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A Method for Using a Thermal Camera to Measure Heat Loss Through Bird Feather Coats

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TITLE:
Using a Thermal Camera to Measure Heat Loss Through Bird Feather Coats

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KEYWORDS:
birds, flat skin, thermal camera, specimens, feathers, temperature, heat, thermoregulation, insulation, performance

SUMMARY:
This protocol describes the quantification of heat transmission through a flat skinned avian specimen using a thermal camera and hot water bath. The method allows obtaining of quantitative, comparative data about the thermal performance of feather coats across species using dried flat skin specimens.

ABSTRACT:
Feathers are essential to insulation and are important factors in the cost of thermoregulation in birds. There is robust literature on the energetic cost of thermoregulation in birds across a variety of ecological circumstances. However, few studies characterize the contribution of feathers to thermoregulation. Several previous studies have established methods for measuring the insulation value of animal pelts, but they require destructive sampling methods that are problematic for birds whose feathers are not distributed evenly across the skin. More information is needed about 1) how the contribution of feathers to thermoregulation varies both across and within species and 2) how feather coats may change over space and time. Reported here is a method for rapidly and directly measuring the thermal performance of feather coats and the skin using dried whole skin specimens, without the need to destroy the skin specimen. This method isolates and measures the thermal gradient across a feather coat, using measurements of heat loss and metabolic cost in live birds that behavioral and physiological thermoregulation studies cannot use. The method employs a thermal camera, which allows the rapid collection of quantitative thermal data to measure heat loss from a stable source through the skin. This protocol can easily be applied to various research questions, is applicable to any avian taxa, and does not require destruction of the skin specimen. Finally, it will further the understanding of the importance of passive thermoregulation in birds by simplifying and accelerating the collection of quantitative data.

INTRODUCTION:

Feathers are the defining characteristic of birds and serve many functions, among the most crucial being insulation[1]. Birds have the highest average core temperatures of any vertebrate group, and feathers insulating them from environmental temperature changes are a vital part of energy balance, especially in cold environments[2]. Despite the importance of feathers, the majority of literature on changes in thermal condition in birds has focused on metabolic responses to temperature variation rather than the function of feathers as insulation[3-10] (for further details, refer to Ward et al.)[11-13]. However, feathers themselves may vary across time, individuals, and species.

The method presented here is useful for quantifying the overall thermal value of the feather coat alone. It removes confounding factors in live birds, such as behavioral thermoregulation[1] and varying amounts of insulating fat. More widespread measurement of the thermal performance of feather coats is necessary to improve the understanding of how feathers contribute to insulation and how this varies among and within species throughout a bird's life history and annual cycle.

Feathers insulate birds by trapping air both between the skin and feathers as well as within the feathers, and they create a physical barrier to heat loss[14]. Feathers consist of a central feather shaft, called a rachis, with projections called barbs[14]. Barbules are small, secondary projections on barbs that interlock with adjacent barbs together to "zip" up the feather and give it structure. Furthermore, down feathers lack a central rachis and have few barbules, therefore forming a loose, insulative mass of barbs over the skin[14]. Feather coats vary across species[15-16], within species[17-18], and within comparable individuals[2,19-24]. However, there is little quantitative information about how variations in number of feathers, the relative abundance of different types of feathers on a bird, or changes in the numbers of barbs/barbules affect the overall thermal performance of a feather coat. Previous studies have focused on determining a single mean value of insulation and thermal conductivity for a given species[11-13].

The feather coat is known to vary among species. For example, most birds have distinct areas of skin from which feathers do (or do not) grow called the pterylae and apteria, respectively[14]. The placement of the pterylae (sometimes called "feather tracts") varies across species and has some value as a taxonomic character[14]. However, some birds (i.e., ratites and penguins) have lost this pterylosis and have a uniform distribution of feathers across the body[14]. Additionally, different species, especially those inhabiting different environments, have different proportions of feather types[15]. For example, birds inhabiting colder climates have more down feathers[15.] and contour feathers with a larger plumulaceous portion[16] than species inhabiting warmer environments.

The microstructure of certain types of feathers may also have an effect on insulation across species[25,26]. Lei et al. compared the microstructure of contour feathers of many Chinese Passerine sparrows and found that species inhabiting colder environments have a higher proportion of plumulaceous barbs in each contour feather, longer barbules, higher node density, and larger nodes than species inhabiting warmer environments[25]. D'alba et al.

compared the microstructure of down feathers of common eiders and graylag geese and described how these differences affect both the cohesive ability of the feathers and ability of the feathers to trap air[26]. Quantitative comparative data about how these variations in feathering affect the overall thermal performance of the feather coat across species is limited (for more details, refer to Taylor and Ward et al.)[11,13].

Within a species, the feather coat's thermal performance may vary. Some species, such as the monk parakeet[17], inhabit very large and diverse geographical ranges. The different thermal stresses posed by these different environments may affect the feather coats of birds within a species regionally, but there are currently no data available on this topic. Additionally, Broggi et al. compared two populations of great tits (*Parus major L.*) in the northern hemisphere. They demonstrated that contour feathers of the more northern population were denser but shorter and less proportionally plumulaceous than those of the more southern population. However, these differences disappeared when birds from both populations were raised in the same place[18].

Furthermore, Broggi et al. explained these findings as a plastic response to differing thermal conditions, but they did not measure the insulation values of these different feather coats[18]. The results also suggest that contour feather density is more important to insulation than the proportion of plumulaceous barbs in contour feathers, but Broggi et al. suggested that northern populations may be unable to produce optimal feathers due to a lack of adequate nutrients[18]. Quantitative measurements of the overall thermal performance of these feather coats would further the understanding of the significance of plumage differences.

Over time, the feather coats of individual birds vary. At least once a year, all birds molt (replace all of their feathers)[19]. As the year goes on, feathers become worn[2,20] and less numerous[18,21-23]. Some birds molt more than once a year, giving them multiple distinct feather coats each year[19]. Middleton showed that American goldfinches, which molt twice a year, have a higher number of feathers and higher proportion of downy feathers in their basic plumage in winter months than they do in their alternate plumage during summer months[24]. These annual differences in the feather coat may allow birds to conserve more heat during colder periods passively or shed more heat passively during warm seasons, but no studies have tested this conclusively.

Although birds thermoregulate behaviorally[1,27] and can acclimate metabolically to different thermal conditions[3-10,26], feathers play an important role in thermoregulation by providing a constant layer of insulation. The method described here is designed to answer questions about the feather coat alone and its role in passive thermoregulation (i.e., how much heat does a living bird retain without modifying its behavior or metabolism?) by isolating the feathers. While active and physiological thermoregulation are ecologically important, it is also important to understand how the feathers alone aid in insulation and how they influence the need for active and physiological thermoregulation.

Previous studies have established methods for quantifying thermal conductivity and thermal insulation of animal pelts[11-13,28]. The method presented here is an extension of the "guarded hot plate" method[11-13,28]. However, the method described here measures the temperature at the outer boundary of the feather coat using a thermal camera, rather than thermocouples. The guarded hot plate method gives very precise estimates of energy flow through a pelt, but it requires construction of a multi-material hot plate, some familiarity with the use of thermocouples and thermopiles, and destructive use of a pelt that must be cut into small pieces. These pieces are then greased to eliminate air between the sample and hot plate apparatus. With the exception of the few birds that lack apteria (e.g., penguins), cutting small squares from bird skins is problematic for comparative purposes, since the location of the cut has large effects on the number of feathers actually attached to (and overlying) the skin. This problem is exacerbated by the variation among taxa in the presence, size, and placement of ptyerlae[14].

Furthermore, while museum specimens can be a potentially rich resource for assessing the variation in insulation among birds, in general, permission to cut and grease skin specimens in scientific collections is unattainable. Additionally, specimens taken from the wild for guarded hot plate measurements cannot be subsequently used as museum specimens. The method presented here differs from the guarded hot plate method in that it can be used with whole dried bird skins, without 1) requiring the destruction of the specimen and 2) greasing the underside of the skin. It uses thermal cameras, which are increasingly affordable (though still relatively expensive), precise, and used for live bird measurements of thermal relations.

This method does not measure energy flow (and therefore thermal conductivity or insulation value) through the skin and feathers directly as the guarded hot plate method does. Instead, it measures the temperature at the outer boundary of a feather coat using a thermal camera. The resulting values represent an integrated measure of the heat lost passively through the skin, feathers, and air trapped between them (compared to a heat source underneath). Specimens prepared as flat skins and measured using the described technique can be stored in collections, and indefinitely provide value for future research. This method provides a standardized, comparable, and relatively simple way to measure feather coat thermal performance in any flat skinned specimen, which is especially useful in inter- and intra-specific comparisons.

PROTOCOL:

This work did not involve any work with live animals and was therefore exempt from animal care review.

1. Set-up and materials (Figure 1)

1.1. If flat skins of the species of interest are not available, use Spaw's protocol[29] to create skins from fresh or frozen specimens. Preen feathers into a neat, natural position, and dry to obtain a constant weight before proceeding with measurements.

1.2. Set up a constant temperature hot water bath.

NOTE: This set-up is quite tall, so it is easiest to place the hot water bath on the floor.

1.3. Place a sheet of clear acrylic glass (**Table of Materials**) over the surface of the constant temperature hot water bath. The glass allows transmission of the heat to the underside of the skin without wetting the specimen.

NOTE: This pilot study used a sheet of acrylic glass (0.125 in thick). The thickness of the glass will not affect the emissivity[30] of the material (it will always be 0.86), but it will affect the absolute temperature at the surface of the glass (i.e., thicker glass will result in a lower temperature). Thus, all measurements should be taken using an acrylic glass sheet of the same thickness.

1.4. Place a piece of foam core board (1 in thick) with a circular hole (0.5 in diameter) over the acrylic glass.

NOTE: The size of the bird should guide the size of the hole and thus the size of uninterrupted feathering receiving heat from the source. Here, a 0.5 in diameter hole is used, because this size is large enough to achieve sufficient heat transmission to the specimen while still being small enough to center the heat under the breast feather tracts (of all birds except for the smallest ones). Regardless of the size of the thermal opening, to obtain a comparable and replicable value for every bird, make sure to perform measurements with holes of the same size.

1.5. Attach a thermal camera to a tripod directly above the set-up at the camera's minimum focusing distance.

NOTE: Here, a FLIR SC655 thermal camera is used (680 px x 480 px resolution, ±2 °C or ±2% accuracy of reading, 40 cm minimum focusing distance). Other cameras may differ in degree resolution.

1.6. Calibrate the camera by entering the following into the thermal camera software:

1.6.1. Find the reflected temperature by placing aluminum foil (shiny side facing up) over the foam with the emissivity value set to 1.0 in the camera calibration software. Take a thermal image. The temperature at the surface of the aluminum foil is the reflected temperature, which should be similar to the ambient temperature of the room.

1.6.2. Set the emissivity value[31] to 0.95.

NOTE: 1) Emissivity is the relative amount of heat that an object emits[32] and ranges from 0 to 1. An object with a high emissivity value emits a large amount of heat, while an object with a low emissivity value radiates little heat[32]. This value represents the emissivity of feathers. 2) This value (0.95) is disputed. Cossins and Bowler claimed that feathers have an emissivity value between 0.90–0.95 but included no evidence[31]. Hammel reported a value of 0.98, but he obtained this value from a frozen specimen, so it may not be accurate[33]. Despite the lack of evidence, 0.95 emissivity is the value most often used in the thermal camera literature (as evidenced by Cossins and Bowler[31]).

1.6.3. Make sure the ambient temperature and humidity in the room are constant. These values should be measured before every measurement and updated in the camera calibration software. The temperature and humidity of all indoor rooms will fluctuate somewhat, so recording these values and updating them in the software reduces errors.

NOTE: Here, FLIR ResearchIR Max software is used. This software does not save data for all images, so it is crucial to record all of these values for each image.

2. Performance of measurements

2.1. Set the constant temperature hot water bath to a target temperature (40 °C is a proxy for the mean internal core temperature of most passerine birds)[34].

NOTE: If working with a species whose resting core temperature is higher (e.g., hummingbirds[34]) or lower (e.g., penguins[34] or ratites[34-35]), it may be appropriate to adjust the hot water bath accordingly. **Figure 3** shows the relationship between the temperature of the hot water bath and temperature at the surface of the acrylic glass (e.g., the actual temperature of the heat source the flat skin is exposed to).

2.1.1. Results obtained from this protocol (see **Figure 5**) suggest that obtaining measurements across a range of temperatures is also informative of thermal performance differences. To accomplish this, follow the protocol using 5 °C increments from 30–55 °C.

2.2. In the thermal camera software, draw a circle/ellipse over the hole in the foam where the heat is escaping. This makes it possible to visualize this area when placing the skin on the foam to ensure that the correct area on the flat skin is measured.

2.3. Place the flat skin specimen on the foam with the area of interest over the hole.

NOTE: Here, the belly region of each bird is measured, because it is not obstructed by other parts of the body such as the wing and is centrally located enough not to be subjected to edge effects. The placement of the skin over the heat hole will vary according to the experimental question. In general, placement directly over a feather tract, and as far away from the skin edge as possible, is recommended. Make sure not to flatten or disorder the feathers when placing the skin. If necessary, preen them into a natural position once the skin is placed.

2.4. Wait 15 min to allow the skin to acclimatize to the heat source. If measurements are made too early, the temperature value at the surface of the feather coat will be too low. Here, the temperature transmission through the skin and feathers stabilized at 15 min, so waiting longer than 15 min will not yield an artificially high result.

2.5. Taking a thermal image of the flat skin

2.5.1. Set the emissivity value[31] to 0.95 before taking the thermal image.

2.6. Remove the skin from the foam and immediately take a thermal image of the set-up without the flat skin on the foam. This quantifies the temperature at the surface of the acrylic glass and calibrates the area of the heat source with the measurement area on the flat skin.

2.6.1. Here, the emissivity of the acrylic glass used[30] is 0.86. Make sure to record this in the thermal camera software before taking the picture without the skin.

NOTE: The temperature displayed by the hot water bath is not necessarily the temperature at the surface of the acrylic glass (**Figure 3**), since its thermal conductance is not perfect. Using the temperature of the glass reduces errors in estimating how warm the underside of the skin is, and is therefore a combined estimation of how much heat is lost through the skin and feathers.

2.7. Place the skin back on the foam in the same position. Repeat steps 2.5–2.6 for a total of five trials.

2.7.1. To place the specimen skin correctly, gently touch the feathers in the target measuring area with one fingertip, then remove the finger and view the thermal image. The residual heat from the finger will remain visible on the thermal image briefly. Check to ensure that the sampling area is within the visible circle drawn in the software, which represents the area of heat radiating through the skin from the hot water bath. If it is not, move the skin until it is. This process is illustrated in **Figure 2**.

NOTE: While fresh skins (when available) may represent the natural thermal performance of the skin in a living bird more closely, using dry skins for these measurements allow comparable, repeatable results on a much larger pool of specimens. Therefore, all measurements should either be taken using skins dried to constant weight in both fresh and dried skin of specimens.

3. Data collection from thermal images

3.1. Each measurement consists of two thermal images: one of the flat skin and one of the acrylic glass. First, open the image of the acrylic glass. Align the circle drawn in the software with the hole in the foam visible in the image. Record the temperature value at the center of the circle.

NOTE: For more details on extracting data from thermal images, see Senior et al.[36].

3.1.1. Make sure to calibrate the camera with the proper values. Set the emissivity[30] to 0.86 and set the ambient temperature and humidity to match the current conditions in the lab, before recording the temperature value.

3.2. Open the thermal image of the flat skin. Without moving the circle, record the temperature value at the center of the circle.

NOTE: Because the circle is not recorded in the image, it is important to calibrate the placement of the circle with the image of the acrylic glass taken in section 2.6.

3.2.1. Make sure to calibrate the camera with the proper values. Set the emissivity[31] to 0.95, and make sure to set the ambient temperature and humidity to the current conditions in the lab, before recording the temperature value.

3.3. Repeat steps 3.1–3.2 for all measurements of all specimens.

4. Calculation of thermal performance

4.1. Subtract the temperature of the surface of the feather coat from the temperature of the acrylic glass. This value represents the heat retained by the feather coat.

REPRESENTATIVE RESULTS:

Representative results from a series of one individual of each of five species, measured at six temperatures, are presented in **Figure 4** and **Figure 5**. These show that small variations in the placement of the skin can result in variations in the readings of up to 1.7 °C. **Figure 4** shows how training of an investigator increases repeatability of the measurements. For example, the same individual house sparrow (*Passer domesticus*) was measured five times at a single target temperature by an inexperienced investigator (**Figure 4A**). After training on a variety of specimens of different species, one investigator (J.G.) measured the same specimen five times at the same target temperature (**Figure 4B**). The estimate of the relationship between the temperature of the acrylic glass and temperature at the surface of the feathers changed by only a small (but perhaps important) amount. As a result, repeatability of the measurements themselves changed by almost four-fold. Repeated practice is therefore highly recommended for operators on a non-sample skin (before taking measurements that will be analyzed) until measurements converge and variation in the measurements stabilize (i.e., no further improvement in reproducibility is seen with additional practice). This is important to achieve before conducting analyses on repeated measures (or the mean of repeated measures) in each specimen.

The data shown in **Figure 5** represents a small pilot sample but suggests that this method for measuring thermal performance of the feather coat is likely to yield important insight into the thermal ecology of birds. To reduce measurement error, only one investigator (J.G.) trained and took the measurements. Although these data represent only a single individual of each of the species listed (house sparrow, eastern phoebe [*Sayornis phoebe*], gray catbird [*Dumetella carolinensis*], eastern bluebird [*Sialia sialis*], and tufted titmouse [*Baeolophus bicolor*]), variation in the slopes of the resulting data demonstrates that the thermal performance of feather coats varies among these individuals. Furthermore, the magnitude of these differences suggests that the variation may be due to species differences.

Moreover, given that a single trained investigator performed all the measurements in **Figure 5**, investigator skill alone does not control for variation in R^2 values. For example, it was particularly difficult to obtain measurements repeatedly in the house sparrow, even after training, compared to the eastern phoebe and eastern bluebird (**Figure 4, Figure 5**). The latter two birds were both hatch-year individuals. Thus, their age class might influence the evenness of their insulation (although, that is speculation without further study), but there is no reason to expect that placement of their skins for the measurement should be any easier to repeat than that of the house sparrow. Thus, some incompletely understood quality of feather coats in the house sparrow may demand further investigation. Similarly, variation in slopes of the lines in **Figure 5** suggests that measuring thermal performance across temperature ranges (e.g., the thermoneutral zone of a species) may be more biologically informative than using a single reference heat level.

FIGURE LEGENDS:

Figure 1: Diagram of the complete thermal camera and hot water bath set-up.

Figure 2: Thermal images representing a method for replicating the same placement over multiple trials. The ellipse has already been placed on the heated area on the acrylic glass. These images show movement of the skin and not the ellipse. Gently and briefly touch a fingertip to the target measurement area on the feather coat. The fingertip will leave a heat mark on the skin for a few seconds. (**A**) Shown is the heat mark outside the ellipse, meaning that the target measurement area is not exposed to the heat. Being careful not to move the foam or acrylic glass (this would cause the ellipse to inaccurately represent the area of heat exposure), adjust the placement of the flat skin and touch the target measurement area again. Continue this process until (**B**) the heat mark is contained within the ellipse.

Figure 3: Relationship between temperature of the hot water bath (display reading) and temperature at the acrylic glass surface (e.g., the actual heat source the flat skin is exposed to). It should be noted that the temperature at the surface of the acrylic glass is consistently slightly higher than the temperature displayed by the hot water bath. Use this figure only to gain an understanding of this relationship, and always measure the temperature at the surface of the acrylic glass for each trial (section 2.6).

Figure 4: Improvement in the repeatability of temperature measurements at the surface of a feather coat in a single bird. These values were obtained from an individual house sparrow (**A**) before and (**B**) after repeatability training by the investigator for measurement performance.

Figure 5: Relationship between temperature at the acrylic glass surface and temperature at the feather surface in single specimens of five bird species. Points on any single graph represent repeated measurements in the same individual at six different target temperatures. It should be noted that although measurements at the reference heating point of 40 °C are

similar, the slope of these lines varies. This suggests that the thermal performance of feather coats in these birds differs (with a slope of 0 being a perfect insulator and slope of 1 being completely non-insulative). It should also be noted that measurement repeatability varies. Even after measurement training of the investigator, variance in repeated measures is highest for the house sparrow and lowest for eastern phoebe and eastern bluebird.

DISCUSSION:

This paper provides a protocol for repeatable, standardized thermal imaging measurements of avian flat skin specimens. This method makes it possible to compare thermal performance of the feather coat among species, within species, between comparable individuals, and at different locations on the bodies of individuals, all without destruction of the specimen.

The availability of necessary materials and equipment may be a limitation of this method. Although thermal cameras are rapidly becoming more accessible and affordable, research-grade thermal cameras still cost tens of thousands of dollars[37]. However, thermal cameras can be used for many practical applications in biology. McCaffery advocates for the use of thermal cameras to investigate ecological questions[28]. Thermal cameras are especially useful for collecting data on free-living organisms in the field because they are long-distance and non-invasive tools. The method presented here makes possible the integration of field and laboratory studies with measurements in the same units, as performed by the same equipment.

The use of thermal camera software other than that used here may necessitate modifications to this protocol, but such changes will only affect the set-up stage (section 1). Studies of smaller birds or certain questions about larger species of birds may require a differently sized holes in the foam layer.

Similarly, the temperature of the hot water bath (step 2.1) may need modification for some species with higher or lower core temperatures if the goal is to measure temperatures with direct biological relevance to the experimental question. In general, water bath temperature standardization at 40 °C across studies will facilitate comparative analysis of the relative thermal performance of different kinds of intact plumages and feather structures. If precise measurement of the energy flux across skin and feathers is required, the guarded hot plate method[11-13,28] is likely a better approach, since it 1) eliminates air between the heat source and skin and 2) measures temperature at the inner surface of the skin directly. However, while this method does not measure or calculate energy transfer directly, it is designed to facilitate rapid and repeatable measurements of whole specimens. Finally, the results demonstrate ample precision in detecting patterns of variation in plumage thermal performance.

This method uses flat skins, which are not currently widely available in most museum collections. Round skins, which are abundantly available in most natural history collections, could be used with this method if demounted, softened, flattened, and redried. However, curators are unlikely to approve such remounting in most cases. To increase the resources for comparative studies of the thermal values of bird feathers, we advocate for widespread adoption of flat skinning in as many species as possible. Added benefits of flat skinning are that flat skins do not require the partial destruction of the skeleton and musculature of a specimen that round skinning does, and higher numbers of flat skins can be stored in the same space that round skins require.

In skins of any particular species, it is essential to develop a technique for accurately placing the skin in the same spot over the heat hole every time. The results obtained here suggest that the technique (as described in step 2.7) minimizes measurement error more rapidly and effectively than practice in skin placement alone. However, it is plausible that especially dense plumages (e.g., penguins[11]) may not lose sufficient heat through the feathers to make it possible to visualize heat holes through the skin and feathers on the thermal image.

Because of the presence of pterylae in most bird species, the arrangement of the feathers over the skin on a specimen will affect the pattern of heat transfer across the feather coat. Therefore, it is important that the feathers are positioned in as close to their natural position in a live bird as possible. Preening the feathers into a neat, natural position is the last step in the protocol for flat skinning a specimen[29]. Thus, if specimens are prepared properly, feather placement should be appropriate to species across specimens. The amount of ptiloerection of the feathers will also affect thermal performance of the feather coat by trapping insulating air in the feather coat. In contrast, in flat skinned specimens, the feathers lay flat on the skin[29], so ptiloerection should effectively be comparable across all specimens.

Although this study focuses on birds, this method may be equally useful for mammal skins. Boonstra et al*.* asserted that bird feathers are more insulative than mammal fur, but this study was a qualitative assessment based on visual analysis of thermal videos[39] rather than a quantitative measure of the heat escaping from comparable body areas. It is believed that the method described here will contribute to an expansion of comparative thermal research and yield great insight into the evolution and ecology of thermoregulatory structures such as feathers[40].

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DISCLOSURES:
The authors have nothing to disclose.

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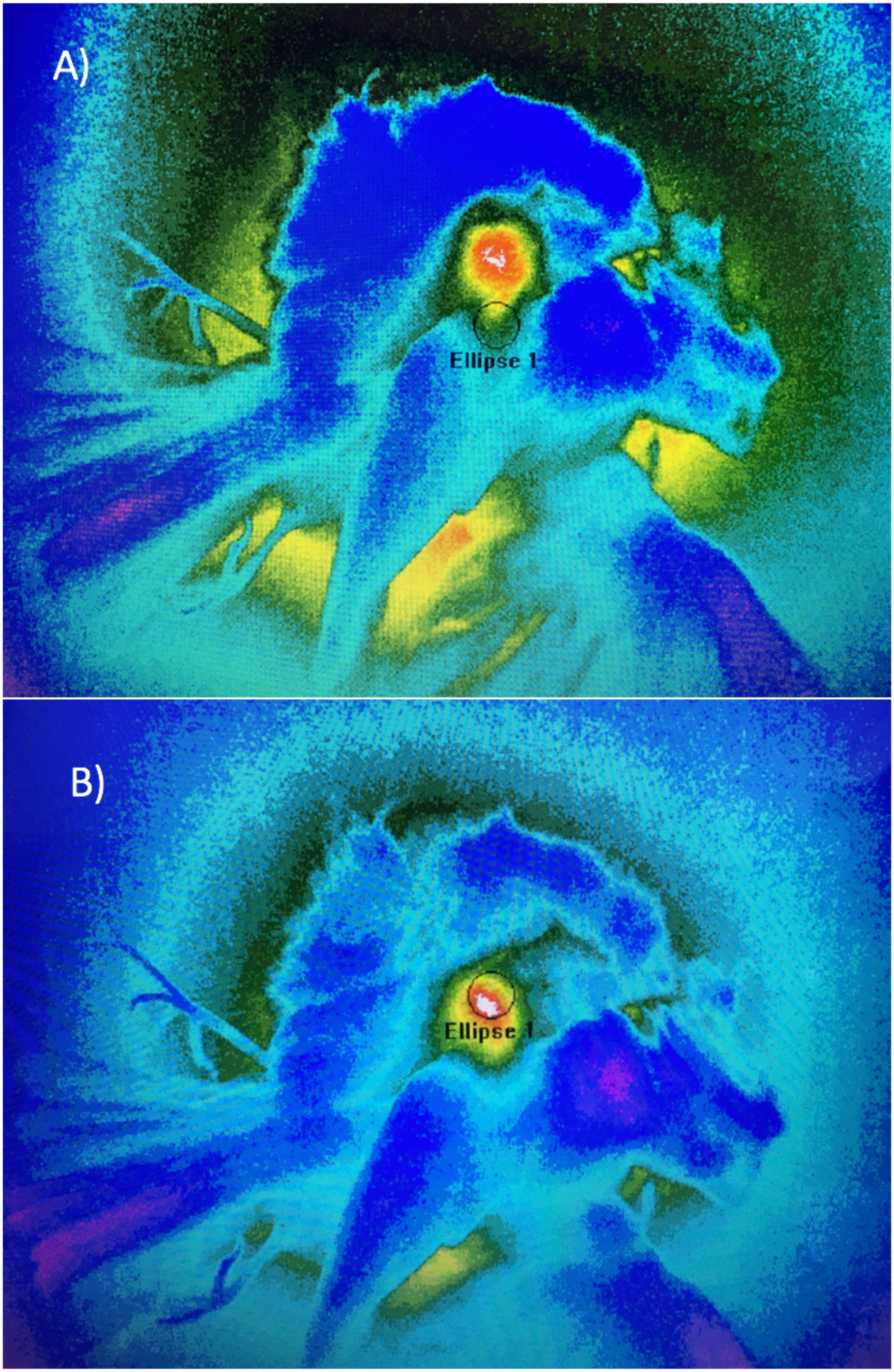
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Figure 1

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Figure 2

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Figure 3

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Figure 5

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Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Aluminum Foil	Reynolds Wrap	109000831	30 square ft.; this exact model need not be used.
Foam Core Board	Foamular	20WE	1 in. x 4 ft. x 8 ft; this exact model need no be used.
General Purpose Water Bath	PolyScience	WB02	Ambiet +5 °C to 100 °C; ±.01 °C
PDF Data logger	Elitech	RC-51H	Built in temperature and humidity sensor
Plexiglass	AdirOffice	1212-3-C	Acrylic glass; 12 in. x 12 in. x 1/8 in.; this exact model need not be u
Thermal Image Analysis Software	FLIR	ResearchIR Max v4.40.7.26 (64-bit)	Allows collection of precise, quantitative thermal data
Thermal Imaging Camera	FLIR	SC655	680x480-pixel resolution, ±2 °C or ±2% accuracy, 40 cm minimum fc
Tripod	The Audubo	The Birder Tripod with Manfrotto 700RC2 Rapid Release Head	65" maximum height; this exact model need not be used.

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Response to Reviewers Comments

The value of thoughtful, well-informed peer review was very much realized in the case of this manuscript, and we are grateful to the two reviewers who drew our attention to important previous work we had unaccountably missed, and issues with our method that needed consideration. We believe their feedback has strengthened the manuscript considerably, and helped us more accurately reflect the contribution this new method makes.

Direct responses to each point raised follow the reviewers' comments.

Editorial comments:

General:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We have proofread the manuscript carefully, and believe it to be error free. One point that we would appreciate editorial clarification on; spellcheckers flag "plexiglass" as needing to be capitalized ("Plexiglass"), presumably because it is a brand name. Our sense is that, in common use, plexiglass is understood to mean a kind of generic kind of material, and therefore should not be capitalized. Does JoVE have an editorial policy on this?

2. Please reduce the number of personal pronouns (we, you, your).

We have eliminated personal pronouns, with the exception of "we advocate" twice, in the Discussion section, where it seems appropriate to acknowledge personal opinions as belonging to the authors.

Protocol:

1. If applicable, please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution.

As this work did not involve live animals at any point, it was exempt from animal care review; we have added a statement saying so at the beginning of the protocol.

2. For each protocol step/substep, please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps.

This requirement was met in the original manuscript, and revisions have not changed it.

Figures:
1. Please cite Figure 1 outside of the Figure Legends section.

Done; Figure 1 is now appropriately cited at the end of the subhead, “1. Setup and Materials”, since it illustrates the entire set up.

2. Figures 3-5: Please use a proper degree symbol (°) instead of a superscript ‘o’.

Done. We have also remade all figures as .SVG files, as requested.

References:
1. Please do not abbreviate journal titles.

This has been corrected throughout the References list.

Table of Materials:
1. Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.

Done. For some materials, it is not imperative to use the exact model listed in the table (*e.g.* tripod); this has been noted in the table.

Reviewers' comments:
Reviewer #1:

General comments:

I agree with the authors that there has been, and is, to [sic] little focus on the insulative value of bird coats, and how this changes within and across species in different latitudes. So for this reason, there is good reason to apply techniques to be able to measure these things. However, there are some points where I disagree about the novelty of this study. There is certainly value in this approach, but the paper and protocol is not quite there yet. I have written some general comments below, which are followed by a list of more itemized, line-specific, remarks. I hope my assessment will be useful.

1. I disagree that this is a new approach, which is a central premise of the paper. The protocol is still useful, but it is basically an extension of the 'guarded hot plate' method that has been used to measure thermal resistance of animal coats (and manmade materials) for a long time. The only major difference here is that you measure the surface temperature using a thermal camera, but then on the other hand you also do not use a highly conductive material to distribute the heat (e.g. copper) and you do not ensure that there are no air pockets between the skin surface and the hot plate. The Plexiglas could be problematic because it has a sluggish thermal response.

We are very grateful to Reviewer 1 for drawing our attention to these important papers and the guarded hot plate method, and for saving us from publishing in ignorance of them. We agree completely that we have converged on a similar method, and it has been very instructive to us to study the difference; we have altered the manuscript in the Introduction and the Discussion sections to acknowledge this, and to point out the differences, and trade-offs between the two methods. If we had found the guarded hot plate method first, we might have attempted it, but we ultimately would have rejected it, because of two problems: a) it requires the destruction of a specimen by cutting it into pieces. This is problematic in almost all birds because of the uneven distribution of attachment of feather quills to the skin – the exact location from which you cut out the square sample to stick down to the hot plate would have an enormous effect on the final result, and results in, *e.g.* Ward et al. 2001, demonstrate that fouling and disarrangement of feathers reduces the value of the measurement. b) it requires that the underside of a specimen be greased to ensure there are no air pockets between the specimen and the hot plate, a step that would not be allowable with dried museum specimens, and that would negate the value of any specimen so treated for potential further use as a museum specimen.

It is accurate that a copper plate is highly conductive, and is generally excellent at distribution of heat; we agree that the guarded hot plate method is likely to be a better, more precise method for estimating the thermal resistance of materials, and we have altered the Discussion to acknowledge that. It is not obvious to us, however, that a sufficiently long waiting period for the temperature of the specimen to stabilize, as detailed in the protocol, for the heat to rise through the plexiglass (even if sluggishly), and the air pocket above the plexiglass, does not ultimately result in even, stable application of heat to the underside of the specimen (and indeed, our work showed that temperature stabilizes after a 15 minute wait period; see line 236 of the manuscript, item 2.4 of the protocol). When some of the skin is in contact with a hot plate, and some of it is not, it will indeed add uncontrolled error to the measurement; however, in our case, ALL of the heat transfer is from air to skin, and any error in the estimate should be consistent across the whole area heated, and therefore comparable from one measurement to the next.

Our overarching goal in the development of this method was not to measure the thermal resistance of the materials the feather coat of birds is made of (though that is a worthy goal), per se, but to develop the means to quantitatively compare the performance (see response to Comment 2, below) of the skin plus the plumage in retaining heat across species, individuals, and areas of the body, integrating all the variation in attachment of feathers to the skin, morphology of feathers, and relative abundance of feather types. We aimed to develop a method to search rapidly across species for patterns that might identify where deeper investigation of materials might be fruitful. We believe that the method we report in this manuscript has value for detection of such patterns without the need to destructively sample specimens, and have altered the manuscript throughout to reflect the relative value of, and distinction between, each method.

These classical approaches can be achieved for a fraction of the cost of a thermal camera. See e.g. Kvadsheim et al. J Therm Biol 1994, Ward et al. British Poultry Sci 2001, Wildlife Biol 2007.

Reviewer 1 is, again, right about this; a good thermal camera is far more expensive than the materials for a guarded hot plate, and we acknowledged the expense as a limitation of the method in the original draft. Having said that: thermal cameras are rapidly becoming more affordable, and more importantly, are now in widespread use to measure the thermal relations of live, wild birds. Our method has the advantage of using a tool that can be used on both a live bird and its pelt alone, and producing measurements, in the same units, that can be directly compared. We have added language in multiple places in the manuscript explicitly identifying these as values of the approach.

2. You do not supply methods for quantifying coat insulation, but only report on thermal gradients. From these data you would need to work out how much energy is retained by the coat (thermal resistance, or its inverse thermal conductivity). Alternatively, you could supply the necessary information to let readers work of heat transfer rate. But without any unit of energy transfer, I am afraid that you do not deliver on your central premise.

Thanks to these comments, it has become apparent to us, as it was not during the writing of the original draft, that the term “insulation value” has been used in earlier work to mean, technically and specifically, the *energy* retained by the materials of the pelt, in units other than °C. The reviewer is correct that we do not supply those methods, or provide those values. We converged on the term “insulation value” with a looser definition based on thermal gradients, i.e., the temperature on the inside (of the skin) vs. the temperature on the outside (of the feathers), and we continue to believe that these temperature gradients are an appropriate and biologically informative (as well as intuitive) measure of how the whole (uncut) feather coat performs thermally across all the variation present in birds. We agree however that, in light of the strict definition of “insulation value”, it is inappropriate to use that term in this paper. We have revised the title, and lines throughout the manuscript, to use the term “thermal performance” instead, a term that we believe more closely reflects our interest in how all the elements of the feather coat (e.g., feather morphology and curvature, placement, density) interact to keep heat inside the bird, rather than focusing on thermal resistance of the materials, alone.

3. I am a bit skeptical that you can accurately measure your reference temperature by simply taking a thermal image of the plexiglass plate, because to work out the heat loss through the plumage you would want a measure of the plate temperature when the skin sample is on.

We acknowledge that ideally we would want a measure of the temperature of the plexiglass while the skin is over it. Measuring it with the skin off adds error through heat loss. We have worked to minimize this error in our own practice by minimizing the amount of time between removing the skin and taking the image of the plexiglass, and we have revised the text in step 2.6 to emphasize the need to take the measurement immediately upon lifting the skin, and to explicitly identify heat loss at this step as a source of error. We have avoided the term “accurate” in the manuscript because we acknowledge, and are open and explicit about, the presence of error in our measurements, as every method has error. The repeatability of our measurements suggests that we have contained the error from this source. But we emphasize, as above, that our aim is to facilitate the uncovering of patterns by *comparable* measurements; we are trading off the greatest possible accuracy in estimating the “true” value of the measurement in any particular case against other goals. We have revised the manuscript in multiple places to reflect this focus on comparison as our goal, as well as to suggest the guarded hot plate method as superior for more exacting measurements.

4. You give a lot of details on setting up the thermal camera. This is important, but will have mostly minor influence on the absolute thermal gradient you measure. What is missing is a method for knowing exactly which temperature the camera is measuring (it is only accurate to +/- 2%). See some guidelines e.g. in Jerem et al. JoVe 2015.

We have read the guidelines presented in Jerem *et al*. JoVe 2015 with interest, but have to admit that we cannot see their relevance for the present study. We have therefore been unable to respond to this comment with changes in the manuscript.

The guidelines presented in Jerem *et al.* address obtaining the correct eye temperature of free living, moving birds, despite blinking by the birds, and filming through a mesh known to influence the temperature reading. They address, at some length, a method for collecting temperatures from many frames of a thermal video recording, creating a spreadsheet for those values, and correcting for/filtering out those that are associated with, e.g., low temperatures recorded when the bird's eye is not visible to the camera, or closed, or obscured by mesh components, plus correcting for the ambient temperature. Our study uses temperatures obtained from *single images* (snapshots) of unmoving materials (the bird pelt; the plexiglass), corrected for ambient temperature by the software that reports the temperatures, as detailed in our protocol in the original manuscript. The importance of entering the ambient temperature and relative humidity, for this correction, is mentioned repeatedly (e.g., at lines 209- 214 and lines 277- 279). That the camera accuracy is limited to +/- 2% is an unavoidable limitation of the equipment, one that we reported explicitly in the original manuscript. Kvadsheim et al. report that the accuracy of the guarded hot plate is +/- 4%, thus it is unclear to us that the degree of error in our measurement is in any way remarkable. We acknowledge, again, that there is error in our measurements, as there is in all measurements, but we are convinced our results show that the method constrains the error so that measurements are comparable, and the error is sufficiently small that patterns of biological interest can be identified.

Moreover, you also do not mention the need to standardize arrangement of the feathers when sampling a skin. Different amounts of 'ptiloerection' will influence heat transfer processes.

We agree absolutely that the arrangement of feathers will have a large effect on the resulting measurements; indeed, the disruption of natural feather arrangement is our chief concern with cutting skins up to measure their insulation performance. When preparing an avian flat-skin specimen, the last step in the protocol is to preen the feathers into a natural position (Spaw 1989), and into a flat (*i.e.* not erected; curved feathers are not flattened) position. We have emphasized this by adding "preen feathers into a neat, natural position" to step 1.1 and by further explaining this in the Discussion. In the Discussion, we also have added wording to explain that feathers are preened down against the skin in flat-skin specimens, so ptiloerection should be comparable across specimens.

Line-specific comments:

1. Line 2: I disagree this is what you report on in the paper.

See response to Comment 2, above. We have changed the title to “A Method for Using a Thermal Camera to Measure the Heat Loss Through Bird Feather Coats” to reflect the fact that we are not taking measurements in units of energy.

2. Line 25: I would be more inclined to say feathers are essential to birds' insulation, but not so much to the way they thermoregulate as this writing implies physiological regulation.

This wording has been changed from “Feathers are essential to the way birds thermoregulate” to “Feathers are essential to insulation, and therefore to the cost of thermoregulation, in birds” to minimize physiological implications.

3. Lines 28-29: I think you are undermining all previous efforts to describe insulation of animal coats here.

Given our previous ignorance of the papers pointed at by this reviewer, we agree that this section was overstating the case for novelty, and have toned down novelty claims throughout the manuscript. We do believe there is still much left to be discovered, but have revised the Introduction to discuss this with more nuance, and more thoroughly describe the available literature, and to clarify the contribution of this particular study in that context.

4. Line 30: Please revise - you only report on the temperature gradient.

Done: see response to Comment 2, above.

5. Line 44: Perhaps 'changes' rather than 'swings'?

“Swings” has been revised to “changes.”

6. Line 46: What does 'thermal flux' mean in this context?

Revised to “changes in thermal condition in birds”

7. Line 47: This is not true.

We have to respectfully disagree; we have re-searched the literature, and find far more literature on metabolic responses to temperature changes, than on insulation values of feathers, specifically. Nonetheless, we acknowledge there is more of a literature than we were aware of on insulation, specifically, and we have added citations at the end of this sentence to reflect that.

8. Lines 51-52: Biologically speaking, would not the sum of these effects (i.e. what you measure in a live animal) be the property of matter?

We are not sure we understand this comment; taken to an extreme logical conclusion, all biological phenomena are the result of the properties of matter. This does not negate the importance of understanding the contribution of feathers which, once grown, do not respond dynamically to thermal challenges the way that, for instance, a bird can change its metabolic rate. We have not revised this passage.

9. Line 53: In-between the feathers?

Yes, insulating air is also trapped between feathers. This detail has been added to this line.

10. Line 54: Why only to convective heat loss?

This has been revised to just “heat loss” to include all kinds of heat loss, including convection, conduction, and radiation.

11. Line 58: I think e.g. some of the papers by Peter Pap and colleagues would say differently.

We are perplexed by this comment; it appears to us that the papers in question (one of the most relevant of which we cited in the original draft) unquestionably support the assertion we made that “feather coats vary across species, within species, and within comparable individuals”. We have left the line unchanged, but have added additional citations from Pap and colleagues.

12. Line 70: You need to remember physiological regulation of heat production and heat dissipation here.

Indeed; that is why we think it is important to be able to characterize the contribution of the feathers, alone. The feathers also have an effect. See lines 113-120. We have left this line unchanged.

13. Line 80: Though, in many birds, heat dissipation is a very active process.

Agreed; but the feathers, and condition of the feathers, also have an effect, and our method attempts to quantify the thermal performance of the feathers alone in order to better understand how birds thermoregulate as a whole. See lines 113-120. We have left this line unchanged.

Reviewer #2:

Major Concerns:

I was struck by the fact that in the method described, the flat-skin specimen is placed over a "air hole" in a thick insulating "foam" resting over a plexiglass sheet. This means that there is radiative heat transfer to the inner surface of skin, something that never happens in living birds. Also, the convective/conductive heat transfer is from the underlying air space, not tissue/blood. While the method aims at measuring the passive thermal properties of the skin/feather preparation, this is very different from the situation in the whole bird. Could differences in the emissivity of the inside of the skin have an effect? If so, this would be artefactual because such differences in emissivity have no effect in living birds.

We agree that this method does not provide a biologically realistic heat source; at the same time we would argue that a hot plate does not either. Given that, across samples, whatever error resulting should be consistent. As revised into the manuscript, and elsewhere in this response, this method holds its value in simplicity, preservation of specimens, and is useful for comparative work. We believe the most important use of this method is the identification of patterns of variation, rather than replicating exactly what is occurring in a live bird.

Additionally, .95 is the accepted emissivity value for feathers and skin, so this should not have an effect, or if does, this is not the only study affected. See the second note on step 1.6.2 in the protocol for further explanation.

Although the method is standardizes as for the placement of the flat-skin specimen, it may be that even the same anatomical location is not comparable between species because there may differences in pterylosis between species. Although feathers cover the skin in a relatively uniform way, the higher amount of solid keratin shafts in pterylae might create variation in results.

This is absolutely a source of error among species. However, on flat skin specimens, feathers are less spread out than they would be on a round skin, so all the feathers in all tracts will be somewhat pushed together, even when the feathers are properly preened during specimen preparation. Still, because the length of feathers across species varies, measuring a point in the middle of a feather tract has less error between comparisons than measuring apteria covered in feathers. Finally, we aim, with this method, to uncover such impacts of variation in pterylosis on thermal performance overall.

The authors make a strong point an fixing a standardized temperature for these tests. Yet, in a contradictory fashion, they show in figure 5 nicely how a range of temperatures can be used to detect differences using the slope of plexiglass/feather surface temperature.

Our intention in including Figure 5 was to demonstrate that the relationship was linear across a range of temperatures, *i.e.,* that anomalous values for a given species would not be obtained as a function of very high or very low plexiglass temperature. However, we can see that the reviewer is right, in that the slopes of the line vary across these species. What this might mean, biologically, at temperatures birds would not ever survive (*e.g.,* 55°C) is not clear, which is why we suggest a target temperature that is typical of the core temperature of many birds, but we have revised the Discussion, and the Figure caption, to address this promising avenue for further investigation.

Minor Concerns:

Lines 45-47. It Is bit strong to say the studies on avian thermoregulation have *ignored* feathers as insulation. For example, heated, skin-covered metal casts have been used, https://www.sciencedirect.com/science/article/abs/pii/0300962980900341 and skin/feather patches have been used to measure passive thermal conduction, https://sora.unm.edu/sites/default/files/journals/auk/v103n01/p0160-p0168.pdf.

That is true, and feathers have not been completely unstudied. We have revised this paragraph to convey that the “majority” of literature on avian thermal biology addresses aspects other than the feathers, alone (which we believe to be true), but to reinforce this, we cited the papers the reviewer helpfully pointed at, along with a few others, at the end of this sentence.

Line 53: Air is trapped not only *between* the feathers and skin, but also within the feathers.

Yes, insulating air is also trapped between feathers. This detail has been added to this line.

Lines 232-233. "…suggests that repeatability of measurements". Please clarify.

This clause was an error and has been removed entirely.

Line 294. "The use of thermal camera software than we used…" should probably read "The use of thermal camera software other than we used…"

This has been changed to "The use of thermal camera software other than that which was used in this study."