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Sea warming affects bream (*Sparus aurata*) tissues and stress proteins (HSP70)

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The increase in CO_2 emissions from anthropogenic sources may not only result in temperature increase on a global scale but also in increased ocean acidification (OA), by lowering the ocean's capacity to absorb additional atmospheric CO_2. As a consequence, changes in ocean chemistry are prone to occur through the imbalancing of sea-atmosphere [1,2] gas exchange, thus affecting O_2 absorption as well.

There are many studies on the potential effects of OA and increased ocean temperatures on the physiology of marine organisms but little is known about changes at histological level and there are still several gaps at molecular level which must be studied for a better understanding of all biological mechanisms involved. With regard to hypoxia, alterations to the stress response can provide information on the organisms' physiological effects and coping strategies triggered by anoxia. For instance, it is known that organisms respond by reducing protein synthesis [3,4]. Once temperature affects physiological, behavioral and ecological processes, there is a need to understand what mechanisms are behind the organisms' response to stress, enhancing our predictive and environmental management capacities considering a climate change scenario. This is of great importance, in particular to countries with a sea-based economy.

The aim of the present study is to assess the stress response of a marine fish, sea bream (*Sparus aurata*) exposed to increasing water temperature and different water acidity (alone or in combination). Here we present preliminary data on temperature effects on *S. aurata* at a cellular and molecular level. In addition, tissue samples from muscle, livers, gills and intestine are examined to evaluate any alterations caused by altering this physical parameter.

Fish were distributed randomly in tanks (n=96) and allowed to acclimate at 18ºC (the same temperature of the hatchery) before the beginning of the bioassays. After assessment of the upper thermal limits (UPL), water temperature was increased at a rate of 1ºC per hour using a thermostatized bath with a constant rate of water-temperature until reaching the endpoint, following the dynamic method of Critical Thermal Maximum (CTM) [5]. Every 2ºC step, fish were euthanized by cervical transection and the selected organs removed and stored at -80ºC until further analysis. Sub-samples were taken and processed for histological examination following standard techniques [6]. Frozen samples were analysed for heat stress proteins (HSP70) as described by Madeira et al. [7]. The histological observations were carried out using a Leica microscope (DMLB model). Preliminary results of the histological examination showed changes in the cellular structure, with visible damage at higher

temperatures in liver and gills (Fig. 1). Regarding HSP70, significant changes were observed throughout the temperature assay. The results indicate that elevated water temperature can be a major stressor that will affect fish due to potential climate changes, thus compelling the need to perform these studies to enhance our predictive and environmental management capacities.

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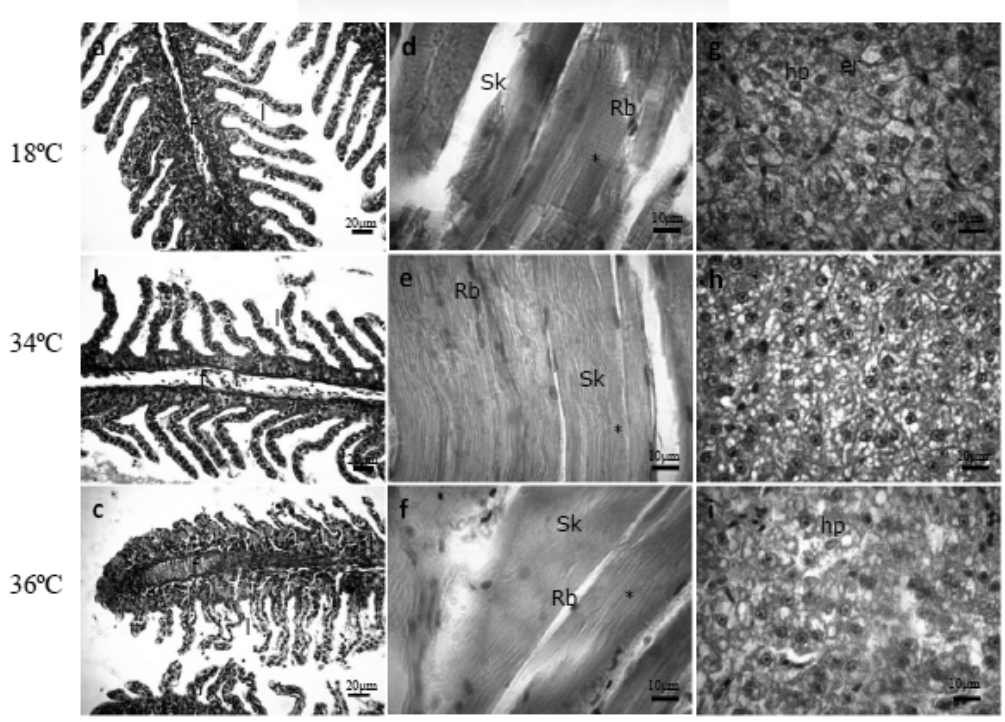

Figure 1. Representative images from sea bream gills (a to c), muscle fibers (d to f), liver (g to i) exposed to increasing temperatures. Legend: Gills - f, filament; l, lamellae. Muscle fibers - Sk, skeletal muscle; cross-striations (*); Rhabdomyopcytes (Rb). Liver—hp, hepatocytes; er, eritrocytes. Staining: H&E.