Measuring Cardiorespiratory Variables on Small Tadpoles Using a Non-invasive Methodology

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Introduction: Cardiorespiratory studies in tadpols species are scarce, reflecting challenges of using invasive tools, for measuring heart rate (f_H) and breathing frequency (f_B) by means of implanting electrodes, cannulae, and others, especially in the case of long-term measurements. This study attempted to improve techniques previously used by Longhini and coworkers (2017) on measuring cardiorespiratory parameters non-invasively in bullfrog tadpoles in a much smaller anuran tadpole specie, *Boana albopunctaca* (Anura: Hylidae) from the Savannah-like Cerrado. This specie occurs in the central, southern and southeastern regions of Brazil in an area where deforestation levels are high.

Objective: To determine the resting values for f_H and f_B of tadpoles (stage 26 from Gosner, 1960) *B. albopunctata* at 25°C using a non-invasive methodology. Additionally, we determined the Maximum Critical Temperature (CT_{max}) by exposing tadpoles to a heating ramp (1°C/4min).

Material and Methods: Pre-metamorphic tadpoles (N=11) of *Boana albopunctata* were collected (SISBIO: 621361) in the Cerrado biome, in Minas Gerais state (20º 14' 43" S; 46º 21' 57" O), and maintained in the lab in aquariums at 25°C. The cardiorespiratory parameters, f_H and f_B, were obtained by a non-invasive electrocardiogram (ECG) and surface electromyogram (EMG) method, respectively. After baseline measurement, animals were handled in order to administer an intraperitoneal injection of Ringer solution (vehicle) to verify the influence of the injections on the basal cardiorespiratory parameters, which were recorded for an additional hour. For that, we used a thin dental needle (30 gauge) connected by a polyethylene tube (PE10, Clay Adams) to a Hamilton syringe (5 µl). The CT_{max}, the temperature at which animals lose the ability to escape from conditions that may ultimately lead to death, was determined by exposing tadpoles acclimatized at two different temperatures (15 and 25°C) to a constant heating rate of

1°C/4min. When the animals began to present loss of equilibrium, we stimulated them with a glass rod, and considered the CT_{max} when tadpoles were motionless after five consecutive taps. Immediately after assessing CT_{max}, we transferred tadpoles to plastic cups with water at ambient temperature (25°C). We validated the CT_{max} for individuals that remained alive for at least 24 h after the test. The data obtained were expressed as mean ± s. e. m.

Results: At 25°C, baseline values for f_H and f_B were 104.6 ± 20.0 and 85.94 ± 18.9, respectively. Cardiorespiratory parameters were not significantly affected by the intraperitoneal Ringer solution injection: f_H (beats.min^{-1}): 103.2 ± 15.6 and f_B (movements.min^{-1}): 85.3 ± 16.1. Animals' acclimatized at 20°C exhibited CT_{max} of 35.88 ± 1.58°C compared to those acclimatized at 30°C, with a CT_{max} of 39.57 ± 0.49°C.

Conclusion: According to our preliminary results, we confirm that the non-invasive methodology adapted for larger tadpoles (e.g Bullfrog; size: ~9 cm) can be improved for measuring cardiorespiratory parameters non-invasively in small tadpoles (size: ~4 cm).After one hour from intraperitoneal ringer injection, f_H and f_B returned to their baseline values. Furthermore, animals acclimated to the higher temperature (30°C) showed greater thermal tolerance during a heating rate protocol.

Keywords: *Boana albopunctaca*, Heart rate, bucal movements frequency, Savannah-like Cerrado.

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