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Annual cycle of soil temperatures in the dry forest of Ankarafantsika, Ambato-Boeny region, West Madagascar

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Breeding is often a challenge, especially for chameleon species kept in small numbers - and one of the biggest challenges is the successful incubation of the eggs. The most logical and supposedly simplest method would be to imitate exactly the ground temperatures of the place of origin. Unfortunately, there is - or was - no data on soil temperatures in Madagascar.

Therefore we set off for Madagascar in 2018, armed with a backpack full of ground thermometers. "Measure as much as possible," was our plan. And we started in the West. After more than a year we had collected much more data in Ankarafantsika than we had planned. We did not follow an exact study design, because the data collection was not originally intended for publication. However, since we now find the data too interesting to let them disappear into a drawer, we finally evaluated them statistically. The following article is the result.

The Ankarafantsika National Park is located in western Madagascar in the Ambato-Boeny region. The dry forest of the national park is divided into two parts by the Route Nationale 4. At the RN4, directly at the park entrance and campground, lies the small village Ampijoroa. Opposite is the approximately 33-hectare lake Ravelobe. The rainy season here lasts from November to March, the dry season from April to October. Among several other reptiles, three chameleon species live in the dry forest of Ankarafantsika: *Furcifer angeli* (Brygoo & Domergue, 1968, [6]), *Furcifer rhinoceratus*

(Gray, 1845, [11]) and *Furcifer oustaleti* (Mocquard, 1894, [14]). Females of these three species can be observed again and again in the dry forest, especially during the rainy season [10]. Of *Furcifer oustaleti*, clutches with up to 72 eggs are known [9, 16], of the other two species there are no published reports on clutches. About the egg-laying sites, there are only isolated reports within the genus *Furcifer*. The depth of egg deposition varied between 9 and 20 cm, depending on the snout-vent-length (SVL) of the female [8, 15].

Between April 01, 2018, and March 17, 2019, daily soil temperatures were measured in the dry forest of Ankarafantsika. The measuring points were located in an imaginary rhombus between -16.30621, 46.80126 west of the RN4, -16.29784, 46.82523 north of Lake Ravelobe, -16.31667, 46.82194 south of the lake, and -16.30621, 46.80126 southwest of the campground (Fig. 1). The survey sites were selected using two methods: First, we measured where females of *Furcifer rhinoceratus* and *Furcifer oustaleti* had been observed laying eggs or where freshly hatched young had just been found. Additionally, randomly selected shady places in the forest were measured. During the dry season - outside the egg-laying season of the chameleons - all these places were measured repeatedly.

The soil at all measuring points consisted of a mixture of laterite, sand, and earth. X4-Life soil testers (Lived non food GmbH, Friedrich-Seele-Str. 20, 38122 Braunschweig, Germany) were used for the measurements (Fig. 2). The soil testers have a measuring range from -9 to 50°C. The thermometers were inserted into the ground for measurement, corresponding to a measuring depth of 20 cm. For each measurement, a waiting period of three to five minutes was observed until the display remained stable. Except for two days within the mentioned period, four measurements were taken daily: One directly after sunrise between 05 and 06 a.m., one at noon between 12 and 01 p.m, one in the late afternoon between 04 and 05 p.m., and the last measurement between 9 and 11 p.m. Sunrise in Madagascar during the year is between 05:15 and 06:30 a.m., sunset is between 05:30 and 06:30 p.m.

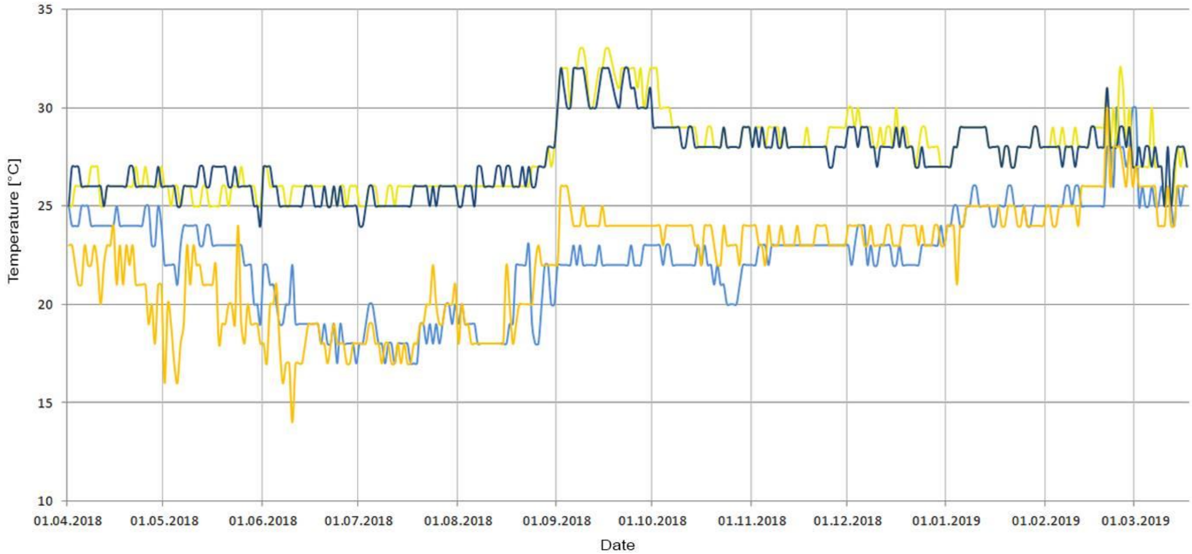
Figure 1: Study area in Ankarafantsika, map data from GoogleMyMaps © 2020 CNES / Airbus, Maxar Technologies.

Figure 2: Measurement of soil temperature next to a burrowing female *Furcifer rhinoceratus* in Ankarafantsika, March 2019.

To track the annual course of soil temperatures, the arithmetic mean and standard deviation for the four measurements were calculated for each month. Paired t-tests were used to compare the temperatures in the rainy and dry seasons. All statistical calculations were performed using Microsoft Excel (Microsoft Ireland Operations Limited, 70 Sir Rogerson's Quay, Dublin, Ireland). $P \geq 0.05$ was determined to be significant.

A total of 1396 soil temperature measurements were made and an estimated 300 different sites were measured. *Furcifer rhinoceratus* was most frequently observed during egg deposition (Fig. 3), similarly often *Furcifer oustaleti*. *Furcifer angeli* was only sporadically detected at all in the investigated region and could not be observed during egg-laying between April 01, 2018, and March 17, 2019. Hatchlings were only found during the rainy season. Table 1 shows an overview of the average soil temperatures of all months at the four different measurement periods. Figure 4 shows the course of all measured soil temperature single values over the whole measuring period.

Figure 3: *Furcifer rhinoceratus* female in Ankarafantsika digging, March 2019.



Temperature profile [°C] of the soil around Ampijoroa, western Madagascar, at a depth of 20 cm between April 2018 and March 2019, including 1396 measured values. Vertical lines indicate the beginning of a new month. The light blue line indicates measurements immediately after sunrise, the yellow line indicates measurements at noon, the dark blue line indicates measurements between 1600 and 1700 in the afternoon, the orange line indicates measurements between 2100 and 2300 at night.

Month	Morning	Noon	Afternoon	Night
April	24.2 ± 1.0	26.1 ± 0.6	26.1 ± 0.4	21.7 ± 2.1
May	22.6 ± 1.6	25.6 ± 0.6	26.1 ± 0.8	19.8 ± 1.8
June	19.2 ± 1.0	25.8 ± 0.5	25.3 ± 0.5	17.9 ± 0.7
July	18.4 ± 0.9	25.8 ± 0.2	25.5 ± 0.6	18.7 ± 1.2
August	19.5 ± 1.9	26.5 ± 2.4	26.8 ± 2.2	20.2 ± 2.6
September	22.3 ± 0.5	31.7 ± 1.2	30.8 ± 1.1	24.2 ± 0.3
October	21.8 ± 0.9	28.8 ± 0.5	28.5 ± 0.5	23.5 ± 0.7
November	22.9 ± 0.4	28.6 ± 0.6	28.1 ± 0.5	23.3 ± 0.5
December	22.7 ± 0.9	28.5 ± 0.8	27.8 ± 0.8	23.5 ± 0.8
January	24.9 ± 0.5	28.2 ± 0.6	28.3 ± 0.6	24.5 ± 0.5
February	26.1 ± 1.7	28.7 ± 1.4	28.1 ± 0.9	26.1 ± 1.3
March	25.6 ± 1.2	26.9 ± 1.1	27.2 ± 1.0	25.3 ± 0.9

Table 1: Average monthly soil temperature data in Ankarafantsika, Ambato-Boeny region, Western Madagascar, between April 2018 and March 2019, presented as mean value ± standard deviation [°C].

During the rainy season, soil temperatures during the night and early morning were significantly higher ($P = 0.04$) than during the dry season in Ankarafantsika. Between noon and afternoon temperatures there were no significant differences in the monthly mean between rainy and dry seasons. Only the soil temperatures of two months with maximum values of the corresponding season, February (rainy season) and June (dry season), differed significantly ($P = 0.05$) at all measured times. Soil temperatures in February were significantly higher than in June but showed a much lower night setback of 4°C maximum. In June, however, the difference between midday and night temperatures was up to 12°C. Figures 5 and 6 each show a weekly summary of the soil temperature trend in Ankarafantsika in comparison, once in June 2018 during the dry season and in February 2019 during the rainy season.

The lowest soil temperatures during the observation period were measured with 14°C at 22:10 on June 10, 2018. The highest soil temperatures in Ankarafantsika were 33°C and were recorded on September 08, 2018. Between these two peaks, there are 19°C.

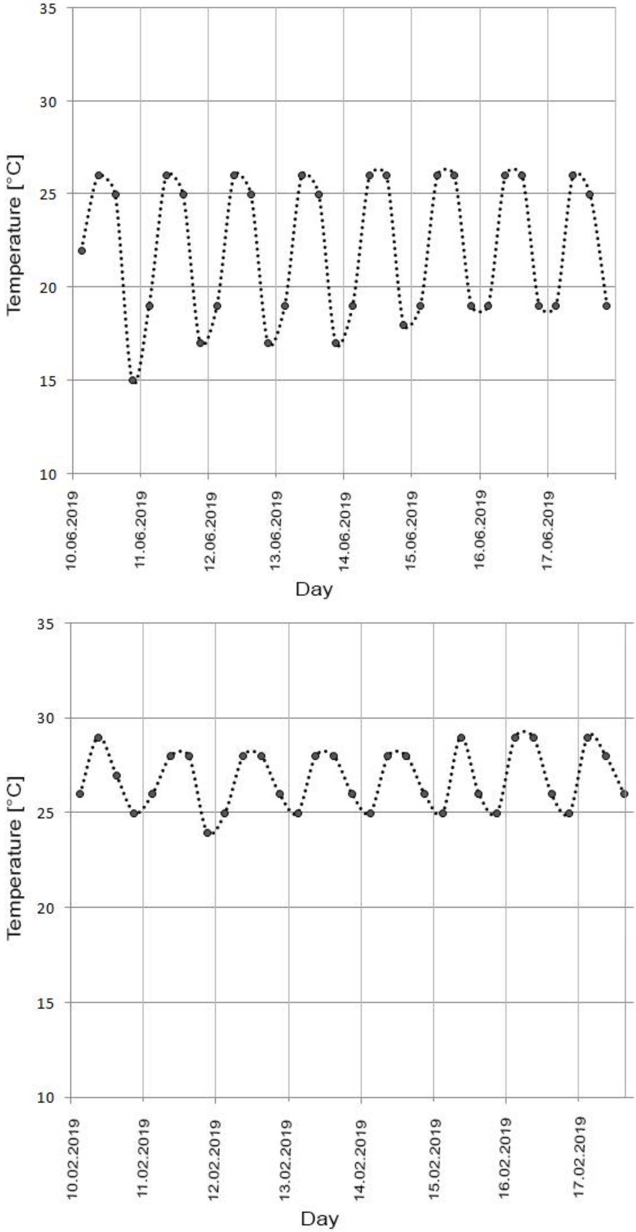


Figure 5: Temperature profile of the soil in Ankarafantsika at a depth of 20 cm during the dry (on top) and the rainy season (bottom).

This massive difference shows that the clutches of *Furcifer angeli, Furcifer rhinoceratus*, and *Furcifer oustaleti* are exposed to strong temperature fluctuations during the year.

What can certainly be discussed in the measurements is the continuous measurement of soil temperatures at a depth of 20 cm. We have already measured several oviposition sites of still digging females in Ankarafantsika, they all lay at about this depth. Therefore we have used the 20 cm as a reference point. Scientific investigations of the depth of dug egg deposition sites of different chameleon species are rare. The depth measurements known so far are mostly based on anecdotal reports [8,15] Further research in this field would be desirable.

The currently available knowledge about the incubation and development of chameleon eggs comes almost exclusively from terraristics. Eggs are mostly incubated with constant day and night temperatures [13], even if night set-back is discussed from time to time. Studies on introduced *Furcifer oustaleti* in Florida (USA), whose clutches were incubated experimentally at 19-23°C instead of the continuous 28°C often recommended in the literature, showed that viable young animals hatch even at lower temperatures [13, 16]. Studies in Malagasy geckos and non-Madagascan chameleons also confirm the influence of incubation temperatures on the survival of the individual animals as well as the incubation period [1, 2, 3, 5, 7]. Comparable studies for Malagasy chameleons are still pending. However, the data currently available suggest that night-time reductions in soil temperature may be more important for the fitness of Madagascan chameleon hatchlings than previously thought.

For years, the entire Ankarafantsika National Park has been massively threatened by out-of-control slash-and-burn agriculture and illegal logging, which further restricts the animals' habitat [4, 17]. Given the increasing destruction of habitat even within the designated national park boundaries in Madagascar, there is a high risk that the soil conditions for chameleon oviposition will continue to change significantly in the future. Climate change, which is already afflicting the tropical island of Madagascar with extreme weather conditions, also contributes to this issue [12]. It would be very interesting to compare the soil temperatures of cleared former forest areas with the data we have collected in the dry forest to investigate the effects of environmental destruction on the incubation of chameleon eggs in western Madagascar. Considering the changing profile of nest temperatures during the year, it is conceivable that incubation of chameleon eggs in non-forest areas could lead to reduced hatching success.

Finally, we would like to express a big and heartfelt thank you to Ralahy Andriamasy (Ndrema), who works as a local guide in Ankarafantsika National Park, for his tireless support during each of our stays in Western Madagascar.

By the way, the next upcoming project will be to measure temperatures directly in the nests of various female chameleons during the year. We want to cover different species as well as different places in Madagascar. We are already testing various special, very small, waterproof thermometers for their suitability and robustness in wet soil. So far the biggest problem seems to be to find the tiny thermometers after a whole year at all.

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