Methods of body temperature assessment in *Conolophus subcristatus* and *Conolophus pallidus* (Galápagos land iguanas)

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Since cardiovascular, respiratory, and metabolic systems of reptiles are affected by temperature, accurate measurements are of great importance in both captive husbandry and research. Ectothermic animals generally have core body temperatures close to ambient temperature but can differ from the immediate environment if they are using sunlight to thermoregulate. Many zoological facilities and exotic pet caregivers have begun using infrared temperature guns to assess ambient temperatures of reptile enclosures but there are currently few studies assessing the efficacy of these devices for measuring the body temperatures of reptiles. *Conolophus pallidus* and *C.subcristatus* are robust land iguanas endemic to the Galápagos archipelago. By comparing the infrared body temperature measurements of land iguanas against virtual simultaneous collection of cloacal temperatures obtained using a thermocouple thermometer, we sought to assess the efficacy of this noninvasive method. We found that internal body temperature can be predicted with a high level of accuracy from three external body temperature sites, providing a good non-invasive method that avoids the capture of animals.

1   **Methods of body temperature assessment in *Conolophus subcristatus* and *Conolophus***
2   ***pallidus* (Galápagos land iguanas).**

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24 **Abstract**
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26 Since cardiovascular, respiratory, and metabolic systems of reptiles are affected by temperature,
27 accurate measurements are of great importance in both captive husbandry and research.
28 Ectothermic animals generally have core body temperatures close to ambient temperature but can
29 differ from the immediate environment if they are using sunlight to thermoregulate. Many
30 zoological facilities and exotic pet caregivers have begun using infrared temperature guns to
31 assess ambient temperatures of reptile enclosures but there are currently few studies assessing
32 the efficacy of these devices for measuring the body temperatures of reptiles. *Conolophus*
33 *pallidus* and *C. subcristatus* are robust land iguanas endemic to the Galápagos archipelago. By
34 comparing the infrared body temperature measurements of land iguanas against virtual
35 simultaneous collection of cloacal temperatures obtained using a thermocouple thermometer, we
36 sought to assess the efficacy of this noninvasive method. We found that internal body
37 temperature can be predicted with a high level of accuracy from three external body temperature
38 sites, providing a good non-invasive method that avoids the capture of animals.

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48 **Introduction**
49   Since cardiovascular, respiratory, and metabolic systems of reptiles are affected by
50 temperature, accurate measurements are of great importance in both captive husbandry and
51 research (Sato et al., 1995; Deen and Hutchinson, 2001; Seebacher and Franklin, 2005; Long,
52 2016). Body temperature assessment is also an important part of veterinary health examinations
53 (Music and Strunk, 2016; Cusack et al., 2018). While ectothermic animals generally have core
54 body temperatures close to ambient temperature, it has been shown that the animal's temperature
55 can differ from the immediate environment (Raske et al., 2012). The size (mass) of the animal
56 would also effect this as smaller animals heat up and cool down more quickly than larger ones
57 (Gillooly et al., 2001; Seebacher and Franklin, 2005).
58
59   Many zoological facilities and exotic pet caregivers have begun using infrared
60 temperature guns to assess ambient temperatures of reptile enclosures (Rizzo, 2015), but there
61 only a few studies assessing the efficacy of these devices for measuring the body temperatures of
62 amphibians and reptiles (Hare et al., 2007; Rowley and Alford, 2007; Halliday & Blouin-
63 Demers, 2017). By comparing the infrared body temperature measurements of land iguanas
64 against virtual simultaneous collection of cloacal temperatures obtained using a thermocouple
65 thermometer, we sought to assess the efficacy of this noninvasive method in a large terrestrial
66 reptile.
67   *Conolophus pallidus* and *C. subcristatus* are land iguanas endemic to the Galápagos
68 archipelago. The former species is restricted to Santa Fe island and both species are classified as
69 vulnerable by the IUCN Red List of Threatened Species (World Conservation Monitoring
70 Centre, 1996a, 1996b). As part of a population health assessment authorized by the Galápagos
71 National Park (GNP), wild iguanas from three islands (North Seymour, South Plazas, and Santa
72 Fe) were captured in July 2018. Veterinary health examinations, that included sampling blood,
73 ectoparasites, and feces, were performed on each animal in accordance with the ethics and
74 animal handling protocols of Galápagos Science Center (GSC) and the Galápagos National Park.
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77 **Materials and Methods**
78   This study was conducted in the Galápagos archipelago of Ecuador in July, 2018 as part
79 of a population health assessment authorized by the Galápagos National Park Service (Permit #
80 PC-70-18 to G.A. Lewbart) and approved by the Universidad San Francisco de Quito ethics and
81 animal handling protocol.
82
83   Twenty-one adult *C. pallidus*, thirty *C. subcristatus*, and one *Amblyryhnchus cristatus* X
84 *C. subcristatus* hybrid weighing between 1.2 and 7.2 kg (mean = 3.9 kg) were captured for health
85 assessments. All data were collected over a period of 4 days, with the ambient environmental
86 temperature ranging from 20.5^0C to 26^0C at the time of examination. Body temperature
87 measurements with a Nubee® infrared temperature gun (Model NUB838OH) were collected
88 from the right axillary space (T_{AXI}), dorsum (T_{DOR}), and right femoral space (T_{FEM}) (Figure 1).
89 The axillary space is the area just caudal to the insertion of the upper arm to the body. The
90 dorsum is an area directly above the axillary location at the most dorsal part of the animal. The
91 femoral space is the area just cranial to the insertion of the rear limb to the body. The device was
92 held approximately 7 cm from each location for 5-10 seconds (until the temperature stabilized).

93   This device has a distant to spot ratio of 8:1. Thus, the area being measured is approximately 1
94   cm. Cloacal temperatures (T_{INT}) were measured using an EBRO® Compact J/K/T/E
95   thermocouple thermometer (Model EW-91219-40; Cole-Parmer, Vernon Hills, IL, USA 60061)
96   inserted approximately 10 cm into the vent. The thermocouple thermometer was held in place
97   until a stable temperature reading could be recorded (approximately 15 seconds).

98   *Statistical analysis*

99   We used repeated-measures ANOVA with Huynd-Feldt (HF) method to correct for
100 departure from sphericity (HF: ε=0.7896778, Mauchly's Tests for sphericity: W=0.54929,
101 P=<0.001) to test for temperature differences between the four methods. We then performed
102 pairwise comparisons using paired t test with Bonferroni-Holm correction. Given non-
103 independence of measurements, we investigated the best predictors of internal (a.k.a. cloacal)
104 body temperature (T_{INT}) in two complementary ways. Running simple linear models, we first
105 looked at simple pairwise correlations using as regressors the three infrared external
106 temperatures: Femoral (T_{FEM}), Dorsal (T_{DOR}), and Axillary (T_{AXI}). Then, we chose the external
107 body temperature with the highest correlation coefficient as the best single predictor of internal
108 temperature. We also extracted the first principal component of the three external temperature
109 measures to be used as a single compound predictor. Statistical analyses were performed in R
110 version 3.5.1 (R Development Team, 2018).

111

112 **Results**

113   Average body temperatures measured at each site are displayed in Table 1. Figure 2
114 graphs all four temperature points for each animal. The average temperature differed
115 significantly between the four methods (Repeated-measures ANOVA: $F_{2.4,120.9}$= 9.8397,
116 P<0.001), however the effect size was small (η^2= 0.01224529). Pairwise comparisons showed
117 significant differences between T_{DOR} - T_{AXI} (t=3.01, DF=51, P=0.012) and all three other
118 pairwise comparisons (P<0.01 for all cases). The non-significant differences included T_{INT} -
119 T_{DOR} (t=1.36, DF=51, P=0.359) and T_{DOR} - T_{FEM} (t=0.68, DF=51, 0.498. Simple pairwise
120 correlation between temperature variables showed that all four measures were highly correlated
121 (Figure 2). Internal body temperature T_{INT} correlated highly and significantly with each of the
122 three methods of recording external body temperature: T_{FEM} (r= 0.967; t= 26.941, DF=50,
123 P<0.001), T_{DOR} (r= 0.928; t= 17.63, DF=50, P<0.001), T_{AXI} (r=0.918; t=16.08, P<0.001).

124 The best single predictor (i.e., with the highest correlation coefficient) of internal body
125 temperature (T_{INT}), running a simple linear model was femoral body temperature (T_{FEM}
126 (r^2=0.936, $F_{1,50}$= 725.8, P<0.001):

127   *T_{INT} = 0.754 (S_E±1.144) + 0.989 (S_E±0.037))* T_{FEM}*

128 where S_E are standard errors of the estimated coefficients.
129
130 The predictive model of internal body temperature (T_{INT}), running a simple linear model using
131 the compound variable (Principal component, PC1: r^2=0.948, $F_{1,50}$= 903.8, P<0.001) from all
132 external body temperatures was (Figure 3):
133
134   T_{INT} = 31.381(S_E± 0.113)+0.587(S_E± 0.020)*PC1
135

136   where S_E are standard errors of the estimated coefficients.
137
138   **Discussion**

139   Thermal biology is a rich and dynamic area of biology that studies the way animals react
140   and adapt to their environment by regulating body temperature. Camacho & Rusch (2017)
141   provide a thorough and comprehensive review of the subject and the various methodologies that
142   have been used to determine body temperature in lizards. Dozens of studies exist in reptiles over
143   a range of topics that include preferred body temperature (PBT), voluntary thermal selection, and
144   absolute thermal tolerance (Tattersal, 2016; Camacho & Rusch, 2017). Many of these studies
145   use standard thermometers or thermocouples, but some use infrared cameras (Luna and Font,
146   2013) and others use infrared guns similar to the one used in our study (Hare et al., 2007;
147   Rowley and Alford, 2007; Halliday & Blouin-Demers, 2017). Our study differs from the
148   previous three works in the important area of animal size. The land iguanas in our study were
149   between 10 times and 100 times larger than the small lizards, amphibians, and snakes in these
150   studies.

151   In our study the differences between the three methods of recording body temperature
152   were small and all three external body temperatures slightly underestimated internal body
153   temperature. Dorsal temperature was the closest to internal temperature, axillary was the lowest,
154   and femoral was in the middle.

155   The best model predictor shows that internal body temperature can be predicted with a
156   high level of accuracy from the three external body temperatures, providing a good non-invasive
157   method that avoids the capture of animals. Femoral temperature, in spite of not being the most
158   accurate approximation to internal body temperature, was the best single predictor to estimate
159   internal body temperature as it was the least variable with the highest correlation coefficient. We
160   speculate this is because the femoral area is closest to the thermocouple location (deep cloaca).

161   One issue to consider, although we did not experience it, would be an animal that has
162   been in a burrow for a period of time and then climbs out into direct sunlight. In this case it's
163   possible, if not likely, that the external temperature might not reflect the true core temperature.
164   This would be an interesting angle to pursue with either this species or other reptiles. Another
165   factor that should be considered is what is the preferred temperature (T_{pref}) for the land iguana.
166   Based on the overall health of the animals in this study it may be similar to the T_{pref} of 25-35
167   degrees C for its mainland relative *Iguana iguana*, the green iguana (Girling and Raiti, 2004).

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169   **Conclusions**

170   This non-invasive method for measuring body temperature using an infrared temperature
171   gun is useful for approximating body temperature in Galápagos land iguanas. The quantitative
172   assessment provides data that can be most likely be used for monitoring the health status of other
173   similar sized lizard species in captive collections or in the field. Further evaluation is warranted
174   for its applicability to other species of large reptiles.
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284 Table 1. Average temperature and standard deviation data for the 52 land iguanas (*Conolophus*
285 *pallidus, C. subcristatus,* and *Amblyryhnchus cristatus* X *C. subcristatus* hybrid) assessed by
286 cloacal thermocouple thermometer and an infrared temperature gun.
287
288 Figure 1. An adult *Conolophus subcristatus* with the right axillary, dorsum, and right femoral
289 locations for infrared temperature measurement depicted. Photo by GA Lewbart.
290
291 Figure 2. Body temperature measurements data for the 52 land iguanas (*Conolophus pallidus, C.*
292 *subcristatus,* and *Amblyryhnchus cristatus* X *C. subcristatus* hybrid) assessed by cloacal
293 thermocouple thermometer and an infrared temperature gun.
294
295 Figure 3. A simple linear model using the compound variable from all external body
296 temperatures for the 52 land iguanas (*Conolophus pallidus, C. subcristatus,* and *Amblyryhnchus*
297 *cristatus* X *C. subcristatus* hybrid) assessed by cloacal thermocouple thermometer and an
298 infrared temperature gun.
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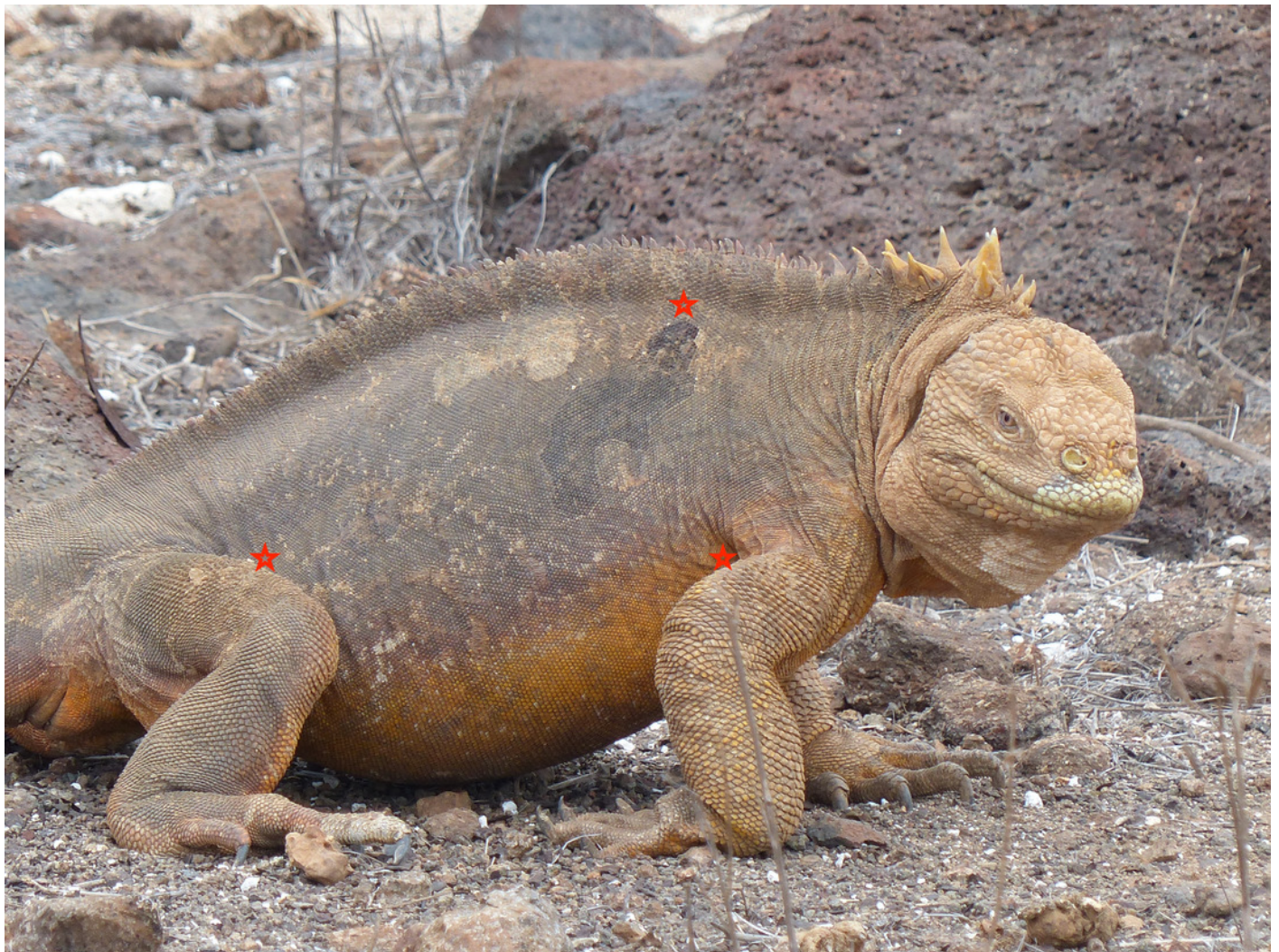
Average temperature values for the land iguanas in this study.

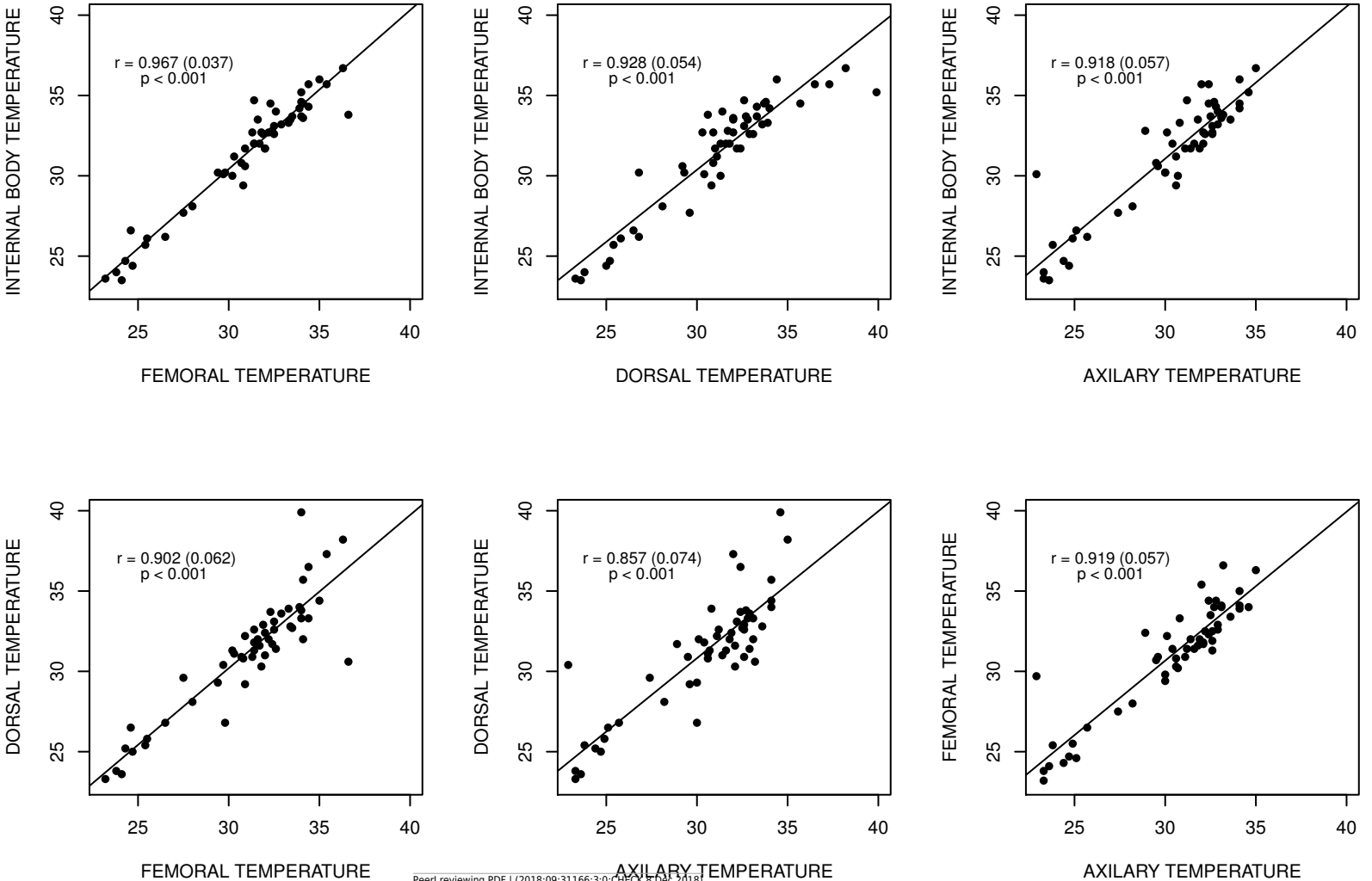
Average temperature and standard deviation data for the 52 land iguanas (*Conolophus pallidus, C. subcristatus,* and *Amblyryhnchus cristatus* X *C. subcristatus* hybrid) assessed by cloacal thermocouple thermometer and an infrared temperature gun.

ANATOMIC LOCATION	AVERAGE TEMPERATURE (°C)	STANDARD DEVIATION
Infrared temperature gun T_{AXI}	30.3	3.4
Infrared temperature gun T_{DORS}	31.1	3.6
Infrared temperature gun T_{FEM}	31.0	3.4
Thermocouple thermometer T_{INT}	31.4	3.5

Figure 1

Anatomical locations for infrared temperature assessment.

An adult *Conolophus subcristatus* with the right axillary, dorsum, and right femoral locations for infrared temperature measurement depicted. Photo by GA Lewbart.

Graphic representation of the land iguana body temperatures.

Body temperature measurements data for the 52 land iguanas (*Conolophus pallidus, C. subcristatus,* and *Amblyryhnchus cristatus* X *C. subcristatus* hybrid) assessed by cloacal thermocouple thermometer and an infrared temperature gun.

Bar graph depicting land iguana body temperatures from the different sites.

A simple linear model using the compound variable from all external body temperatures for the 52 land iguanas (*Conolophus pallidus*, *C. subcristatus*, and *Amblyryhnchus cristatus* X *C. subcristatus* hybrid) assessed by cloacal thermocouple thermometer and an infrared temperature gun.