Deliverable Report

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Objective: Report on the prevention/treatment of Chronic Ulcerative Dermatopathy (CUD) in meagre (*Argyrosomus regius*): Based on the results from the study of CUD, this deliverable will comprise a set of recommendations for the prevention and treatment of the disease.

Chronic Ulcerative Dermatopathy (CUD) is a newly described condition affecting the lateral line canals of many cultured fishes, both freshwater and seawater. It has been described in the Australian freshwater fish Murray cod (*Maccullochella peelii peelii*) in sites supplied by groundwater (Baily et al., 2005; Schultz et al., 2011, 2008). The disease results in focal erosion, ulceration and loss of epidermis around the lateral line canals of the head and trunk, and fin erosion. It has been associated with reduced growth rates, increased mortalities and significant reduction of the marketability due to the severe disfigurement of the affected fish (Baily et al., 2005; Schultz et al., 2008). The same condition was also reported for goldfish (*Carassius auratus*) after exposure to freshwater groundwater (Baily et al., 2005). Concerning seawater species CUD was reported to affect the sharpsnout sea bream (*Diplodus puntazzo*) when reared in saline groundwater (Katharios et al., 2011). Both for Murray cod and sharpsnout seabream the authors reported that the lesions resolve if fish were transferred to natural freshwater and seawater, respectively, and they could not associate the disease with any infectious agent, concluding that the development of the disease is correlated with the use of groundwater sources. However, the aetiology is still unknown since they couldn't establish the exact component of the water which results in the development of the disease (Baily et al., 2005; Katharios et al., 2011; Schultz et al., 2011, 2008).

Meagre (*Argyrosomus regius*) is one of the CUD-sensitive fish species. The disease affects 100% of the population and results in ulceration of the skin overlying the lateral line canals. However it is not associated with mortalities (Rigos and Katharios, 2010) although, if the rearing in borehole water is prolonged, then there is severe disfigurement of the fish resulting in reduced acceptability of the juveniles for on-growing in sea cages (**Figure 1**).

Figure 1. Severe disfigurement of CUD-affected meagre following prolonged rearing in borehole water.

As we described in ***D24.7 Diagnostics protocol for Chronic Ulcerative Dermatopathy in meagre and aetiological factors***, two parallel rearing trials of meagre in borehole and natural seawater were conducted in order to study the development of CUD in meagre using histology and SEM, and to investigate osteoclast activity using molecular markers. The results indicate that CUD in meagre can be induced with the use of borehole water, which is in agreement with the conclusions of Baily et al. (2005) and Schultz et al. (2008) for Murray cod and those of Katharios et al. (2011) for sharpsnout sea bream. Furthermore, the lesions resolved if fish are transferred to natural seawater. The results from histology and SEM confirmed that the lesions were limited to the lateral line organ in the head.

Physicochemical analysis of the two water sources showed that the pH was lower and CO_2 higher in borehole water in comparison to natural seawater. Katharios et al. (2011) hypothesized that borehole water, which is rich in CO_2, as indicated by a lower pH compared to that of natural seawater, increases the enzymatic activity of osteoclasts that would cause the lesions seen in the fish. The qPCR results from this task are in agreement with this hypothesis since there is indeed overexpression of the genes cathepsin K and TRAP, which are related to osteoclast activity, in the fish reared in borehole water compared to those reared in natural sea water. However, results of a second rearing trial that we performed in order to investigate whether CO_2 in borehole water is the aetiological agent causing the development of CUD lesions in meagre suggested that neither pH nor CO_2 are involved in the development of CUD lesions.

Metal analysis of the head of meagre reared in the two different water sources showed that the concentration of copper was significantly higher in the head of meagre reared in borehole water than in the head of meagre reared in natural sea water. Eisler and Gardner (1973) found that copper alone or in combination with zinc or cadmium damages the epithelium of canals in the head of mummichogs (*Fundulus heteroclitus*). However, concentrations of all metals were comparable to published data from other farmed and wild fish species that have no lesions (Alasalvar et al., 2002; Kalantzi et al., 2016, 2013; Zotos and Vouzanidou, 2012). Nevertheless, a toxic agent as a causative factor for the development of CUD cannot be ruled out, because the low pH of the borehole water and the long exposure times of the fish could lead to increased metal toxicity.

Although CUD is directly associated with the use of borehole water, the causative agent is still unknown for meagre, as well as for Murray cod and sharpsnout seabream. For all species the lesions resolve when the fish are transferred to natural freshwater or seawater (Baily et al., 2005; Katharios et al., 2011). Furthermore, for Murray cod, Schultz et al. (2011) found that the retention of groundwater into a vegetated earthen pond or in

a tank containing biofilms growing on an artificial macrophyte (Aquamats TM) for 72 h prevented the development of CUD. The maturation of water through biofiltration in recirculation systems could be correlated with the resolution of the lesions. In the Cretaquarium (Heraklion, Crete, Greece), the public aquarium of HCMR, we have observed that the development of the lesions occurs when the fish are in tanks where flow-through water is used, whereas in tanks where there is recirculation of the water (also borehole water) there is a much slower development of the lesions and in some cases reversal of the condition (unpublished personal observations).

Thus, our recommendations for prevention of CUD in meagre are:

- to avoid borehole seawater if natural sea water sources are available for the rearing of meagre.
- to monitor the physicochemical parameters of water (low pH and increased CO_2 should be avoided with degassing).
- water pre-treatment is advisable if applicable.
- early transfer of the juveniles to sea cages for on-growing, since the earlier the transfer the faster the recovery of the lesions .

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