New species for EU aquaculture

Deliverable Report

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Task Title:	Chronic Ulcerative Dermatopathy		
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Objective: The deliverable is a report that contains the information about the development of Chronic Ulcerative Dermatopathy (CUD) in meagre (*Argyrosomus regius*) and the description of the pathology**.**

Introduction

The lateral line is a mechanosensory system found in all fishes and in the larvae of aquatic amphibians, which is used for the detection of water movements and/or pressure fluctuations (Bleckmann and Zelick, 2009; Webb and Shirey, 2003). The receptors of the lateral line that detect water flow are called neuromasts and they are distributed on the head, the trunk and the tail of the fish. Neuromasts can be either superficial in the skin or enclosed in the fluid-filled canals of the lateral line that open to the environment through a series of pores (Bleckmann and Zelick, 2009; Webb, 1989). A schematic appearance of the lateral line system, the canals and the neuromasts is presented in **Figure 1**. It has been demonstrated that the lateral line canals develop through a bone remodeling process with the implication of both osteoblasts for bone apposition and osteoclasts for bone resorption (Wada et al., 2014).

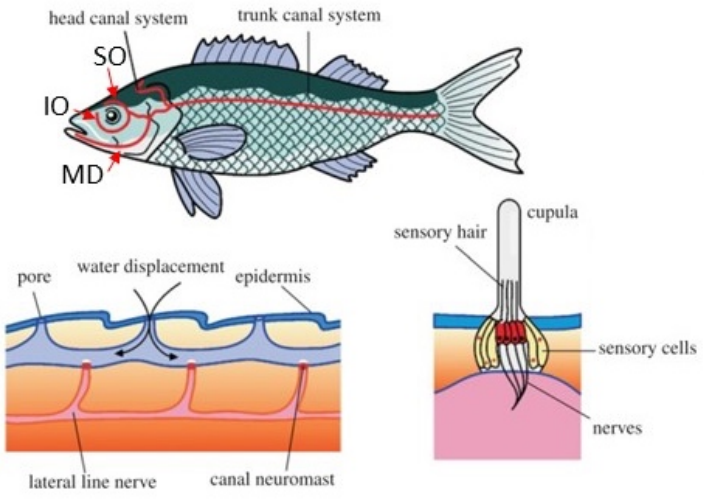

Figure 1. Lateral line system in fish. Structure of lateral line canal and of a neuromast. SO: supraorbital canal, IO: infraorbital canal, MD; mandibular canal (from Dagamseh et al., 2013 with modifications).

Chronic Ulcerative Dermatopathy (CUD) is a newly described condition affecting the lateral line canals of many cultured fishes both freshwater and marine. 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 the trunk, and fin erosion. It has been associated with reduced growth rates, increased mortalities and significant reduction of 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 marine species, CUD was reported to affect the sharpsnout sea bream, *Diplodus puntazzo*, after culture in saline groundwater (Katharios et al., 2011). For the sharpsnout seabream, the authors suggested that there is an indication of osteoclastic enzymatic activity in the affected fish. The enzymes implicated in bone remodeling of the lateral line canals are the tartrate resistance acid phosphatase (TRAP) and cathepsin K for bone resorption, and vATPase for bone apposition. Both for Murray cod and sharpsnout seabream, the authors reported that the lesions resolve if fish are transferred to natural freshwater and seawater respectively and they could not associate the disease with any infectious agent. The final conclusion of both studies was that the development of the disease is correlated with the use of groundwater sources. However, the aetiology is still unknown since they could not establish the exact component of the water which results to the development of the disease (Baily et al., 2005; Katharios et al., 2011; Schultz et al., 2011, 2008). A similar condition under the term 'lateral line depigmentation' has been reported in channel catfish. In this case the authors concluded that the causative agent for development of the disease was the exposure of fish to chronic nutritional stress by 12 months of fasting (Corrales et al., 2009).

Meagre (*Argyrosomus regius*) is one of the sensitive CUD fish species. The disease affects 100% of the population and results in ulceration of the skin overlying the lateral line canals, however is not associated with mortalities (Rigos and Katharios, 2010). The aim of this study is to describe the disease in meagre using histology and SEM and to investigate osteoclast activity using molecular markers. Through this study, the final goal is to investigate the aetiology of the disease and suggest preventive measures.

Materials and methods

Rearing trials

Two parallel rearing trials of meagre in borehole and natural seawater were conducted in order to study the development of CUD. Eggs produced in May 2015 at the facilities of the Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Crete, Greece were used for the rearing trial, which was performed in duplicate 40m^3 tanks (HCMR). The rearing trial lasted from 1-56 days post hatching (dph). Every day, measurements of pH, CO_2, O_2 and T were made in the two water sources. Fish were sampled according to the timeline presented in **Table 1** for each type of analysis**.**

Table 1. Sampling timeline for the CUD experiment

	Days post hatching (dph)																			
	1	2	3	4	5	6	7	9	11	13	15	17	19	21	26	31	36	41	46	51	56
SEM																					
Histology																					
qPCR																					

Gene expression of cathepsin K, TRAP and vATPase

Ten fish from each tank were snap-frozen in liquid nitrogen and stored at -80^{o}C until analysis. For qPCR, total RNA was isolated from the head of meagre by using the Nucleospin RNA plus Kit (Macherey-Nagel)

according to the manufacturer's instructions, and cDNAs were synthesized from 1 μg RNA by using a QuantiTect Reverse transcription kit (Qiagen). The sets of degenerate oligonucleotide primers used for cathepsin K, TRAP and vATPase were the following and were determined by Prof. C. Secombes' team (P5. UNIABDN):

CathK	F	ACGCTCACTCCAAATCCAACTG
	R	CCGTGCCGCTACAATTCATCA
TRAP	F	CGTAATTGCTGCCATCTCTGT
	R	CTGTTCTCCTGTGCTTAGCCTAC
vATPase	F	TGTATGCCTGTTATGCCATTG
	R	TCCTGAGCGATGAAGTTCTT

The mRNA expression of genes encoding for CathK, TRAP and vATPase was determined with quantitative polymerase chain reaction (qPCR) assays using the KAPA SYBRH FAST qPCR Kit (Kapa Biosystems). Reactions were cycled and the resulting fluorescence was detected with a Mini Thermal Cycler (Bio-Rad) under the following cycling parameters: 95°C for 3 min (DNA Polymerase activation step), 95°C for 15s (denaturation step), 60°C for 30s (annealing step), 72°C for 20s (extension step), 36 cycles (step 2–step 4). Levels of CathK, TRAP and vATPase mRNA were normalized based on the reference gene b-actin. A relative standard curve was constructed for each gene, using 4 serial dilutions (1:5) of a pool of all cDNA samples.

Scanning electron microscopy (SEM):

Three fish from each tank were fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.4) for 1 or 2 days (depending on the size of the fish) and then stored in sodium cacodylate buffer at 4°C. The samples were then washed with sodium cacodylate buffer, post-fixed with OsO_4 and dehydrated in an ascending alcohol series, mounted on stubs and sputter-coated with gold-palladium. Samples were viewed using a JEOL JSM-6390LV scanning electronic microscope at 15 kV at the Electron Microscopy Laboratory of the University of Crete.

Histology:

Three fish from each tank were fixed in 4% formaldehyde: 1% glutaraldehyde. Subsequently they were dehydrated in gradually increased ethanol solutions (70-96%) and then embedded in glycol methacrylate resin (Technovit 7100, Heraeus Kulzer). Sections of 4 μm were obtained with a microtome (RM 2035, Leica, Germany). After drying, slides were stained with methylene blue/azure II/basic fuchsin according to Bennett et al. (1976) and examined under a light microscope.

Metal analysis

Concentrations of 22 metals were determined in 9 heads from fish reared in borehole seawater and natural seawater respectively by Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) using the protocols described in detail by Kalantzi et al. (2013). The analysis was conducted at HCMR's lab under the supervision of Dr. Tsapakis.

Recovery trial

For the recovery trial one group of 4-month-old meagre with visible lesions associated with CUD were transferred from the facilities of HCMR in Heraklion to cages in Souda, Chania and were monitored macroscopically for the next 5 months.

Second rearing trial

A second rearing trial was performed, in order to investigate whether CO_2 in borehole water is the aetiological agent that causes the development of CUD lesions. Eggs produced in June 2016 at the facilities of the Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Crete, Greece were used for the rearing trial, which was performed in $40m^3$ tanks (HCMR). In this trial, we used two parallel rearing tanks supplied with natural sea water. In one of these tanks we adjusted the pH to

7.4 with the use of a pH-controller which was connected to a CO_2 feeding pump. With this experimental design we wanted to replicate the pH and CO_2 conditions of the borehole water without including any other chemical or environmental factor in the trial. The trial lasted from 1-60 dph. Every day, measurements of pH, CO_2, O_2 and T were made in the two tanks. Every 7 days, 10 fish were sampled from each tank for SEM, histology and qPCR respectively.

Results

At the end of the rearing trial all the fish reared in borehole water had visible lesions associated with CUD in comparison with the fish reared in natural sea water (**Figure 2).**

Figure 2. Meagre reared in natural seawater (left) and borehole water (right). All fish reared in borehole water had visible lesions on the head associated with CUD.

Growth performance

The average length and weight of the fish of the different water sources at the end of the rearing trial (56 dph) are presented in **Figure 3**. The growth performance of the fish was not affected by the different source of water ($p>0.05$)

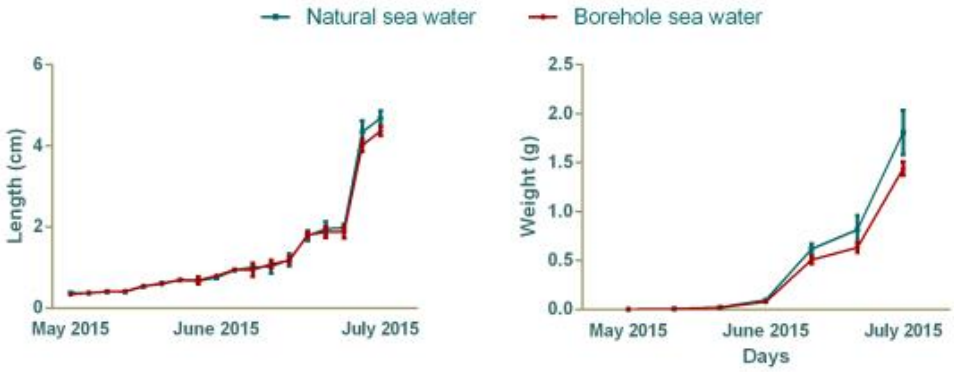

Figure 3. Average length and weight of meagre reared in borehole and natural seawater. The values are mean±SD.

Physicochemical analysis of water

The physicochemical analysis of the two water sources is presented in **Figure 4**. The pH was lower and CO_2 higher in borehole water in comparison to natural sea water, while T was higher in seawater from June, but O_2 levels did not differ between the two sources.

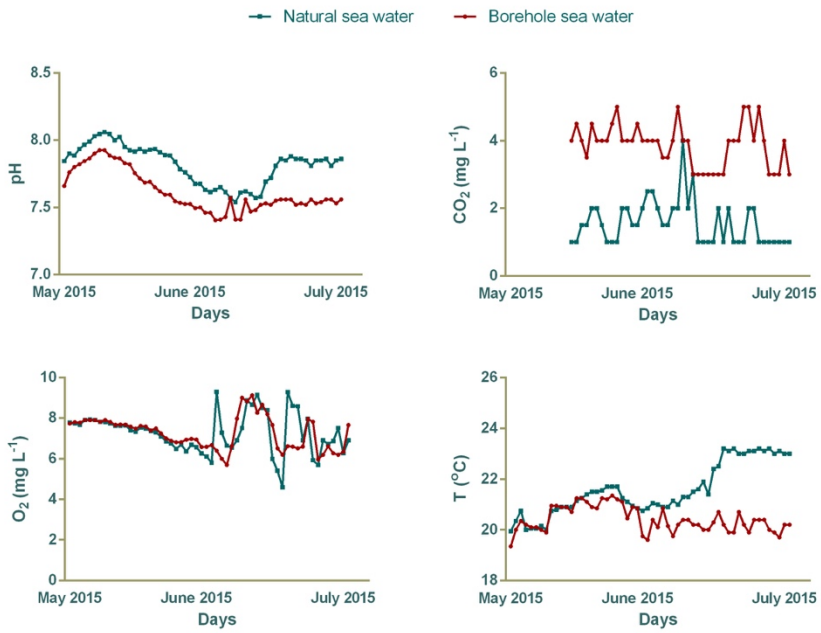

Figure 4. Physicochemical analysis of two different sources of water.

Expression of CathK, TRAp and vATPase

Expression profile of CathK, TRAP and vATPase in the head tissues of the fish reared in different water sources was significantly different at the end of the rearing trial (56 dph). In particular, cathepsin K and TRAP expression was 2.7 and 2.1 times higher, respectively, in the fish of the borehole water group compared to the seawater group ($t(17)=2.26$, $p=0.037$ for cathepsin K and $t(17)=2.41$, $p=0.028$ for TRAP). The expression of vATPase did not exhibit significant differences between the two water sources ($t(17)= -.219$, $p=0.830$) (**Figure 5**).

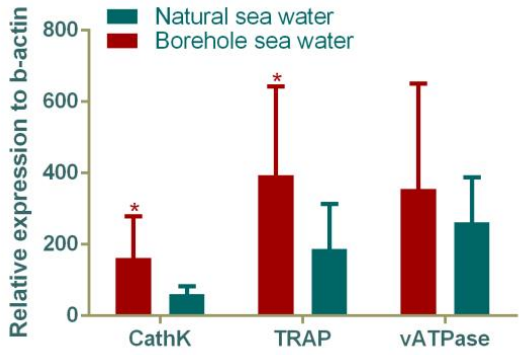

Figure 5. Relative expression of CathK, TRAP and vATPase in heads of meagre reared in borehole and natural sea water at the end of the rearing trial (56dph). Values are means+SD, (*) indicates statistically significant differences between the two water sources ($p<0.05$).

Scanning Electron Microscopy (SEM)

Based on the SEM observations, it was evident that the main affected areas were the supraorbital commissure (SOCom) which joins the left and right supraorbital canals (SO), the infraorbital canal (IO), the mandibular canal (MD) and the area of the nostril (R). The supraorbital canal (SO) of both healthy and CUD-affected fish was outlined by a distinct series of pores **(Figure 6A & B).** Instead, both the IO and the MD canals of CUD-affected fish were wide-open with no pore-bearing canal roof **(Figure 6B, D &F)**. The canals had a groove-like appearance where damaged canalized neuromasts were exposed **(Figure 7)**. However, the nares of CUD-affected fish often had wider openings than healthy individuals but the olfactory rosette was not affected (**Figure 6E, F**).

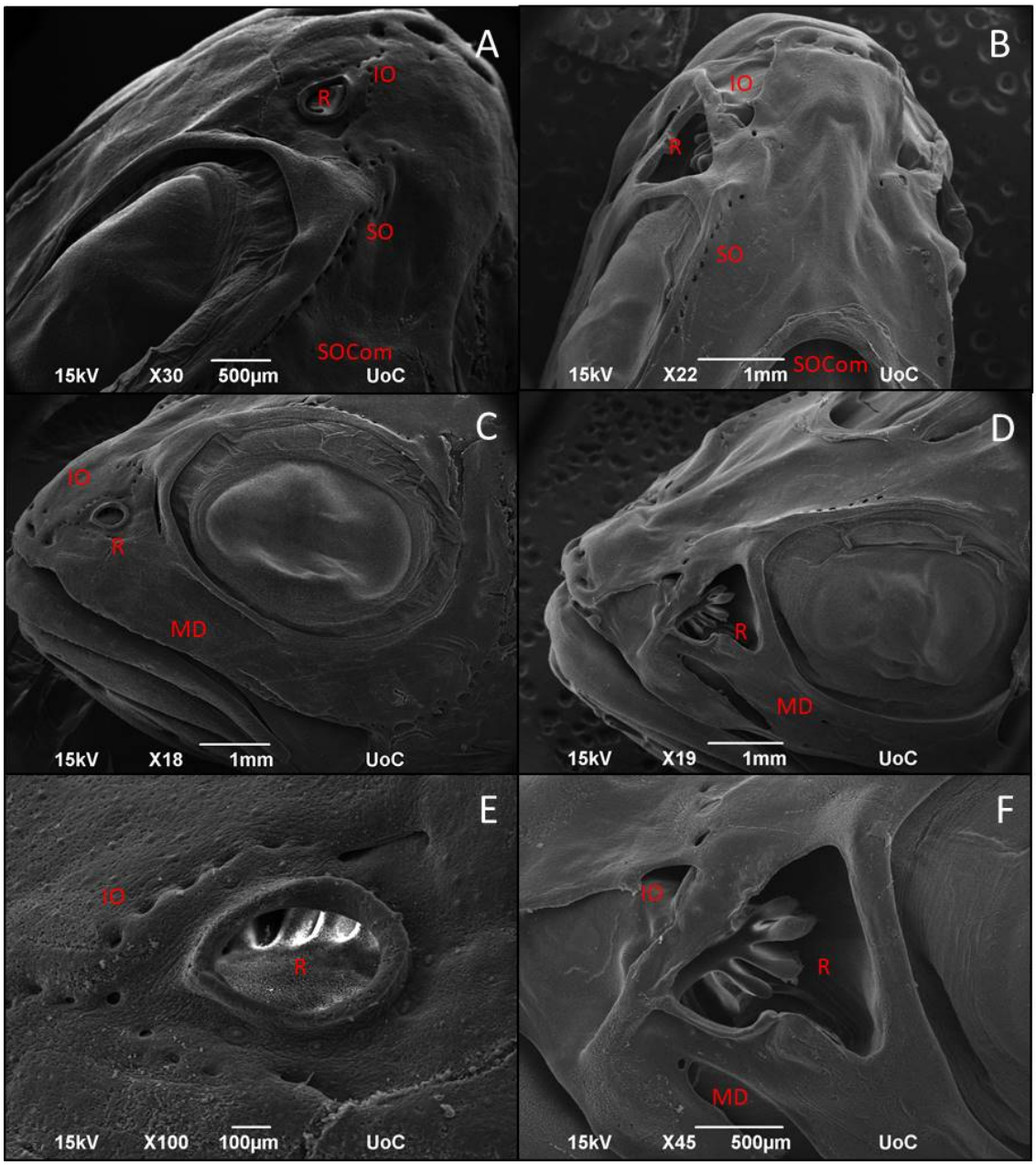

Figure 6. SEM micrographs of healthy and CUD-affected juvenile meagre (56 dph). Dorsal view showing the supraorbital canal (SO), the infraorbital canal (IO) and supraorbital commissure (SOCom) of healthy **(A)** and CUD-affected meagre **(B)**. Lateral view of healthy (**C**) and CUD-affected meagre (**D**) showing the infraorbital canal, the nostril (R) and the mandibular canal (MD). Higher magnification of the nostril (R) with the infraorbital canal (IO) and the mandibular canal (MD) of healthy (**E**) and CUD-affected meagre (**F**).

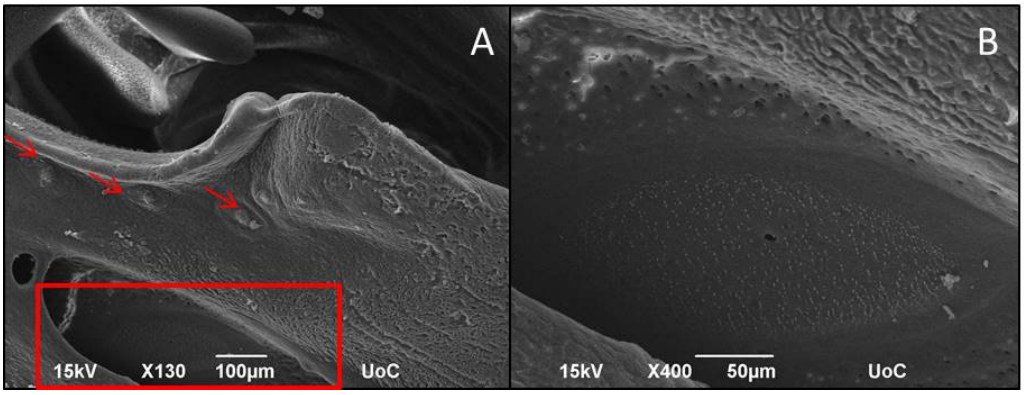

Figure 7. SEM micrographs of CUD-affected juvenile meagre (56 dph). **A.** Lateral view of the nostril with the opened mandibular canal (framed area). Arrows indicate normal superficial neuromasts around the nostril. **B.** Higher magnification of the framed area showing an exposed neuromast.

Histology

From the comparative histological analysis of meagre reared in borehole and natural seawater no differences were observed until 41 dph. F**igure 8** shows an infraorbital canal **(A & B)** and a mandibular canal **(C & D)** of meagre reared in natural seawater and in borehole water on 56 dph. In the meagre reared in natural seawater, the canals were completely developed. Instead, in meagre from borehole water we observed erosion, ulceration and loss of the basal membrane while the neuromasts were exposed to the external environment. The lesions were initially manifested as hydropic swelling and hyperplasia of the epidermis before becoming ulcerative (**Figure 9**).

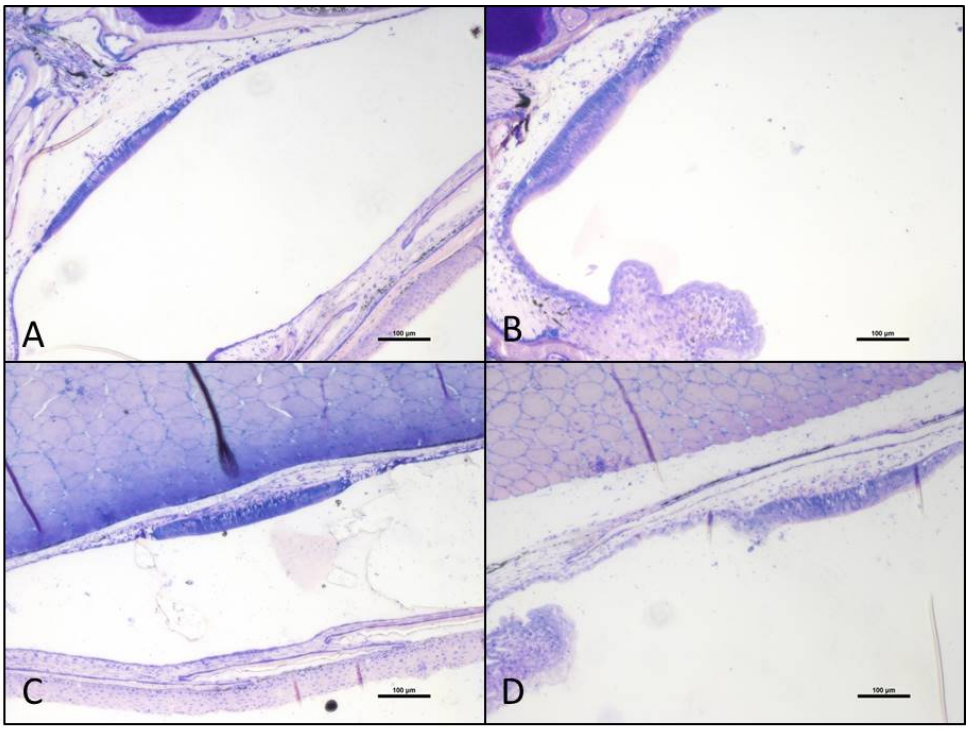

Figure 8. Cross sections of an infraorbital canal of healthy **(A)** and CUD-affected meagre **(B)** and a mandibular canal of healthy **(C)** and CUD-affected meagre **(D)**.

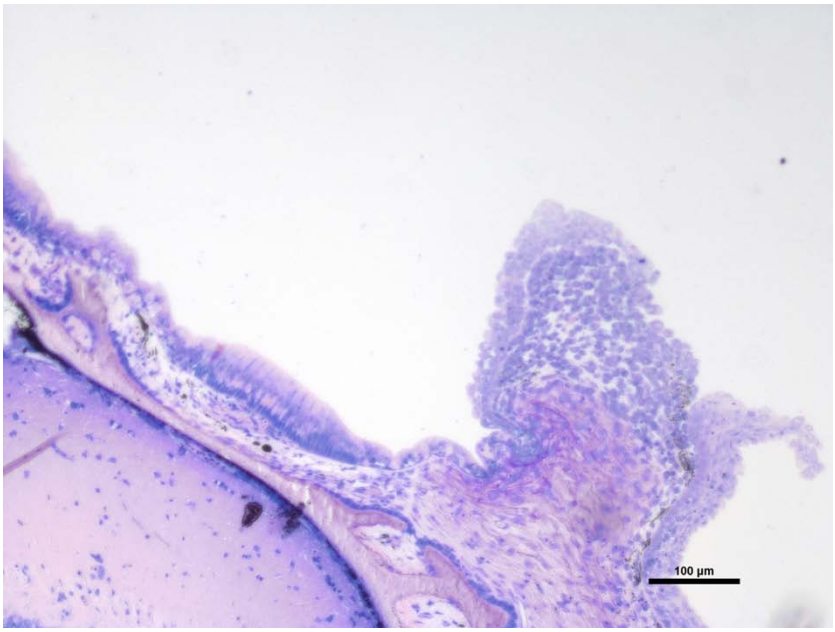

Figure 9. Oedemic lesion of the epidermis overlaying the lateral line canals of CUD-affected meagre.

Metal concentrations in fish head

Mean metal concentrations in the heads of meagre reared in borehole seawater and natural seawater are summarized in **Table 2**. Meagre reared in borehole seawater were found to have significantly higher concentrations of Lithium (Li), Chromium (Cr), Manganese (Mn), Iron (Fe), Cobalt (Co), Copper (Cu) and Barium (Ba) compared to fish reared in natural seawater. Aluminium (Al), Vanadium (V), Cadmium (Cd), Caesium (Cs) and Lead (Pb) was detected only in fish reared in borehole water.

Table 2. Mean metal concentrations in the head of meagre reared in borehole and natural seawater. The values are mean±SD (bdl: below detection limit). Different letters indicate statistically significant differences between the two water sources.

	Borehole seawater	**Natural seawater**
Mg (mg/g)	1.33±0.53	0.86±0.03
P (mg/g)	49.26±4.95	39.27±6.83
K (mg/g)	0.75±0.11	0.83±0.13
Ca (mg/g)	92.44±10.61	85.40±2.53
Mn (mg/g)	0.03±0.01	0.02±0.00
Na (mg/g)	16.25±1.05	14.57±2.48
Li (mg/kg)	0.16±0.04^{a}	0.07±0.00^{b}
Cr (mg/kg)	0.89±0.20^{a}	0.52±0.09^{b}
Co (mg/kg)	0.15±0.05^{a}	0.08±0.01^{b}
Ni (mg/kg)	3.44±0.57	2.82±0.14
Zn (mg/kg)	65.72±9.67	55.69±1.82
Se (mg/kg)	0.65±0.02	0.61±0.07
Rb (mg/kg)	0.15±0.02	0.12±0.03
Hg (mg/kg)	0.07±0.01	0.07±0.01
Al (mg/kg)	6.31±5.67	bdl
V (mg/kg)	0.22±0.03	bdl
Cu (mg/kg)	1.70±0.51^{a}	0.85±0.25^{b}

Mo (mg/kg)	0.10±0.03	0.08±0.04
Cd (mg/kg)	0.01±0.00	bdl
Ba (mg/kg)	2.14±0.28[a]	1.12±0.13[b]
Pb (mg/kg)	0.62±0.10	bdl

Development of the condition (Diagnostics)

As stated earlier, the first detectable signs of the disease are at 46 dph. Macroscopically, the fish of this age had a white epidermis overlaying the head lateral line canals. However, lesions were evident with both SEM and histology. We monitored the development of the lateral line organ throughout the rearing trial in order to detect the first signs of the disease that would be helpful for diagnostic purposes.

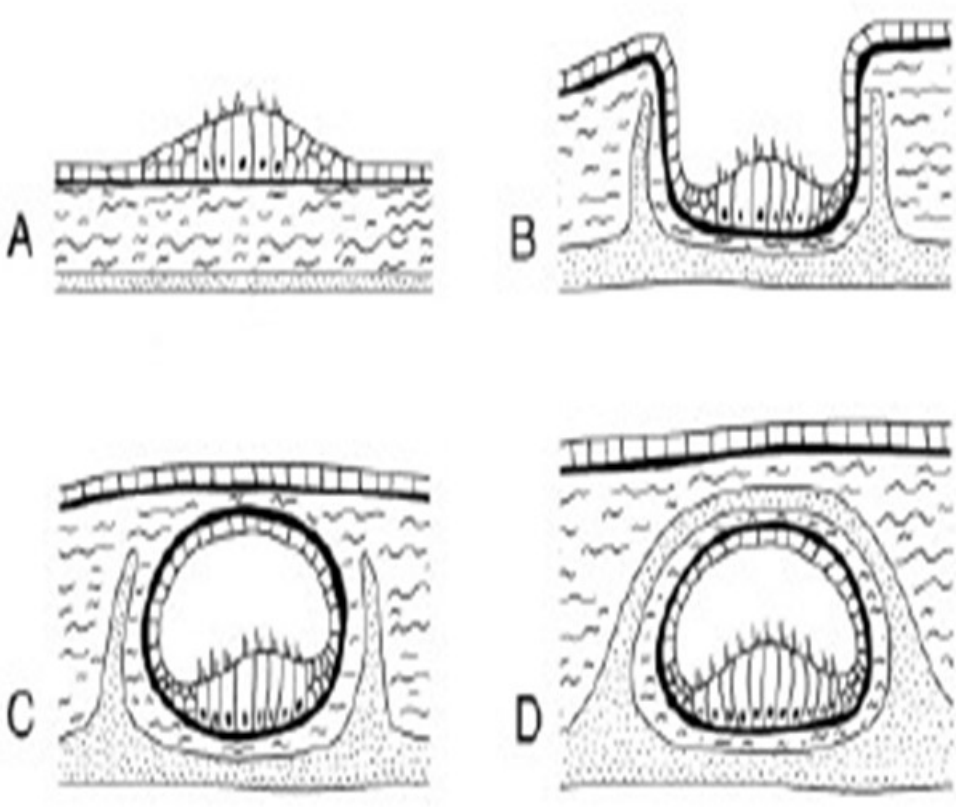

Figure 10. The ontogenesis of the head lateral line canals according to Tarby and Webb (2003). At hatching, the neuromast lays on the surface of the epidermis. As the fish grows, the neuromast becomes embedded within a groove that eventually is enclosed by an overlaying epidermal roof which at the end of ontogenesis is ossified.

The development of the lateral line organ in meagre follows the pattern described in other fish species **(Figures 10-13)**. At hatching the neuromast is located at the surface of the epidermis **(Figure 11)**.

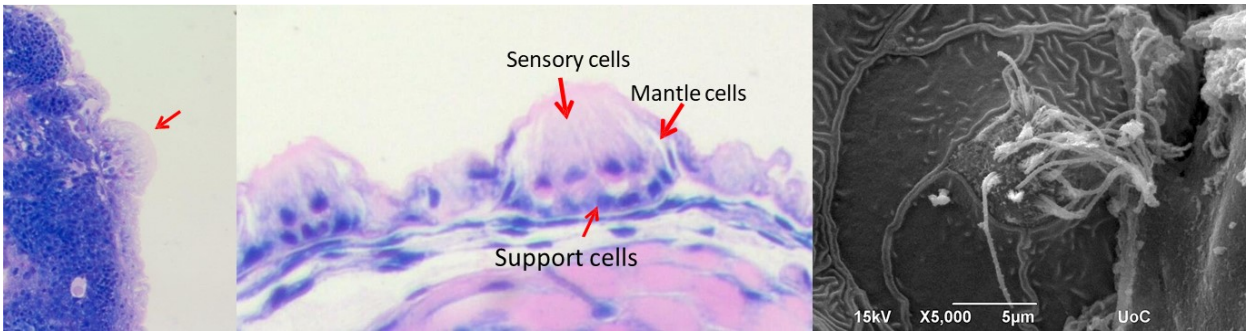

Figure 11. Left: a superficial neuromast on the epidermis of a newly hatched meagre larva (1dph); Middle: the structure of the neuromast of a 3 dph meagre larva; Right: the neuromast as seen with SEM.

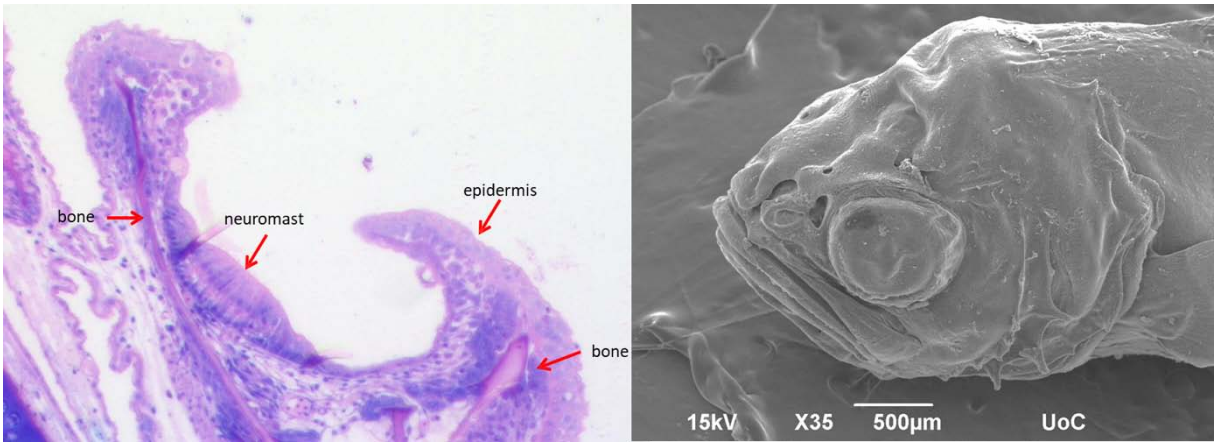

Figure 12. Between 17-25 dph, the neuromasts sink within the grooves in which the canals will be later formed. At this stage, the roof is still incomplete which is normal (pictures from meagre grown in seawater).

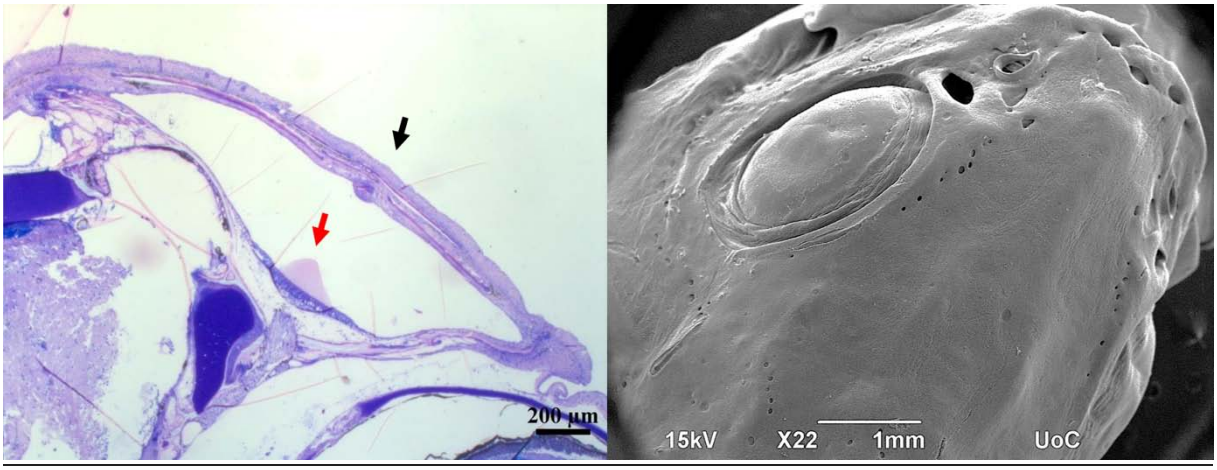

Figure 13. At 40 dph, the lateral line canals are fully formed and complete. Left: cross section of the supraorbital canal in the head of a normal meagre, showing the neuromast with the cupula (red arrow) and the fully formed canal roof (black arrow). Fish of the same age as observed with SEM, note the pores at the epidermis outlining the underlying canals.

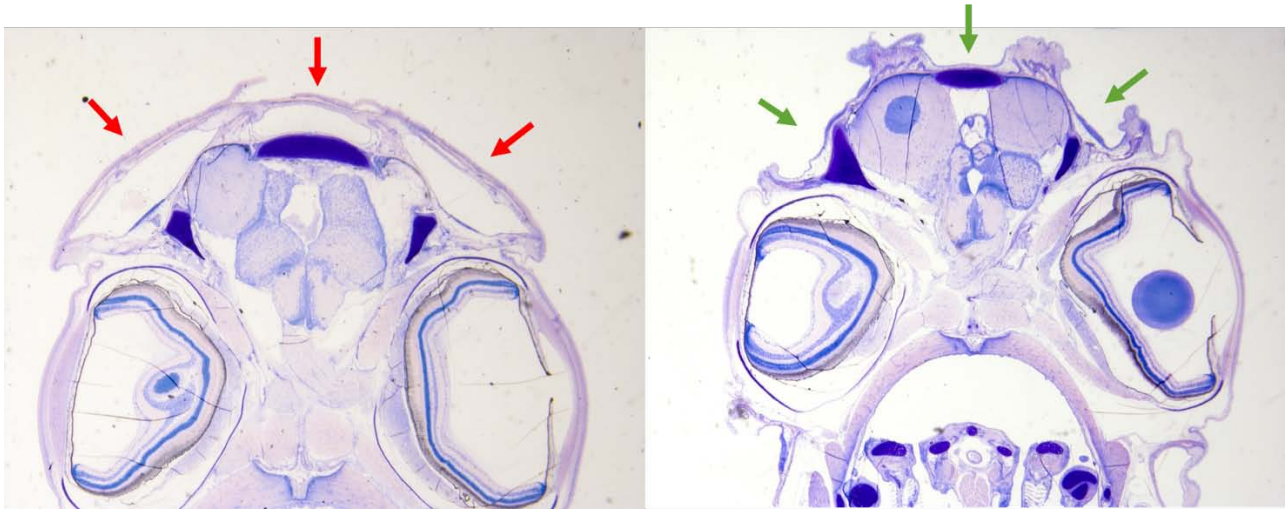

Figure 14. Comparison of cross sections of meagre heads at 46 dph grown in natural seawater (left) and borehole water (right). The arrows indicate the position of the head lateral line canals which are completely uncovered in the CUD-affected fish.

Recovery trial

The transfer from borehole water to natural sea water of CUD affected meagre led to almost full recovery of the lesions within 5 months (**Figure 15**).

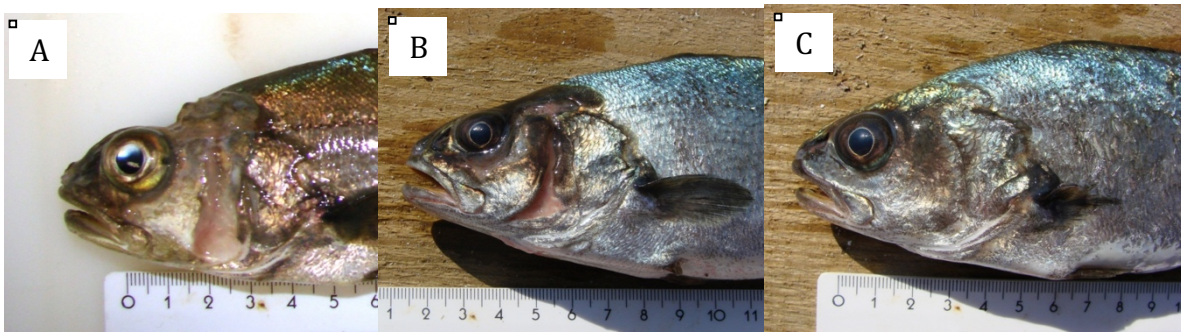

Figure 15. A. Nine—month-old meagre reared solely in borehole seawater. **B. Nine**—month-old meagre transferred to natural seawater for 5 months, with partial resolution of the lesions. **C. Nine**—month-old meagre transferred to natural seawater for 5 months, with complete resolution of the lesions.

Second rearing trial

At the end of the rearing trial (60 dph) none of the fish that reared in natural sea water + CO_2 had visible lesions associated with CUD. (**Figure 16).**

Figure 16. Meagre reared in natural seawater (left) and natural seawater+CO_2 (right). None of the fish reared in natural seawater+CO_2 had visible lesions on the head associated with CUD.

Growth performance

The average length and weight of the fish of the different water sources at the end of the rearing trial (60 dph) are presented in **Figure 17**. The growth performance of the fish was not affected by the different source of water ($p>0.05$)

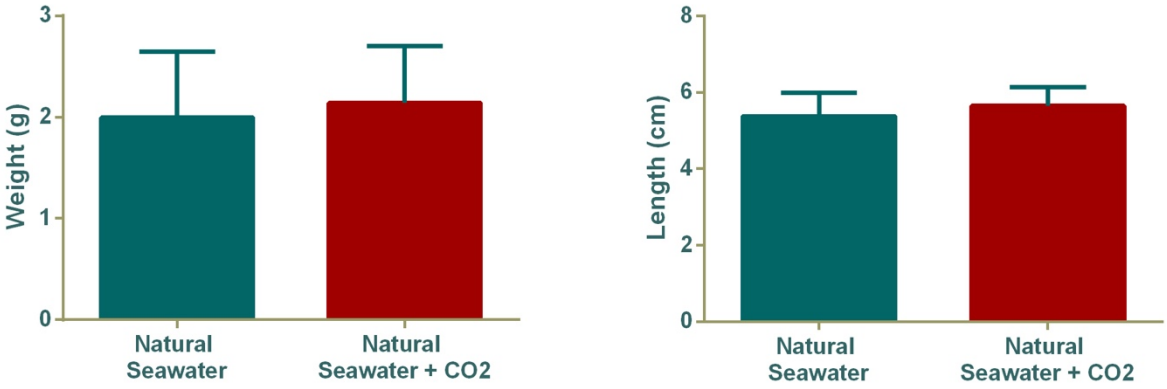

Figure 17. Average length and weight of meagre reared in natural seawater and natural seawater+CO_2 at the end of the rearing trial (60dph). The values are mean±SD.

Physicochemical analysis of water

The physicochemical analysis of the two water sources is presented in **Figure 18**. The pH was lower and CO_2 higher in natural seawater+CO_2 in comparison to natural sea water, while T and O_2 levels did not differ between the two sources.

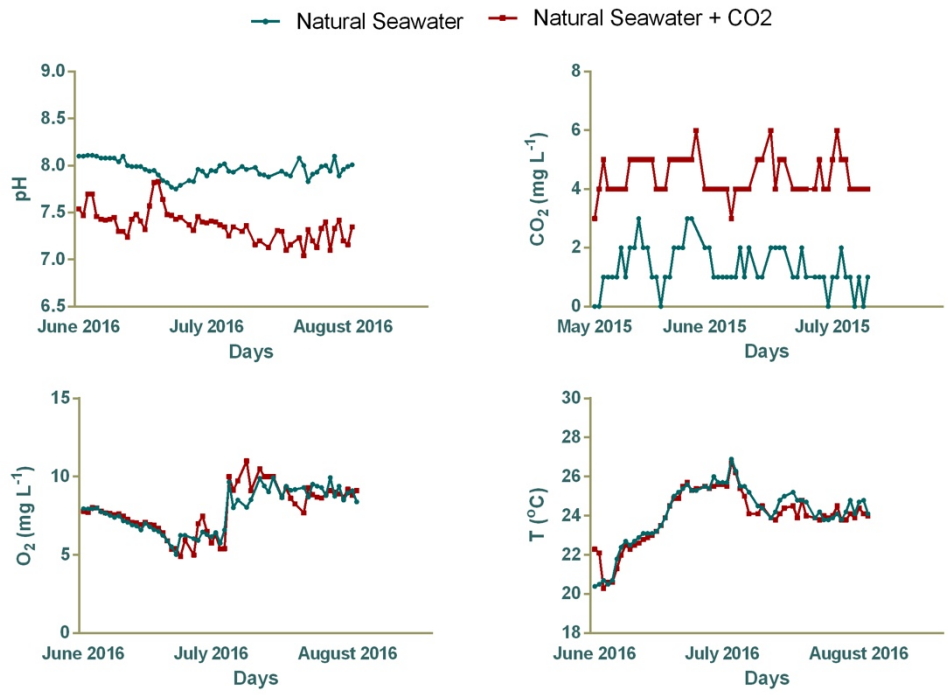

Figure 18. Physicochemical analysis of two different sources of water.

Histology and SEM analyses are still ongoing, however first indications suggest that neither pH nor CO_2 are the factors affecting the development of CUD lesions.

Discussion

The results indicated that CUD in meagre is induced by 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 of Katharios et al. (2011) for sharpsnout seabream. Furthermore, another similarity with Murray cod and sharpsnout seabream is that the lesions resolve 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.

From the physicochemical analysis of the two water sources it is noteworthy that the pH was lower and CO_2 higher in borehole water in comparison with natural seawater. Katharios et al. (2011) hypothesized that borehole water which is rich in CO_2, as indicated also by the lower pH compared to the pH of natural seawater, increases the enzymatic activity of the osteoclasts. The CO_2 activates the osteoclasts, which are in close proximity with the environment, such as the osteoclasts of the lateral line canals. In this scenario there would be an environmentally induced imbalance between osteoclasts (bone resorbing cells) and osteoblasts (bone depositing cells) that would cause the lesions seen in the fish, located exclusively in the lateral line canals. The qPCR results from this task are in agreement with this hypothesis since there is an overexpression of the genes cathepsin K and TRAP that are related to osteoclast activity, in the fish reared in borehole water compared to those reared in natural sea water, while vATPase which is the osteoblastic marker remained unchanged.

Based on these results, we performed a second rearing trial that was not included in the DOW of DIVERSIFY in order to investigate whether CO_2 in borehole water is the aetiological agent that causes the development of CUD lesions. In this trial, we used 2 parallel rearing tanks supplied with natural sea water. In one of these tanks we adjusted the pH to 7.4 by infusing CO_2. We cultured meagre from eggs to 60 dph.

Although the analysis of this trial has not yet been finalized, the lack of lesions in the head and the trunk of the fish following visual examination, suggests that neither pH nor CO_2 are the factors affecting the development of CUD lesions.

Eisler and Gardner (1973) found that copper alone or in combination with zinc or cadmium damages the epithelium of canals in the head of mummichog (*Fundulus heteroclitus*). The facilities and the water sources we used for this trial were the same that Katharios et al. (2011) used for the study of CUD in sharpsnout sea bream. From the heavy metal analysis of water samples, they found that borehole water had higher concentrations of copper, lead, nickel and zinc than natural seawater, however these levels were within the acceptable limits for marine aquaculture and much lower than the toxic limits. Our results from the 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. However, concentrations of all metals where comparable to published data from other farmed and wild fish species where lesions are absent (Alasalvar et al., 2002; Kalantzi et al., 2016, 2013; Zotos and Vouzanidou, 2012). Nevertheless, metal toxicity as a causative factor for the development of CUD cannot be ruled out because of the lower pH of the borehole water and the longer exposure times of the fish.

Furthermore, another interesting similarity between CUD-affected meagre and CUD-affected Murray cod is the presence of the enigmatic rodlet cells. Schultz et al. (2014) found a significantly greater number of rodlet cells in the gills, kidneys and intestines of CUD-affected Murray cod and assumed that it was a response to a toxicant in the groundwater. In this task we didn't examine the soft tissues of meagre. However, in trials to investigate the causes of systemic granulomatosis we used meagre reared in borehole water with visible lesions associated with CUD. As we have described in deliverables 24.1, 24.2 and 24.5, rodlet cells in meagre are present in large numbers, aligned like epithelial cells in the peritoneal membranes, liver, pancreas, intestine and kidney. In both meagre and Murray cod, no pathogens were identified in any tissue, so the secretory nature or rodlet cells might be connected to defense mechanisms of fish against a toxicant in the water. However, this hypothesis cannot be fully supported since no data exist on the presence of these cells in normal (not affected by either systemic granulomatosis or CUD) or wild meagre.

Although the disease 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 for 72 h prevents the development of CUD. Thus, it is recommended to avoid borehole seawater for the rearing of meagre if natural sea water sources are available and to pay careful attention to the source of the water used. Alternatively, the residence time of meagre in borehole water should be reduced to the minimum necessary, and fish should be moved to natural seawater (*e.g.* in sea cages) as soon as possible once the nursery phase is completed, in order to allow the tissue regeneration process to complete before marketing the fish.

Bibliography

Alasalvar, C., Taylor, K.D.., Zubcov, E., Shahidi, F., Alexis, M., 2002. Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and trace mineral composition. Food Chem. 79, 145–150.

Baily, J.E., Bretherton, M.J., Gavine, F.M., Ferguson, H.W., Turnbull, J.F., 2005. The pathology of chronic erosive dermatopathy in Murray cod, *Maccullochella peelii peelii* (Mitchell). J. Fish Dis. 28, 3–12. doi:10.1111/j.1365-2761.2004.00586.x

Bennett, H.S., Wyrick, A.D., Lee, S.W., McNeil, J.H., 1976. Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains. Stain Technol. 51, 71–97.

Bleckmann, H., Zelick, R., 2009. Lateral line system of fish. Integr. Zool. 4, 13–25.

Corrales, J., Ullal, A., Noga, E.J., 2009. Lateral line depigmentation (LLD) in channel catfish, *Ictalurus*

punctatus (Rafinesque). J. Fish Dis. 32, 705–712. doi:10.1111/j.1365-2761.2009.01069.x
Dagamseh, A., Wiegerink, R., Lammerink, T., Krijnen, G., 2013. Imaging dipole flow sources using an artificial lateral-line system made of biomimetic hair flow sensors. J. R. Sociey Interface 10, 1–9.
Eisler, R., Gardner, G.R., 1973. Acute toxicology to an estuarine teleost of mixtures of cadmium, copper and zinc salts. J. Fish Biol. 5, 131–142.
Kalantzi, I., Black, K.D., Pergantis, S.A., Shimmield, T.M., Papageorgiou, N., Sevastou, K., Karakassis, I., 2013. Metals and other elements in tissues of wild fish from fish farms and comparison with farmed species in sites with oxic and anoxic sediments. Food Chem. 141, 680–694.
Kalantzi, I., Pergantis, S.A., Black, K.D., Shimmield, T.M., Papageorgiou, N., Tsapakis, M., Karakassis, I., 2016. Metals in tissues of seabass and seabream reared in sites with oxic and anoxic substrata and risk assessment for consumers. Food Chem. 194, 659–670.
Katharios, P., Papadaki, M., Ternengo, S., Kantham, P.K., Zeri, C., Petraki, P.E., Divanach, P., 2011. Chronic ulcerative dermatopathy in cultured marine fishes. Comparative study in sharpsnout sea bream, *Diplodus puntazzo* (Walbaum). J. Fish Dis. 34, 459–474.
Rigos, G., Katharios, P., 2010. Pathological obstacles of newly-introduced fish species in Mediterranean mariculture: A review. Rev. Fish Biol. Fish. 20, 47–70.
Schultz, A.G., Healy, J.M., Jones, P.J., Toop, T., 2008. Osmoregulatory balance in Murray cod, *Maccullochella peelii peelii* (Mitchell), affected with chronic ulcerative dermatopathy. Aquaculture 280, 45–52.
Schultz, A.G., Jones, P.L., Toop, T., 2014. Rodlet cells in Murray cod, *Maccullochella peelii peelii* (Mitchell), affected with chronic ulcerative dermatopathy. J. Fish Dis. 37, 219–228. doi:10.1111/jfd.12099
Schultz, A.G., Shigdar, S.L., Jones, P.L., Ward, A.C., Toop, T., 2011. Groundwater pre-treatment prevents the onset of chronic ulcerative dermatopathy in juvenile Murray cod, *Maccullochella peelii peelii* (Mitchell). Aquaculture 312, 19–25. doi:10.1016/j.aquaculture.2010.12.013
Tarby, M.L., Webb, J.F., 2003. Development of the supraorbital and mandibular lateral line canals in the cichlid, *Archocentrus nigrofasciatus*. J. Morphol. 255, 44–57. doi:10.1002/jmor.10045
Wada, H., Iwasaki, M., Kawakami, K., 2014. Development of the lateral line canal system through a bone remodeling process in zebrafish. Dev. Biol. 392, 1–14.
Webb, J.F., 1989. Neuromast morphology and lateral line trunk canal ontogeny in two species of cichlids: an SEM study. J. Morphol. 202, 53–68. doi:10.1002/jmor.1052020105
Webb, J.F., Shirey, J.E., 2003. Postembryonic Development of the Cranial Lateral Line Canals and Neuromasts in Zebrafish. Dev. Dyn. 228, 370–385. doi:10.1002/dvdy.10385
Zotos, A., Vouzanidou, M., 2012. Seasonal changes in composition, fatty acid, cholesterol and mineral content of six highly commercial fish species of Greece. Food Sci. Technol. Int. 18, 139–49.

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