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3D printed models are an accurate, cost-effective, and reproducible tool for quantifying terrestrial thermal environments

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ABSTRACT

Predicting ecological responses to rapid environmental change has become one of the greatest challenges of modern biology. One of the major hurdles in forecasting these responses is accurately quantifying the thermal environments that organisms experience. The distribution of temperatures available within an organism's habitat is typically measured using data loggers called operative temperature models (OTMs) that are designed to mimic certain properties of heat exchange in the focal organism. The gold standard for OTM construction in studies of terrestrial ectotherms has been the use of copper electroforming which creates anatomically accurate models that equilibrate quickly to ambient thermal conditions. However, electroformed models require the use of caustic chemicals, are often brittle, and their production is expensive and time intensive. This has resulted in many researchers resorting to the use of simplified OTMs that can yield substantial measurement errors. 3D printing offers the prospect of robust, easily replicated, morphologically accurate, and cost-effective OTMs that capture the benefits but alleviate the problems associated with electroforming. Here, we validate the use of OTMs that were 3D printed using several materials across eight lizard species of different body sizes and living in habitats ranging from deserts to tropical forests. We show that 3D printed OTMs have low thermal inertia and predict the live animal's equilibration temperature with high accuracy across a wide range of body sizes and microhabitats. Finally, we developed a free online repository and database of 3D scans (https://www.3dotm.org/) to increase the accessibility of this tool to researchers around the world and facilitate ease of production of 3D printed models. 3D printing of OTMs is generalizable to taxa beyond lizards. If widely adopted, this approach promises greater accuracy and reproducibility in studies of terrestrial thermal ecology and should lead to improved forecasts of the biological impacts of climate change.

1. Introduction

Humans are causing rapid shifts in climate with consequences for terrestrial ectotherms that have already been observed in many ecosystems (Deutsch et al., 2008; Sinervo et al., 2010; Thomas et al., 2004). Although most climate data available to biologists are measured at coarse scales (e.g., the frequently leveraged WorldClim dataset; Fick and Hijmans, 2017), temperature variation at fine scales (i.e., microsites) can have significant effects on the equilibrium body temperatures of ectotherms and therefore can have important ecological and evolutionary consequences (Alujević et al., 2023; Fey et al., 2019; Pincebourde and Suppo, 2016; Potter et al., 2009; Sears et al., 2016; Sears and

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Angilletta, 2007). Thus, to understand why populations are changing, and to predict future changes, it is imperative to be able to accurately quantify thermal landscapes at appropriate scales for diverse organisms that live in a wide range of habitats.

Thermal environments measured in biologically meaningful ways and at biologically relevant scales represent null distributions of available temperatures. These "operative temperature distributions" provide valuable information about habitat quality and the energetic requirements for individuals to perform well in their local environment. Classically, an operative temperature represents an instantaneous estimate of the equilibrium body temperature that an individual animal would achieve in a given microsite if heat exchange properties of the environment remain constant (Bakken, 1976; Bakken and Gates, 1975; Taylor et al., 2021). The quantification of operative temperatures has been essential for studying the thermal ecology, physiology, behavior, and evolution of both ectotherms and endotherms (Bakken, 1992; Dzialowski, 2005), and for predicting the responses of ecosystems to rapid environmental change (Gunderson and Leal, 2012; Huey et al., 2012; Logan et al., 2013, 2015).

Historically, several approaches have been used to quantify operative temperatures. One approach has been to combine meteorological and spatial data (often collected via remote sensing) with biophysical equations to model rates of heat exchange for particular organisms in specific environments (Kearney et al., 2009; Kearney and Porter, 2009; Porter et al., 1973). However, the computational skill and data access required for biophysical modeling of real environments can be prohibitive and has limited the use of this technique to certain laboratories and regions of the globe, and regardless, the accuracy of such computationally determined operative temperature distributions should be verified with *in situ* measurements. The most common way thermal environments are quantified for small terrestrial vertebrates is the deployment of physical models (temperature data loggers) that mimic certain properties of the study organism. These "operative temperature models" (OTMs) provide a measure of the thermal environment at the scale of the organism by integrating the conductive, convective, and radiative avenues of heat transfer between the organism and the environment (Bakken, 1976).

Over the past several decades of research in biophysical ecology, OTMs have been built using a wide range of techniques, including taxidermy mounts (Bozinovic et al., 2000; Hayes and Shonkwiler, 1996; Sharpe and Van Horne, 1999; Stoutjesdijk, 2002; Tieleman and Williams, 2002) and animal carcasses (Bishop and Armbruster, 1999; Frears et al., 1999; Ide, 2002; Kingsolver, 2000; Klok and Chown, 1999; Merrick and Smith, 2004). Nevertheless, the gold standard for OTM construction has been to use the process of electroforming to create copper casts of the study species (Bakken, 1992; Dzialowski, 2005). OTMs built through copper electroforming tend to accurately mimic the morphology of the organism of interest while also having minimal heat capacity (i.e., low thermal inertia; Angilletta, 2009; Christian et al., 2006; Porter et al., 1973). In combination with proper painting of the model's surface to match the spectral reflectance of the study species, these properties allow the model to rapidly and accurately equilibrate to changes in operative temperature (Dzialowski, 2005).

Despite their ability to produce accurate operative temperature datasets, copper electroformed OTMs have well-known drawbacks. First, they require the use of caustic chemicals that can be hazardous to work with. Secondly, the equipment and materials required to construct many models can be expensive. Third, electroforming is a time-intensive process that can require specialized training, and the OTMs are often brittle and thus frequently need to be replaced. Because of these problems, many researchers have resorted to the use of simplified OTMs in the form of metal or plastic pipes (Caetano et al., 2020; Logan et al., 2015, 2016, 2019, 2021; Neel et al., 2021; Piantoni et al., 2019), or spheres (Milling et al., 2018; Olsoy et al., 2022). Others have abandoned the use of OTMs all together and deployed fully or partially exposed temperature loggers in the field (Cox et al., 2020; Nicholson et al., 2022; Price-Rees et al., 2013; Williams et al., 2022). While these approaches to measuring operative temperatures are sometimes sufficient in environments that are uncomplicated with respect to heat transfer (e.g., under dense forest canopies or in fossorial habitats), in more radiatively complex environments they tend to produce large measurement errors (Dzialowski, 2005). This is because simplified models rarely match the shape, volume, surface area, posture, or texture of the organism of interest and therefore tend to respond to changes in air temperature, wind speed, and radiation differently than either morphologically accurate models, or the organism itself. Moreover, the objects used to create simple OTMs often have substantial thermal inertia and thus struggle to capture "instantaneous" estimates of operative temperature. These problems are often ignored, downplayed, or simply misunderstood by the authors of these studies (Blouin-Demers and Weatherhead, 2001; Lutterschmidt and Reinert, 2012). Dzialowski (2005) showed that basic calibration of models with live animals was either not done at all or not reported in more than 60% of studies that used OTMs. These issues with the ways that many biologists build and calibrate OTMs undermine our ability to understand the thermal ecology of terrestrial organisms and to predict how they may respond to environmental change (Bakken and Angilletta, 2014).

Recent technological advancements and cost reductions with three dimensional (3D) printing offer a unique opportunity for producing morphologically accurate, robust, easily replicated, and cost-effective OTMs that capture the benefits and alleviate the problems of electroforming. Watson and Francis (2015) compared the frequency distributions of operative temperatures produced by copper electroformed and 3D printed models for the Texas horned lizard (*Phrynosoma cornutum*). They found that these model types produced effectively indistinguishable frequency distributions of operative temperature in both open and closed habitats. While this study represents the first evidence that 3D printing may be a viable alternative to electroforming in thermal ecological studies, it only included an assessment of model performance for a limited number of environments using models printed with only one material (acrylonitrile butadiene styrene, or "ABS"), and direct comparisons with live lizards were not made. Possibly as a result of the limited scope of this previous experiment, 3D printed OTMs have yet to be widely adopted by researchers.

Here, we evaluate the ability of 3D printed OTMs to produce accurate estimates of operative temperature in studies of terrestrial thermal ecology. We assessed the performance of 3D printed OTMs against eight lizard species that differed both in body size and the habitat they occupy, and we validated models against live individuals in multiple microsites in nature. We focused our efforts on lizards because this group has a long history of being used for studies of terrestrial thermal ecology, thermal adaptation, and forecasting the responses of biological communities to climate change (e.g., Gunderson and Leal, 2012; Jiang et al., 2023; Logan et al., 2013; Sinervo et al., 2010). Moreover, lizard biologists have frequently used OTMs for thermal studies and many pioneering biophysical ecologists have studied lizards (e.g., Angilletta, 2001; Dunham et al., 1989; Hertz et al., 1993; Huey and Slatkin, 1976; Kearney and Porter, 2004; Sears et al., 2016). If 3D printed models provide accurate estimates of operative temperatures, the broad adoption of this approach beyond lizards would allow for more accurate assessment of animal responses to climate change and increased reproducibility and comparison across studies.

2. Methods and analyses

2.1. Species used for model validation

We tested 3D printed OTM performance in eight lizard species that differed nearly 15-fold in body size and occupied diverse habitats (Table 1). These focal organisms included one species that is restricted to tropical forest: the yellow-chinned anole (*Anolis gundlachi*); three tropical species that occur in forest-edge and open habitats: the crested anole

Table 1
Lizard species included in this study with corresponding collection location, habitat information, trial location, average reflectance (measured with a spectrometer), body mass, and the number of validation trials included in analyses.

Species	Location	Macrohabitat	Microhabitat	Mean mass (g)	Reflectance (%)	No. trials
Bush anole (*Anolis pulchellus*)	Guanica, Puerto Rico	Sub-tropical dry forest	Shaded grass in open woodland	2.97	25.99	7
Brown anole (*Anolis sagrei*)	Miami, Florida	Sub-tropical forest-edge	Low vegetation, low tree trunk, ground	3.88	14.90	29
Crested anole (*Anolis cristatellus*)	Guanica and El Verde, Puerto Rico	Sub-tropical dry and moist forest	Shaded grass, low tree trunks	4.10	11.44	6
Yellow-chinned anole (*Anolis gundlachi*)	Toro Negro, Carite, and El Verde, Puerto Rico	Sub-tropical moist and wet forest	Forrest logs, low tree trunks, ground	5.14	13.98	14
Southern prairie lizard (*Sceloporus consobrinus*)	Sherwood, Arkansas	Xeric grassland	Rock outcrops, trees, ground	7.20	9.79	6
Common wall lizard (*Podarcis muralis*)	Cincinnati, Ohio	Deciduous woodland	Stone walls, rock piles	7.40	7.15	12
Western fence lizard (*Sceloporus occidentalis*)	Reno, Nevada	High-elevation desert	Rock outcrops, low vegetation	21.0	17.23	19
Eastern collared lizard (*Crotaphytus colllaris*)	Mountain View, Arkansas	Xeric woodland	Open rocky glades	30.2	18.32	6

(*Anolis cristatellus*), the bush anole (*Anolis pulchellus*), and the brown anole (*Anolis sagrei*); a non-native species that has colonized anthropogenic structures and urban areas in eastern North America: the common wall lizard (*Podarcis muralis*); a xeric grassland species: the southern prairie lizard (*Sceloporus consubrinus*); a species that occupies rocky glades in the Ozarks of central North America: the eastern collard lizard (*Crotophytus collaris*); and a species that lives on rocky outcrops in the Great Basin Desert, among other habitats in western Northern America: the western fence lizard (*Sceloporus occidentalis*). All of these species have been the focus of thermal studies in the past (Bodensteiner et al., 2021; Brewster et al., 2020; Campbell-Staton et al., 2020, 2021; Firth et al., 1989; Gunderson et al., 2020; Leibold et al., 2022; Logan et al., 2014; Oufiero and Van Sant, 2018). Research was conducted under the following permits: Ohio Division of Wildlife Wild Animal Permit #23-014, Arkansas Game and Fish Commission Scientific Collection Permit #052420211, Texas Wildlife Diversity Permit #2022-IC-021 and Nevada Department of Wildlife Permit #229931. Procedures were approved by: Ohio Wesleyan University IACUC protocol #12-2020-02, University of Central Arkansas IACUC protocol #20-002, University of Texas at Arlington IACUC protocol #A2019.0007, University of Nevada Reno IACUC protocol #21-02-1129 and Florida International University #IACUC-20-033-AM04.

2.2. Development of 3D printed OTMs

We 3D scanned deceased individuals (well-preserved museum specimens or recently sacrificed and frozen specimens) of our focal lizard species using an Artec Spider 3D scanner (Artec3D, Senningerberg, Luxembourg; Table 1) at the University of Nevada, Reno library Makerspace. Lizards were scanned in a position they commonly assume in the field, in which their entire ventral surface was in contact with the substrate. Scans were assembled using the Artec Studio software to generate stereolithography (STL) files. Using the Raise3D ideaMaker software (Raise3D, Irvine, CA, USA), STL files were converted into g-code files that contain 3D printing commands. For each species, we printed three models made from different materials: acrylonitrile butadiene styrene (ABS), polylactic acid (PLA), and copper-infused PLA (Premium Metallic PLA; 65% copper; Fig. 1A). We chose these materials because ABS and PLA are two of the most common and affordable materials for 3D printing, and copper-infused polymer is the closest match to the material properties of copper-electroformed models. We printed all models using a Raise3D Pro2 printer with a 0.6 mm shell thickness, a layer height of 0.15 mm, and an infill density of 0% (i.e., they were hollow). Models were painted to match the reflectance of each lizard species which we measured on live, recently frozen, or ethanol-preserved specimens using a spectrometer (Ocean Optics, Ocean Insight, Orlando, FL). We measured reflectance across multiple positions on each lizard's dorsal surface. For each spectral measurement, we calculated mean reflectance for the interval 350–980 nm, then averaged the values across different positions of the body to obtain a single reflectance value per species (Table 1 and A1). Our spectrophotometer could not measure the near-infrared wavelengths beyond 1025 nm. This limitation may have introduced some error in assessing the reflectance of our research animals, although the close match we observed between live lizard and model temperatures in natural conditions (see below) suggest that we were able to sufficiently approximate the reflectance of our focal species.

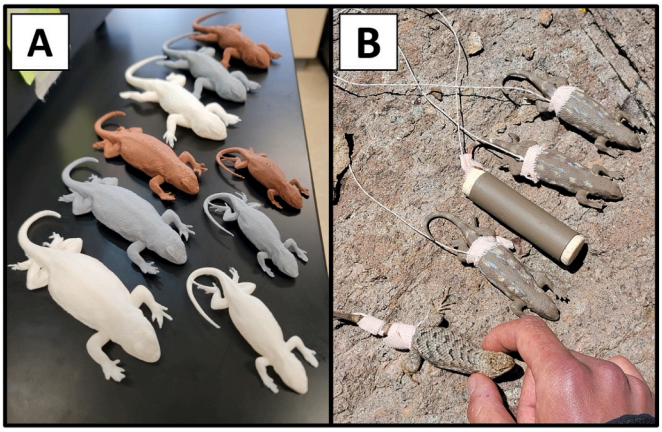

Fig. 1. A) 3D printed operative temperature models for three species of desert-dwelling spiny lizards (*Sceloporus magister*, *S. occidentalis*, and *S. graciosus*) printed using ABS (grey), PLA (white), and copper PLA (brown); B) A field-validation trial comparing 3D printed OTMs and a traditional copper pipe-based OTM with a live western fence lizard. Photo credit: Samir A. K. Gulati. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.3. Field comparisons of OTMs and live animals

Because of the diversity of locations, species, and laboratories that worked to validate models, some methodological details varied slightly among species (see the Online Supplementary Information). Regardless, the core aspects of our validation methodology were consistent across species. Generally, we compared 3D printed OTMs of the three commonly available material types to live individuals of each lizard species in their respective habitats (Fig. 1B). Following the capture of a

lizard in the field, we recorded body mass, snout-vent-length, and sex. We then inserted a thermocouple into the lizard's cloaca and secured it with medical tape. We placed the lizard into a cloth bag and then placed the bag in a cooler that was kept in the shade. We inserted a thermocouple into a small hole drilled on the side of each OTM, ensuring it was centrally suspended within the model's cavity, while also ensuring that the sensor tip did not make contact with the OTM walls. We then secured it in place using medical tape. OTMs were then placed in the same cooler as the live lizard with all thermocouples connected to a temperature reader for a minimum of 10 min or until the live lizard's body temperature approximated the OTM temperatures (<2 °C difference). Once the lizard and OTMs were at the same starting temperature, we simultaneously moved all of them to a randomly selected "shaded" or "sun-exposed" microsite within the surrounding habitat. Trial start time and general weather conditions were recorded at the beginning of each trial. For field-validation conducted using the western fence lizard (*S. occidentalis*), we also included a simple copper pipe-based model (Type M copper, wall thickness 1.15 mm) because of the ubiquity of this type of model in studies of lizard thermal ecology (Brewster et al., 2021; Brewster and Beaupre, 2019; Logan et al., 2019; Moore et al., 2018). Lizard body temperatures and OTM temperatures were logged or recorded every 10 s until the lizard reached equilibrium temperature with the environment. After completion of a trial, lizards were marked (to avoid pseudoreplication) and released at their original capture location (but see Supplementary Information for *P. muralis*).

2.4. Assessing OTM performance

We fitted asymptotic regression models using the 'drm' function in the R package 'drc' (Ritz et al., 2015) to raw data in order to generate individual heating and cooling curves for live lizards and OTMs. Curve fit was visually inspected and trials that could not be fitted (due to clearly identifiable problems associated with experimenter error, equipment malfunction, or sudden anomalous weather conditions) were discarded. From each curve, we extracted 'equilibration temperature' and 'time to equilibration' as the temperature and total elapsed time, respectively, at which the curve reached an asymptote. Since environmental conditions can change discontinuously during trials and heat flux is more rapid when starting differentials between ambient conditions and live lizards or models are large, equilibration time is a function of trial conditions and the difference between the trial starting temperature and equilibration temperature. Thus, to obtain comparable equilibration times, we standardized them across trials by dividing the time to equilibration by the total temperature change from the beginning of the trial to the point where equilibration temperature was reached. This gave us a rate of change per degree Celsius (min/°C) for every trial. To test if OTMs equilibrate to the same temperature as live lizards in natural conditions, we regressed lizard equilibration temperature against each OTM's equilibration temperature. We also calculated 'OTM accuracy' as the deviation between OTM and lizard equilibration temperature, with higher deviations indicating lower OTM accuracy. We then ran a linear mixed-effects model with 'OTM type', 'species', and their interaction as fixed predictors, 'trial number' as a random intercept to account for the three model types being run concurrently, and 'OTM accuracy' as the response variable using the 'nlme' package (Pinheiro et al., 2017). We tested for differences in OTM performance between sun exposure conditions (shaded versus sun-exposed microsites) in a separate linear mixed-effects model with 'OTM type', 'sun exposure', and their interaction as fixed predictors, 'trial number' as a random intercept, and 'OTM accuracy' as the response variable. We compared 'time to equilibration' between live animals and each of the models by fitting a linear mixed-effects model with 'OTM type', 'species', and their interaction as fixed predictors, 'trial number' as a random intercept, and log-transformed 'time to equilibration' as the response variable. Lastly, we tested 3D printed OTM performance relative to a simple copper pipe-based OTM in the western fence lizard. We ran two linear mixed-effects models with 'OTM type' as a fixed predictor, 'trial number' as a random intercept, and either 'OTM accuracy' or log-transformed 'time to equilibration' as the response variable. The distribution of residuals was inspected for normality and best fit statistical models were selected using a backward stepwise approach with likelihood ratio tests. All figures were generated using the R package 'ggplot2' (Wickham, 2016) and statistical tests were conducted in R v.4.2.2 (R Core Team, 2013).

2.5. Development of online database and repository of 3D scans

To make the 3D printed OTM method broadly accessible, we created an open access database of animal scans that are available at www.3dotm.org. This database currently contains high quality 3D scans of 19 different lizard species in the easy-to-use format of stereolithographic files (.stl), along with metadata corresponding to each file. When possible, these metadata include the geographic location at point of capture, as well as sex, body size, and habitat information. We encourage readers to submit their own 3D scan files to the website curator following the instructions on the "Contribute to 3Dotm.org" section to grow this resource for the community. While this database only includes scans of lizards as of the date of this publication, we anticipate that many other groups of organisms will eventually be represented.

3. Results

We generated 295 curves across 99 trials for our eight focal lizard species (Table 1). 3D printed OTMs predicted lizard equilibration temperature with high accuracy ($R^2 = 0.9$, $p < 0.0001$), and they performed well irrespective of species and model type (Table A1,Fig. 2Figs. 2 and 3). Deviations in equilibration temperatures between live animals and 3D printed OTMs were negligible, with the absolute mean deviation (absolute value of the raw mean deviation) for ABS, PLA, and copper PLA being 0.27 ± 1.92 °C, 0.01 ± 1.87 °C, and 0.08 ± 1.88 °C, respectively (mean ± standard deviations presented here and below). All OTM types performed accurately in both shaded and sun-exposed microsites, although variability was higher in sun-exposed sites. The absolute mean deviation between live lizards and model temperatures in shaded microsites was 0.1 ± 1.06 °C. The absolute mean deviation between live lizards and model temperatures in sun-exposed microsites was 0.1 ± 2.73 °C (Table A2 and Fig. 4). All 3D printed OTMs, irrespective of material, had substantially faster equilibration times than live lizards (mean equilibration time of live lizards = 2.39 ± 1.84 min/°C; ABS = 1.23 ± 1.28 min/°C; PLA = 1.33 ± 1.71 min/°C; and copper PLA = 1.18 ± 1.13 min/°C), and there were no significant differences between model types (Table A3 and Fig. 5). There was no significant difference between the equilibration temperatures achieved by 3D printed OTMs and a simple copper pipe-based OTM in western fence lizard trials (Table A4 and Figure A1A), but the copper pipe OTM took as long as the live lizard to equilibrate (3.25 ± 4.05 min/°C and 3.06 ± 2.94 min/°C, respectively; Table A5 and Figure A1B).

4. Discussion

The measurement of terrestrial thermal environments has suffered from inconsistent construction and validation of OTMs which has arisen partly because of the difficulty in producing these objects using traditional methods. Here, we validated the use of 3D printed OTMs for studies of the thermal ecology of terrestrial organisms, which largely overcome these drawbacks and challenges. We tested 3D printed OTMs in eight lizard species that range substantially in body size and occur in disparate micro- and macrohabitats. Across these diverse conditions, 3D printed OTMs equilibrated rapidly to the same body temperatures as live animals, suggesting that this method is as accurate as copper-electroforming and offers substantial advantages to traditional methods.

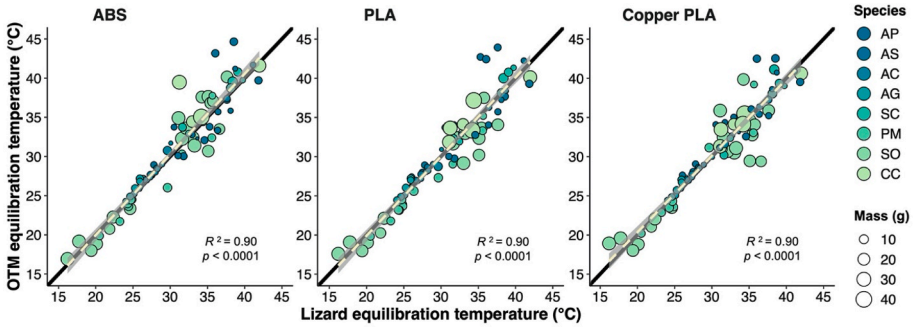

Fig. 2. Relationships between the equilibration temperatures of OTMs 3D printed with three different materials (ABS, PLA, and copper PLA) and live lizards (all species are pooled). Color represents species (AP = *Anolis pulchellus*, AS = *A. sagrei*, AC = *A. cristatellus*, AG = *A. gundlachi*, PM = *Podarcis muralis*, SC = *Sceloporus consobrinus*, SO=*S. occidentalis,* CC = *Crotaphytus collaris*) where species with lower average body mass are shown in dark blue and higher body mass in light green. Dot size corresponds to the body masses of individual lizards. Black lines are the 1:1 slope and yellow dashed lines are the best-fit relationship. Grey shaded areas are the 95% confidence interval. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

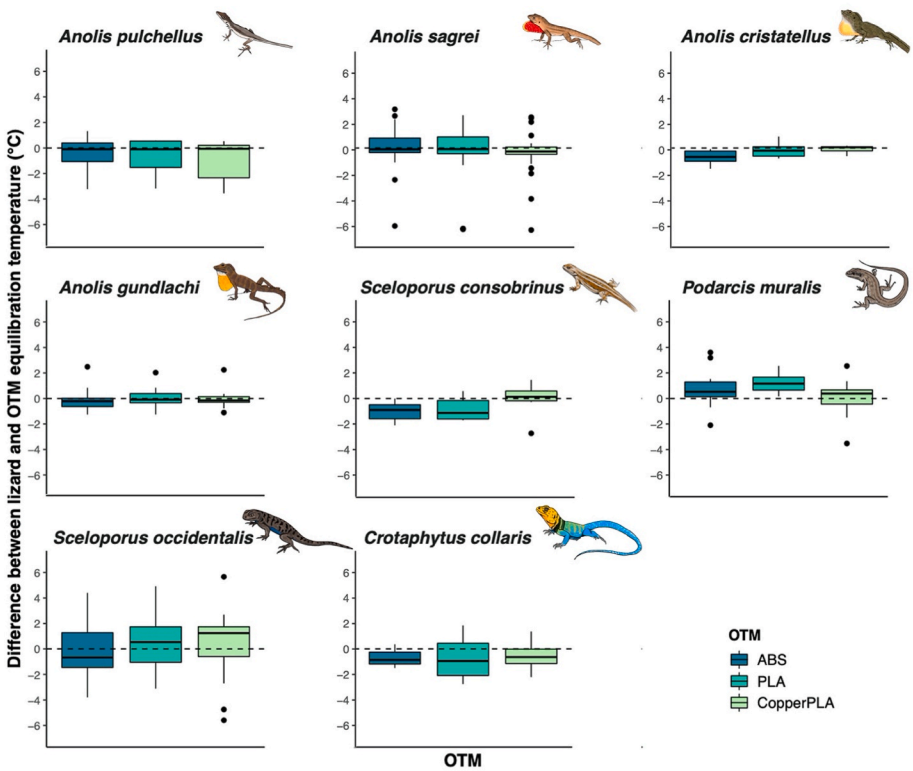

Fig. 3. Model accuracy expressed as the deviation between each model and lizard equilibration temperature for each study species. Species are ordered from smallest to largest mean body mass. There were no significant differences between OTM types across species. Boxplots are represented with medians and whiskers (within 1.5 times interquartile range). Illustration credit: Guillermo Garcia-Costoya.

The accuracy and reliability of OTMs rests on the premise that they equilibrate to the same temperature as the live animal when both are under the same set of ambient environmental conditions. Irrespective of the material they were printed with, our 3D printed OTMs predicted the equilibration temperature of the live organism with high accuracy, and this generally held true across species, habitat types, and sun-exposure conditions. Historically, copper electroformed casts have been shown to provide the most accurate measure of operative temperature in terrestrial ectotherms like lizards due to the properties of copper most closely matching the key heat exchange dynamics of a live organism (Bakken and Gates, 1975; Dzialowski, 2005; Walsberg and Wolf, 1996). While effective, copper electroforming can be an expensive, resource intensive, and time consuming process, often making it an impractical approach for large-scale field studies (Watson and Francis, 2015). Nevertheless, given the history of success using copper for accurate OTM production, we had expected that the OTMs that we 3D printed using copper-infused polymer would perform the best. Instead, we found that copper-based and non-copper polymers performed very similarly. This suggests that, for 3D printing, material type is less important than morphological accuracy.

An ideal OTM should not just equilibrate to the same temperature that a live animal would achieve in a given microsite under a given set of environmental conditions, but it should do so rapidly. In other words, OTMs should have low heat capacity to capture a relatively "instantaneous" estimate of operative temperature and thus be able to track those temperatures in real time as conditions change (Bakken and Gates, 1975). We designed our 3D printed OTMs to have minimal heat capacity by giving them a shell thickness of less than 1 mm and making them

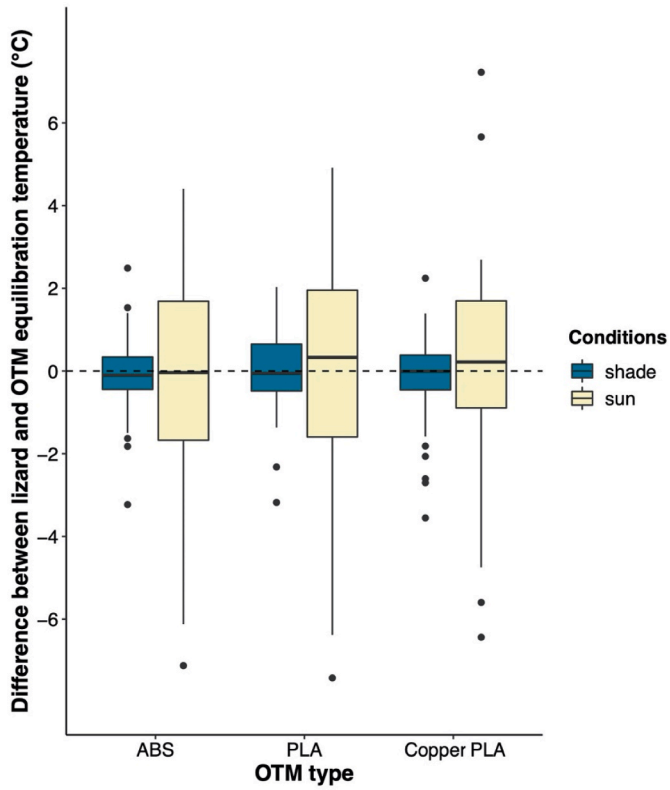

Fig. 4. Model accuracy expressed as the deviation between each model and lizard equilibration temperature in sun-exposed vs. shaded microsites. All lizard species are pooled. There were no significant differences in model performance based on sun-exposure. Boxplots are represented with medians and whiskers (within 1.5 times interquartile range).

hollow. As a result, 3D printed OTMs equilibrated much more rapidly than live animals, and this was true for lizards that were less than 2 g in mass and those that were greater than 20 g, and for models printed with any of the three test materials. On the other hand, the copper pipe OTM (a commonly used design in modern thermal ecology studies) had virtually the same heat capacity as a live western fence lizard. Thus, while copper pipe OTMs might produce accurate estimates of equilibration temperature, these models do not effectively track moment-to-moment changes in the operative thermal environment. It is important to note that we used a type of commercially available copper pipe that has the thinnest available wall thickness, so equilibration times are unlikely to be improved by using a different product. The importance of considering a model's thermal inertia is proportional to the size of the animal; when constructing OTMs for larger organisms, it is likely that OTMs will have greater thermal inertia (O'Connor, 2000; O'Connor et al., 2000; Shine and Kearney, 2001; Zimmerman et al., 1994). While we did not test 3D printed OTM accuracy for very large terrestrial ectotherms (e.g., >40 g in body mass), the benefit of using 3D printing is that model properties such as wall thickness can be easily manipulated to optimize model accuracy, even in larger animals. Another consideration for larger organisms is the positioning of the temperature sensors within the OTM, where it may be necessary to include multiple sensors to capture thermal variation within the model. Still, above a certain threshold of body mass, it is likely that OTMs constructed following any method would decline in accuracy due to both higher thermal inertia and thermal gradients within the OTMs themselves (Buttemer and Dawson, 1993; O'Connor, 2000; O'Connor et al., 2000). Data generated from OTMs may also be less relevant to larger species because of the high degree of regional heterothermy that is likely present in these species. Techniques such as biophysical modeling of heat flux in specific microenvironments may be more appropriate than OTM deployment for very large organisms.

Watson and Francis (2015) showed that models 3D printed using ABS were lighter, more durable, cheaper, and easier to replicate than

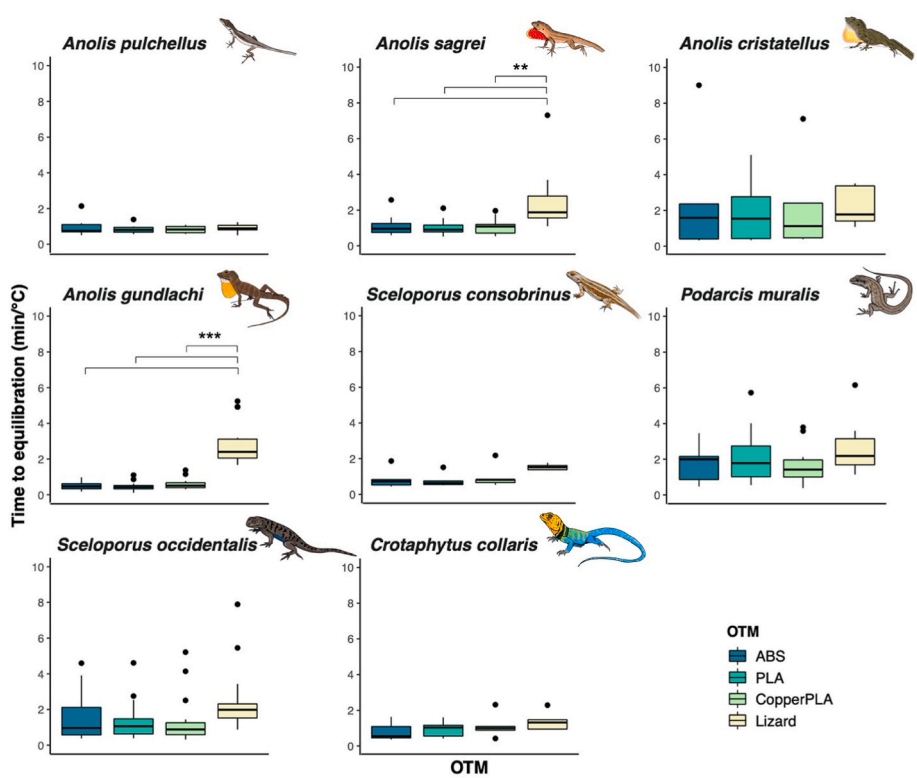

Fig. 5. Time to equilibration across models printed using different materials for each of our study species. Asterisks denote statistically significant differences (**p < 0.01; ***p < 0.001). Boxplots are represented with medians and whiskers (within 1.5 times interquartile range). Illustration credit: Guillermo Garcia-Costoya.

copper electroformed casts, and that both types of models produce similar operative temperature distributions when deployed in the same habitat, suggesting that the 3D printed OTM method may be viable. We tested ABS but included additional materials like PLA and copper-infused PLA, and found that these were comparable in price and production time to ABS. 3D printing using copper-infused PLA does, however, require more specialized equipment that is capable of printing metal. Conveniently, our data suggest that there is no need to use metal-infused materials to produce accurate and physically robust OTMs, since ABS and PLA perform almost identically to copper PLA, and all reproduce the body temperatures of live animals across a broad range of microsites and ambient conditions (when painted to match the reflectance of the focal organism). In addition, ABS and PLA polymers are sturdier, cheaper, and easier to print with current 3D printer technology compared to copper PLA. These materials are physically robust, and they can endure high temperatures (melting points are ~200 °C for ABS and ~150 °C for PLA).

While 3D printed OTMs appear to be robust and accurate, there are additional benefits of their use. First, it is easy to generate models that are morphologically accurate by scanning live or well-preserved museum specimens in natural postures using 3D scanners and accompanying software available at many university and public libraries. Although the Artec scanner we used for this study will be prohibitively expensive for many labs to acquire, there are cheaper versions of 3D scanners available, or one can even use a range of smartphone apps to generate printable 3D files (e.g., Qlone, Adobe Substance 3D Sampler). Alternatively, scans can be obtained from an already available museum or database. Many natural history museums are now producing 3D scans either following similar methods to ours or by using CT scans or photogrammetry (e.g., MorphoSource; https://www.morphosource.org/). These high-resolution scans allow researchers to replicate the texture of the organism's surface (e.g., the rugosity of scales; Fig. 1A) in substantially greater detail than is possible with electroforming. This is important as skin and scale texture can impact emissivity and other variables which mediate heat exchange (Achenbach, 1977), although this has yet to be empirically tested in the context of animal thermoregulation. Second, 3D scan files can be readily modified using the software built into most 3D printers to produce models with various specifications, including overall size, wall thickness, and infill (the degree of hollowness). Additionally, free CAD software (e.g., FreeCAD, Tinkercad, Blender, Fusion 360, etc.) can be used to modify original files by changing the position of the limbs or torso to generate models with different body postures which have been shown in some species to greatly influence equilibrium body temperatures (Brewster and Beaupre, 2019). Thus, a single 3D scan file can be used to produce different models that might represent different sexes, ontogenetic stages, or thermoregulatory behaviors of a given species.

While 3D printing of OTMs is straightforward when shape files are already available for the organism of interest, the process of 3D scanning specimens to generate printable files represents an additional layer of time, financial commitment, and complexity. For this reason, we created an online database of 3D scans (www.3dotm.org) where users can download (for free) printable files (plus metadata) for a number of lizard species that have functioned as focal organisms in thermal ecology studies in the past. The files that are available at the date of publication are those that we have produced ourselves, although we hope that researchers or members of the public that have 3D scans of any terrestrial organism will submit those scans to the website's curator via the "Contribute to 3Dotm.org" section of the website. As the number of scans grows in this way, we anticipate that this resource will aid in the adoption of 3D printed OTMs because 3D printers are now commercially available for relatively affordable prices (often below $1000), and many academic institutions and publicly funded maker-spaces now have 3D printers that are available for use. Further, this database may become useful to researchers outside of the field of thermal ecology because 3D printed models can be employed in other ways. For example, 3D printing can be used to generate molds for clay model studies of predation rates, or as tools in scientific outreach and education.

We focused on lizards in our validation of 3D printed OTMs because they are one of the most commonly studied groups in thermal biology. Studies of lizard biophysical ecology and physiology date back many decades, and have been used to develop fundamental theory in the fields of thermal ecology, thermal adaptation, and global change biology (Hertz et al., 1993; Huey and Hertz, 1984; Huey and Kingsolver, 1989; Huey and Slatkin, 1976). Lizards have played a central role in these fields because they often employ easily observable and quantifiable behavioral mechanisms to cope with variation in their thermal environments, many are widely distributed and have relatively short generation times for vertebrates, and they are frequently easy to maintain and breed in captivity. Despite our focus on lizards for validation, the 3D printed OTM approach is potentially suitable for a wide range of organisms. For studies on insects, OTMs are often made of carcasses or environmental temperatures are measured using bare temperature loggers such as iButtons or Tidbits (e.g., Ide, 2002; Kingsolver, 2000; Merrick and Smith, 2004). Small mammal researchers have utilized unheated taxidermic mounts or improvised spheres (e.g., Bozinovic et al., 2000; Stoutjesdijk, 2002; Tieleman and Williams, 2002). 3D printing may be a viable, sustainable, and much more reproducible alternative to these approaches for arthropods and mammals. 3D printed OTMs may also be adaptable to studies of amphibians, as novel 3D printing techniques are emerging that use materials such as agar which permit heat exchange via evaporative water loss to be taken into account (Wang et al., 2021).

The accurate quantification of thermal environments has direct implications for forecasting organismal responses to rapid environmental change (Bakken and Angilletta, 2014). Errors in estimation of operative temperature can reduce the accuracy of thermoregulatory indices which generate systematic biases in our interpretations and predictions of how organisms might compensate for changing environments. Many of these errors are a product of poor design of operative temperature models or a lack of calibration of these models against live animals in realistic environmental conditions (Dzialowski, 2005). While it has been known for decades what properties are needed to develop reliable OTMs, lack of a viable alternative to electroforming has slowed progress in this area of research. The effort and expenditure required to produce OTMs of sufficiently high quality has often resulted in the sacrifice of accuracy for convenience, yet technological advancements with 3D printing offer a logistically straightforward, accurate, robust, and reproducible alternative to traditional methods of OTM construction. The implementation of 3D printing in OTM design and construction represents a compelling new approach that will allow researchers to produce biologically realistic estimates of operative temperature that are comparable across studies.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2023.103762.

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