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### Why does male *Calotes nigrilabris* has a Blackcheek?

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#### Abstract

The reason for the *Calotes nigrilabris* adult males to have black-cheek/lips was investigated from August 2016 to August 2017 in the grasslands of the Horton Plains National Park, which is located on the southern plateau of the central highlands of Sri Lanka. Line transects and visual encounter surveys were carried out to find individual lizards and the surface cheek/lip and trunk temperatures were recorded along with the ambient temperatures and perch light conditions. There was a significant, positive relationship (p<0.05) between ambient temperature and the body surface temperatures of lizards (Pearson correlation; r = 0.939) which indicated ectothermic thermoregulatory behavior. Adult males showed a significant difference between the cheek/lip and trunk temperatures when compared to adult females (Kruskal-Wallis test, p>0.05). The results indicate that the presence of the black stripe on adult male *C. nigrilabris* results in a thermoregulatory advantage for them which allow them to absorb more heat energy, especially when perching under direct sunlight.

Keywords: Thermoregulation, Black-cheeked lizard, agamids, body coloration, melanism

#### 1. Introduction

Calotes nigrilabris is an endemic agamid species restricted to a few localities in the central highlands of Sri Lanka. It is one of the 3 lizard species that inhabit the cold climatic conditions (average annual temperature of ~15 °C) of Horton Plains National Park. C. nigrilabris males have broad black bands on the lips and cheeks which extend to the posterior part of the head (Fig -1); females with whitish/yellowish lip areas (Fig -2)<sup>[1]</sup>. Due to this unique characteristic C. nigrilabris is known as the Black-cheeked lizard in English and "Kalu kopul katussa" in Sinhala <sup>[2]</sup>. When it was first discovered by Peters (1860) it has influenced the scientific naming of this species as well, where "nigri" ~ black and "labris" ~ lips/labrum in Latin [3]. However, no explanation has been given for this unique morphological characteristic of black lips and cheeks in adult males<sup>[4]</sup>. This species can be distinguished from other Calotes using several characteristics such as backward or downward directed lateral body scales, a row of continuous spines above the tympanum and dorsal scales being smaller than the ventral scales <sup>[1]</sup>. The general body coloration is green and usually darker than that of other Calotes. Body coloration is an important aspect of lizard identification and taxonomy. The pigment cell morphology of reptiles is different from mammals and birds. Only the deepest pigment cell layer of lizards produces melanin <sup>[5, 6]</sup>. Aggregation or dispersal of granules within dermal melanophores results in skin lightening or darkening respectively, enabling the lizards to undergo short term color change <sup>[5]</sup>. Occurrence of individuals with darker pigmentation is called melanism <sup>[6]</sup>. Thermal melanism, cryptic coloration, protection from UV radiation and sexual selection are some of the hypotheses related with the occurrence of melanism <sup>[8,-10]</sup>. According to thermal melanism hyposthesis, under low temperature conditions, individuals with darker body color are at an advantage as they heat up faster at a given solar radiation level <sup>[11, 12]</sup>. These differences could have important implications for evolutionary fitness of the animal as a result of increased success in territorial defending, mate finding, feeding and escaping predators  $^{[10]}$ . Hence, microhabitat utilization and the individual fitness level of C. nigrilabris may also be affected by the color variations (melanism) within the species. The main objective of this study was to investigate the reason behind black-colored lips of male C. nigrilabris. Thermoregulation of this species was also analyzed.



Fig 1: Adult male *C. nigrilabris* with broad black colored cheek/lip area



Fig 2: Adult Female *C. nigrilabris* without the Black Coloration on Lips

#### 2. Materials and Methods

Ten 200m line transects were marked in the grassland habitat of Horton Plains National Park. Each transect was traversed three days a month from August 2016 to August 2017 between 08.00 hrs to 17.00 hrs. When lizards were first sighted the maturity stage was determined based on the categorization of Jayasekara *et al.*<sup>[13]</sup>. Only the adult males and females were considered for the study. After observing the perch light condition (categorized as direct sunlight or shade) for more than fifteen minutes, temperature of the cheek/lip region and temperature of the trunk (area between the limbs, head and tail) of the lizards were recorded using Extech IR201A Infra-Red thermometer. Ambient temperature was recorded using weather tracker (Kestrel 4000 pocket weather meter, USA). Each temperature reading was replicated and the average value was recorded.

#### 3. Results

103 adult males and 109 adult females were recorded during the study. There was a significant positive relationship between ambient temperature and the body surface temperatures of lizards (Pearson correlation; r = 0.939, p<0.05) which indicated ectothermic thermoregulatory behavior. Trunk-surface temperature of *C. nigrilabris* ranged from 11.3 °C to 32.6 °C with an average temperature of 20.72 °C. Cheek/lip region surface temperature ranged from 12.2 °C to 33.4 °C with an average temperature of 22.4 °C. General body surface temperature was maintained at a slightly lower value than the ambient temperature (Fig - 3).

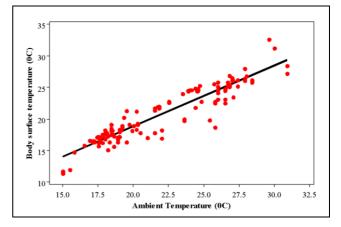


Fig 3: Relationship between Body Surface Temperature and Ambient Temperature of C. Nigrilabris

Temperature difference between cheek/lip and trunk surface was significant between *C. nigrilabris* adult males and adult females (Kruskal-Wallis test, p < 0.05) when perching in direct sunlight. Cheek/lip temperature of adult males was high compared to the trunk temperature. Therefore, the temperature difference between cheek/lip-trunk recorded a more positive value. In adult females, temperatures of both regions were very close to each other. Therefore, the temperature difference was very low (Fig - 4).

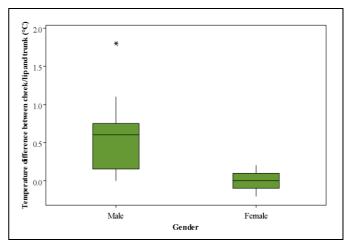


Fig 4: Temperature Difference between Surface Cheek/Lip Temperature and Surface Trunk Temperature of the Two Genders (In Sun Light)

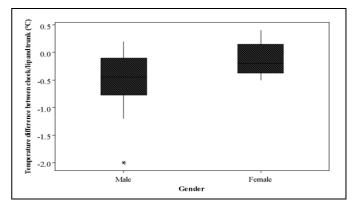


Fig 5: Temperature Difference between Surface Cheek/Lip Temperature and Surface Trunk Temperature of the Two Genders (in shade)

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The temperature difference of cheek/lip and trunk regions of adult males and adult females was not significant when in shade (Kruskal-Wallis test, p>0.05). However, adult males maintained their cheek/lip temperature lower than the trunk temperature. Hence, the temperature difference values of males were more negative. Adult females maintained very much similar temperatures in the two regions. (Fig - 5)

#### 4. Discussion

# 4.1 Relationship between ambient temperature and body surface temperature of *C. nigrilabris*

The strong positive correlation of body temperature of *C. nigrilabris* with ambient temperature indicated a high level of thermoconformity. However, the observed average body surface temperature was lower than that of ambient temperature. This result implies that *C. nigrilabris* performs some amount of behavioral thermoregulation to keep its body temperatures within optimum levels or within the critical range. This may help lizards to avoid dangerously extreme body temperatures and achieve some control over metabolic processes <sup>[14]</sup>.

## 4.2 Temperature difference between cheek/lip and trunk regions of lizards perching in direct sunlight and shade

When adult male and female of C. nigrilabris were considered there were some morphometric and color differences between the two genders. This was specifically prominent in the anterior region where males had broader swollen cheeks with more black coloration. The intensity of this characteristic varied between individuals. No explanation has been given so far for this secondary sexual characteristic of adult male [4]. The analysis of surface temperature difference in two body regions (cheek/lip and trunk) showed there was a significant difference between the two sexes. The higher cheek/lip temperature compared to rest of the body observed in adult male when perching in sunlight can be attributed to the black coloration found on its cheek/lip area. Therefore living in relatively cold temperatures of HPNP and other motane areas of Sri Lanka, C. nigrilabris adult males seem to have benefited from this special characteristic feature. Furthermore, adult male individuals that have broader black stripes may have a selective advantage over those with smaller stripes as they can absorb heat energy more efficiently. Higher heat absorption in the jaw region may facilitate the better performance of their jaw muscles enabling them to start feeding within a short period of basking. Usually males with broader black stripes also possess broader swollen cheeks. Verwaijen [15] suggests that lizards with larger head regions (increased head length and wider jaws) attain greater bite force which in turn increases their prey handling capacity. These two secondary sexual characteristics will also affect the territorial behavior of C. nigrilabris which was considered as a highly territorial lizard by Amarasinghe et al. [16]. Interestingly in the present study, not a single territorial behavior was observed from the grasslands of HPNP. The reason for this observation could be the availability of enough resources within the microhabitats of grasslands and better maturity stage structure <sup>[13]</sup> which reduces the need of fighting for resources and mates.

Adult females didn't show much difference between cheek/lip and trunk surface temperatures either in sunlight or shade. Adult females do not possess the black colored bands or swollen cheeks. Unlike males, the thermal energy requirement of females may not be very high due to their small body size and relatively higher surface–mass ratios when compared to males. That is because smaller organisms have shorter equilibration times and achieve lower equilibrium temperatures compared to larger ones, under same conditions <sup>[10, 17].</sup> Before the courtship behavior adult females were seen in contrastingly different coloration. Most of the body coloration was dark blackish green and ventral side of the head region was vibrant orange. This may be a result of rising hormone levels in the body <sup>[6, 10]</sup>. It may also increase the heat absorption before the courtship.

#### 5. Conclusions

The results of the present study indicate that the presence of the black stripe on adult male *C. nigrilabris* results in a thermoregulatory advantage allowing them to absorb more heat energy, especially when perching under direct sunlight. This compensate for the thermal energy requirements of the more active adult males and it may also help in the territorial defense, prey catching and mate finding. Therefore, adult males with a broader black stripe have a selective advantage. Furthermore, *C. nigrilabris* shows a high level of thermo conformity and some amount of behavioral thermoregulation.

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