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Enhancing Stress Assessment in Sledge Reindeer (*Rangifer tarandus*): A Pilot Study on Infrared Thermal Imaging and its Opportunities for Advancement as a Welfare Assessment Tool

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Abstract – Measuring immediate physiological stress responses in animals can be challenging; saliva and blood sampling, while invasive, may also generate confounding stress responses, and equipping animals with heart rate sensors is not always feasible. Nevertheless, emerging technologies offer a non-invasive and contactless method to measure additional body surface temperature changes induced by acute stress, using infrared thermal measurement of the eye caruncle region. Contactless temperature measurement has the potential to assess the emotional state of animals affected by human physical contact. Reindeer, being highly sensitive to touch and naturally avoiding physical contact, exemplify this case. With growing interest in safari tours involving sledging reindeer, there is a need to investigate how these animals are affected by human interactions and how they adapt to daily close contact. In this pilot experiment, we evaluated the efficacy of thermal imaging in measuring medial canthus temperature fluctuations in non-habituated sledging reindeer while being petted by unfamiliar humans. Our findings support the hypothesis that medial canthus temperature significantly decreases during petting and increases once the interaction ceases, aligning with established stress response patterns found in the literature. This pilot experiment underscores the potential of infrared thermal imaging for non-invasively monitoring physiological responses to stress in tamed reindeer. Moreover, it sets the foundation for refining methodologies and experimental designs using infrared thermal imaging in animal welfare research.

Keywords – Infrared Thermal imaging, Emotion, Ungulates, Thermal camera, Eye temperature

In recent years, Nordic countries have strategically focused a significant proportion of their marketing efforts on animal tourism, with reindeer sledge safari tours emerging as a key attraction. In 2018, Finnish Lapland alone involved approximately 650 reindeer across 34 farms dedicated to tourism, contributing over 23% to the annual revenues generated by animal-based services in Finnish tourism (around €15 million per year) (García-Rosell & Tallberg, 2021). As the importance of animal tourism, and in particular reindeer-related activities, continues to rise, the number of reindeer employed for such purposes is expected to increase in the foreseeable future. Amid this growth, discussions surrounding the welfare and ethical considerations of utilizing animals in tourist attractions have gained considerable momentum. Animal welfare is now a pivotal criterion employed by tour operators to showcase responsible treatment of animals. One notable aspect of tourism is that working animals not only interact with their familiar handlers but also engage daily with numerous strangers, which involves forced physical contact with tourists that are eager to interact with the animals by petting them or feeding them. Interactions with inexperienced tourists can result in a higher occurrence of inappropriate human behaviors towards working animals, leading to an accumulation of negative interactions (Carr & Broom, 2018). Farm animals in petting zoos have been observed to avoid contact with visitors (Anderson et al., 2002) and to negatively react to improper grooming from visitors (Ramont et al. 2021). This is of relevance for reindeer. Despite the intricate social structures observed in large herds of reindeer, where hundreds of individuals coexist (Body et al., 2015a; b), they rarely engage in affiliative behaviors involving direct physical contact, such as allogrooming. Indeed, reindeer are sensitive to touch and naturally avoid direct contact with both their conspecifics and humans. This raises the question of how interactions with tourists can impact reindeer and how they adapt to varying human encounters when compelled to engage closely with people daily. Investigation into these dynamics becomes crucial for ensuring the well-being of the reindeer subjected to regular human interaction in the tourism sector. Working sledge reindeer, habituated to human presence and light handling, can exhibit curiosity towards humans, easily approach them, follow individuals leading them on a rope (Liehrmann, 2023) and show great aptitude in following human indications to find food (Liehrmann et al., 2023). However, reindeer herders still describe their sledge reindeer as very sensitive to human touch and their handling must be as gentle as possible (Kauppinen, 2019; personal observation and communication with reindeer herders). Therefore, measuring their immediate stress response presents a challenge.

As awareness grows regarding the ethical treatment of animals, there is an escalating recognition of a need to assess stress responses rapidly and efficiently in animals. Although measuring immediate physiological stress in animals can be challenging, behavioral observations alone may lack precision and invasive methods like saliva and blood sampling pose limitations as they may themselves trigger stress for the animal., Similarly, equipping animals with devices such as heart rate sensors is not always feasible. However, emerging new technologies, such as the infrared thermal measurement of body parts, offer an alternative for measuring immediate responses to stimuli. The benefits of using an infrared thermal camera lie in its mobility, wireless design, and non-invasive nature, thereby eliminating the need for direct contact with animals. Studies in humans (Ioannou et al., 2013) and other primates (Kano et al., 2016) have demonstrated that emotional stressors lead to a decrease in nose temperature. This phenomenon is attributed to arousal induced by emotional stimuli, triggering a vasoconstriction reaction in the sympathetic nervous system. This reaction reduces blood flow in highly innervated peripheral body areas, resulting in a temperature decrease. In brief, when the blood flow is redirected to the core, the body's peripheral areas can be expected to cool (Jerem & Romero, 2023; Vinkers et al., 2013). Then, following a stress event, the parasympathetic nervous system takes over, promoting vasodilation to restore the initial temperature (Ioannou et al., 2013). These temperature changes occur rapidly, varying among individuals and in response to stimulus intensity. The decrease can commence within seconds (Jerem et al., 2019) and may persist for several minutes before returning to baseline (Kano et al., 2016; Stewart et al., 2008). Consequently, variation in temperature in specific areas could serve as an indicator of arousal in animals. However, in many mammals, the nasal area is often covered by fur, making it challenging to assess its exact temperature with infrared thermal imaging. An alternative solution involves measuring the temperature of the eye region, which is also highly innervated. This method was successfully used in cows (Stewart et al., 2008) and certain birds (Jerem et al., 2019, Ouyang et al., 2021), finding similar patterns of a sudden decrease of the temperature during a stressor followed by an increase during the recovery. However, a recent study indicated that the base of the bill maybe be more suitable to use with birds (Tabh et al., 2021). In horses, Kim and Cho (2021) confirmed that the medial canthus (hairless area in the corner of the eye) was the most reliable location for measuring skin temperature using thermal imaging. These new technologies offer interesting opportunities to assess animal stress responses in contexts not previously feasible, such as those where the collection of blood or saliva samples is not possible. The contactless and remote measurement of the medial canthus offers a new opportunity that has not been used to-date on species like reindeer when investigating the effects of potential stressors on animal arousal.

In this preliminary investigation, we aim to evaluate the efficacy of thermal imaging as a non-invasive tool for measuring emotional fluctuations in reindeer before, during, and after physical contact with an unfamiliar human. Acknowledging the reluctance of reindeer to human touch, we designed an experiment involving eight sledge reindeer non-habituated to tourist touch. Throughout the experiment, we recorded both the reindeers' behavioral responses (to confirm that petting was a source of stress) and the temperature of the eye caruncle while the reindeer were petted by an unfamiliar person. According to what has been observed in previous studies (Jerem et al., 2019; Stewart et al., 2008), we hypothesized that the temperature in the medial canthus would decrease during the stressor and then increase back to baseline levels once the stressor stopped.

Method

Ethics Statement

The reindeer owner provided an informed consent for the use their reindeer via the completion of an information form provided in Finnish. Data were stored according to the EU General Data Protection Regulation Act 12 to 14 (2016/679). The owner had the right to withdraw their consent. This study was evaluated by the Project Authorization Board of the Southern Finland Regional Administration Agency (ESAVI) responsible for the Legal Security and Permits (decision: ESAVI/5563/2022).

Subjects

For this study, we worked with eight castrated male reindeer from the Reindeer Journey farm based in Finnish Lapland. The herders associated with this farm boast a heritage of generations long reindeer herding and possess expertise as professional sledding reindeer trainers. Our study subjects, aged between 4 and 12 years (mean ± se = 6 ± 2), comprised active working sledding reindeer or individuals undergoing training for such roles. Sledding safaris involving reindeer typically entail a brief introduction of the animals to tourist groups, including interactions with well-habituated reindeer through petting, followed by a sled tour. During these safaris, the first reindeer is led by a herder walking alongside the animal, while the following reindeer is attached to the sledge of the first reindeer and so on (this is called a "*raito*" formation). The pace does not exceed walking speeds, and the safari duration ranges from 20 min to 3 hr. Crucially, our study focused exclusively on reindeer that have not been habituated yet by the herders for being petted by tourists. This deliberate selection aimed to ensure the observation of a stress response when these reindeer were subjected to petting by an unfamiliar experimenter.

Material and Procedures

The experiments were conducted on March 16th, 20th, and 23rd, 2022, from 13:00 to 15:00 in the Finnish Lapland at a reindeer park located in Levi, where the working reindeer spend the tourist seasons. The tests were performed in a place familiar to the reindeer—a paddock just next to their enclosure—where they could see the other reindeer, ensuring they did not suffer from isolation. In their enclosures, the reindeer wore a halter on their head with a rope attached to it. This is a common practice, so the herder just needs to grab the rope on the ground without having to put the halter on and off every time, and thus limiting unnecessary direct physical contact with the reindeer. After the herder caught the reindeer from their enclosure, they lead them to the experimental enclosure to perform the experiment:

The experiment involved three phases:

1. ***Baseline:*** The reindeer was held by the herder on a short leash and stayed still for one minute. The temperatures recorded during this phase were used to generate a baseline temperature for each individual.

2. ***Test:*** Following the baseline phase, an experimenter, unfamiliar to the reindeer, approached and petted the reindeer while the herder continued to hold the reindeer on a short leash. The petting was done with one hand on top of the shoulders of the animal and slowly sliding down to the rump; the movement was repeated for one minute. Then, the experimenter stepped away from the reindeer.
3. ***Recovery:*** The reindeer was held by the herder and stayed still for 45 seconds (we shortened the timing to limit the frustration due to the length of the test).

To confirm that the petting was a source of stress for the reindeer, the whole experiment was also video recorded for further behavioral analyses using the software program BORIS (Friard & Gamba, 2016). We recorded the number of avoidance behaviors during each phase, such as pulling on the rope, and the avoidance behaviors oriented at the experimenter, such as stepping away from the experimenter and lowering the back to avoid hand touch during the petting phase (Table 1).

Table 1

Ethogram for Reindeer Avoidance Behaviors

Behavior	Definition
Rope pulling	Animal tries to get some distance from the handler by pulling on the rope with a fast head movement or strongly shaking its head.
Stepping away	Animal takes a step away from the petting experimenter by moving forward, backward or taking a step aside to the left (the experimenter stands on the right of the reindeer).
Avoids hand touch	Animal lowers its back when it feels the hand of the experimenter on top of it.

During the three phases, an experimenter took infrared thermographic images using an infrared radiation camera (FLIR E6 TX, FLIR Systems Inc., Wilsonville, OR, USA, f = 1.5, spatial resolution = 3.4 mrad, thermal sensitivity < 0.06, Calibration certificate < 6 months). The emissivity (ε) was set up at 0.98 which is the emissivity for mammalian hairless skin (McCafferty, 2007). The experimenter held the camera with an angle of approximately 90◦ from the eye of the reindeer and at a distance of 50 to 80 cm from the reindeer's head. The same experimenter (OL) took all images and recorded as many photos as possible during the whole experiment. To avoid potential bias from the brain lateralization, images were always taken from the right eye of the reindeer. In our case, reindeer are habituated to having tourists with cameras surrounding them during the safari tours; the presence of the experimenter using the thermal camera was not likely to affect the reindeers' behavior during the experiments.

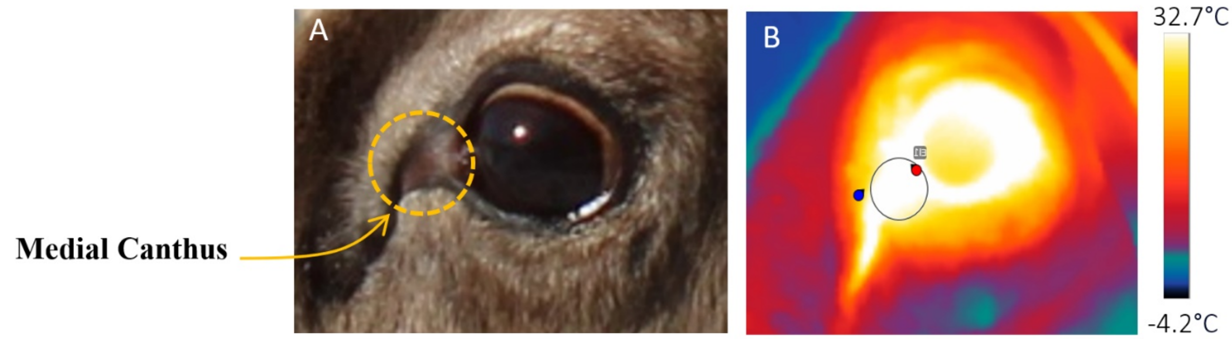
Before every test, the ambient air temperature (4.7 ± 2.8 °C) and the relative humidity (60.46 ± 10.34 %) were recorded. The test area was in a forest providing a good shelter from the wind; therefore, the wind speed was always < 0.5 Km/h, which we considered as insignificant (Church et al., 2014). Moreover, we made sure to always perform the test in the shade to avoid potential perturbations from the solar radiation that could affect the temperature registered by the thermal camera. Each of the eight reindeer were tested at least once and four of them were tested a second time on another day (not all the reindeer could be repeated due to the time schedule of the herder) resulting in a total sample size of 12.

Picture Selection and Temperature Extraction

A total of 324 infrared thermal images were taken, and because the reindeer were allowed to move their head during the measurements, a considerable number of images were not usable. The selection criteria were that the angle was approximately 90◦ and that the eye was well open. After this selection, the total of remaining images was 286 with a range of 13 to 32 pictures per test (8 ± 3 images per test phase). The FLIR Thermal Studio Suite (Teledyne FLIR LLC, Oregon, United States) was used to analyze the images and extract the maximum temperature values from the medial canthus region. The air temperature and relative humidity values recorded before each test were entered into the program, to obtain corrected temperature values from the FLIR software (Figure 1) (Kim & Cho, 2021).

Figure 1

Details of the Reindeer Medial Canthus



Note. A) Image of a reindeer eye. B) Thermographic image of a reindeer eye and the area of interest from which we extracted the maximal temperature in Celsius circled using the FLIR thermal studio program.

Statistical Analyses

All analyses were carried out using the statistical software R, version 3.6.3 (R Core Team, 2022), and figures were created using the 'ggplot2' package (Wickham, 2016).

We first confirmed that the chosen stimulus (being petted by a stranger) promoted a clear behavioral stress response using a Generalized Linear Mixed Model (GLMM) from the package *glmmTMB* (Brooks et al., 2017). We defined the response variable as the count of avoidance behavior weighted by the duration of the test phase to obtain a number of avoidance behavior per minute (Poisson distribution). Distributions, within-group variance, and homoscedasticity of the residuals for the model were checked using the package *DHARMa* (Hartig, 2021). The test phase was included as an independent 3-level variable and the identity of the reindeer as a random factor to account for repeated measures. A post hoc analysis was then conducted using the emmeans function (Length, 2021) to extract the contrast between the test phases.

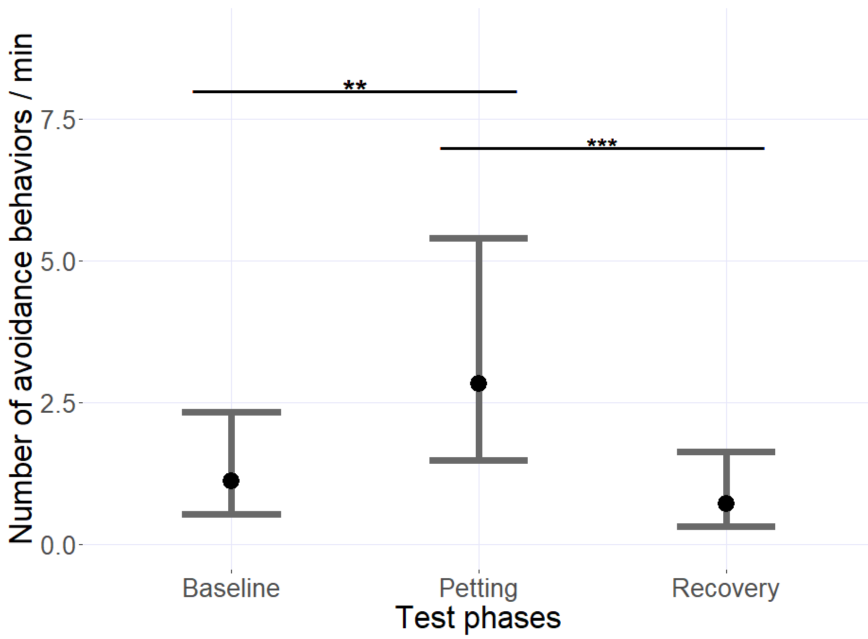
We defined the eye temperature baseline for each individual by calculating the mean of the maximum medial canthus temperature point extracted from the pictures taken during the control phase. We checked with bivariate analyses using Spearman correlation that there was no correlation between the baseline eye temperature with the air ambient temperature ($r_s = -0.46$, $p = .165$, N = 12) and with the relative humidity ($r_s = 0.45$, $p = .197$, N = 12). Because there were no correlations, the air temperature and the relative humidity were not considered as influencing the recorded eye temperature and were not included in the final models for the analyses. We calculated the maximum medial canthus temperature difference from the baseline for each picture and used it as response variable in our investigation. As we are interested in the progression of the temperature true time in each phase we built a Linear Mixed Model (LMM) using the package *glmmTMB* (Brooks et al., 2017) assessing the effect of the experiment phases (Test phase /Recovery phase) in interaction with the time since the Test phase started. The reindeer identity was nested in the date of the test and implemented as a random factor to account for individual variation and repetitions. Distributions, within-group variance and homoscedasticity of the residuals for the LMM were checked using the package *DHARMa* (Hartig, 2021).

Results

We first confirmed the stress response generated from being petted by a stranger. The reindeer tried to remove themselves from the situation (pulling on the rope, head shaking) significantly more often during the petting (test phase) than during the baseline phase (estimate ± SE = 0.39 ± 0.11; $t = -3.49$; $p = .004$) and during the recovery (estimate ± SE = 3.93 ± 1.24; $t = 4.34$; $p < .001$) (Figure 2). Additionally, we observed numerous attempts of the reindeer to avoid the human's hand and to step away from the experimenter, confirming that the presence of the experimenter and the action of petting was uncomfortable for them and therefore a source of stress.

Figure 2

Rate of Reindeer Avoidance Behaviors During the Test Phases

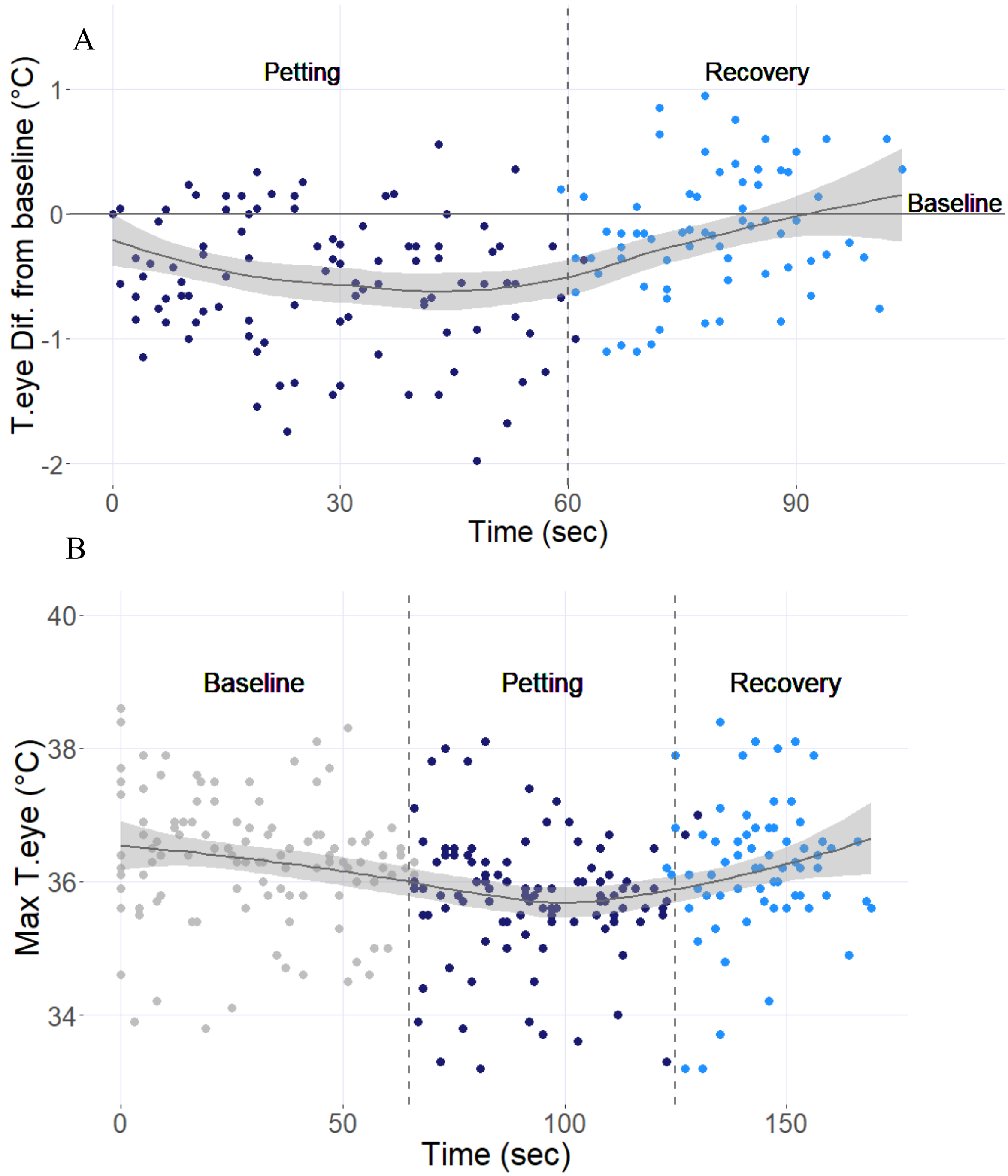


Note. Number of avoidance behaviors per minute (pulling on the rope and head shaking) during the three different phases of the test. The error bars represent the 95% confidence intervals. '**' Indicates significant differences with a p-value < .01; '***' indicates significant differences with a p-value < .001. The means and their associated 95% confidence intervals were extracted from the corresponding model using the function emmeans from the "emmeans" package in R.

Eye temperature decreased over time during the petting phase (mean ± SE = -0.44 ± 0.49 °C in the first 15 sec of the petting; mean ± SE = -0.73 ± 0.63 °C in the last 15 seconds of the petting) and increased over time during the recovery phase (mean ± SE = -0.30 ± 0.58 °C in the first 15 sec of the recovery phase; mean ± SE = -0.06 ± 0.57 °C in the last 15 seconds of the recovery phase). This was confirmed by the IRT model showing that there is a significant interaction between the experiment phase and the time (estimate ± SE = -0.009 ± 0.004; $t = -2.55$; $p = .011$) (Figure 3A). The data with the raw temperatures recorded in the three test phases are presented in Figure 3B. Figure 4A provides examples of the temperature variation pattern for four of the tested reindeer; only four individuals are presented to enhance clarity of the figure and they were selected to highlight the various forms of the observed patterns. All individuals' patterns are shown in Figure 4B.

Figure 3

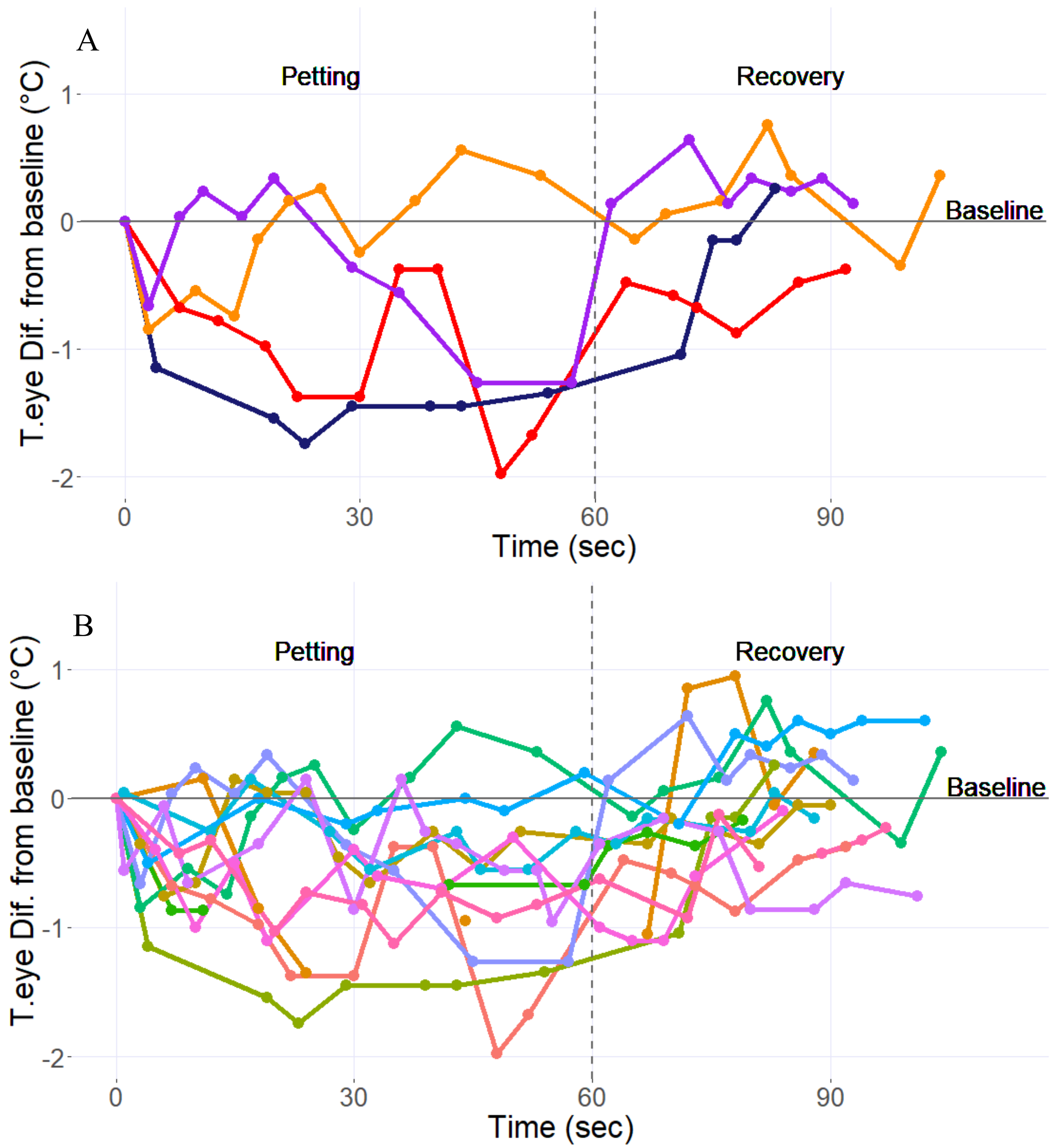
Medial Canthus Maximal Temperature According to Time and Test Phases



Note. A) Progression of the medial canthus maximal temperature difference from the baseline according to time and the experimental phases. Petting phase and Recovery phase. 0 is the baseline and refers to the individuals' mean temperature during the control phase. Temperatures below 0 are colder than the individual baseline and temperature over 0 are warmer than the individual baseline. The curve is fitted with ggplot2 using a Local Polynomial Regression Fitting method (loess). The grey area represents the standard error. B) representation of the raw measurement of the medial canthus maximal temperature according to time and the test phases. The curve is fitted with ggplot2 using a Local Polynomial Regression Fitting method (loess). The gray area represents the standard error.

Figure 4

Individual Medial Canthus Maximal Temperature According to Time and Test Phases



Note. A) examples of the individual progression of the medial canthus maximal temperature difference from the baseline according to time and the experimental phases. Each colour corresponds to a different individual. B) individual progression of the medial canthus maximal temperature difference from the baseline according to time and the experimental phases. Individual pattern of all the tested reindeer. Each colour corresponds to a different individual. 0 is the baseline and refers to the individuals' mean temperature during the control phase. Temperatures below 0 are colder than the individual baseline and temperature over 0 are warmer than the individual baseline.

Discussion

In this pilot experiment, our objective was to assess the feasibility and reliability of utilizing infrared thermal imaging to record the medial canthus temperature as a non-contact method for evaluating stress in reindeer. Working sledge reindeer, while habituated to human presence and light handling still have a notable sensitivity to touch, and engaging in physical contact with humans can induce stress, as our results confirmed. The reindeer demonstrated significantly more avoidance behaviors, such as pulling on the rope to remove themselves from the situation when being petted by a stranger as compared to baseline. Additionally, they displayed behaviors like bending and curving their back to avoid touch and stepping away from the experimenter, highlighting their discomfort. This confirms that the chosen stimulus elicited a stress reaction in reindeer. Furthermore, it underscores the complexity of measuring physiological stress responses in reindeer, as procedures involving physical contact (e.g., blood and salivary sampling) may trigger strong behavioral and physiological reactions, complicating the process of stress assessment and potentially confounding hormonal measurements. In numerous laboratory species (e.g., rodents, rabbits, birds, non-human primates), basic handling procedures were shown to affect parameters such as heart rate, behavior, corticosterone and prolactin levels (Balcombe et al., 2004). In this context, the utilization of infrared thermal imaging emerges as a possible alternative for collecting physiological data without the need for direct physical interaction with the animals.

The results regarding the development of the medial canthus temperature during and after a stressor were in accordance with our hypothesis. Overall, the medial canthus temperature significantly decreased during petting and then started to increase when the experimenter stopped petting the animal and walked away. This is consistent with previous response patterns to stressor found in the literature. Cows subjected to an injection of epinephrine (an adrenaline like neurotransmitter that plays a key role in the "fight or flight" response) presented a fast and strong decrease in their eye temperature during the injection process followed by an increase back to baseline (Stewart et al., 2010). In their study, Kano et al. (2016) observed a decrease in the nasal temperature of chimpanzees while viewing a video depicting conspecifics engaged in a conflict. Subsequently, the temperature increased after the cessation of the video. Our initial experiment indicates that employing the thermal camera in outdoor conditions allows us to observe a comparable physiological response to stressor in reindeer. As this was a pilot study, we acknowledge that further investigation including additional sources of confounding factors would benefit our efforts to distinguish whether it was the presence of the unfamiliar person or the petting itself that elicited the stress response observed during the test phase. However, we advise being careful when employing a "no stimulus" control when conducting experiments outdoors, the lack of stimulation from the experimenter increases the likelihood of animals becoming more attentive or sensitive to external disturbances, potentially introducing confounding variables.

We also highlight the importance of continuous recording when assessing temperature changes during an emotional stimulus rather than recording the temperature before and after an event. A few existing studies focused on recording temperature before and after a stressor. Dai et al. (2015) took images of the eye temperature of horses before and after a fearful novel object test. They found a significant increase of the eye region temperature after the test. However, there is no information regarding the exact exhibited thermal pattern throughout the whole test. A few other studies on horses reported positive correlation between eye temperature and heart rate (Bartolomé et al., 2013; Evans et al., 2024; Redaelli et al., 2019). Again, in these studies, the measurements were punctual before and after a stressor (training, show jumping competition). Therefore, there is now an assumption that higher eye temperature measurement is associated with stress, when increases of temperature in peripheral areas could on the contrary be associated with soothing and appeasement (Ioannou et al., 2013). Taking a single point temperature measurement after a stressful event may not always be relevant, as it does not provide information on the intensity of the response nor how long the process lasted until full recovery. Continuous measurement has the potential to identify the lowest and highest temperature points across time providing a better understanding of the overall response. Moreover, the magnitude of the skin temperature change was shown to reflect the intensity of acute stressor in hens (Herborn et al., 2015). In our study, we could not always discern a consistent decrease or increase when looking at the individual patterns of the temperature changes through time. We note a significant variability among consecutive data points for the same individual. This variability could potentially be attributed to the camera employed in our study. Notably, this camera is designed for capturing still images rather than recording videos. Consequently, it introduces certain drawbacks to our data collection process. The camera necessitates refocusing before each shot, consuming approximately one or two seconds, thereby impeding the efficient collection of numerous and evenly spaced data points. Moreover, any subtle shifts in the focal point might account for slight temperature variations between successive pictures. Enhancing the efficacy of our data collection process is essential, and we propose adopting a thermal camera capable of recording videos. This strategic shift will notably augment the quantity of usable images for subsequent analyses, particularly when dealing with highly mobile animals. Video recording should not only increase data volume, but also contribute to improving consistency in the recorded temperature, offering a more comprehensive and accurate depiction of the experimental conditions.

This pilot experiment demonstrated the potential of infrared thermal imaging measurement of the medial canthus as a tool for a non-invasive monitoring of physiological responses to stressors in tamed reindeer. We also demonstrated that it is feasible with a low-budget infrared thermal camera (costing less than €3000); however, for more advanced research with a larger budget, we recommend opting for a thermal camera that supports thermal videos at a greater resolution. This would enhance data collection by providing researchers with a greater number and quality of thermal images collected to analyze. Additionally, the higher resolution would either yield a clearer image or enable filming from a greater distance, offering more flexibility in observational perspectives.

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