THE EFFECT OF DENSITY-RELATED STRESS ON SKELETAL DEVELOPMENT IN ZEBRAFISH, *DANIO RERIO* (HAMILTON, 1822)

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The maintenance of welfare in reared fish is becoming a key factor in several fields, such as aquaculture, ornamental fish industry and research. Welfare refers to the physical and mental state of the farmed animals in relation to their environment and it implies the absence of suffering and stress and the achievement of a generally good quality of life for farmed animals. Stress refers to the physiological response of animal to threatening situation, and it is a necessary mechanism to re-establish the homeostasis. Not all stressing conditions negatively affect organism functioning: very low levels of stress (eustress) can be adaptive, while higher or prolonged levels of stress (distress) may have maladaptive effects (*i.e*. disfunctions).

In my PhD work, the term stress is used from the perspective of distress, *i.e.* the stress against which the animal cannot cope with to restore the equilibrium of its internal environment.

In aquaculture, high stocking density seems to exert negative effects in terms of energetic, growth and survival and it is postulated their involvement in the onset of skeletal malformations. So far, it is not clear if high densities directly influence bone modelling and remodelling molecular pathways and the onset of skeletal anomalies, or if the latter are mechanically induced by the altered swimming performance produced by too-close interactions among fish. In this PhD work, the study of skeletal anomalies and some physiological and behavioural responses to stress in zebrafish reared under three stocking densities is carrying out. The aims of this project are: i) determining the level of stress induced by different rearing densities, ii) assessing if rearing density-related stress has some effects on the animals' swimming performance of individuals and iii) investigating the impact of different rearing densities on skeletal growth, at anatomical and histochemical level. Zebrafish were reared from 30 to 90 dpf at three different stocking densities in a RAS: i. high (32 ind/L), ii. medium (8 ind/L) and iii. low density (2 ind/L). In order to evaluate if differences in skeletal anomalies occurrences exist between the three experimental groups, a sub-sample from each group was whole-mount stained for bone with Alizarin Red and for cartilage with Alcian Blue. On each fish, the meristic counts and the types and number of anomalies are registered. The obtained characterization of the density-related malformations in this species is deeper analysed throughout histological sections, ISH and IHC. In addition, the behaviour of fish from each density condition is analysed through the novel tank diving test in order to detect density-related effects on swimming behaviour. Finally, the level of stress is analysed throughout physiological biomarkers as the whole-body cortisol concentration, liver glycogen content and some haematic parameters, in order to identify stress-related biochemical changes in over- and low-crowded zebrafish.