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Standard metabolic rate and daily activity patterns of *Australoheros facetus* (Jenyns, 1842)

Juan Manuel Molina*, Roman Ubrig, Juan Pons, Gabriela Blasina

Instituto Argentino de Oceanografía (IADO), CONICET/UNS, Bahía Blanca, Argentina

*jmmolina@criba.edu.ar

INTRODUCTION & AIM

Cichlids are freshwater fishes widely distributed across tropical and temperate regions of the world, known for their remarkable adaptive radiation in the great East African lakes. Southamerican cichlids are also diverse, and have radiated into astonishing variations, and occupy many diverse habitats (Rican et al., 2021), from tropical amazonian streams, reaching into the northern Patagonian riverine systems (Almiron et al., 1997).

The neotropical cichlid *Australoheros facetus* (Jenyns, 1842) (Fig. 1), commonly known as *chanchita* or *chanchito (*Spanish and Portuguese word for piglet, respectively*)*, and chameleon cichlid in English, demonstrates adaptability to diverse freshwater habitats (Baduy et al., 2020). This species extends to 38°44' S, being one of the southernmost distributed cichlid species within South America, second only to *Crenicicla scotti* that inhabits Patagonian rivers (Almiron et al., 1997).

The study of fish metabolic rates, provides critical insights into their adaptations, energetics, and ecological interactions, and can be applied directly in widely different research fields (Clark, 2022). In this sense, providing these metabolic estimates will prove an ideal complement to past and future research on the physiology of *A. facetus* focused on unraveling its interactions with the environment, its adaptability and its effectiveness at invading foreign habitats.

Figure 1*: Australoheros facetus*

METHOD

Individuals of *A. facetus* were sourced from the Napostá Grande river, using traps, baited and set close to submerged vegetation on the margins of the river at shallow depths (less than 1 m), during spring (September-November in the southern hemisphere) of 2023. Napostá Grande river is located in southern Buenos Aires province, Argentina. This river is part of the Ventania basin, that discharges in the southwest Atlantic.

Fish were then quickly transported to an acclimation closed recirculation system, located in the nearby Fish ecology and biology laboratory (at the National Institute of Oceanography). The fish were left for one month in these acclimation tanks to recover from the stress originated from the capture and transportation. During this period, they were fed commercial feed for omnivorous fish (every other day, 3% w/w tank biomass), and kept at natural temperature and photoperiod (Sept 2023: 17.9±0.28°C; 13 Light hours (L) and 11 dark hours (D); Oct 2023: 19.9±0.11°C; 14L:10D; Nov 2023: 22.9±0.11°C; 16L:9D). Details in table 1.

An intermittent flow respirometry array to measure oxygen uptake was used to estimate the metabolic rates (MRs) of *A. facetus*, using the guidelines outlined in Clark (2022). This array consisted of four flow-through transparent acrylic respiration chambers (1.5L and 1.8L chambers, between 30-45 respirometer:fish volume ratio). Each chambers was connected to a circulation and a flush pump, and the whole set-up was placed inside a 200 L measuring tank. Dissolved oxygen concentrations were measured in each chamber using an optical oxygen sensor probe (Pyroscience GmbH, Germany). The circulation pump kept the water inside the chamber moving steadily and in a laminar fashion, and was always on, and the flush pump was used to flush the chamber with freshly oxygenated water from the measuring tank, and was activated and deactivated. To be able to measure oxygen uptake, the flush pump was turned off, allowing the fish to consume the oxygen in the chamber from 100% to a minimum of 85% O_2 saturation. The flush pump was then activated by means of an electrical switch. The system was configured to measure for six minutes and flush for four minutes. Animals measured for oxygen consumption were left unfed 48 h prior to the measurement to ensure a post absorptive state (Clark 2022). Standard metabolic rate (SMR) is considered to be the MR of a post-absorptive fish in a quiescent and calm state. Maximum metabolic rate (MMR) was determined using an exhaustive chase protocols. Measurements were performed for 48 h based on suggestions from the literature, to determine SMR, and 72 h for MMR. These experiments were run at 20 °C, with fish previously acclimated to this temperature in the acclimation tanks (18-23 °C).

MR data were grouped, obtaining a median, standard deviation and 1st and 3rd quartiles per hour. SMR and RMR were calculated following the recommendations of Clark (2022) for standardized metabolic measurements and calculations. SMR was considered the median value of the quantiles that place it below the lowest 20% of the night (inactive phase) observations (q 0.2) for each fish. For RMR it was the median of the quantiles that place it above the highest 80% of the daylight (active phase) observations (q 0.8) for each fish. MMR was determined employing the rolling regression method with a window width of 1 minute. Aerobic scope were calculated as the differences between MMR and SMR.

RESULTS & DISCUSSION

At 20°C the SMR of *A. facetus* was estimated at 99.23mg O_2 kg^{-1}h^{-1}. Figure 2 shows the hourly variations in the MR of the 15 individuals that took part in the SMR/RMR determination for the total duration of 48 hours. The daily pattern of higher activity (RMR) during the daylight hours and quiescence (mainly SMR) during the night, is evident for each 24 h period. Mean MR was 132.91±59.05mg O_2 kg^{-1}h^{-1} during the day and 117.87±49.87 mg O_2 kg^{-1}h^{-1} during the night. The high values obtained during the first 10 hours of the experiment is in accordance with the acclimation-to-respirometer estimation. Despite the variability in the data, evidenced by quantile dispersion, it is evident that the activity pattern is diurnal, without a marked peak of oxygen consumption, followed by a decrease in oxygen consumption as the night ensues (Fig. 2). Exhaustive exercise produced an increase in the MR of *A. facetus* (n=8). MMR was estimated to be 227.66mg O_2 kg^{-1}h^{-1}, within the first 3 hours of measurement, producing an aerobic scope of 128.43mg O_2 kg^{-1}h^{-1} (129.4%) at 20 °C. Figure 3 shows the behavior of the MR post-exercise according to the hours of the day, and the subsequent decrease of MR to pre-exercise levels after a period of 30 to 40 hours (Fig. 3).

Table 1: Standard, routine and maximum metabolic rate (SMR and MMR in mg O_2 kg^{-1}h^{-1}) of *Australoheros facetus* subjected to exhaustive chase protocol, as well as aerobic scope (AS), calculated as difference. W= weight in grams, TL= total length in millimeters. Median values are provided in the last row.

Fish	W	TL	MMR	SMR	AS
1	15.5	9	373.6	160.4	213
2	17	9.5	255.7	119.4	136
3	24	10.1	224.3	83.4	141
4	27.5	11.2	231.1	107.9	123
5	29	11	222.4	101.5	121
6	40	11.5	238.1	109	129
7	51	12.6	185.2	57.44	128
8	63	16.2	139.2	44.79	94.4
Median	28.3	11.1	227.7	104.7	123

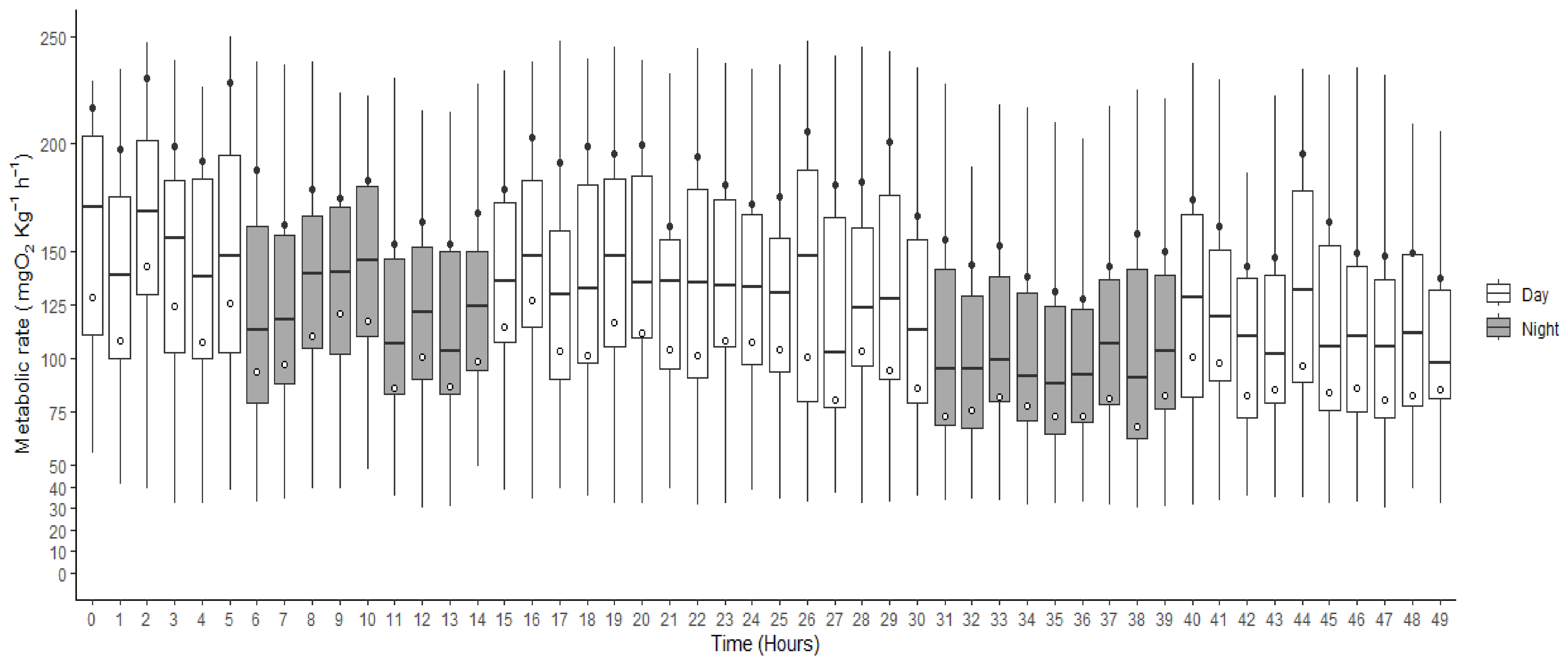

Figure 2*:* Metabolic rates of *Australoheros facetus* in the standard metabolic rate trial

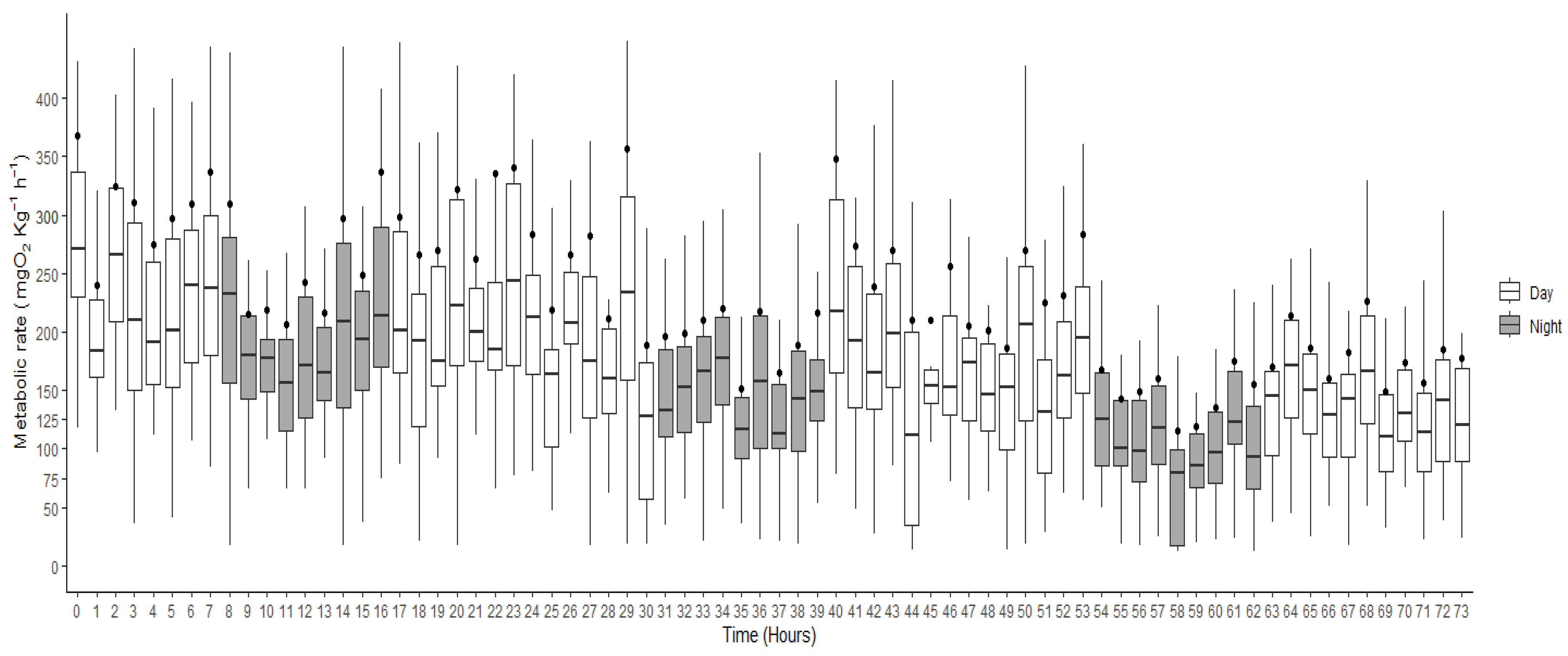

Figure 3*:* Metabolic rates of *Australoheros facetus* in the maximum metabolic rate trial

CONCLUSION

In this study we profiled the metabolic performance of *A. facetus*, providing estimates of SMR, MMR and aerobic scope for the first time for this species. Aside from these physiological parameters, reference methodological information is also provided, for use in future studies concerning *A. facetus* as well as other cichlids.

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