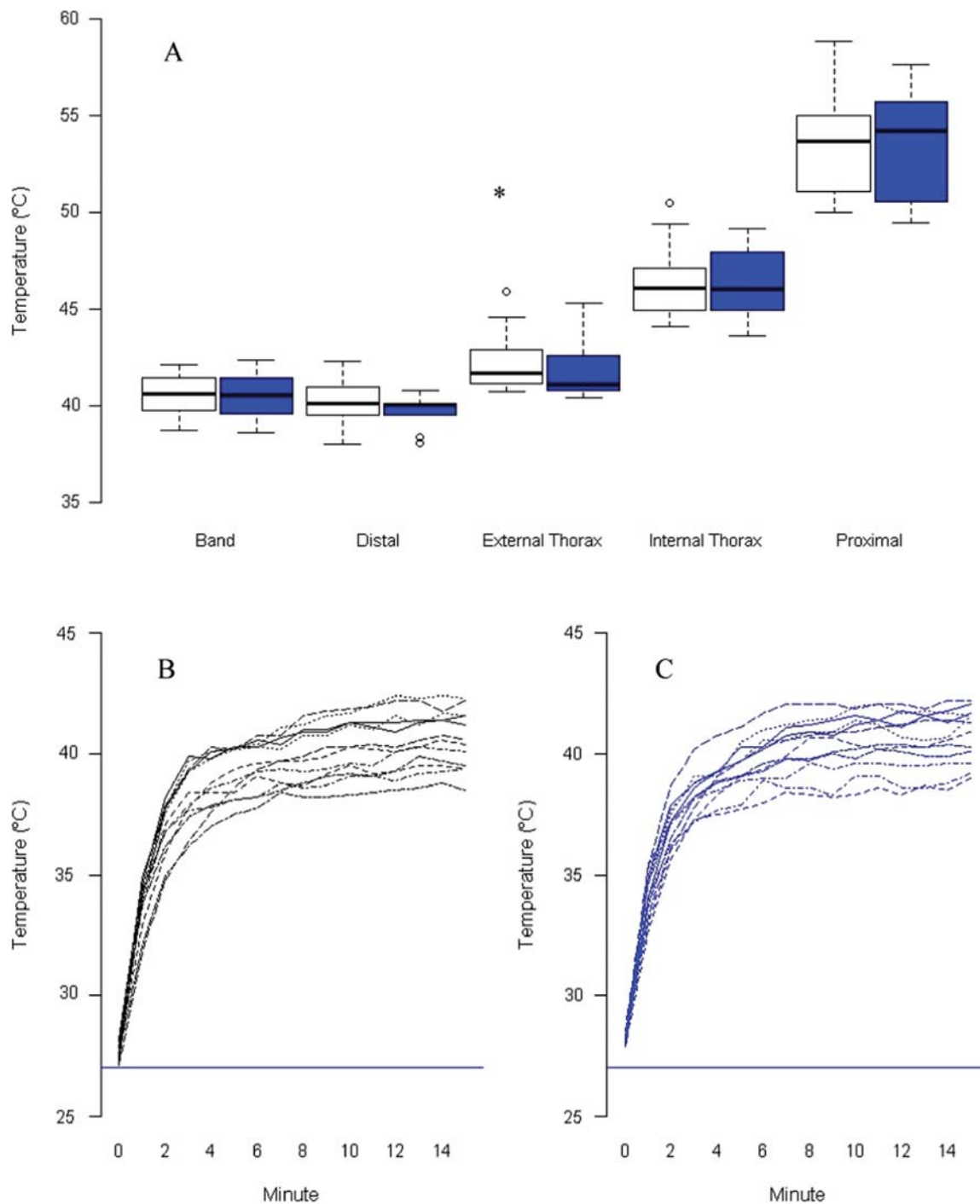
differences between the two treatments for equilibrium temperatures. Our results support previous findings by Kammer and Bracchi (1973) and Wasserthal (1975) in that coloration further than 3 mm away from the thorax has a minimal effect on internal thoracic temperatures. And like Wasserthal (1975) and Kammer and Bracchi (1973), we demonstrated that only the proximal portion of the wing is important for internal thoracic temperatures.

It must be noted that although we found an increased rate of heating after the darkened band manipulation, this result may be an order effect and caused by a decreased thermal inertia from evaporative water loss during the first heating trial. And again, this supports our initial hypothesis that the bands have minimal thermal effect. That is, when the plane of *Anartia fatima* dorsal wing surfaces are perpendicular to a light source mimicking the sun, with very little air moving across their bodies, and even accounting for a decreased thermal inertia, darkening their wing band does not allow them to reach a higher thoracic temperature, and it increases their rate of heating by less than 0.5 °C/min.

Surprisingly, there was no significant effect of femur length, mass, and non-band wing total reflectance on equilibrium temperature and heating rate, although these metrics have been shown to have a strong effect on these variables in other studies (Berwaerts et al., 2001; Gilchrist, 1990; Kingsolver, 1987; Watt, 1968). In the Baltimore checkerspot (*Euphydryas phaeton*), females are better able to maintain body temperatures than males, which are half the size of females (Gilchrist, 1990). It is likely that we did not find a difference in thermoregulatory ability dependent upon size in *A. fatima* due to the similarity in size of the collected individuals. Although there is variation in size between individuals, *A. fatima* does not have large size variation between individuals of the same sex. However, females and males do differ in size and thus, there may be differences in thermal properties of males and females. Lastly, Huey et al. (2003) provide a theoretical construct on how thermoregulatory function may not be a leading driver of morphology because organisms can mediate morphological constraints through behaviors.

The position of the butterflies, whether to the right or left of center in the paired trials, had a significant effect on equilibrium temperature and distal wing temperature (Table 1), indicating that the lamp provided uneven heating. We provided for this effect experimentally by randomizing the assigned position of each butterfly and statistically by including it as a random factor in our model to account for the variance. Similarly, to account for the variance produced by taking temperatures before and after heating, we included pre- and post-comparisons as a fixed factor in our model; its significance verifying that each part of the butterflies increased in temperature (Table 1).

Our findings support both experimental and observational studies showing that wings play a minimal role in thermoregulation of internal thoracic temperature (Kammer and Bracchi, 1973; Wasserthal, 1975). The results of wing ablation in our experiment showed that removing the distal portion of the wing, including the band, made no difference compared to intact wings. We only found a thermal difference when we removed the entire wing from an individual and this could be due to water loss from the thorax, more than a result of radiation absorption of the wing. Furthermore, due to the small sample size of the wing ablation experiment ( $n = 4$ ), we may have not detected differences between the natural wing and the wing partially removed. Kammer and Bracchi (1973) showed that wing circulation does not play a role in heat conduction from the wings, and melanization had no effect on heating rates in the small lycaenid butterfly, *Polyommatus icarus* (De Keyser et al., 2015). Our results support the overall conclusion that absorption of energy by the thorax is more important than absorption by the whole wing, and that the thorax is the primary driver of temperature in lighter and darker butterflies



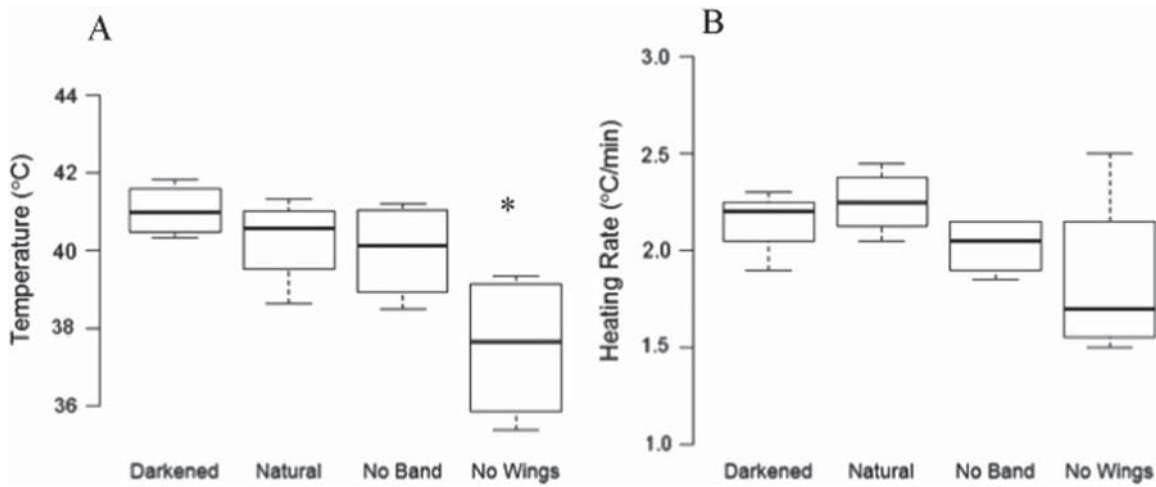
**Fig. 4.** (A) Equilibrium temperature recordings for each location for the un-manipulated treatment (uncolored) and for the Sharpie darkened treatment (blue). The internal temperature recordings over the 15 min trials for each individual for the un-manipulated treatment (B) and for the Sharpie darkened treatment (C). Blue line is air temperature. Asterisk indicates a significant difference ( $p < 0.05$ ) between natural and manipulated conditions (Table 1). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

under natural conditions (Heinrich, 1972).

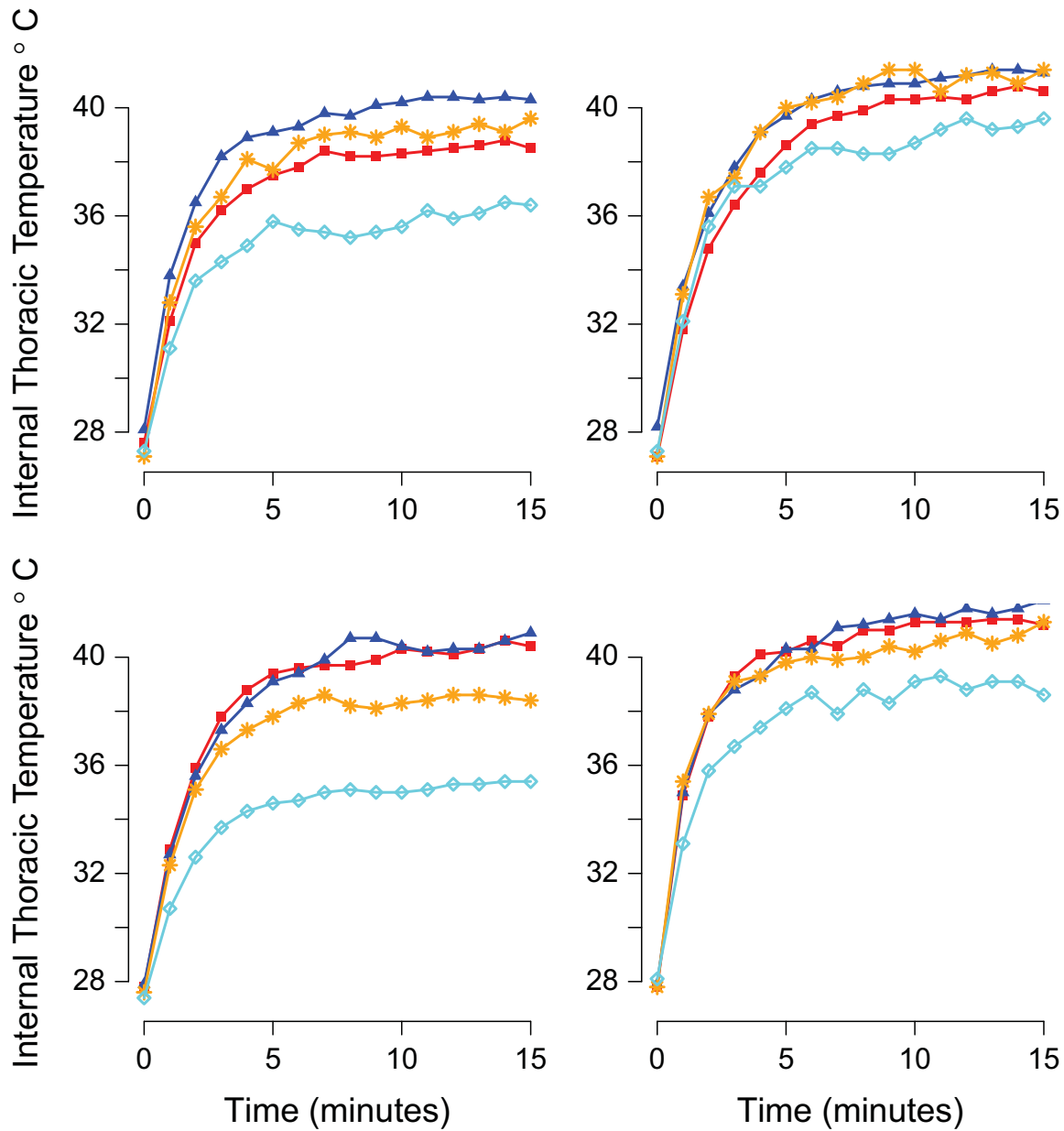
In a natural environment, the effects of convection are likely much stronger than any band heating effect. Convection can reduce heat load dramatically so that even in a species where there is a dark morph, for example in the speckled wood butterfly (*Pararge aegeria*), the dark morph engages on longer flights that make it consistently have a lower body temperature than the lighter morph, despite heating faster under controlled conditions (Van Dyck and Matthysen, 1998). We attempted to repeat our experiment in the meadow adjacent to the field station and were

unable to get butterflies to reach an internal thoracic equilibrium temperature (i.e. the internal temperature reaches a horizontal asymptote) due to the effects of a mild breeze.

Two observations, that minimal convection removed the thermal effect of the band when we attempted to reproduce the experiment in a meadow, and that ablation up to and including the band did not affect equilibrium temperature (Table 1; Fig. 5A) or rate of heating (Table 1; Fig. 5B), warrant further research on the ecological role of the band in *A. fatima*. Although no published research has investigated thermoregulatory behavior in *A. fatima*,



**Fig. 5.** Equilibrium temperatures (A) and heating rates (B) for the four treatment groups in experiment two: wing ablation. Asterisk indicates a significant difference ( $p < 0.05$ ) between butterflies with the band darkened and butterflies without wings (Table 2).



**Fig. 6.** The four treatments of the wing ablation experiment for the four individuals. Each graph shows the four treatments groups for one individual. The treatment groups are: not manipulated (red squares), darkened band (blue triangles), band and distal brown cut away (orange asterisks), wing cut away (cyan diamonds).

**Table 2**

Results from post-hoc analysis, using holm alpha correction, of paired t test comparisons of internal equilibrium temperatures and heating rates when *A. fatima* butterflies (n=4) had their natural wings, the wing band darkened, the wing ablated just past the band, and the wing removed.

	Natural wing	Band darkened	Ablated to band
<b>Ablation equilibrium temperature</b>			
Natural wing	–	–	–
Band darkened	0.23	–	–
Ablated to band	0.67	0.23	–
Wing removed	0.16	<b>0.03</b>	0.07
<b>Ablation heating rate</b>			
Natural wing	–	–	–
Band darkened	0.94	–	–
Ablated to band	0.19	0.40	–
Wing removed	0.75	0.94	0.94

*A. fatima* individuals do perch with wings held at varying and ever changing angles and this may serve a thermoregulatory function as shown in other butterflies (Barton et al., 2014; Berwaerts et al., 2001). Of course, the slower rate of heating due to the occurrence of a band, coupled with changes in wing position could be adaptive for *A. fatima*. *Anartia fatima* occupies open tropical habitats that are exposed to bright sunshine where the daily ambient temperatures can exceed 37 °C and in captive settings, individuals are very susceptible to dying from heat stress and dehydration (Seymour personal observation). Having a band that would slow the rate of overheating would allow *A. fatima* to spend more time searching for nectar sources, mates and host plants. Alternatively, the band may function in warming *A. fatima* individuals through reflective basking, in which solar radiation is reflected from light wing patches onto the thorax through behavioral adjustment of body position (Kingsolver, 1987). Now that there is evidence that a wing band has thermal effects, further research into the effects of a light band on thermoregulation in the field is needed.

Bands can serve ecological functions other than just thermoregulation. Emmel (1972) demonstrated that males use the wing band for selecting mates and Taylor (1973) showed that the total reflectance and color of the band can be informative of individual age as the wing band fades over time. Recently, Seymore and Aiello (2015) supported the role of the band functioning as disruptive crypsis, as individual models with a natural band were less likely to be attacked than models that had the band moved to the wing margin. Therefore, *Anartia fatima* has many selective pressures acting upon its coloration and the three pressures mentioned above (thermoregulation, mate choice, and crypsis) may all contribute to advantages conferred by the wing band.

## 5. Conclusions

We tested the thermoregulatory role of the wing band in *Anartia fatima* and found that the band had an effect on the heating rate of the thorax but no effect on internal thoracic temperature equilibrium. We further supported that wings have a minimal effect on thermal properties by showing that when wings are removed, the thorax has a lower internal temperature. The equilibrium findings align with previous studies showing that wing coloration has a minimal effect on internal thoracic temperatures. However, the effect of band on heating rate is a novel find and urges further research into the thermoregulatory role of wing bands in butterflies, especially behavioral experiments in the field.

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