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Assessment of HSP70 and catalase in *Brachidontes rodriguezii* (d'Orbigny, 1842) a mussel from the Argentinean coast

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Introduction: Intertidal ecosystems are complex environments of great importance for the ecological balance of coastal zones and are vulnerable areas to effects caused by natural stressors of global relevance such as temperature. Mussels, are often exposed to drastic variations of temperature and can show intracellular mechanisms of compensation that allow them to counteract exposure in the short and medium term at high temperatures. Heat shock proteins are chaperones that allow thermal stability of proteins against abrupt changes in temperature and catalase is an antioxidant enzyme that aid cells to fight against oxidative stress caused by diverse environmental stressors [1,2].
Materials and methods: Mussel samples were homogenized in PBS buffer (phosphate saline solution, pH =7.4) and centrifuged (10,000×g for 15 min at 4 °C). Afterwards, the supernatants were transferred to 1.5 ml microtubes and stored at −80 °C until analysis. The HSP70 (Heat shock Proteins) levels were determined in individuals from both sexes, exposed at different temperatures (20 °C, 24 °C, 26 °C y 28 °C) for 7 and 14 days. Organisms sampled at T0 were also analysed. The HSP70 levels were determined by ELISA (Enzyme Linked Immunosorbent Assay) as described by Madeira et al. [2] and catalase following the method described in Diniz et al. [3].
Results and discussion: The preliminary results show an absence of significant differences between sexes of HSP70 levels at T0, 20 °C and 24 °C. However, at 26 °C and 28 °C, a differentiated response was observed in organisms exposed at higher temperatures, where the females had higher levels of HSP70 in the short term (7 days) compared to male individuals ($p < 0.05$). In the medium term (14 days) it was observed that females have similar or lower values than males.
Conclusions: The results show the ability to evaluate thermal stress by determining biomarkers such as HSP70 or antioxidant enzymes in tissues of *B. rodriguezii* providing valuable information to a better understanding of the effects of natural stressors on marine organisms. It also establishes that the sex of animals is a major factor of importance when evaluating this type of responses related to thermal stress.

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Catostylus tagi: survival and maintenance trials of planula and polyps

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Introduction: Besides the knowledge of life cycles in nature, the sustained use of aquatic biological resources also requires the development of know-how to reproduce and maintain them in captivity [1]. The scyphozoan *Catostylus tagi*, native to the Tagus and Sado estuaries, has already shown applicability in the areas of gastronomy and drugs for humans and has potential to be used as food for aquaculture organisms [2]. In this work we started to investigate the benthic life stages of *C. tagi* and their maintenance in laboratory.
Materials and methods: In September 2017, gonads of 20 specimens of *C. tagi* (12 males and 8 females) were collected in the Tagus estuary. Pairwise samples of male and female gonads were placed in containers with 80 mL of Tagus water and different substrate for *in vitro* fertilization. The settlement, growth and survival of the polyps were monitored daily by microscope. The survival rate was calculated as the daily percentage of live polyps and planulae, relative to the total number of initial planulae (Table 1). Polyps were cultured under constant conditions of temperature (19 ± 1 °C) and salinity (34 ± 1 ‰) and kept in the dark to minimize the algae growth. When the polyps developed 6 tentacles, they were fed every 2 days, alternating between macerated female mussel gonads and rotifers supplied *ad libitum*. Waste particles and food remnants were removed daily from the containers. Between 20-50% of the water in each container was replaced with filtered Tagus water after the polyps had been fed. Statistical approach was made by Mann-Whitney U test, considering $p < 0.05$.
Results: Polyp survival rate was high on the second day (~81%), but with a marked fall on the day after, and a trend for stability with some decrease on the following days (~50%) (Figure 1). The highest survival rate was observed in containers that had substrate, specifically wood and plastic, while the lowest rate was observed in containers without substrate. Sand and shell were the substrates with the lowest results, the latter having a survival rate below 50% in the last days.
Discussion and conclusions: At the conditions of temperature and salinity used in this trial, statistical comparison confirmed that the survival rate of the polyps was significantly different in environments with and without substrate. Other authors observed similar rates of growth and survival in related species [3].

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Table 1. Survival data of *Catostylus tagi* polyps during the first 8 days of settlement.

Containers*	Sept 12nd Pla/Pol**	Sept 13rd Pla/Pol	Sept 14th Pla/Pol	Sept 15th Pla/Pol	Sept 16th Pla/Pol	Sept 20th Pla/Pol
10.4 Plastic	10/37	3/46	1/40	0/41	0/41	0/32
10.4 Wood	2/36	0/35	0/35	0/28	0/28	0/28
10.4 Sand	20/2	15/2	8/4	6/6	6/6	6/6
10.4 Shell	14/6	16/6	5/4	3/7	3/7	3/6
10.2;10.5;10.7	21/0	20/0	8/0	7/1	7/1	3/3
2.1 15h46 Wood	43/0	35/0	29/0	27/2	26/3	23/6
2.7 15h46	13/0	8/1	3/0	4/2	4/2	1/5
2.7 16h46	41/0	23/2	8/4	2/4	1/5	0/5
3.2; 3.3; 3.6; 3.7	12/0	9/0	9/0	9/0	9/0	6/0

*The identification of the containers was based on the number of male and female used. The name plastic, wood, sand or shell, refers to the type of substrate. The time indication was applied in the event of a test repeat. The denomination of several pairs indicates the fertilization trials that were assembled in the same container **Pla = planula; Pol = polyp.

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