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### PHYSIOLOGICAL ANALYSIS AFTER ${\rm CO_2}$ EXPOSURE FROM HATCHING STAGE IN RAINBOW TROUT

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Reduction of chronic stress consequences is of major relevance in aquaculture due to its supposed impact on fish health and welfare. Among important stressors, chronic exposure to high-CO2 has been shown to affect fish growth, feed conversion efficiency and nephrocalcinosis (Fivelstad 2013). Several studies are performed to measure the effect of CO<sub>2</sub>-induced ocean acidification on marine system, however less is known about CO<sub>2</sub> in freshwater (Ou et al 2015). The aim of the present study was to assess in freshwater rainbow trout the effects of a chronic exposure to moderate CO<sub>2</sub> level on fish health status and coping ability through a multivariate analysis covering endocrine and physiological parameters. Fish health status and coping ability was evaluated before and after a 24hours-challenge.

Experiment was conducted in INRA PEIMA experimental infrastructure. At hatching stage, fish were divided in 6 flow-through tanks. All tanks used running spring water with different  $CO_2$  concentrations (more or less degassed): control group: 3 tanks with 0-3mg/l  $CO_2$  (pH~6.6) and  $CO_2$  group: 3 tanks with 8-15mg/l (pH~6) (figure).  $O_2$  was 10-11mg/l. Six month later, part of fish was sampled and the other part was exposed for 24 hours to bad water quality by reducing water renewal and increasing density.

Growth performance was followed during 6 months. Blood and various tissues including gill, head-kidney, pituitary and brain were collected. Gill functions were assessed by measuring blood parameters (ion levels, pH, hematocrit, complement and lysozyme activities) and gene expression levels in gill tissues. In the same fish, we measured mRNA levels of major genes regulating HPI (hypothalamo-pituitary-interrenal) axis activity in head-kidney, pituitary and brain. In addition, before and after 24h-challenge, responsiveness of the HPI axis was also assessed on sub-groups of control or hypercapnia group by submitting trout to acute netting and handling stress (duration: 4 minutes) with a 1-hour recovering period in control conditions. Then, blood was collected for further analysis of plasma cortisol levels.

Measurements of the various parameters of the biological functions as described above are presently in progress. Preliminary results show a clear effect of  $CO_2$  on growth. Furthermore, gill responses to a 24h-challenge are different between control and  $CO_2$  groups. Overall, these analyses will give us a comprehensive description of the health and welfare status of trout exposed to our  $CO_2$  condition.

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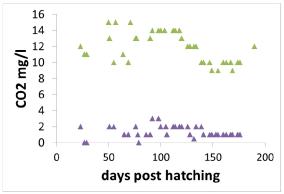


Figure: Evolution of CO<sub>2</sub> in water in control tanks (violet) and CO2 tanks (green) during experiment.

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