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Critical thermal maximum and minimum in *Australoheros facetus*, a neotropical invader in the Iberian Peninsula

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Australoheros facetus, or chanchito, is a neotropical cichlid native to rivers and streams in southeastern Brazil, Paraguay, Uruguay and northeastern Argentina. In the Iberian Peninsula the species was introduced in the wild probably via aquarists and is now invasive in streams of the Sado and Guadiana basins, in the Arade and Odelouca rivers and in related dams of Southern Portugal. Interestingly, in Portugal this species was first reported in the Vouga River drainage in 1943 (Helling, 1943), 300km north of its current distribution. The riverine systems occupied by the species display wide seasonal amplitude in temperature (between 11 and 27ºC in Vascão river, compiled from http://snirh.pt/ and personal observation) and flow regimes, a likely deterrent for a sub-tropical fish. It is hypothesized that the main reason why the species survived and established in Mediterranean habitats is its better tolerance to low winter temperatures but also to warm waters during hot dry summers, when compared to native fishes. If true, this invasive fish may further expand its geographical distribution through thermal tolerance mechanisms, which would further favor them in future climate change scenarios.

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The critical thermal maximum and minimum (CT_{max} and CT_{min}) are a measure of the thermal point at which physiological processes start to break down and is determined using a constant linear temperature change upward or downward from the acclimation temperature (Cox, 1974). In this work, we provide CT_{max} and CT_{min} for *Australoheros facetus*, and assess some physiological mechanisms of thermal regulation using metabolic substrates and cortisol as stress indicators.

Six groups of fish (N=20 fish per duplicate, totalizing N=240) were acclimated to either 12ºC or 24ºC (TA) for 15 days prior the experiments. Then, two groups were maintained at those control temperatures (TA) to simulate seasonal averages, while two groups were heated (A-from 12ºC and B-from 24ºC) and two others were cooled (C- from 12ºC and D-from 24ºC), at a mean rate of 3ºC per hour until loss of equilibrium was observed in at least 50% of fish of each group. Fish (n=10-12 per group) were anesthetized in 3L of water from the same tank where they were collected using 0.33% MS-222 • sodium bicarbonate. Blood samples were taken using heparinized 26G needles and 1 ml syringes and then fish were euthanized by cervical section. Cortisol was analysed in denatured plasma through radioimmunoassay (Guerreiro *et al*., 2006). Lactate and glucose were measured in duplicate using colorimetric commercial kits from Spinreact (Barcelona, Spain) adapted to microplates.

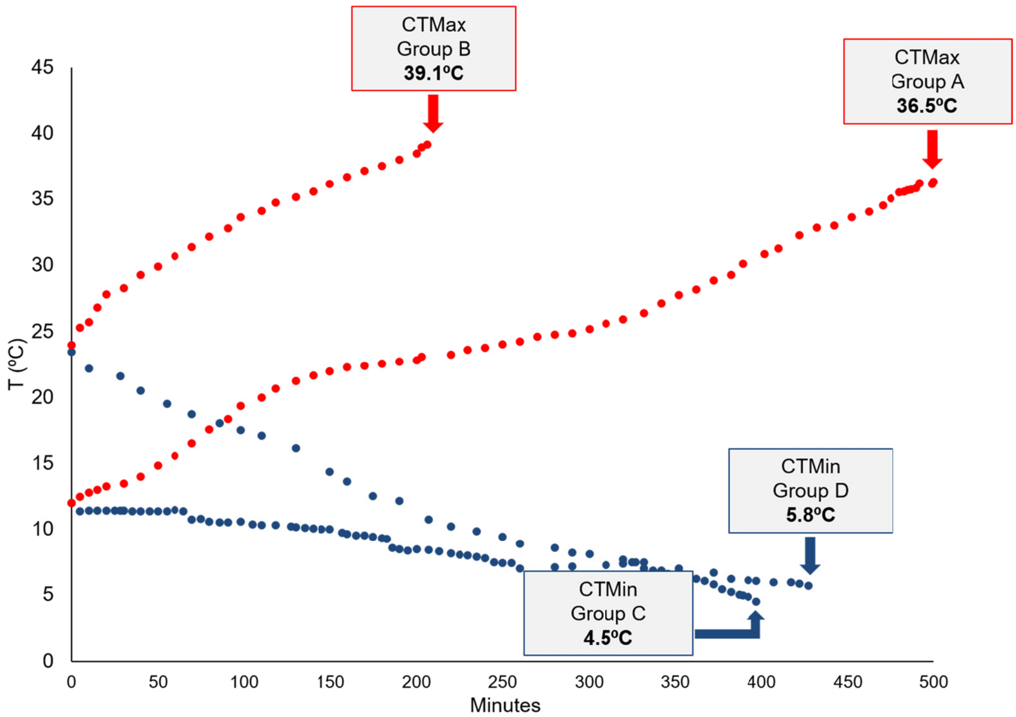

FIGURE 1. CT_{max} and CT_{min} of *Australoheros facetus* acclimated to different temperatures. In red groups that were heated: Group A from 12ºC and Group B from 24ºC. In blue groups that were cooled: Group C from 12ºC and Group D from 24ºC.

CT_{max} and CT_{min} values were 36.5ºC and 4.5ºC, respectively, for the 12ºC-TA groups, and 39.1ºC and 5.8ºC for the 24ºC-TA groups (Fig. 1). No significant differences in plasma cortisol, glucose and lactate were observed between the two control groups. There were no differences between groups A and B nor between groups C and D, but significant changes were found between controls and heated/cooled groups. Cortisol was almost two-fold higher in cooled than in heated groups (one-way ANOVA, F=5.67, p<0.001), but not always different from the control groups. Contrary, glucose and lactate showed higher values in heated groups than the control or cooled groups (glucose: Kruskal-Wallis H=42.18, p<0.001; lactate: Kruskall-Wallis H=51.99, p<0.001).

Australoheros facetus overall high tolerance to temperature is related to acclimation temperature (TA), as previously reported for other temperate fishes (Beitinger *et al.*, 2000). Such differences in CT_{max} and CT_{min} probably reflect a cellular metabolic adaptation providing the fish with a safety margin for extreme events in relation to its habitat seasonal optima, as annual fluctuations usually afford sufficient time for acclimation. In the present case, such amplitude may well confer the *A. facetus* a large advantage in Mediterranean streams.

Interestingly, the temperature of acclimation itself did not influence plasmatic parameters, showing successful rheostasis. Similar situations were previously reported for sturgeons (Zhang & Kieffer, 2014). But the relatively fast increases or decreases in water temperature evoked substantial responses on stress hormones and energetic substrates. Changes in temperature are a major factor stimulating cortisol release in fish. In the present study, we found higher cortisol in cooled rather than in warmed fish. Whether this means that a drop in water temperature can be more stressful than rapid warming remains to be confirmed. High levels of cortisol in fish were also reported in fishes that were subjected to cold exposure (e.g. He *et al.*, 2015). On the other hand, biochemical reactions are greatly affected by temperature and increases in glucose and lactate could reflect a change in energy utilization, expected to occur at higher temperatures.

Together with data from on-going studies, these results can partially explain the current distribution patterns and more importantly be used as indicators for the prediction of fish seasonal and geographical distribution and used in the control and management of this invasive species.

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