



Warm up and spread your wings: thermoregulation for flight in the stag beetle *Lucanus cervus*

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ABSTRACT

Flight in insects is a highly energy-demanding activity, requiring precise thermoregulation to ensure optimal muscle performance. In the European stag beetle *Lucanus cervus*, males exhibit a significant degree of morphological variation, with larger individuals possessing exaggerated mandibles that may hinder agility and increase energetic costs. This study investigates the thermoregulatory mechanisms employed by *L. cervus* males to prepare for and recover from flight, using thermal imaging. Field observations revealed a distinct pre-flight warmup phase characterized by increased pterothoracic temperature. The temperature in this region, housing the primary flight muscles, reached over 30 °C, 24 while abdominal temperatures remained close to ambient temperatures. Thermal imaging across three flight phases (i.e., stand-by, pre-flight, and post-flight) showed a significant temperature increase in the pterothorax. μ CT analysis unveiled a sophisticated tracheal system in which the detailed submillimetric features of the air channels are consistently reproduced across individuals, while the spatial architecture at the whole-body scale varies individually. These observations suggest that tracheal organization may play a role in heat transfer between body regions, potentially contributing to the preservation of muscle efficiency and the protection of vital organs from overheating. However, the functional significance of this variation remains to be experimentally verified. Additionally, field data indicated peak flight activity between 19:30 and 21:00. These findings provide the first evidence of thermoregulatory behavior in *L. cervus*, highlighting the role of morphological and physiological adaptations in flight performance.

1. Introduction

Flight is one of the most energetically demanding activities in insects, requiring precise thermoregulation of the thoracic musculature to ensure optimal performance. The thoracic temperature during flight reflects the balance between metabolic heat production by flight muscles and heat exchange with the environment (Heinrich, 1980; May & Casey, 1988; Harrison et al., 1996; Harrison and Fewell, 2002). The aerodynamic and biomechanical constraints on insect flight have been extensively characterized (Ellington, 1984a, 1984b; Dudley, 2002), providing a fundamental framework for understanding how insects

modulate metabolic heat production through adjustments in wing kinematics, including alterations in wingbeat frequency to regulate body temperature during flight (Roberts and Harrison, 1999; Harrison and Fewell, 2002). The thermal performance properties of flight muscle further explain how metabolic performance changes with temperature (Glass and Harrison, 2024a; Glass et al., 2024b; Glass et al., 2024c; Glass and Harrison, 2024b). More recent work has begun to integrate these biomechanical and physiological dimensions with biophysical approaches. Thoracic temperatures are actively elevated during pre-flight warming documented across diverse insect taxa including bees, butterflies, and beetles (Oertli, 1989; Stone, 1994; Heinrich, 1995;

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Bartholomew and Casey, 1977; Scholtz, 2000; Verdú et al., 2004, 2006, 2012, 2019; Zermoglio et al., 2018) and heat generated in the thorax can be redistributed to other body regions to facilitate dissipation during flight (Sepúlveda-Rodríguez et al., 2024). Additionally, airflow generated during flapping flight may contribute to convective heat loss (Glass et al., 2026). Understanding insect flight thermoregulation therefore requires integrating perspectives from thermal physiology, aerodynamics, and biophysics. The thermal performance properties of flight muscle have also been proposed as a key determinant of how metabolic performance changes with temperature (Glass and Harrison, 2024a; Glass et al., 2024b; Glass et al., 2024c; Glass and Harrison, 2024b; Glass & Harrison, 2024 a,b). Most recently, it has been demonstrated that the airflow induced by flapping wings during hovering flight may contribute meaningfully to convective heat loss, directly influencing the thermal balance of flying insects (Glass et al., 2026). Taken together, these advances highlight a rapidly developing research framework in which flight kinematics, muscle physiology, and physical heat-exchange processes interact to shape body temperatures during flight a framework within which the present study on *Lucanus cervus* can be productively situated. Despite the wealth of information available for other taxa, thermoregulation during flight has never been studied in the European stag beetle *Lucanus cervus* (Coleoptera: Lucanidae), the largest saproxylic beetle in Europe and a charismatic flagship species protected under the European Habitats Directive both females and males use active flight for dispersal and mating (Romiti et al., 2017; Giannetti et al., 2023, 2024; De Zan et al., 2023). Behavioural thermoregulation, including basking, shade-seeking, and metabolic modulation, may further contribute to optimizing physiological performance across diverse environmental conditions (Merrick and Smith, 2004; Verdú et al., 2004, 2006, 2012, 2019; Goyens et al., 2015a; Goyens et al., 2015b; Goyens et al., 2016; Zermoglio et al., 2018). Despite this growing body of knowledge, no study has so far been carried out on the thermoregulatory activity of *L. cervus*. In the present study, we analysed thermoregulation activity and the presence of pre-flight warming in the stag beetle *Lucanus cervus*. To obtain information on daily activity patterns, we conducted thermal monitoring using a thermographic camera. Specifically, we addressed two main objectives: (1) to determine whether *L. cervus* exhibits active pre-flight warming through endothermic muscle contractions prior to flight, characterising the thermal dynamics of this behavior across body regions; (2) and we also performed descriptive analysis with micro-computed tomography (μ CT) to explore the mechanism of pre-flight activity and the morphological and physiological traits that enable to maintain effective thermoregulatory performance during flight.

2. Materials and methods

2.1. Study area

Field experiments were conducted in Northern Italy (Carrega Regional Park, Parma Province, Emilia-Romagna Region). The area has been protected since 1982, when the first Regional Natural Park was established, and it is designated as a Special Area of Conservation (SAC IT4020001) under the EU Habitats Directive. It covers approximately 1270 ha between the Taro River and the Baganza Stream, largely dominated by deciduous woodland that represents a significant forest remnant within the highly modified agricultural landscape of the Po Plain. Before its designation as a protected area, the woodland was managed as coppice for roughly a century. Within this Park, we selected Bosco delle Capannelle (44.730389, 10.211556), a 0.15 km² area with an elevation ranging from 130 to 155 m characterized by a deciduous oak forest dominated by *Quercus cerris* L. and *Q. Robur* L., as our main study site based on previous assessments of a high density of *L. cervus* individuals (Giannetti et al., 2023, 2024, 2026).

2.2. Thermoregulation of flight activity

Inside the study area, we identified 10 transects (10 × 2 m). Each transect was enclosed on the sides with garden shade cloth and covered on top with a transparent sheet. We monitored male beetles four times per week from June 12, to July 13, 2024, and again from June 12, to July 13, 2025, by two operators. Within each transect, male *Lucanus cervus* individuals were identified and marked following Giannetti et al. (2023). Each male ($n = 16$) was followed for the entire flight time. We captured three thermal images per individual representing three phases: stand-by, pre-flight, and post-flight: stand-by (1 h before flight), pre-flight (at the moment of elytra opening and wing deployment), and post-flight (after landing, wing folding, and elytra closure).

To perform the measurements, we characterized the flight phases:

- Stand-by. Individuals remain motionless on their substrate of choice for several hours. In this phase, the insect is either perpendicular to the resting surface or completely lying on the substrate.

- Pre-flight. Individuals begin to move upward, selecting leaf-less portions of vegetation. During this phase, individuals turn laterally toward the most open frontal take-off point, flexing their hind legs. The body becomes inclined relatively to the resting surface, the elytra open, wings unfold, the head slightly bends, and take-off occurs.

- Post-flight. After flying for a variable amount of time, individuals land on different parts of the vegetation and fold their wings and elytra.

Temperature measurements of male bodies were conducted with the thermal camera (FLIR-FlukeTiR 110 thermal camera [Detector resolution: 160 × 120 (19,200 pixels); Thermal sensitivity: ≤ 0.08 °C at 30 °C target temperature (80 mK); Temperature measurement range: -20 °C to $+150$ °C (-4 °F to $+302$ °F). Furthermore, using SmartView software, temperatures were extracted by measuring along the whole body, a point in the head, thorax, prothorax, pterothorax, and abdomen regions (Fig. 1a). Specifically, we considered the pterothorax as the portion extending from the junction of the prothorax to the midpoint of the elytra, which coincides with the beginning of the abdominal segments (Raš et al., 2024). Moreover ambient temperature was simultaneously recorded using a datalogger deployed within the transect.

2.3. Thermal camera to analyze daily flight activity

To analyze pterothorax temperature at different times of the day, field measurements using a thermal camera were conducted. Within the study area, operators walked along four transects, each 500 m long, three times per week from 7:00 PM to 12:00 AM during June and July of 2023 and 2024. For this analysis we used independent stag beetle males ($n = 120$). Each individual was measured at a distinct location along the transect by the operators, ensuring that no individual was recorded more than once. For each one we acquired a single thermal image at the time of discovery, and recorded air temperature using a datalogger (Elitech rc-5+ te).

2.4. Micro-computed tomography to describe the internal structure of the pterothorax

For the micro-computed tomography (μ CT) analysis two males of different sizes were selected, corresponding to the larger and smaller morphotypes (A: Mandible Length: 17.03 mm; Head Length: 12.0 mm; Head Width: 20.06 mm; Pronotum Length: 9.61 mm; Elytron Length: 29.16 mm / B: Mandible Length: 12.06 mm; Head Length: 11.0 mm; Head Width: 10.03 mm; Pronotum Length: 5.9 mm; Elytron Length: 17.06 mm (Romiti et al., 2015, 2016)). The samples were maintained in ice-cold insect Ringer's solution (Verdú et al., 2012) and at the time of μ CT they were dried, enclosed in Parafilm and taped to a sample holder. μ CT scans were carried out at a nominal pixel size of 16 μ m by means of a Bruker SkyScan μ CT 1276 scanner (Skyscan, Kontich, Belgium) at 55 kV and 72 μ A, using a 0.25 mm aluminium filter. Two images were captured and averaged every 0.4° through 180° of rotation, applying a camera

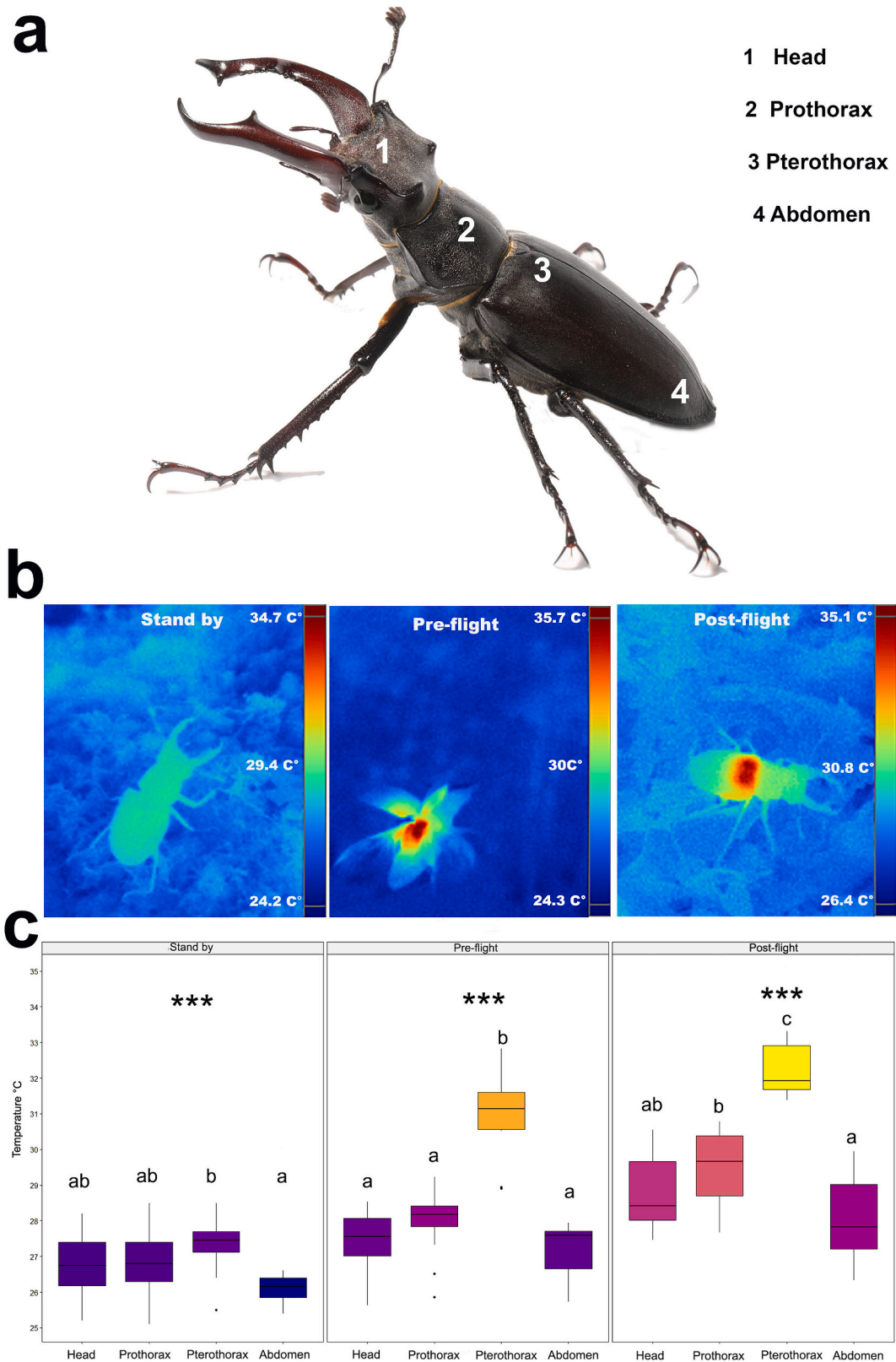


Fig. 1. a) Male of *Lucanus cervus* showing the body sections investigated in our research; b) example of thermal images of one male during the three phases of flight (stand-by, pre-flight, and post-flight phases); c) the graph shows the results of the linear mixed-effects model (LMM) describing temperature differences among body regions during the three phases of flight. Notably, the significant interaction between body region and flight phase is primarily driven by the localized and substantial temperature increase in the pterothorax during the pre-flight and post-flight phases. In the boxplots, the horizontal line represents the median, the box spans the interquartile range (IQR, 25th to 75th percentiles), and the whiskers extend to the most extreme data points within $1.5 \times \text{IQR}$ from the box edges. Individual isolated dots represent outlier values. The letters on the box plots show the results of post-hoc pairwise comparisons (estimated marginal means with Bonferroni correction); boxes sharing the same letter are not statistically different. (***) if $p < 0.001$.

pixel binning of 2×2 . Multiple connected scans were required in order to image the whole body of each insect, yielding a total scan time of 1.5–2 h. The reconstruction was performed with the SkyScan NRecon 2.2 software using the InstaRecon reconstruction engine, ring artifact reduction, beam hardening correction, matching and fusion of the scans. Image analysis was executed by means of the Skyscan CT Analyser 1.20.8.0+ (CTAn) software. In particular, the body tissues, including hypodense liquids and hyperdense deposits in the abdomen, were segmented by means of a global thresholding of the attenuation coefficient, set equal or greater than 2.358×10^{-2} (1/mm). The whole-body mask of the insects, i.e. all the volume enclosed by the external body surface and constituting the Volume of Interest (VOI) of the analysis, was thus obtained resorting to despeckle operations. The airways segmentation resulted from the subtraction of the tissue mask from the whole-body mask. Finally, morphometric, thickness and separation analyses in 3D and 2D were performed for the airways lumen inside the VOI (or the Region of interest in 2D, ROI). Volume rendered 3D images of the beetles' whole body and the internal airways were generated using the SkyScan CT-Vox (CTVox). Color-coded 2D histograms of the airways thickness and separation (i.e. body thickness, used afterwards) distribution along the Body Length (%) of the two insects were obtained from the 3D thickness maps of CTAn, by means of a custom-made script written in MATLAB23b. For aiding an easier visualization, the histogram matrix of each insect was rescaled to unit height, interpolated to achieve the same matrix size, smoothed and the contrast was enhanced by saturating the top 1–2.5% values.

2.5. Statistical analysis

Statistical analyses were performed by using the software R 4.0.3 (R Core Team, 2024). To account for the hierarchical structure of the data, arising from repeated measurements taken on the same individuals across different body regions and flight phases, we employed a Linear Mixed-Effects Model (LMM). In the model, 'body region', 'flight phase', and their interaction were treated as fixed effects, while 'individual identity' was included as a random intercept to account for within-subject correlations. Model adequacy was verified through residual diagnostics: visual inspection of Q-Q plots and a formal Shapiro-Wilk test were conducted to confirm that the assumptions of normality and homoscedasticity of the residuals were adequately met. Post-hoc pairwise comparisons with Bonferroni correction were used to assess specific differences between groups to analyze the relationship between body temperature in the three considered flight phases. To analyze the relationship between body temperature and detection time in flying specimens, a Generalized Additive Model (GAM) was employed (Hodgson et al., 2011). This approach allows for the description of non-linear relationships between variables, avoiding the imposition of a predefined functional form. Since temperature may vary over time in a curvilinear and non-constant manner, the GAM enables modeling of hourly variation through a penalized smoothing function using a cubic regression spline as the smoothing basis. To ensure the smooth term was not artificially constrained during model fitting, the maximum number of knots (k) was evaluated and set to 20. The adequacy of the model was assessed using the *gam.check* function, evaluating the significance of the smooth term, the proportion of deviance explained, and yielding an unconstrained fitted smooth term with 13.41 effective degrees of freedom (EDF). A paired Wilcoxon signed-rank test was conducted to comparing each beetle's surface temperature with the concurrently recorded ambient temperature.

3. Results

3.1. Thermoregulation of flight activity

The analysis was carried out on 16 males of *L. cervus* that performed an average fly-time (i.e., considering all the three phases) of 7.13 min

within the transect. While this sample size is relatively small for drawing broad generalizations at the species level, the repeated-measures design across multiple body regions yielded high statistical robustness. During continuous sampling observations, males did not perform any visible abdominal or wing movement during the pre-flight phase, despite thermographic analysis showing a significant localized increase in temperature. As explicitly suggested for other endothermic insects, this heat production likely reflects undetected flight muscle activity (i.e., shivering thermogenesis) to reach optimal flight temperatures without macroscopic wing kinematics. Specifically, we registered an increase of temperature in the pterothorax in the pre- and post-flight phases (Fig. 1 b,c,d) (Supplementary S1). The Linear Mixed-Effects Model (LMM) revealed a highly significant interaction between body region and flight phase ($F = 16.12, P < 0.001$), alongside significant main effects for body region ($F = 120.62, P < 0.001$) and flight phase ($F = 113.13, P < 0.001$). A simulation-based power analysis confirmed that the experimental design possessed >99% statistical power to detect this interaction, supported by exceptionally large effect sizes (Marginal $R^2 = 0.770$; Conditional $R^2 = 0.785$); Table 1; Fig. 1). However, we acknowledge that while this repeated-measures design provides robust statistical power to detect within-individual thermal changes across flight phases, the relatively small sample size of individuals ($N = 16$) limits broader inferences regarding population-level variability or morph-specific thermal differences.

The abdomen showed lower temperatures during all phases. Post-hoc pairwise comparisons with Bonferroni correction revealed that during the stand-by phase, there was no significant difference in temperature between the prothorax and the pterothorax ($P = 0.44$). However, during both the pre-flight and post-flight phases, the pterothorax became significantly hotter than the prothorax ($P < 0.001$ in both comparisons), confirming a localized heat generation mechanism specific to the flight muscle region.

3.2. Thermal camera to analyze daily flight activity

The results of GAM showed a statistically significant difference between the scan sampling (Edf = 13.41, $F = 36.51, p < 0.001, R^2 = 0.83$). Moreover, the GAM model showed a significant non-linear relationship between temperature and time. The estimated curve showed an irregular trend with period of increase and decrease in temperature. The check of the smoothing parameter (k -index = 0.83) confirms an adequate flexibility of the model. Specifically, we recorded a high temperature from 19:30 to 20:00 PM (Mean $\pm$ standard error: $31.6^\circ\text{C} \pm 0.52$) and from 20:00 to 20:30 PM (Mean $\pm$ standard error: $32.2^\circ\text{C} \pm 0.16$) (Fig. 2). According to previous results, the temperatures recorded from 19:30 to 20:00 PM may characterize specimens in pre-flight and post flight phases while, on the contrary, the temperatures recorded from 20:00 to 21:00 may be characteristic of specimens that have just flown (Supplementary S1). Moreover revealed that active males were markedly warmer than the environment ($V = 7021, P < 0.001$). This significant divergence definitively confirms the active thermoregulatory profile of the species during the evening flight period.

Table 1
Average temperatures across different body parts during the three flight phases of each 16 males (mean $\pm$ standard error). Ambient temperature was simultaneously measured using a datalogger placed within the transect.

Flight phases	Head C°	Prothorax C°	Pterothorax C°	Abdomen C°	Ambient temperature C°
Stand by	26.79 ± 0.19	26.76 $\pm$ 0.23	27.36 $\pm$ 0.19	26.20 $\pm$ 0.09	25.55 $\pm$ 0.17
Pre-flight	27.27 ± 0.19	27.77 $\pm$ 0.22	31.54 $\pm$ 0.35	27.10 $\pm$ 0.18	25.45 $\pm$ 0.19
Post-flight	28.31 ± 0.3	28.85 $\pm$ 0.36	32.23 $\pm$ 0.15	27.72 $\pm$ 0.30	25.75 $\pm$ 0.18

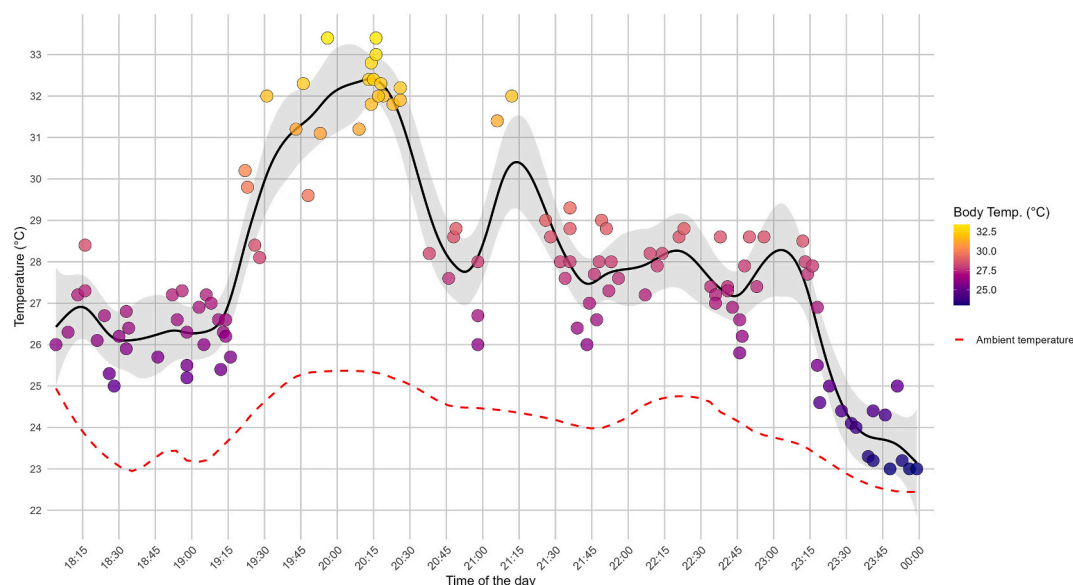


Fig. 2. The figure illustrates the temperature trend measured on the pterothorax of 120 individual male stag beetles from 18:00 to 24:00; in relation to ambient temperature (dashed line). The GAM analysis describes the pterothorax temperature trend across the sampled individuals, revealing a temperature increase in the time interval between 19:00 and 20:00.

3.3. Micro-computed tomography

The high-resolution μ CT images allowed for a very good 3D discrimination of internal body components from the air-filled tracheal system in the two scanned individuals (Supplementary materials and

Supplementary images Fig. S1, S2, S3). Overall, the analysis confirmed a highly developed respiratory architecture, with the Airways Volume accounting for approximately 27–34% of the Whole Body Volume. A highly detailed, quantitative description of all 2D and 3D morphometric parameters (including surface-to-volume ratios, structural thickness,

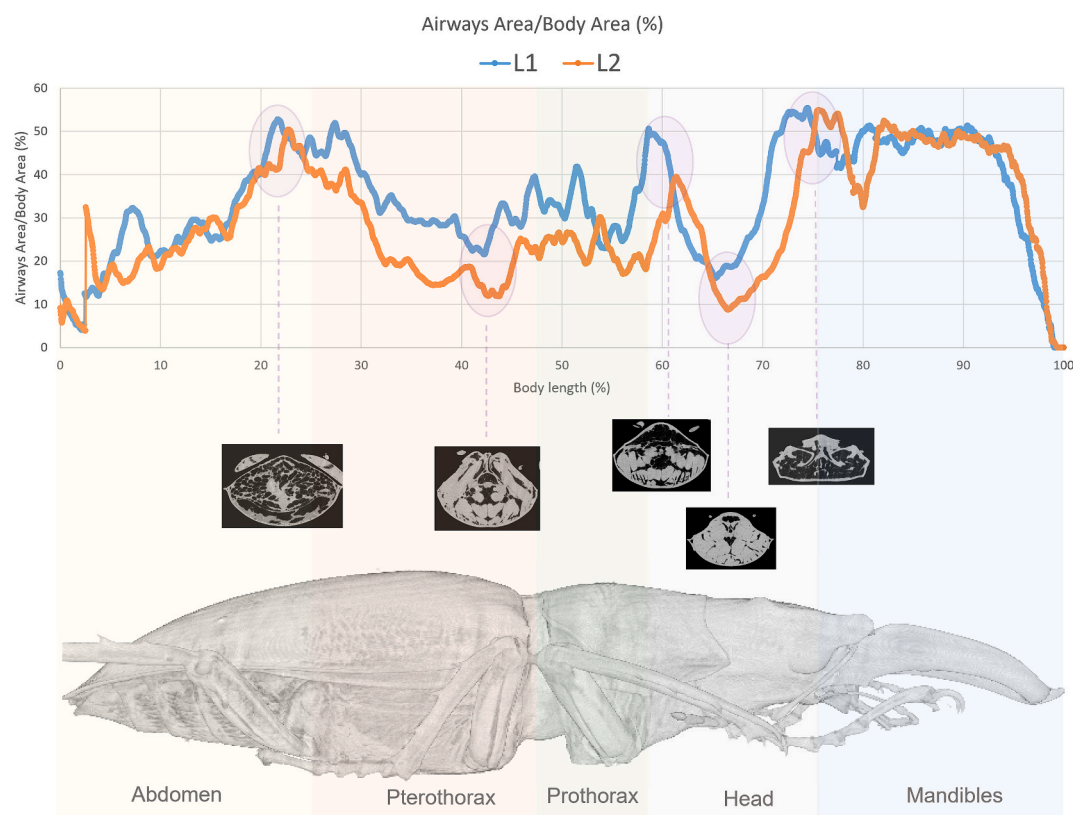


Fig. 3. Airways area/body area (%) in L1 and L2 obtained by the 2D μ CT analysis and expressed as a function of the Body Length (%), from 0%, corresponding to the endpoint of elytra, to 100% at the apex of the mandibles. μ CT images of the most distinctive features are displayed below the main graph at the same body level. The position of the five main body regions (abdomen, pterothorax, prothorax, head, mandibles) with respect to the Body Length (%) is highlighted with different colors over a 3D rendered body of one of the two insects, whose long axis is aligned to the x-axis of the graph, at the bottom of the figure.

and connectivity indexes) is fully reported in the Supplementary Tables 1 and 2. Biologically, the structural distribution of the airways along the body length revealed a highly heterogeneous network (Fig. 3).

In regions characterized by highly developed musculature, specifically the head and the pterothorax, the relative airways area reached its minima, as air is confined to fewer, well-separated cavities that directly supply the muscle bundles. Conversely, prominent peaks in airway density were localized in the boundary regions (e.g., between head and prothorax) and primarily in the abdomen. The abdominal region is characterized by a complex network of channels and an extended ventral air sac (Supplementary material fig. S3). This specific ventral compartment features very thin separating walls and irregularly distributed hypodense fluids of likely oily nature. While the average Airways Thickness across the entire body was consistently homogeneous (around 0.3–0.6 mm), it distinctly peaked over 1.1 mm in the abdomen due to the presence of these large ventral cavities (Fig. 4). Interestingly, body thickness and airways separation analyses highlighted wider muscular groups and increased spatial separation in the smaller individual, particularly in the thoracic regions (Fig. 5).

4. Discussion

The results made it possible to describe for the first time a warm-up behavior in *L. cervus* that aids the heating of the wing muscles, ensuring better flight condition capability. Pre-flight warming is a widespread behavior among bees and many butterfly species (Heinrich, 1995; Stone, 1994). This process involves a rapid and sustained increase in body temperature to achieve the optimal flight temperature. This warming is generally driven by the activity of thoracic muscles, generating heat by contraction (Bartholomew and Casey, 1977). This phenomenon has been observed in several other species, like the beetle *Cyclocephala signaticollis* (Scarabaeidae). For instance, in *C. signaticollis*, thoracic temperature can rise by approximately 9 °C above ambient temperature, averaging 34 °C to 39 °C (Zermoglio et al., 2018). A similar increase has

been documented in other beetles, such as *Strategus aloeus* (Scarabaeidae) and *Stenodontes molarium* (Cerambycidae), where thoracic temperature exceeds the ambient temperature by 5–7 °C during flight activity (Bartholomew and Casey, 1977). Heat transfer was observed in *Scarabaeus cicatricosus* (Scarabaeidae), which actively transfers heat from the thorax to the abdomen, where it can be dissipated more effectively through the body surface (Verdú et al., 2004; Verdú et al., 2012). The absence of differences in temperature between pre- and post-flight phases shows that this mechanism is aimed at reducing overheating, thus ensuring improved flight duration and capacity by maintaining a constant temperature even during prolonged flights. In *S. cicatricosus*, analyses using infrared light revealed a clear and effective heat flow between the thorax and abdomen as its main mechanism of thermo-regulation (Verdú et al., 2004). The relative differences between L2 and L1, particularly in the Airways Percentage of the Whole Body Volume (Obj.V/TV), indicate that the internal air-filled structures do not scale linearly with body size. The smaller individual shows a markedly lower Obj.V/TV and a higher Air Surface/Volume ratio (Obj.S/Obj.V), consistent with a general reduction in the size of the tracheal system. In contrast, the Number of Objects (Obj.N), as discrete air elements including both connected airways and unconnected cavities, decreases only slightly and their density (Obj.N/TV) limitedly increases from 3.07 to 3.34 1/mm³ (+9%). The spatial development across the body and the architecture of the air channels at the millimetric and submillimetric levels are comparable across individuals. Notably, in the smallest individual the Airways Area/Body Area (%) is appreciably reduced in the region corresponding to 25–70% of the Body Length (%), covering the pterothorax, prothorax and part of the head, while it is comparable to the larger individual in the abdomen and mandibles. In the same regions, airways separation is augmented. Overall, this pattern suggests that inter-individual variations in tracheal system volume occur mainly between the pterothorax and the head. These structural differences might suggest that heat dissipation capacity could vary along the body length; however, any inference about reduced capacity at the prothorax/

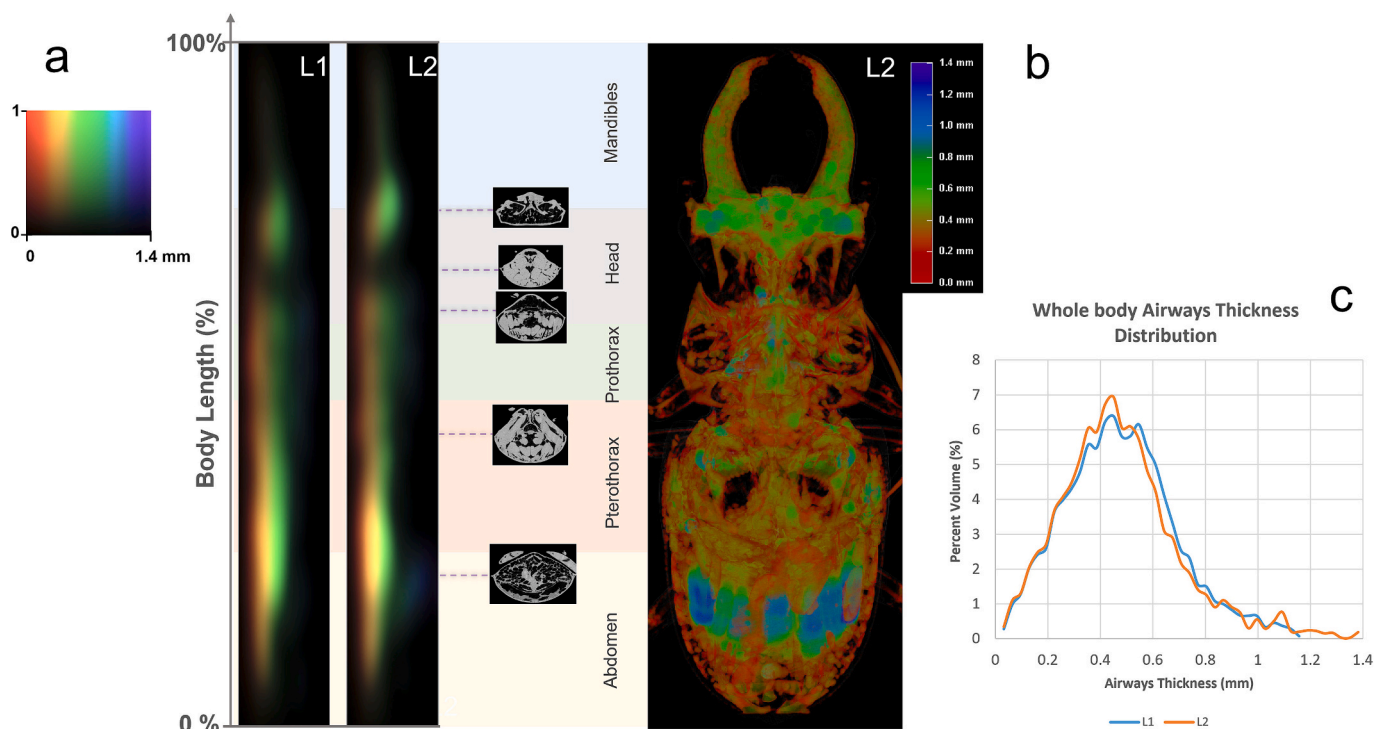


Fig. 4. a) 2D color-coded Airways Lumen Thickness distribution, including the relative colormap on the left. The colormap is common to the 3D color-coded rendering of the distribution in b); c) the Airways Lumen Thickness distribution in the whole body in both L1 and L2, indicating that the air channel size is very similar in the two insects.

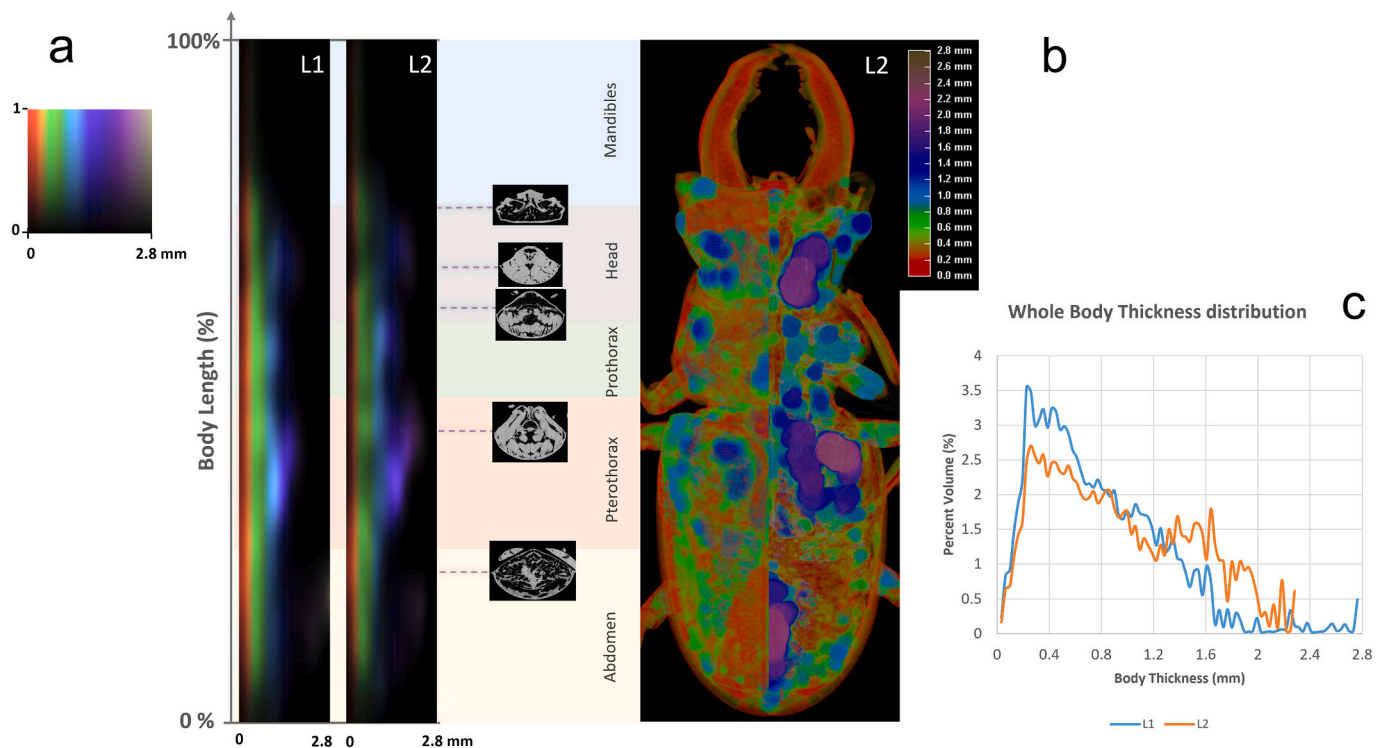


Fig. 5. a) 2D color-coded Air Separation Distribution, corresponding to the Body Thickness Distribution in the VOI, including the relative colormap on the left. The colormap is common to the 3D color-coded rendering of the distribution in b), where a cutting shape is applied to the right side of the insect body to undisclose the distribution values inside the body. c) the Body Thickness Distribution in the whole body in both L1 and L2, indicating that thickness values shift to higher values in L2.

pterothorax boundary requires further analysis of direct measurements of internal heat transfer or airflow. The air channel thickness distributions of the two individuals closely match across the body length, peaking around 0.48 mm. In both individuals, at the abdominal level, a network of small elliptic cavities is observed in the dorsal and lateral regions, coexisting with a large ventral air compartment partially filled with liquid. Whether this extended air sac represents an additional ventilation pathway, and whether it plays any role in heat dissipation during flight, cannot be determined from morphological data alone and requires direct functional investigation. Taken together, the morphological data provide a structural description of the airway network of *L. cervus*, though whether this organization reflects adaptations to body density or heat transfer needs cannot be determined from the present observations alone. Such an architecture could potentially contribute to limiting temperature increases in the head and abdomen, thereby reducing heat-dependent risk to the reproductive and nervous systems. However, since the present study does not directly measure heat transfer dynamics or airflow within the tracheal system, these observations should be regarded as a plausible hypothesis warranting future experimental investigation.

Moreover in our study, thermal trends of *L. cervus* specimens were recorded from 19:30 to 21:30, with a decrease in temperature and the eventual disappearance of thermal traces approaching midnight. Our results imply a narrower flight window in our study area than reported for this species elsewhere (e.g., [Tini et al., 2017](#)). The peak values correspond to the phases in which males were most frequently observed warming up or immediately after flight. Crepuscular thermoregulatory patterns have been documented in other large beetles, such as *Scarabaeus cicatricosus*, which maintains thoracic temperatures well above ambient during dusk flight ([Verdú et al., 2004, 2012](#)), and *Cyclocephala signaticollis*, where individuals show rapid thoracic heating before take-off ([Zermoglio et al., 2018](#)). Infrared thermography is a non invasive technique that allows to gather key behavioural and physiological

information without causing harm to species with a conservation status. Thermal imaging proved to be a highly valuable, non-invasive tool for monitoring *L. cervus*, allowing precise quantification of body temperature during different behavioural phases and across the daily flight activity. The ability to detect subtle thermal differences, demonstrates the sensitivity of thermography to describe physiological processes that are otherwise difficult to observe in the field. Similar applications have been successfully employed in other insects ([Verdú et al., 2004, 2012](#); [Kovac et al., 2009](#); [Zermoglio et al., 2018](#)). In a conservation context, thermography and new technologies, offers a powerful approach for studying endangered or elusive species, as it enables behavioural and physiological monitoring without handling or disturbing individuals, an essential requirement for species protected under the EU Habitats Directive ([Almstedt et al., 2023](#); [Giannetti et al., 2024](#); [Giannetti et al., 2025](#); [Palazzetti et al., 2025](#)). Moreover, thermal data can help identify environmental conditions that optimize flight performance and mating activity, supporting habitat management strategies aimed at preserving microclimatic conditions critical for reproduction. The integration of thermography with behavioural observations could provide a robust framework for understanding how environmental temperature shapes activity patterns, a key factor in predicting species responses to climate change ([Sunday et al., 2014](#); [Pincebourde and Casas, 2015](#)).

5. Conclusion

This study provides the first comprehensive evidence that *L. cervus* relies on an integrated thermoregulatory strategy combining behavioural warm-up and physiological heat control, to sustain flight. While haemolymph circulation is traditionally recognized as a primary thermoregulatory mechanism in flying insects, the tracheal system may represent a complementary heat dissipation pathway. These two mechanisms are not mutually exclusive, and their relative contribution in *L. cervus* remain to be assessed. Future studies combining thermal

imaging with haemolymph flow measurements would be required to disentangle their respective roles. Thermal imaging showed a clear pre-flight heating phase and a stable thoracic temperature maintained across flight stages, while μ CT analyses uncovered a highly developed yet individually modulated tracheal system, which may be able to redistribute heat along the body and protect temperature-sensitive organs. These results demonstrate that thermoregulation in *L. cervus* is not a passive consequence of muscle activity, but a coordinated process shaped by the species' morphological polymorphism, energetic constraints, and ecological demands. By linking field-based thermal monitoring with high-resolution internal imaging, our work establishes a methodological framework applicable to other large beetles and saproxylic taxa, offering new insights into how internal anatomy mediates performance under variable microclimatic conditions. From a conservation perspective, understanding the thermal ecology of *L. cervus* is crucial for anticipating its responses to habitat alteration and climate warming, as flight capability underpins dispersal, mating success, and population connectivity.

CRedit authorship contribution statement

Daniele Giannetti: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Antonio Verolino:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Enrico Schifani:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Formal analysis, Data curation. **Marta Filibian:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Roberta Gioia:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Emanuele Fior:** Writing – review & editing, Visualization, Validation, Supervision. **Silvia Gisondi:** Writing – review & editing, Visualization, Validation, Supervision. **Alessandro Campanaro:** Writing – review & editing, Visualization, Validation, Supervision. **Donato A. Grasso:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jinsphys.2026.105012>.

Data availability

Data will be made available on request.

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