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Assessment of the Critical Thermal Maximum (CT_{max}) of Iberian chubs

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Abstract

The upper thermal tolerance of organisms is usually reported as the Critical Thermal Maximum, or CT_{max}. Here, we describe an adapted protocol to assess the CT_{max} in the Iberian freshwater fish of the genus *Squalius*, commonly known as Iberian chubs. The protocol involves exposing individuals to a controlled and progressive increase in water temperature until the point of loss of equilibrium, i.e., uncontrolled and disorganized swimming followed by loss of dorsoventral equilibrium. This point is recognized as the upper thermal physiological tolerance of individuals (i.e., CT_{max}). Although we applied this protocol exclusively to the Iberian chubs, it can potentially be adapted to other freshwater fish.

Materials

5L water bath
thermometer
aeration water pump (or equivalent)

For fish measurement:
precision scale (accuracy of at least 0.1 g)
graduated ruler

Optional for fin collection:
scissors
ethanol 70% (v/v) for disinfection
1.5 mL tube with 100% (v/v) molecular biology grade ethanol



- 1 Prepare a 5L water bath with dechlorinated water and appropriate artificial aeration (e.g., water aeration pump).
- 2 Warm the water to approximately  19-20 °C .
- 3 Put 2 fish at a time in the water bath and wait 10 minutes.
Note: Record the initial time and water temperature.
- 4 Increase water temperature by  3 °C every  00:10:00
- 5 When fish reach the state of "*Loss of equilibrium*"*, remove them from the water bath, record the water temperature and time, and put them in an aquaria with cold water (around  20 °C) and wait for recovery.

*Loss of equilibrium was defined as uncontrolled and disorganised swimming followed by loss of dorsoventral equilibrium for ten seconds (Becker and Genoay, 1979).

Note: the temperature recorded at the state of "*Loss of equilibrium*" corresponds to the CTmax of the individual.
- 6 Repeat the procedure for all the fish.
- 7 After fish recover from the experiment, measure and weigh the fish.
- 8 Optional: with properly disinfected scissors, obtain a small fin clip to a tube with  100 % (v/v) molecular biology grade ethanol. This sample can be used for further DNA extraction and genotyping.

Protocol references

Becker, C.D., Genoway, R.G. Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Environ Biol Fish* **4**, 245–256 (1979). <https://doi.org/10.1007/BF00005481>