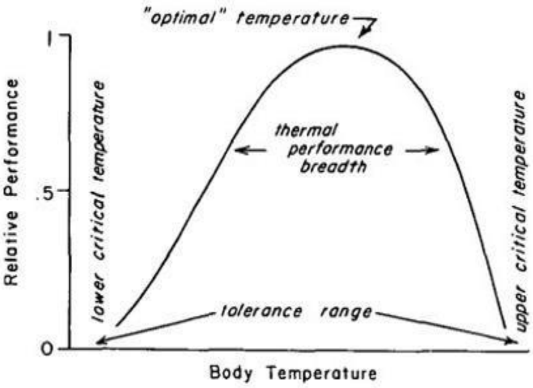
Assessing the effects of climate change on amphibians through the studyof thermal plasticity

INTRODUCTION

Heat waves and extreme cold events have increased in intensity, frequency and duration. These trends are expected to worsen as global warming increases. This could lead to temperatures reaching critical limits (CT) for organisms more frequently.

As the impact of climate change on biodiversity has highlighted the importance of thermal physiology, acclimatisation plasticity is considered the most direct and effective mechanism that ectothermic organisms can use to cope with temperature variation. In vulnerability assessments, critical temperature (CT) limits are the most widely used to understand the response of species to climate warming. As can be seen in Graph 1, the study of the thermal plasticity of a species takes the form of an analysis of the critical upper and lower limits (CT_{MAX} e CT_{MIN}).



Graph1 Hypothetical performance curve of an ectotherm as a function of body temperature

OBJECTIVES AND METHODOLOGICAL FRAMEWORK:

This project serves as the *kontìnuum* of my Master's thesis, which focused on the thermal analysis of some anuran species. Taking the data already obtained as a starting point, this PhD project aims to investigate the physiological consequences of exposure to heat/cold waves by the following objectives:

1. Analysing the post-metamorphic consequences of exposing tadpoles to critical temperatures (developmental speed, morphological abnormalities, size and growth at metamorphosis).
2. Conducting biological analyses by examining source environments (hydroperiod stability and amplitude, monitoring water and air temperatures, examining habitat variability over the years).
3. Investigating the CT and thermal plasticity of an invasive species to define the extent of its thermal niche.

Larvae will be sampled directly in the field in thermally diverse environments: temporary, permanent and semi-permanent habitats. This is because it is assumed that environments subject to periods of drought are more thermally unstable. This phenomenon could consequently influence the plasticity of populations.

To monitor temperature fluctuations, data loggers (HOBO pendant) will be placed at each sampling site to record temperatures (°C) every 15 minutes in water and air.

Both temperature limits will be determined using the dynamic Hutchison method. This method consists of exposing each animal to a constant rate of heating or cooling (ΔT = 0.25° C min-1) until an *end point* is reached. CT are recorded as the temperature of the water next to the tadpole, measured with a quick-recording thermometer.

This PhD project will consist of 3 protocols, in each of which different species will be used and different processes will be tested. *Bufo bufo* (Linnaeus, 1758) (BBUF) and *Pelophylax* kl *esculentus* (Linnaeus, 1758) (PKES) will be analysed in the first; *Hyla intermedia* (Boulenger, 1882) (HINT) and *Rana italica* (Dubois, 1987) (RITA) in the second and *Lithobates catesbeianus* (Shaw, 1802) (LTCA) in the third.

The protocols differ in the type of acclimatisation to which the larvae are exposed before being thermally analysed. CTs are always measured using the dynamic Hutchison method.

Monitoring will be carried out by means of biometric measurements, analysing: size at complete metamorphosis (weight and SVL), any malformations, size and weight at emergence of hind and upper limbs, growth speed, body size and condition at metamorphosis, jumping ability at neometamorphosis.

During the monitoring period, it will be possible to distinguish individuals using VIE (Visible Implant Elastomer) labels.

Protocol 1

The objective of this part is to analyse the post-metamorphic consequences of extreme temperature waves experienced by individuals in the larval stage.

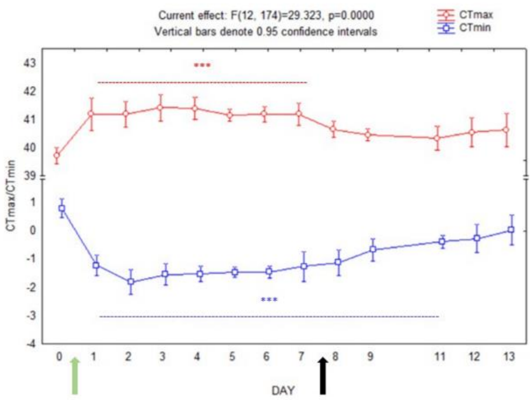
For 3 days, 30 tadpoles are acclimatised for each of the following temperatures: 9°C, 15°C and 25°C and CT assessed. Then the analysed tadpoles are grown in tanks with temperature as average temperature of the source habitats. Animals are monitored throughout the metamorphosis period.

In addition, a further 30 tadpoles will be monitored for the same purpose. They will be grown in tanks with constant temperatures reflecting the critical temperature reached for each acclimatisation temperature (9°C, 15°C, 25°C).

Protocol 2

In order to detect possible physiological and morphological dysfunctions following exposure to temperature peaks, the individuals will be reared in tanks with fluctuating temperatures. These fluctuations cyclically retrace the heat/cooling waves detected with the heat curve description from previous studies Graph2.

The individuals will be reared by exposing them cyclically to temperature peaks with growth/decrease of 0.5°C every half hour. Between one wave and the next, a pause period will follow in which the tank will return to a standard temperature of 20°C.



Graph2 *Example: Temperatures recorded in HINT after exposure to the same temperature for different times*

Protocol 3

To study the critical limits and thermal plasticity of LTCA, 48 larvae will be acclimatised for 3 days at each constant temperature of 9° C, 15° C, 25° C. Another 48 will be acclimatised at fluctuating temperatures of 16.5°-25°, 17.5°-30°, 17.5°-35°. Both CT, for both acclimatisation types, will be determined by testing 24 larvae for each acclimatisation type using the dynamic Hutchison method. Subsequently, the tadpoles will be transferred to water at a temperature of 20° C to allow for recovery. The remaining untested larvae will be used for biometric measurements.

COLLABORATIONS AND COSTS AND PROGRAMME OF ACTIVITIES

The project will be developed in collaboration with the Fondazione Bioparco di Roma and the Estación Biológica de Doñana, which have knowledge of the topics covered, technical staff already trained in the techniques used in the experiments, and machinery to carry out the experimental part.

All the necessary equipment is already present in the Bioparco's laboratories and the necessary software is open access; therefore, no special expenses are foreseen for this study.

The following Graph3 provides an estimate of the project development time.

	2023	2024					2025					2026	
Action	O-D	J-F	M-AP	M-AG	S-O	N-D	J-F	M-AP	M-AG	S-O	N-D	J-F	M-S
Data Collection													
Ther. mod.													
Sampling													
Field monit.													
Lab set-up													
Breeding													
CT													
Monitoring													
Analyses													
Ther. mod.													
Field monit.													
Breeding													
CT													
Devel. monit.													
Writing													
Thesis writing													

Graph3 *Graphical representation of labour distribution*