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Ambient Temperature and Humidity, But Not Sex, Age, or Time of Day Influence Inactive Chimpanzee (*Pan Troglodytes*) Nasal Temperature: Important Methodological and Reporting Considerations

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Abstract – Thermal infrared imaging provides noninvasive autonomic monitoring in freely moving subjects, leading to research on its potential to distinguish mental or emotional states in non-human primates and as a physiological measure of welfare. Unfortunately, methodological variations and the absence of standard reporting conventions may hinder comparisons across studies and species. In an effort to identify variables that may be particularly important for researchers to consider procedurally or standardize, we evaluated the influence of environmental, subject, and image variables on baseline nasal temperature. The current study employed a simple, regular methodology for recording thermal imaging data in 16 female and 14 male adult free-ranging sanctuary-housed chimpanzees (*Pan troglodytes*) across different ambient temperatures. Over three months, thermal images were captured from 1-5 m distance from the subject after each chimpanzee was inactive for two minutes. Of the 608 images collected, 540 (M = 18.00 images per chimpanzee, SD = 3.49, *Range* = 12-27) were deemed to be suitable for analysis as they were not taken in direct sunlight and met, or exceeded, our pre-set minimum pixel number in the region of interest (ROI) of the photograph. Separate stepwise multiple regressions revealed that mean and minimum nasal temperature were each influenced by ambient temperature and humidity, and that minimum nasal temperature was also affected by ROI pixel number. Nasal temperature measures were not influenced by subject sex or age, time of day, indoor or outdoor location, or subject distance from the camera. These findings highlight the importance of controlling for differences in environmental variables procedurally or statistically across conditions in thermal imaging studies as well as standardized reporting of ROI pixel number data.

Keywords – Infrared thermography, Chimpanzee, Nasal skin temperature, Sanctuary, Ambient temperature, Methodological reporting

Infrared thermal imaging can detect, visualize, and measure changes in facial temperature and so has been investigated as a non-invasive proxy for internal psychological states in wild and captive non-human primates (see Table 1). An underlying assumption of this research is that distinct internal states are associated with alterations in blood flow that change blood pressure in different areas of the body. Lower nasal temperatures, for example, have been observed in wild free-ranging male chimpanzees (*Pan troglodytes*) feeding on meat in competitive contexts (Barrault et al., 2022), in situations with a high potential for aggression (de Vevey et al., 2020), and in wild male and female chimpanzees after exposure to conspecifics' vocalizations (Dezecache, Zuberbühler et al., 2017).

Facial temperature changes from environmental or social stimuli have been evaluated under an array of experimental procedures. In chimpanzees, decreased nasal temperature occurred along with excitement behavior and heart-rate variability to sounds and videos of fighting conspecifics (Kano et al., 2016), and to a staged, realistic injury in a familiar human (Sato et al., 2019). Rhesus macaques (*Macaca mulatta*) showed decreased nasal temperature to a threatening person in a laboratory coat with a catching net (Nakayama et al., 2005), to video and audio clips of unknown monkeys (Kuraoka & Nakamura, 2011), and during feeding events (Ioannou et al., 2015). In marmosets (*Callithrix jacchus*), decreased nasal temperature after negative arousing stimuli, such as playback of aggressive vocalizations, was negatively correlated with tail piloerection, and nasal temperature was increased in males, but decreased in females, in response to playback of food calls (Ermatinger et al., 2019); sex differences similarly observed when playback of third-party social interactions occurred (Brügger et al., 2021). On the other hand, in research on a variety of primate species, decreased nose tip temperature was associated with behavioral indicators of a positive emotional state induced by human-initiated play (Chotard et al., 2018). Overall, these findings suggest that arousal generally leads to reduced nasal temperature in primates, but patterns for distinguishing distinct internal states and conditions remain unclear. Moreover, methodological variations across studies may complicate progress in identifying potential patterns.

The majority of research in non-human primates has focused on the nasal region with some studies including multiple facial or head areas (Chotard et al., 2017; Dezecache, Zuberbühler et al., 2017; Ioannou et al., 2015; Nakayama et al., 2005). However, the measures evaluated in the region of interest (ROI) of the thermal images vary across studies and have included pixel-by-pixel changes across conditions (Kuraoka & Nakamura, 2011; Nakayama et al., 2005), minimum ROI temperature (Brügger et al., 2021; Ermatinger et al., 2019; Heintz et al., 2019; Kano et al., 2016), and mean ROI temperature (Barrault et al., 2022; Chotard et al. 2017; de Vevey et al., 2022; Dezecache, Wilke et al., 2017; Dezecache, Zuberbühler et al., 2017; Ioannou et al., 2015; Sato et al., 2019). Furthermore, while Barrault et al. (2022) and Dezecache, Wilke et al. (2017) used between-subject designs, within-subject comparisons are common with delays before experimental conditions are introduced to minimize any effects carrying over from previous conditions (Chotard et al., 2018; Ioannou et al., 2015; Nakayama et al., 2005; Sato et al., 2019), to hours (de Vevey et al., 2022), and to days (Brügger et al., 2021; Ermatinger et al., 2019; Kano et al., 2016; Kuraoka & Nakamura, 2011).

To gather data with thermal cameras accurately, environmental variables, such as air temperature, humidity, and the subject's distance from the camera, all need to be considered (Kuraoka & Nakamura, 2022). Handheld thermal cameras vary in resolution (see Table 1), but even those with lower resolutions can be used with stationary subjects at close, fixed distances (i.e., ~1 m; Kano et al., 2015; Kuraoka & Nakamura, 2022) because the subject's face or other ROIs can occupy the entire image. Atmospheric gases can disrupt thermal readings, and Speakman and Ward (1998) recommended that distances exceeding 10 m from the subject be avoided because atmospheric disruption becomes too great for precise measurements. To ensure subjects remain close to the camera, researchers have utilized head immobilization devices (Kuraoka & Nakamura, 2011; Nakayama et al., 2005), food in combination with an experimenter lightly holding trained subjects' heads (Sato et al., 2019), or food/liquids to encourage the subject to voluntarily target to a required position (Brügger et al., 2021; Ermatinger et al., 2019; Heintz et al., 2019; Kano et al., 2016; Hopper et al., 2021 provides a review of such techniques, as used in eye-tracking protocols). Alternatively, images from freely moving captive animals in outdoor areas (Ioannou et al., 2015; Ross et al., 2021) and in a combination of indoor and outdoor locations (Chotard et al., 2017) have been utilized. In addition, images have also been taken of wild free-ranging animals, with researchers in wild settings using an upper limit of 15 m distance from the camera for safety and health reasons (Barrault et al., 2022; de Vevey et al., 2022; Dezecache, Wilke et al., 2017; Dezecache, Zuberbühler et al., 2017).

Table 1

Infrared Thermal Imaging Studies in Non-human Primates Listed Chronologically

Citation	Study Conditions	Species (Sex; Age)	Comparisons and General Findings	Distance to Subject	Ambient Temp	Emissivity	Camera and Resolution (Pixels)
Nakayama et al., 2005	Indoor; Experimental	Rhesus macaque (4 females; 5–8 yrs)	Decreased nasal temperature to potentially threatening person	23-25 cm	22-26°C	Not provided	NEC Sanei TH5100; 254 x 238 (60,452)
Kuraoka & Nakamura, 2011	Indoor; Experimental	Rhesus macaque (6 males; 4-14 yrs)	Decreased nasal temperature to video clips of a raging monkey, aggressive threat and scream stimuli (but not coo stimuli), and threatening faces and voices.	35 cm	24-26°C	Not provided	NEC Sanei TH9100MLN; 320 x 240 (76,800)
Ioannou et al., 2015	Outdoor; Experimental	Rhesus macaque (1 juvenile and 1 adult female; 1 juvenile and 2 adult males)	Decreased facial temperatures in the upper lip, nose, and orbital region during feeding compared to playing with a toy and teasing with food.	~1 m	6-6.5°C	Not provided	ThermoPro™TP8; 384 × 288 (110,592)
Kano et al., 2016	Indoor; Experimental	Chimpanzee (7 females and 5 males; 6–39 yrs)	Decreased nasal temperature to video or audio of fighting conspecifics with smaller decreases to orangutan long-call and no-sound conditions.	~1 m	18-25°C	Not provided	FLIR T650sc; 640 x 480 (307,200)
Chotard et al., 2017	Outdoor except for the marmosets; Experimental	Marmosets (7), white-throated capuchins (2), rhesus macaques (6), Bornean gibbons (1), western lowland gorillas (2)	Decreased nose tip temperature to combined toy and tickling; increased upper lip temperature to combined food delay and teasing.	~1 m	2-27°C, varied by species	Not provided	ThermoPro™TP8; 384 x 288 (110,592)
Dezecache, Wilke et al., 2017	Outdoor; Opportunistic	Chimpanzee (20 adult females; age not specified)	Pregnant and non-pregnant females had similar skin temperature patterns.	1-15 m	Data provided for each image**; Temperature and humidity controlled for in statistical analysis.	.98	Testo (881-2)* 160 x 120 (19,200)
Dezecache Zuberbühler et al., 2017	Outdoor; Opportunistic	Chimpanzee (3 females, 11-54 yrs; 11 males, 14-35 yrs)	Decreased nose temperatures and increased ear temperatures after conspecifics' vocalizations	< 15 m	Not provided; Temperature and humidity controlled for in ROI analysis.	.98	Testo (881-2)* 160 x 120 (19,200)
Ermatinger et al., 2019	Indoor; Experimental	Marmoset (9 females, 8 males; 3-14 yrs)	Increased nasal temperature in males to preferred food, food call playbacks, and control stimulus. Decreased nasal	1 m	Not provided	.98	FLIR T620 640 x 480 (307,200)

Citation	Study Conditions	Species (Sex; Age)	Comparisons and General Findings	Distance to Subject	Ambient Temp	Emissivity	Camera and Resolution (Pixels)
			temperature in females to food call playbacks				
Heintz et al., 2019	Indoor; Experimental	Western lowland gorilla (3 males, 19-21 yrs)	Nasal temperature decreased in two gorillas but increased in the third during human interactions	~1 m	Not provided	Not provided	FLIR T650sc 640 x 480 (307,200)
Sato et al., 2019	Indoor; Experimental	Chimpanzee (5 females, 1 male; 15 ± 6.0 yrs)	Decreased nasal temperature to overt (staged) injury in a familiar human	~1 m	20.4°C**	.95	Tobii X300 640 x 480 (307,200)
Brügger et al., 2021	Indoor; Experimental	Marmoset (11 females, 10 males; ~2-16 yrs**)	Sex-based differences in nasal temperature to playbacks of positive or negative third-party interactions	1 m**	Not provided	.98**	FLIR T620 640 x 480 (307,200)
Ross et al., 2021	Outdoor and indoor; Opportunistic	Chimpanzee (16 females, 13 males; 20-40 yrs.)	Decreased nasal temperature in active compared to inactive behaviors	1-5 m	43-96°F**; Temperature controlled for in statistical analysis.	.98	FLIR TI-85 320 x 240 (76,800)
Barrault et al., 2022	Outdoor; Opportunistic	Chimpanzee (9 females, 15–37 yrs; 10 males, 15–42 yrs.)	Decreased nasal temperature in competitive compared to cooperative social feeding	2-15 m	25.5-31.1°C**; Temperature and humidity controlled for in statistical analysis.	.98	Testo (881-2)* 160 x 120 (19,200)***
de Vevey et al., 2022	Outdoor; Opportunistic	Chimpanzee (9 adult males, age not specified.)	Nasal temperature decreased in competitive but increased in cooperative events	7-15 m	18-31°C**; Temperature controlled for in statistical analysis	.98	Testo (881-2)* 160 x 120 (19,200)

Note. *Used an additional telescopic lens (9 ° × 7 °/0.5 m); **Information provided in article's supplementary material or online database; ***Information on product website (Testo.com, n.d.)

Another important camera feature to consider is precise thermal sensitivity, or the ability to differentiate between slight changes in temperature, because reported changes in nasal temperatures tend to be relatively small, typically around 1°C (de Vevey et al., 2022; Kano et al., 2015; Nakayama et al., 2005). The ambient temperature of the environment must also be within the operating range of the camera. Given these factors, Kuraoka and Nakamura (2022) recommended that researchers use a camera with a minimum sensitivity of 0.1°C and suggested an environmental temperature of no more than 30°C. Despite this, the extent to which temperature elevations or a broad ambient temperature range can affect a camera's precision remains unclear. Nakayama et al. (2005) evaluated room temperature differences at the beginning and end of their sessions and found no correlation with baseline nasal temperature of rhesus macaques. However, associations between ambient temperature and nasal temperature have been reported in free-ranging animals, in both wild and captive settings (Barrault et al., 2022; de Vevey et al., 2022; Ross et al., 2021), leading researchers to conduct statistical analyses that controlled or accounted for ambient temperature (see Table 1). Such findings are consistent with McFarland et al.'s (2020) conclusion that ambient temperature may have significant impacts on both the camera and the subject.

Despite the growing use of facial temperature to evaluate emotional or mental states in non-human primates, methodological variations and the absence of standard reporting conventions may complicate attempts to compare across studies. Furthermore, to our knowledge, the potential impact of circadian rhythm-based variations in body temperatures in chimpanzees (Fowler et al., 1999) on thermal imaging data has not yet been investigated. Thus, the aim of the current study was to identify variables in thermal imaging studies that may be particularly important for researchers to standardize or consider procedurally, and to report. To that end, we evaluated the effects of environmental (i.e., ambient temperature, humidity, indoor or outdoor location), subject (i.e., age, sex, and time of day as a proxy for circadian rhythm-based variations in body temperature), and image (i.e., ROI pixel number and subject distance from the camera) variables on baseline nasal temperature. We also investigated if analyses of mean and minimum nasal temperature would yield distinct results.

Method

Ethics Statement

This research was approved by the Lincoln Park Zoo Research Committee, the Chimp Haven Sanctuary Chimpanzee Care Committee (SCCC), and by the National Institutes of Health (NIH). Research adhered to the American Society of Primatologists' Principles for the Ethical Treatment of Non-Human Primates.

Subjects and Housing

We collected data on 16 female and 14 male adult chimpanzees, housed at Chimp Haven, Louisiana, USA, in nine social groups ranging in size from five to 20 individuals. Subjects were selected to balance ages across sexes (females: median age = 34.0, range = 22-45; males: median age = 35.5, range = 25-55), so not all individuals in the subjects' social groups were included in the study. During data collection, chimpanzee social groups had access to enclosures that differed in size and design (Fultz et al., 2022). Outdoor enclosures ranged in area from 96.4 m^2 to 20,234.3 m^2, and included climbing and resting structures as well as enrichment items (e.g., hammocks, plastic toys), and had natural grass substrate. Attached ventilated indoor areas ranged in size from 62.2 m^2 to 131.9 m^2 and included hammocks and elevated platforms, as well as nesting materials such as hay, pine straw, wood wool, and blankets. Enclosures were surrounded by metal mesh, concrete walls with metal mesh windows, or flex mesh, requiring thermal photographs to be collected through the mesh. All chimpanzees were fed twice daily with fresh produce and commercially available primate diet, were provided with daily enrichment, and water was available ad libitum. No changes were made to the chimpanzees' standard care routine to accommodate data collection, and all photographs were taken opportunistically and noninvasively.

Thermal Images

Thermal image photographs of the chimpanzees' facial regions were collected with a FLIR E60 thermal imaging camera (320 x 240 pixels; FLIR Systems) that measured changes in thermal radiation emitted by an object. Based on previous reports of thermal imaging of free-ranging chimpanzees (e.g., de Vevey et al., 2022; Dezecache, Zuberbühler et al., 2017; Ross et al., 2021), emissivity was set to 0.98. Reflective temperature was set to 25°C (77°F), which was the reflective temperature of the fluorescent lighting in the chimpanzees' indoor spaces and consistent with Ross et al. (2021). In the absence of standard reporting of reflective temperature setting differences between indoor and outdoor locations (Chotard et al., 2018; Ross et al., 2021), emissivity and reflective temperature settings were held constant for indoor and outdoor data collection in the current study. Photographs collected in direct sunlight were not retained in the analysis.

Data on date, time, ambient temperature, humidity, whether the photograph occurred indoors or outdoors, the subject's estimated distance from the camera, and notes on the behavioral follow of the subject were recorded with each photograph. Ambient temperature and humidity were measured with a ThermoPro TP49 thermometer hygrometer. For safety reasons, photographs were collected at a minimum distance of approximately 1 m from the mesh barriers of the chimpanzees' home enclosures. Distance from the subject to the camera was estimated beginning at 1 m and increasing in 1 m increments by an observer trained in estimating distances using landmarks and floor plans of the enclosures.

Sampling Procedure and Behavioral Observation

Data collection occurred from June to August 2021 between 0820 and 1520 Monday through Friday. Because chimpanzees were separated into groups across a large sanctuary, a quasi-random sampling procedure based on building location was used to maximize efficiency of data collection. Once in a building, data collection proceeded with those groups exhibiting the least amount of movement based, in part, on the absence of recent interactions with caregivers (e.g., feeding). Focal individuals were chosen randomly and were followed for 5 min. If a 2 min period of inactivity occurred, a thermal image was collected at the end of the 2 min period of inactivity. Inactivity could include sleeping or awake states with minor body position shifts but excluded any locomotor activity or arm or leg movements. If the subject's face was unobstructed in the image, sampling proceeded to the next individual. If the subject's face was obstructed, the follow continued up to 5 min until another photograph could be taken. If the 5 min follow did not include 2 min of inactivity or yield a usable image, another individual from the group was selected to follow until all focal individuals in that social group had been sampled on that day.

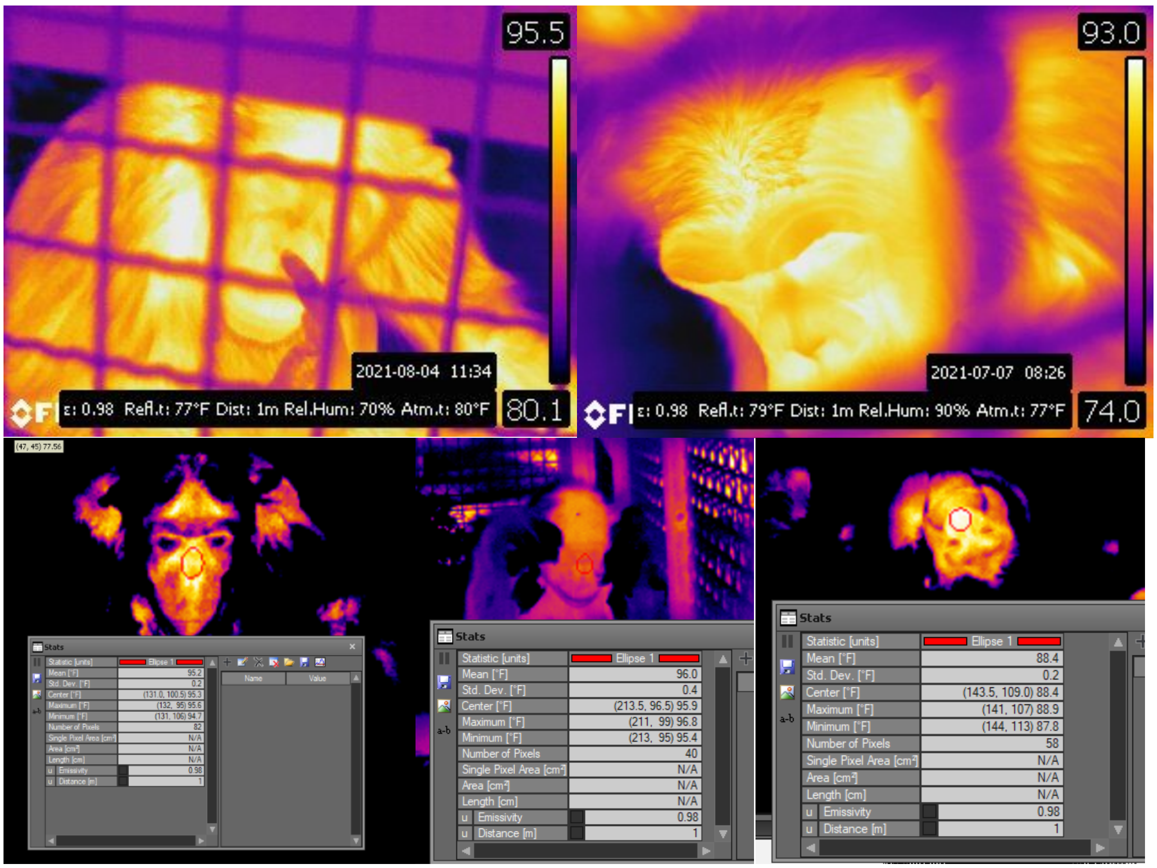
Once all 30 chimpanzees had been followed at least once during each day of data collection, refollows occurred for chimpanzees with no usable image for that day. At the end of each week, individuals with fewer usable photographs were intentionally followed until equal numbers of photographs had been captured for all 30 chimpanzees over the course of a week before resuming the randomized schedule. In addition, to avoid confounds between subject-linked characteristics (i.e., age and sex) and ambient temperature during image collection, if an individual had not had photographs taken evenly across different ambient temperatures, then that individual was prioritized with follows captured within the missing temperature ranges.

Thermal Image Processing

Photographs were processed via FLIR IR max software. The ellipse tool was used to isolate the ROI around the chimpanzee's nose, including the bridge, tip, and sides above the nostrils (see Figure 1). Maximum, minimum, mean, and standard deviation of the temperature within the ROI and ROI pixel number were recorded. Both mean and minimum nasal temperatures within the ROI were selected to compare for analysis. Because every pixel captured within the ROI equals one temperature reading, a higher number of pixels within the ROI reflects a more accurate temperature reading. For this study, we set a minimum of 25 pixels in the ROI as the criteria for image inclusion in analysis, consistent with Ross et al. (2021). Before the abstraction of temperature data from the photographs, intra-rater reliability tests were conducted pseudo-randomly by selecting the first photographs from 15 different chimpanzees that fit pixel criteria for ROI pixel number. Maximum, minimum, mean, and standard deviation of the ROI temperature were repeated three times for each image and each measure was analyzed using two-way mixed-effects, absolute-agreement, single-rater model intraclass correlation coefficients (ICC), yielding a high degree of intra-rater agreement for all measures, ICCs = .959-.998, 95% CIs = .907, .999.

Figure 1

Examples of Thermal Photographs of Chimpanzees



Note. The top row of images shows raw thermal photographs without the ROI isolated. The bottom row is screen shots of FLIR IR max software showing images with the ROI isolated.

Statistical Analysis

Mean and minimum nasal temperature data underwent reflection and log10 transformation to reduce skewness prior to stepwise regression analyses. Stepwise regression was selected to evaluate the unique contribution of each predictor variable incrementally and included the predictor variables of ambient temperature (°C), humidity (%), location (inside = 1; outside = 2), subject age (in years), subject sex (female = 1; male = 2), time of day as a proxy for circadian rhythm-based variations in body temperatures (7 hourly increments; 0820-1520), ROI pixel number, and subject distance from camera (1-5 m). All analyses were conducted using IBM SPSS Statistics 29.0.1.0.

Results

Descriptive Statistics

Data collection yielded 608 photographs, of which 540 fit pre-established inclusion criteria of an unobstructed image of the subject's nasal region, not collected in direct sunlight, and with a ROI that contained ≥ 25 pixels. Table 2 presents descriptive statistics for the image dataset. Overall, ROI pixel number displayed high variance across photographs due to variability in distance from the subject and the size of chimpanzees' nasal regions. Photographs were collected primarily within 2 m of the subjects, and inside rather than outside (Table 2).

Table 2

Image, Environmental, and Subject Descriptive Data

Image Data	
ROI pixel number	Mean ± SD = 129.7 ± 131.6; Range = 26 - 974
Distance from subject (m)	Mean ± SD = 1.6 ± .7, Range = 1 - 5
Environmental Data	
Ambient temp (□C)	Mean ± SD = 27.1 ± 2.7, Range = 18.9 - 33.9
Humidity (%)	Mean ± SD = 76.4 ± 11.8, Range = 42 - 99
Location	Inside = 494 (91.5% of the sample)
Subject Data	
Mean nasal temperature (□C)	Mean ± SD = 33.6 ± 1.7, Range = 26.4 - 36.3
Minimum nasal temperature (□C)	Mean ± SD = 32.8 ± 1.9; Range = 24.9 – 35.9
Sex	Female = 268 images (49.6% of the sample)

Separate stepwise multiple regressions were conducted to determine which environmental (i.e., ambient temperature, humidity, location), subject (i.e., age, sex, time of day), and image (i.e., ROI pixel number, subject distance from the camera) variables were predictors for mean and minimum nasal temperature. Both mean and minimum nasal temperature data underwent reflection and log10 transformation prior to analysis. Tolerance statistics met or exceeded .640 for all predictor variables. Residual scatterplots showed no systemic, differential patterns or point clusters, indicating that assumptions of linearity, normality, and homoscedasticity were met. Regression results revealed an overall model for mean nasal temperature of two significant predictors, $R^2 = .284$, $R^2_{adj} = .281$, $F(2, 537) = 106.395$, $p < .001$, and of three significant predictors for minimum nasal temperature, $R^2 = .267$, $R^2_{adj} = .263$, $F(3, 536) = 65.056$, $p < .001$. These models accounted for 28.1% and 26.3%, respectively, of the variance in mean and minimum nasal temperatures. For mean nasal temperature, ambient temperature ($\beta = -.566$, $t(537) = -14.398$, $p < .001$) and humidity ($\beta = -.124$, $t(537) = -3.156$, $p < .005$) significantly contributed to the model. For minimum nasal temperature, ambient temperature ($\beta = -.551$, $t(536) = -13.784$, $p < .001$), humidity ($\beta = -.134$, $t(536) = -3.345$, $p < .001$), and ROI pixel number ($\beta = .085$, $t(536) = 2.287$, $p = .023$) significantly contributed to the model. Due to reflection of nasal temperature data prior to regression analyses, these results indicate that nasal temperatures increased as ambient temperature and humidity increased and that minimum nasal temperature decreased as ROI pixel number increased.

Discussion

We found that higher nasal temperatures recorded with thermal imaging were associated with higher ambient temperatures and humidity levels in inactive captive chimpanzees, and that minimum nasal skin temperature measures were further influenced by the ROI pixel number. The location of the subject (indoors *vs.* outdoors), subject age, subject sex, time of day, and subject distance from the camera did not affect either mean or minimum nasal skin temperature. These findings, obtained from a large sample of chimpanzees balanced for sex across a relatively wide adult age range, are significant to thermal imaging research. They indicate that researchers should continue to evaluate the potential impact of ROI pixel number on minimum nasal temperature determination but that comparisons of mean nasal temperature data across sex, age, and time of day may be possible when ambient temperature and humidity differences are controlled. Our observed relationship between ambient and nasal skin temperature is consistent with prior findings in freely moving chimpanzees in captive and wild settings (Barrault et al., 2022; de Vevey et al., 2022; Dezecache, Zuberbühler et al., 2017; Ross et al., 2021). Our finding that humidity also influenced nasal skin temperature replicates Dezecache, Wilke, et al. (2017), but contradicts other prior reports (Barrault et al., 2022; de Vevey et al., 2022; Ross et al., 2021). Such environmental effects on nasal temperature are not surprising given that the main physiological function of the nose is to condition inhaled air, and the nasal cavity is where the exchange of heat and moisture mostly occurs (Naclerio et al., 2007).

Our finding that sex did not influence chimpanzee nasal skin temperature is consistent with Barrault et al.'s (2022) results in wild chimpanzees. Studies that included both sexes have tended toward smaller sample sizes than in the current work (e.g., Chotard et al., 2018; Ioannou et al., 2015; Kano et al., 2015; Sato et al., 2019). As documented in Table 1, thermal imaging studies in non-human primates have often focused on only one sex, and with limited age ranges included in sampling. Thus, the current study's large sample, balanced for sex across a wide age range, is uncommon in the thermal imaging literature and, to our knowledge, is the first study to demonstrate that subject variables such as sex, age, and time of day as a proxy for circadian rhythm-based variations in body temperatures did not influence nasal skin temperature in inactive chimpanzees. However, it should be noted that, unlike Sato et al. (2019) or de Vevey et al. (2022), the chimpanzees in the current sample were all mature adults at or over 22 years of age. Thus, our findings may not be generalizable to younger chimpanzees, highlighting the need for researchers to compare thermal imaging results in adults and non-adult chimpanzees (and in other species).

Chimpanzee nasal region temperature was not systematically influenced by distance from the subject, but minimum nasal temperature decreased as ROI pixel number increased; a surprising finding given that distance and ROI pixel number are related (Ioannou et al., 2014; Kuraoka & Nakamura, 2011) because the closer the camera is to the subject, the larger the proportion of the ROI is in the photograph and larger proportions result in higher pixel numbers and resolution. The current finding may be related to the pixel sample size in the ROI, with lower pixel numbers restricting the sample's range, yielding higher minimum nasal temperatures in the ROI. Despite variations in distance from the subject in the current study, all photographs occurred within 5 m of the subjects, a distance within the recommended limit of 10 m (Speakman & Ward, 1998). Of the thermal imaging field studies, de Vevey et al. (2022) alone reported an analysis of distance, which included an upper limit of 15 m, and found no significant effect of distance on nasal temperature; results consistent with the current study.

Both our current study and Ross et al. (2021) used a FLIR TI-85 thermal imaging camera with a resolution of 320 x 240 (i.e., 76,800 pixels), with other studies using cameras of varying resolutions (see Table 1). However, Dezecache, Wilke et al. (2017), Dezecache, Zuberbühler et al. (2017), de Vevey et al. (2022), and Baurralt et al. (2022) all used the same camera model, the Testo (881-2). Given the use of cameras of different resolutions or telescopic lenses, across studies, and our finding that minimum nasal temperature was influenced by ROI pixel number, researchers should consider reporting data on the range and/or mean number of pixels captured within their ROI as well as adopting and reporting pre-determined minimum ROI pixel criteria. For example, FLIR Technologies, a thermal equipment manufacturer, acknowledges that temperature differences can be determined with only a few pixels but that the ability of infrared cameras to accurately represent the temperature of the ROI is a product of the ratio between distance and the size of the object (Teledyne FLIR LLC, 2020). ROI pixel reporting standards may better facilitate comparisons across studies, especially for images of the same body area.

As previously noted, researchers differ in their analysis of the ROI region, with some utilizing minimum ROI temperature (Brügger et al., 2021; Ermatinger et al., 2019; Heintz et al., 2019; Kano et al., 2016) while others used mean ROI temperature (Barrault et al., 2022; Chotard et al. 2017; de Vevey et al., 2022; Dezecache, Wilke et al., 2017; Dezecache, Zuberbühler et al., 2017; Ioannou et al., 2015; Sato et al., 2019). We found that both minimum and mean nasal temperature were influenced by ambient temperature and humidity, but that minimum nasal temperature was further impacted by ROI pixel number, suggesting that comparisons between studies utilizing mean nasal temperatures are feasible and that researchers should further evaluate the extent to which ROI pixel number influences minimum nasal temperature measures. We employed an ellipse tool to isolate the ROI in images (the nasal region), replicating a previously published methodology (Ross et al., 2021). However, we did not investigate the impact of differing methodologies used to delineate the ROI. Determining if using a diamond (Barrault et al., 2022; de Vevey et al., 2022), triangle (Heintz et al., 2019), or circle (Sato et al., 2019) around the ROI alters nasal temperature determination may be an important step within the thermal imaging field as might open access to FLIR or other manufacturers' algorithms for temperature determination.

The current findings apply only to nasal temperatures in adult chimpanzees that have been inactive for two minutes. Previous studies in free-ranging chimpanzees have utilized different procedures to avoid the effects of locomotion on nasal temperature, such as requiring that subjects be stationary for at least one minute (Barrault et al., 2022), move less than 10 consecutive meters for a least one minute (Dezecache, Zuberbühler et al., 2017), or to be categorized based on their movement in the previous five minutes (de Vevey et al., 2022). Such timeframes are shorter than baseline time parameters in studies in which excitement or stress due to separation from home groups or placement to immobilize the head require longer acclimation periods (Kano et al., 2015; Kurakoa & Nakamura, 2011; Nakayama et al., 2005). In the current study, subjects were not separated from their group, and our sampling procedure was designed to avoid groups that were likely to be frenetic (e.g., during bouts of play, feeding, or agonism).

Conclusion

The current findings indicate that, consistent with prior studies in free-ranging wild and sanctuary-housed chimpanzees, ambient temperature (Barrault et al., 2022; de Vevey et al., 2022; Dezecache, Zuberbühler et al., 2017; Ross et al., 2021), and humidity (Dezecache Wilke et al., 2017) affect nasal skin temperature. Our findings underscore the importance of reporting ranges of and controlling for environmental variations in thermal imaging studies, particularly when between- or within-subject conditions are separated by hours or days. Our findings also indicate that ROI pixel number impacts minimum nasal temperature determination, perhaps because of the restricted sample range. In contrast, nasal skin temperature was not influenced by subject sex, age, time of day, and location (indoor *vs*. outdoor). We hope these findings facilitate research exploring skin temperature differences across distinct mental and emotional states in non-human primates. Given Hopper et al.'s (2021) recommendation that methodologies that impact data quality and inclusion be reported so that readers can interpret findings and draw comparisons across studies, we also recommend that thermal imaging researchers standardize reporting the mean and/or range for ROI pixel number and their minimum ROI pixel number criteria for inclusion, to enhance comparability across studies.

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