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Infrared thermography as a measurement tool for non-invasive stress assessment in canaries

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ABSTRACT
Infrared thermography (IRT) is a non-contact and thermal imaging technique increasingly applied in engineering, biomedical, and veterinary sciences. By detecting infrared radiation emitted from living organisms, IRT enables real-time mapping of surface temperature distributions, offering physiological insights often inaccessible to conventional diagnostic methods. Unlike other imaging modalities that rely on electromagnetic radiation to visualize internal structures, IRT provides dynamic thermal sequences and continuous monitoring of physiological processes without discomfort for the subject. This study explores the application of IRT in canaries (*Serinus Canaria*), to quantitatively evaluate acute stress responses through surface thermal variations in different anatomical regions. Thermal images were acquired before and after handling, and three regions of interest (ROIs), namely the beak, the periocular area, and the body surface, were analyzed. The hypothesis was that handling-induced stress would produce measurable and region-specific peripheral temperature changes. Using a high-sensitivity thermal camera under controlled environmental conditions, temperature values were recorded before and after standardized gentle manipulation. Paired statistical analysis revealed significant post-handling temperature increases, with the highest relative variation observed in the beak region (3.52 %), followed by the body surface (2.99 %), and the periocular region (1.02 %). A one-way ANOVA performed on post-stress temperatures demonstrated a significant effect of region of interest (F (2,57) = 16.84, p < 0.001), with a large effect size (η^2 = 0.37). Post-hoc Tukey analysis confirmed that the beak exhibited significantly higher temperatures compared to both the body and the periocular regions. These findings demonstrate that IRT provides statistically robust and region-dependent thermal markers for acute handling stress. Beyond biological implications, this work emphasizes the importance of ROI selection, measurement repeatability, and quantitative statistical validation, positioning IRT as a rigorous and non-invasive measurement technique for stress assessment in small avian species.

Section: RESEARCH PAPER

Keywords: infrared thermography; thermal imaging; measurement challenges; monitoring biological parameters; non-invasive stress assessment; Serinus Canaria; animal welfare monitoring

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1. INTRODUCTION

The domestic canary (*Serinus Canaria*) is a small passerine bird (Figure 1) widely used as a model species in behavioural and physiological studies. Due to its manageable size, sensitivity to environmental stimuli, and long history of domestication, it represents a suitable subject for investigating stress responses using non-invasive measurement techniques. Adult canaries typically measure 12–13.5 cm in length, with a wingspan of approximately 20 cm and a body mass ranging from 15 to 20 g.

From a physiological perspective, canaries exhibit highly efficient thermoregulatory mechanisms, driven by their metabolic rate. Heat exchange occurs predominantly through peripheral, densely vascularized regions, including the beak and periocular area, commonly referred to as “thermal windows”. These regions allow rapid modulation of blood flow in response to environmental and physiological stimuli, making them particularly suitable for detecting stress-induced thermal variations. As a result, surface temperature variations in these areas can reflect acute or chronic stress responses. The sensitivity of these thermal windows, combined with the species' long history of habituation to human handling, makes the domestic canary an ideal model for investigating avian thermoregulation and welfare using non-invasive imaging techniques [1], [2].

Infrared thermography (IRT) is diffusely applied in biomedical and veterinary sciences as a non-contact imaging technique [3]–[6]. It has been increasingly used in animal production and welfare studies [7]–[10], and some systematic reviews and meta-analyses have confirmed its reliability for thermal stress assessment in poultry when specific body regions are properly selected [11].

Recent studies have demonstrated the potential of IRT to detect acute stress responses in various avian species, including domestic pigeons and budgerigars. In 2021, Tabh et al. [12] highlighted the importance of selecting appropriate anatomical regions of interest (ROIs), showing that the bill provides more robust and consistent thermal responses to stress than the periocular region, which is more susceptible to measurement errors due to head orientation. These findings underscore the need for standardized acquisition protocols and careful consideration of anatomical and geometrical factors when applying IRT for physiological monitoring.

Recent advances have expanded thermal imaging toward automated and non-invasive monitoring of physiological parameters in livestock, including real-time vital sign monitoring in calves using thermal imaging technologies [13].

IRT is a non-contact, non-invasive imaging modality that detects infrared radiation emitted by surfaces, producing high-resolution thermal maps in real time. It provides quantitative measurements of surface temperature, offering insights into physiological processes, such as blood circulation, metabolic activity, and stress responses. Unlike traditional methods, such as behavioural observation or blood sampling, IRT minimizes additional stress and allows repeated measurements over time, which is particularly valuable for sensitive species such as canaries. Peripheral temperature variations are closely linked to physiological mechanisms, including vasoconstriction and vasodilation, which are modulated by acute stress or emotional arousal.

Previous research in avian species has demonstrated that exposure to acute stressors, such as handling or novel environments, induces rapid and measurable increases in surface temperature within specific thermal windows, reflecting peripheral hyperthermic response [1], [2]. Heat transfer mechanisms in canaries have been previously investigated [14]. Convective heat transfer at the feather–air interface can be interpreted within the framework of laminar boundary layer theory [15]. Recovery from stress is accompanied by gradual re-warming as blood flow returns to baseline levels. These physiological patterns highlight the potential of IRT as a sensitive, real-time indicator of the psychophysiological state in small birds. The use of IRT also enables longitudinal monitoring, providing an opportunity to study temporal dynamics of stress responses while minimizing disturbance to the animals.

The application of IRT in small birds presents both opportunities and challenges. High-resolution, high-sensitivity cameras capable of detecting subtle temperature variations (in the order of hundredths of a degree Celsius) are required, along with careful control of confounding factors such as ambient temperature, emissivity, and motion artifacts. Biological surfaces are often small and non-uniform, and animal movement or head orientation can introduce variability, affecting measurement repeatability and accuracy. Despite these challenges, IRT offers

Figure 1. A typical example of *Serinus Canaria*.

the opportunity to obtain objective, repeatable, and quantitative data on physiological stress, advancing the standards of welfare assessment and enabling the development of non-invasive protocols that respect natural behaviour [4], [7], [16].

The aim of this study is to evaluate the applicability of infrared thermography as a quantitative measurement tool for non-invasive stress assessment in domestic canaries, with particular focus on the metrological reliability of different ROIs. To this end, a two-phase experimental protocol was designed: baseline thermal images were acquired while birds were at rest, followed by post-handling thermographic measurements to capture acute stress-induced thermal responses. The analysis focused on peripheral regions, namely the beak, the periocular area, and the body surface, to quantify the hyperthermic response associated with acute handling stress [17]–[21], under controlled environmental and acquisition conditions in order to ensure measurement repeatability and comparability.

2. MATERIALS AND METHODS

2.1. Experimental subjects and environmental conditions

The study involved 20 canaries *(Serinus Canaria)*. All subjects were treated as a single experimental population, without stratification by sex or age, but considering similar length (12.5 ± 0.5 cm) and weight (17 ± 2 g), in order to emphasize the measurement capability, sensitivity, and reproducibility of IRT at the acute stress assessment, rather than inter-individual physiological variability. Measurements were performed in a thermostatically controlled room, with ambient temperature and relative humidity monitored using a calibrated thermo-hygrometer (TESTO 608-H2, Testo SE & Co. KGaA, Lenzkirch, Germany). Stable environmental conditions (25 ± 1 °C and 50 ± 5 % RH) were maintained throughout the experiment to minimize fluctuations that could alter thermographic measurements. Prior to image acquisition, each bird was allowed to acclimatize for 30 min in order to stabilize body temperature and reduce potential handling-related stress.

2.2. Equipment and experimental setup

The experimental setup was designed to ensure the welfare of the animals and the reliability of the data collected. Specifically, visible and thermal recordings were obtained using an infrared camera (FLIR T650sc, FLIR Systems Inc., Wilsonville, OR, USA), selected for its technical performance (thermal sensitivity < 0.02 °C at 30 °C, measurement range of –40 °C to 2000 °C, thermal resolution of 640 × 480 pixels, visible resolution of 5 Mpixel). The infrared camera was previously calibrated by the manufacturer. Therefore, a warm-up time of 15 min and a non-uniformity correction procedure was conducted, before starting infrared image acquisition, in accordance with the same

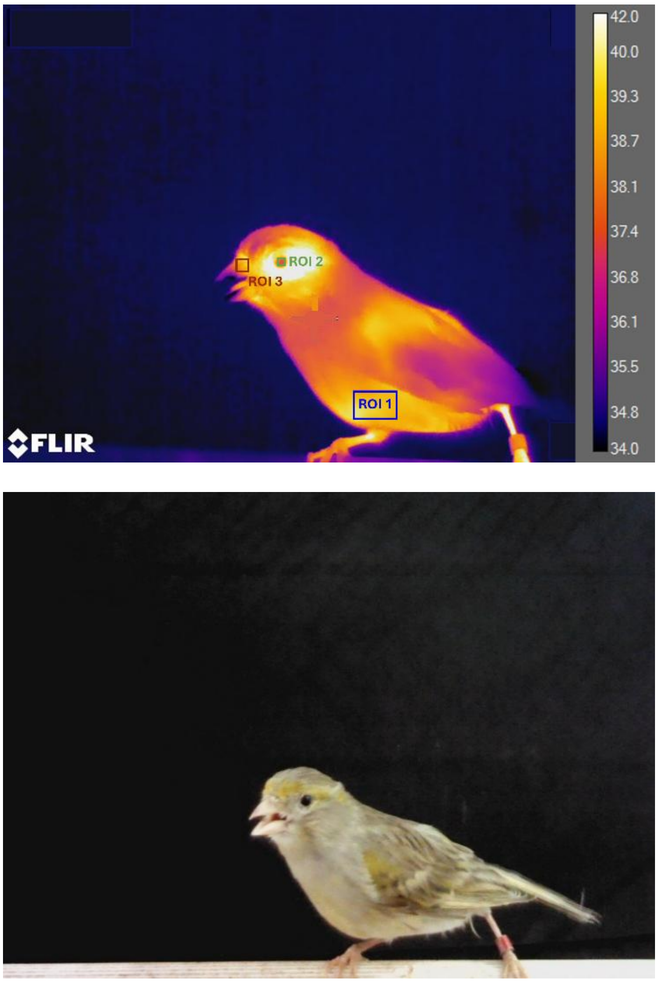

Figure 2. Thermographic and corresponding visible image of a canary, illustrating the ROIs (ROI 1: body surface, ROI 2: eye, ROI 3: beak) selected for infrared temperature measurements under controlled acquisition conditions.

manufacturer's guidelines. An emissivity value typical of avian plumage was adopted [22] and was set to 0.95.

The canaries were filmed through a hole in the cage at approximately 30 cm from the camera and parallel to the ground. The camera was mounted on a tripod at a fixed height of 120 cm above the ground and positioned in front of a metal cage (60 × 40 cm^2). A black matte panel was placed behind the cage to minimize infrared reflections and optimize image visible and thermal contrast. To reduce potential stress from the equipment, the thermal camera was installed in the room one day prior to data collection, allowing the animals to habituate to its presence.

2.3. Data acquisition protocol

The experimental protocol was structured in two distinct phases. The objective was to compare body temperatures at rest with those induced by a stress factor. For each subject, two thermal video recordings were acquired, each lasting approximately 10 s at a frame rate of 30 Hz. The first sequence was acquired under resting conditions (i.e. baseline), capturing the bird in a state of rest and relaxation, while the second was obtained immediately after a standardized stressor consisting of 3 min of gentle manual handling. This protocol was designed to evaluate acute changes in surface temperature between baseline and post-stress conditions. The approach allowed for the assessment of acute thermal changes in response to stress in a reasonably minimally invasive manner. To minimize the effect of head orientation on temperature readings, cranial landmarks were used to estimate yaw, pitch, and roll. Corrections were applied when evaluating the periocular region, which is sensitive to acquisition angle, while beak temperature proved less affected by orientation. This procedure allowed for the correction of possible temperature reading errors in the periocular region, while beak measurements proved to be independent of orientation.

The measurement uncertainty was evaluated according to standard metrological principles [23], [24]. The measurement approach follows instrumentation and measurement principles as promoted by the IMEKO community [25].

The present experimental protocol was approved by the Department of Veterinary Sciences, University of Messina, Animal Experiments Local Ethics Committee (Decision no: 03/2025 bis).

2.4. Data analysis

Thermal data were extracted from predefined quadrilateral ROIs (Figure 2):

- ROI 1 – Body surface (30 mm × 15 mm), centered in the thoraco-abdominal patch under the wing avoiding specular hotspots;
- ROI 2 – Periocular region (eye, 3 mm × 3 mm), centered in the periocular annulus;
- ROI 3 – Beak (5 mm × 5 mm), aligned from the beak attachment to the skull and limited to the inside of the same beak.

For each ROI, selected with the previous criteria, the mean and standard deviation of the surface temperature were computed across 90 consecutive video frames and exported for further statistical analysis by means of the software of the used infrared camera (FLIR ResearchIR, FLIR Systems Inc., Wilsonville, OR, USA).

Statistical comparisons between baseline and post-handling values were performed using paired t-tests ($p < 0.05$ considered significant). Results were expressed as mean ± standard deviation.

The normality of the data distribution was verified prior to statistical analysis. The dataset's histogram visually forms a bell-shaped curve, it indicates an approximately Gaussian (Normal) distribution, characterized by symmetry around its center (mean), with data tapering off equally in both tails.

Statistical analysis included paired t-tests for baseline–post comparisons and one-way ANOVA on post-handling temperature values to evaluate regional differences. ROI measurements were treated as repeated measures within the same individual. When significant effects were observed, Tukey's honestly significant difference (HSD) test was applied for multiple comparisons [26], [27]. Effect size was calculated using eta squared (η^2).

Measurement uncertainty was estimated considering instrument accuracy, emissivity variability, and environmental contributions.

3. RESULTS

Figure 3 shows the comparison between baseline and after-stress temperature values for the three analyzed ROIs. Bars represent mean values, while error bars indicate standard deviation, reflecting both biological variability and measurement-

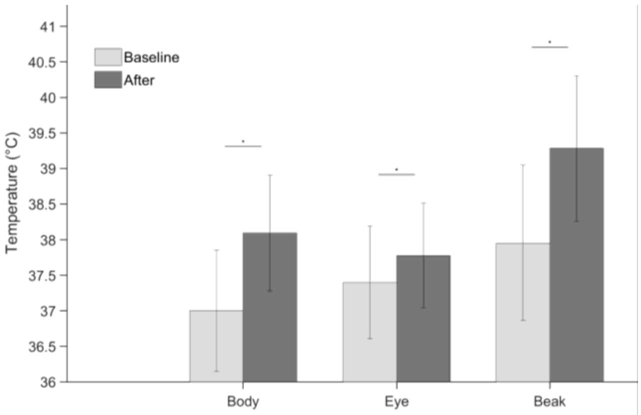

Figure 3. Comparison between baseline and after-stress temperature values for the three ROIs: bars represent mean values, while error bars indicate standard deviation. A paired t-test was performed for each ROI. An asterisk (*) denotes statistically significant differences ($p < 0.05$).

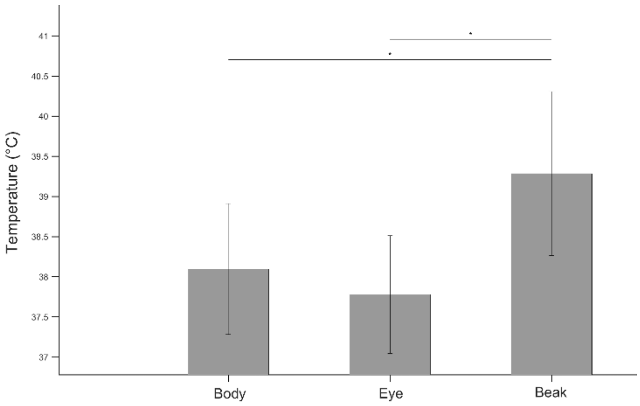

Figure 4. One-way ANOVA and HSD test of after-stress temperature values across the investigated ROIs: bars represent mean values and error bars indicate standard deviation. Horizontal lines and asterisks (*) indicate pairs of ROIs showing statistically significant differences ($p < 0.05$).

related dispersion. A statistically significant increase after stress was observed for the ROIs marked with an asterisk ($p < 0.05$, paired t-test). The magnitude of the thermal response is visually consistent with the statistical analysis, with a pronounced increase at the beak, a moderate increase at the periocular region, and relatively small changes at the body surface.

Paired t-tests revealed significant post-handling temperature increases in the body and beak regions, while the periocular region showed a smaller variation. The relative temperature increases differed among regions, with the beak showing the highest percentage variation (3.52 %), followed by the body (2.99 %) and the periocular region (1.02 %). Detailed percentage increase values are reported in Table 1.

The higher percentage observed in the beak suggests a stronger peripheral vasomotor response to acute handling stress.

As shown in Figure 4, a one-way ANOVA performed on the after-stress temperatures revealed a statistically significant effect

Table 1. Percentage temperature increase of the investigated ROIs from baseline to post-handling conditions.

ROI	Mean % increase	SD (%)
Body	2.99	2.58
Eye	1.02	0.54
Beak	3.52	1.66

Table 2. One-way ANOVA results among the three ROIs in the post-handling conditions.

Source	SS	df	MS	F	p-value
Between	25.20	2	12.60	16.84	1.78×10^{-6}
Within	42.64	57	0.74	-	-

Effect size: $\eta^2 = 0.371$

Table 3. HSD comparisons.

Comparison	Mean Difference (°C)	95% CI Lower	95% CI Upper	p-value
Body - Eye	0.31	-0.34	0.97	0.49
Body - Beak	-1.19	-1.85	-0.53	< 0.001
Eye -Beak	-1.50	-2.16	-0.85	< 0.001

of ROI ($p < 0.05$), indicating that at least two regions exhibited different thermal responses following the stress condition. Post-hoc HSD analysis identified significant differences between specific pairs of ROIs, confirming that peripheral regions, and particularly the beak, exhibited stronger thermal responses to handling stress.

Consequently, another one-way ANOVA was also carried out to evaluate temperature differences among the three ROIs in the post-handling conditions. The analysis revealed a significant effect of region (F (2,57) = 16.84, $p < 0.001$), with a large effect size ($\eta^2 = 0.37$), as summarized in Table 2. That indicates that approximately 37 % of the total variance in the after-stress condition can be attributed to anatomical region differences, corresponding to a large effect size.

HSD analysis was conducted to identify pairwise differences among regions. It revealed that the beak temperature was significantly higher than both the body surface ($mean\ difference = 1.19$ °C, $p < 0.001$) and the periocular region ($mean\ difference = 1.51$ °C, $p < 0.001$). No statistically significant difference was observed between the body and the periocular regions ($p = 0.487$). The beak region showed significantly higher temperatures compared to both the body and the periocular regions, while no significant difference was observed between the body and the periocular areas (Table 3).

Thermal imaging analysis revealed that manual handling, although reasonably minimally invasive, induced acute and statistically significant changes in the surface temperature in specific body regions of the canaries. The magnitude and repeatability of these changes varied across the investigated ROIs. Specifically:

- Body surface (ROI 1) temperature shows statistically significant differences between baseline and post-handling conditions. However, these changes are characterized by high dispersion, indicating limited sensitivity and poor reliability of the central body regions for detecting acute stress-induced thermal responses.
- For eye (ROI 2) temperature, a statistically significant increase in the periocular temperature is observed after handling ($p < 0.001$). Despite its sensitivity to stress, periocular measurements exhibit increased variability, mainly due to head orientation and acquisition geometry, which affected measurement repeatability. Measuring body temperature in free-ranging birds presents intrinsic methodological challenges related to positioning, feather insulation, and environmental exposure [28].

- Beak (ROI 3) temperature exhibited a clear and statistically significant increase following handling (average $\Delta T \approx 1.3$ °C, $p < 0.001$). Compared to other ROIs, the beak provided more stable and repeatable measurements, showing reduced sensitivity to orientation-related errors.

4. DISCUSSION

The results demonstrate that distal and less insulated regions provide the most sensitive indicators of acute physiological responses to stress. Among the investigated ROIs, the beak proved to be the most reliable ROI due to:

- its higher vascularization,
- greater magnitude of temperature changes,
- lower susceptibility to measurement errors and artefacts induced by orientation.

Although periocular measurements showed a measurable increase (approximately 0.4 °C), they were more strongly influenced by head orientation, limiting reproducibility. In contrast, body surface temperature exhibited higher dispersion, despite statistical significance, reducing its suitability as a robust stress marker.

The beak proved to be the most reliable and orientation-independent ROI for standardized thermographic stress assessment in canaries. The observed increase in beak temperature following handling is consistent with previous studies reporting peripheral hyperthermic responses in birds under acute stress conditions [12]. Importantly, the measured temperature variations exceeded the estimated measurement uncertainty, in accordance with established metrological principles regarding measurement uncertainty and traceability in thermal measurements [23], [24], confirming that the detected changes are attributable to physiological responses rather than instrumental noise.

A recent systematic review and meta-analysis confirmed the diagnostic value of specific body regions in poultry thermal stress assessment, highlighting the importance of ROI selection and acquisition standardization [11].

The observed increase in peripheral temperature following handling reflects a stress-induced hyperthermic response, likely associated with increased peripheral blood flow and heightened sympathetic activation. Such responses have been reported in situations involving acute arousal, physical restraint, or emotional activation in birds [17], [18], [29], and may differ from cooling responses observed under other stress paradigms. The present findings align with those of [12], which demonstrated that the periocular region, although sensitive to stress, is significantly affected by the angle of incidence during thermographic imaging, leading to potential underestimation of surface temperatures. In contrast, the beak showed minimal susceptibility to orientation-related errors, confirming its suitability as a robust ROI.

Peripheral thermal responses are known to reflect complex autonomic regulation mechanisms, including transient vasoconstriction or vasodilation depending on the stress context [30].

Central body regions are less sensitive to acute stress-induced thermal changes, which is consistent with findings showing dynamic modulation of body surface temperature in birds under energetic constraints [31], [32]. Measuring core temperature in free-ranging birds presents significant methodological challenges, further supporting the use of peripheral surface thermography as a practical alternative [28].

The large effect size observed in the ANOVA analysis ($\eta^2 = 0.37$) further confirms that the anatomical region plays a major role in determining the magnitude of the thermal response. The absence of significant differences between the body and the periocular regions, combined with the strong divergence of the beak, suggests that distal vascularized structures provide amplified and more detectable stress-related signals. The percentage-based analysis corroborates this interpretation, highlighting the beak as the region exhibiting the highest relative thermal variation.

By integrating orientation correction techniques and focusing on thermally responsive regions, this study addresses key metrological challenges and contributes to the standardization of IRT protocols in avian stress research. From a measurement perspective, the large dispersion observed at the body surface limits its suitability as a reliable ROI for stress assessment, despite statistical significance. It is worth noting that stress-related thermal responses may differ in direction depending on experimental conditions, species, and stressor characteristics, highlighting the importance of interpreting IRT data within a well-defined physiological and experimental framework. From a physiological perspective, the high statistical significance of thermal variations in the periocular and beak regions supports their role as sensitive indicators of acute stress. These findings agree with previous research reporting ocular temperature as a reliable stress marker in budgerigars [18], and the sensitivity of the beak and the periocular regions in other passerines [17]. Jerem et al. [19] and Tabh et al. [12] further emphasized that both the choice of ROI and the acquisition angle strongly affect measurement reliability. Tattersall et al. [5] also identified peripheral regions as privileged windows into avian thermal physiology. Collectively, these studies corroborate the present findings and reinforce the potential of IRT for assessing animal welfare. From a measurement perspective, the results highlight the importance of selecting ROIs that combine sensitivity with robustness. The quantification of surface temperature variations in small biological structures highlights the importance of measuring repeatability. In this study, the use of predefined ROIs and controlled environmental conditions reduced variability and enhanced comparability between individuals.

Methodologically, the reliability of IRT measurements depends on the emissivity setting, camera calibration, and environmental stability, which together ensure repeatability and quantitative accuracy. These aspects directly influence the accuracy and reproducibility of the acquired data.

One of the main challenges is related to measurement uncertainty, which is influenced by animal movement, head orientation, and the limited size of avian thermal windows. Future studies should integrate uncertainty analysis to establish confidence intervals for thermal data. The sensitivity of the infrared camera (< 0.02 °C) enabled the detection of small yet significant thermal changes, demonstrating that IRT can operate within a rigorous metrological framework. While IRT is often used qualitatively [5], [10], this work emphasizes its potential as a quantitative measurement technique. Finally, the limited and inconsistent changes detected in body surface temperature confirm that central regions, due to higher thermal inertia and insulation, are less suitable for acute stress detection. This further validates the use of distal, vascularized regions for reliable stress monitoring. Peripheral temperature dynamics have been shown to vary depending on the emotional context and anticipation state, sometimes resulting in transient drops prior to stress-related increases [30]. The experimental protocol adopted here contributes to the standardization of thermographic measurements in small birds, providing reference conditions (camera distance, acquisition duration, ROI selection) that can be replicated in future studies. The analysis intentionally avoided biological stratification in order to emphasize the measurement capability of IRT rather than inter-individual variability.

From a measurement science perspective, the observed temperature variations exceed the instrumental uncertainty of the thermal camera, confirming the robustness of the detected stress-induced changes [23]–[25].

The methodological framework is consistent with recent applications of IRT in biomedical research [33], [34].

In summary, this study demonstrates that IRT is a valuable, objective, and non-invasive tool for monitoring handling stress. By identifying the beak as the most robust ROI, it provides a methodological basis for standardizing thermographic protocols in ornithological research and opens perspectives for broader applications in animal physiology and environmental biomonitoring. This confirms that the value of IRT lies not in the direction of the physiological response itself, but in its ability to reliably detect and quantify stress-induced thermal variations under controlled conditions. In line with the principles promoted within the IMEKO community, measurement standardization is essential to ensure traceability, repeatability, and inter-laboratory comparability [25].

From a metrological perspective, IRT must be considered as a measurement system characterized by defined scales, units, and uncertainty contributions [23].

A limitation of the present study is the absence of concurrent physiological markers, which could further support the interpretation of thermal responses. Future studies should combine thermographic measurements with validated physiological or inflammatory biomarkers, as demonstrated in recent veterinary investigations [34]. From a metrological perspective, the observed dispersion of the measured temperatures incorporates both biological variability and measurement uncertainty, emphasizing the importance of standardized acquisition conditions and ROI selection.

5. CONCLUSIONS

The proposed approach contributes to extending the measurement frontiers of IRT from traditional engineering and biomedical applications to animal welfare science, addressing both metrological challenges and practical implications.

The study was conducted on a homogeneous experimental population, without stratification by sex or age, in order to emphasize the measurement capacity, sensitivity, and reproducibility of the IRT in assessing acute stress rather than an interindividual biological variability.

Temperature variations in the beak and periocular regions proved to be sensitive and reproducible biomarkers of handling stress, consistent with a stress-induced peripheral hyperthermic response commonly observed in birds under acute stress conditions [17]–[21]. In contrast, the limited and inconsistent variations observed at the body surface confirm that central regions are less suitable for detecting rapid stress-related thermal changes.

These results highlight the importance of selecting distal, vascularized regions as reliable ROIs for stress assessment. Controlled acquisition conditions, predefined ROIs, and careful consideration of measurement geometry were shown to improve the repeatability and comparability of the thermal data. Among the investigated regions, the beak emerged as the most robust thermographic marker of acute handling stress.

While IRT is often applied as a qualitative imaging technique, the present study demonstrates its potential as a robust, quantitative, and fully non-invasive measurement method for detecting acute stress in small birds, provided that metrological constraints and standardized acquisition protocols are respected. By consolidating methodological criteria for ROI selection, calibration, and repeatability, this work supports the use of IRT as an objective tool for animal welfare monitoring and contributes to its standardization within ethology and animal husbandry.

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