SHORT TERM SCIENTIFIC MISSION (STSM) SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number:CA18239
STSM title: Acute responses of Freshwater Mussels
STSM start and end date: 30/03/2022 to 09/04/2022
Grantee name: Paulo José Soares Castro

PURPOSE OF THE STSM:

(max.200 words)

This STSM was included in the framework of the EdgeOmics project (PTDC/CTA-AMB/3065/2020), and the aim of this travel was to share, prepare and test protocols of some of the tasks involved on the project, mainly the critical thermal maximum (CTM), and network with Dr. Martin Österling's team, in Sweden. The CTM is a metric used to measure the maximum thermal tolerance of an organism, characterized by the onset of behavior incapacitation, when exposed to a determined increasing rate of temperature. One objective of the study is to determine the influence of environmental context in thermal resistance. In mussels, the CTM is characterized by the partial or full extension of the foot, separation of the siphons and mantle, relaxation of the adductor mussels and unresponsiveness. To accurately analyze mussel behavior, hall effect sensors are glued to one side of the shell, and a magnet to the other. The signal of the sensor changes based on the distance it is from the magnet, due to the effect of the magnetic field in the electric potential difference. Using Arduino Uno the behavior can be monitored during the experiment.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

(max.500 words)

On the first day at the University of Karlstad was mainly to know the team, supervisor Dr. Martin Osterling, 2 PhD students, Raviv and Sebastian, the wet lab technician Niclas and 2 bachelor students, Sara and Ellenor that were developing their bachelor projects. Also visited the facilities and engaged on discussions about the work that was being developed by PhD students. Additionally, I participated in meetings for the bachelor students' projects, to discuss experimental designs applying the CTM methodology. In the second day, we mainly reviewed some of the technical material functions and prepared the electronic material, soldering the sensors connections to the Arduino board, connecting to the Arduino IDE (Fig.1. a, b).
Early in the next week, we sampled mussels in the field at the Alsterälven river for experiments and testing the setup, namely *Anadonta anatina* and *Unio tumidus*, with the team and bachelors' students (Fig.1. c). During these two days we also calibrated the sensors, by creating a known correlation curve between sensor signal and distance from the magnet, for each sensor. Additionally, during Monday and Tuesday we checked for gravid females of *A. anatina* (Fig.1. d). During Wednesday morning, I presented a small workshop on "EcoEletronics" for the team where several subjects were discussed: 1) how Arduino works and process information; 2) electronic circuits and their main components; 3) different sensors and potential applications; 4) basic functions and syntax on how to program on Arduino IDE. Moreover, we started to setup and performed some trials and tests on the PID controller temperature profiles and the system for the CTM, to check if everything was working properly (Fig.1. f).

Fig.1. Image recordings from activities performed during the STSM

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The last 2 days were focused on optimizing the system, performing trials measuring the behavior of mussels in response to temperature increase, and helping to perform one experiment from the students that tested the CTMs of *A. anatina* with 2 treatments, gravid females, and non-gravid mussels (Fig.1. e, g, h, j, k). The main objectives of the STSM were successfully accomplished, we developed the skills and knowledge needed to understand and perform critical thermal maximum assays. Indeed, it was very motivating to collaborate with the students and learn from them and their own projects, as the team is very knowledgeable and easy to work with.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Here I present some of the results from the trials to test the system. By using the hall effect sensors, we can record the behavior of the mussels in response to their thermal maximum. In figure 2 the temperature profile is represented by degrees per unit of time. The aim is to increase temperature at a rate (±0.3ºC/min) that does not allow acclimation by the organism but at the same time internal body temperature does not lag from water temperature. This was accomplished using a PID (proportional, integrative, and derivative) controller to control temperature profile with precision. The derivation of temperature change over time is explained in 99% by the correlation between these variables (r^2 = 0.99) (Table 1). Furthermore, in this example, temperature changed at a rate of 0.28ºC/min, as expected.

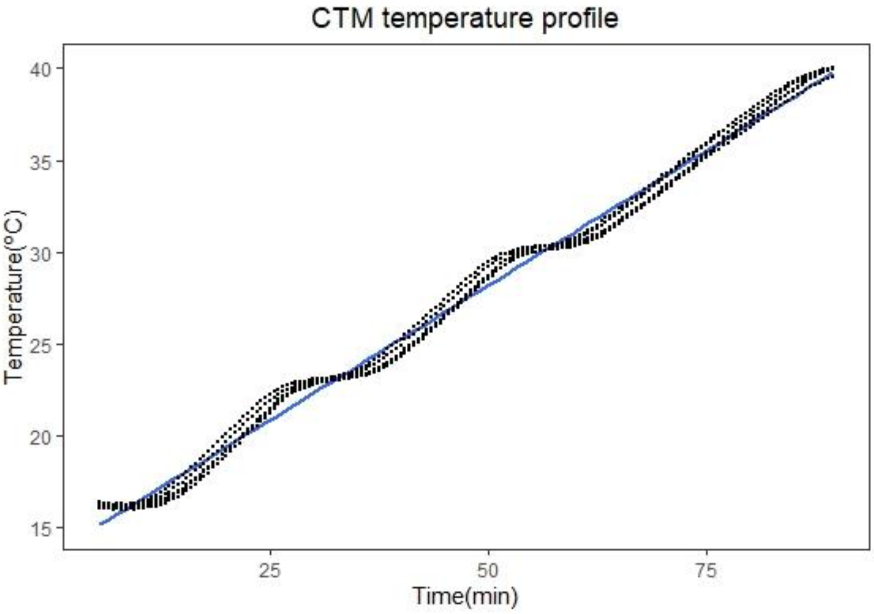

Fig. 2. Critical Thermal Maximum temperature profile achieved using a PID controller with a temperature increase of 0,28ºC per minute.

Table 1. Regression Liner Model coefficients of the temperature profile during the Critical Thermal Maximum trials.

	Estimate	Std. Error	t-value	Pr(>|t|)	R-squared
(Intercept)	14,35	0	273	0	0,99
Time	0,28	0	331	0	

In figure 3 is represented the behavior of 4 individuals from *A. anatina* (C and D) and *U. tumidus* (A and B) during the CTM. By measuring the minimum distance value from the magnet and the sensor when glued to the mussel, the data can be transformed in valve opening. Over time all the mussels gradually increased their maximum opening as the adductor muscles started to relax and they lose the ability to close their shells. When the mussel exhibits extreme stress (relaxation of the foot and adductor muscles), after 2 minutes of no significant movement the mussel is physically probed to check for unresponsiveness. If so, the mussel is collected from the tank and held in a recovery bath and returned to acclimation temperature. This moment is clear in the figure 3 as a sudden spike to ~100% opening, corresponding to the removal of the sensor. The CTM of *A. anatina* was several degrees lower than *U. tumidus*, 31 and 31.8 for *A. anatina*, and 36.8 and 40.8 for *U. tumidus* (not statistically tested). Moreover, these results were corroborated by both bachelor's students' projects.

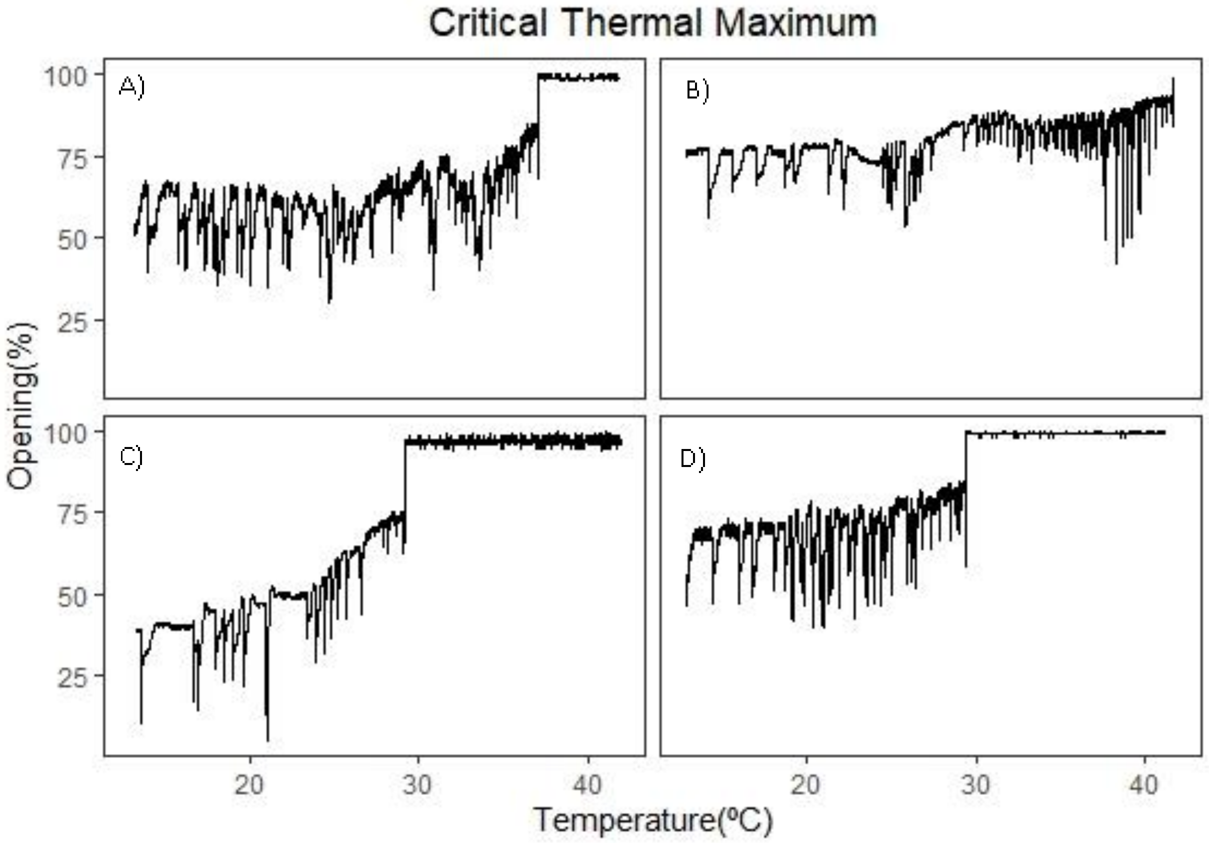

Fig. 3. Valvometry of *Unio tumidus* (A and B) and *Anodonta anatina* (C and D) to the critical thermal maximum. The valve movement is represented as opening percentage.

FUTURE COLLABORATIONS (if applicable)

This collaboration was very fruitful, I was very welcomed in Karlstad by the team. I found the ecological dynamics of the ecosystems and species very interesting when I visited some lakes and river sites and was able to learn much information in very little time. The team was also very interested on the technology and how could it be applied in other studies. Thus, I am looking forward to keeping in touch with the team and developing new ideas.