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Impact of high water carbon dioxide levels on Atlantic salmon smolts (*Salmo salar* L.): Effects on fish performance, vertebrae composition and structure

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Abstract

The role of high carbon dioxide (CO_2) levels on fish performance, bone structure/composition and as a potential cause of spinal deformities was studied. Two groups of fish were exposed to a low (control) and a high level of CO_2 for 135 days during the freshwater period. After smoltification, the fish were transferred to seawater and followed up for 517 days until they reached harvest weight (3.1 kg BW).

Differences in body weight between the control and high CO_2 groups were observed. At the end of the freshwater period, average weight in the group exposed to high CO_2 levels was 20.9% lower than in the control group. Specific growth rates (SGR) from the start of the experiment (10 g BW) to smolt stage were 1.63±0.04 and 1.36±0.01 for the control group and the high CO_2 group, respectively. Differences in body weight were maintained during the initial stages of the seawater period, but were not observed at harvest weight.

Nephrocalcinosis was not observed in any of the experimental groups at the end of the freshwater period and no external signs of spinal deformities were observed either at smolt stage or at harvest weight. X-rays revealed mild abnormalities in some vertebrae bodies, which could not be related to any experimental group. Despite the lack of signs of pathological bone alterations, the histological examination suggested that the exposure to high CO_2levels resulted in an increase in trabeculae volume and a higher rate of bone remodeling at the end of the freshwater period. Furthermore, fish exposed to a high CO_2 level presented a higher bone ash content at the end of the freshwater period. These differences could not be observed at the end of the grow-out period.

Keywords: Atlantic salmon; Carbon dioxide; Growth; Minerals; Bone histology; Deformities

1. Introduction

In recent years, due to the fast growth of the industry, the need for higher numbers of smolts has resulted in a significant increase in fish densities in the tanks during

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the freshwater period. This has been achieved by the addition of oxygen (O_2) to the water, and has resulted in a significant increase in the concentration of carbon dioxide (CO_2) reaching levels up to 40 mg/l. CO_2 in the water depends on water flow and CO_2 excretion rate (Fivelstad et al., 2004a). High levels of CO_2 in the water will reduce pH and increase the toxicity of aluminum causing hypertrophy and hyperplasia of the gill epithelium (Fivelstad et al., 2003). Effects of long-term CO_2 exposure in rainbow trout (Eddy et al., 1977; Smart, 1979, 1981) and Atlantic salmon smolts (Fivelstad et al., 1999) in freshwater include reduced feed conversion ratio and reduced growth. When fish are exposed to high levels of CO_2 for long periods, blood CO_2 will increase (hypercapnia) and blood pH will decrease resulting in respiratory acidosis (Eddy et al., 1977; Ultsch, 1996). Fish compensate for acidosis by increasing the plasma bicarbonate levels and excreting phosphate via the kidney (Lloyd and Swift, 1967) and might also mobilise ions from the bones (Storset et al., 1997). Thus, the question remains whether these compensation mechanisms can induce bone demineralization and thereby cause spinal deformities. Exposing salmonids to high levels of CO_2 is associated with nephrocalcinosis, a condition characterized by the presence of mineral deposits in the kidney tubules (Smart, 1981; Fivelstad et al., 1999). The most abundant minerals in these deposits are Ca, P and Mg (Smart, 1979). In the trial by Fivelstad et al. (1999), Atlantic salmon were exposed for 62 days to different concentrations of CO_2, during the fresh water phase. The percentage of fish presenting nephrocalcinosis was significantly higher in the group exposed to 32 mg/l CO_2 compared to the groups exposed to 7 and 19 mg/l CO_2. Likewise, in the study by Smart (1979), significantly higher Ca and P concentrations were found in the kidney of fish exposed to high CO_2 levels. Such a pathological accumulation of minerals in the kidney clearly reflects a disturbance in mineral homeostasis, which could also affect the bone.

The purpose of the present study was to evaluate the impact of high CO_2 levels in freshwater on growth performance, bone structure and development of spinal deformities in Atlantic salmon both during the freshwater period and at the end of the grow-out period in the sea.

2. Material and methods

2.1. Experimental design

Atlantic salmon parrs (average weight of 10 g) were exposed to two water CO_2 regimes (a standard and a high CO_2 level) for 134 days during the freshwater period until they reached smolt stage. Fish were observed in seawater until an average weight of 3.1 kg was achieved. Growth performance was assessed at different intervals throughout the study in freshwater and at the end of the seawater period. Bone samples from the vertebral column were analyzed by radiology and histology and for mineral composition at the end of the freshwater phase only.

2.2. Rearing conditions

Atlantic salmon with known history and the same genetic background were distributed in six indoor, experimental tanks (volume of 125 l) at Lerang Research Station (Stavanger, Norway), corresponding to 3 tanks per experimental treatment. Each tank contained 60 fish of an average weight of 10 g. The two experimental groups were exposed to the following CO_2 conditions:

- Control group (no CO_2 addition): CO_2 levels corresponded to the CO_2 generated by the fish (max. 7 mg CO_2/l).
- High CO_2 group: CO_2 was added gradually according to fish size in order to mimic the conditions to which fish may be exposed to under commercial conditions. The targeted CO_2 levels in the water were 10, 20 and 35 mg/l during periods 1, 2 and 3 respectively (Table 1). In the present experiment, carbon dioxide of a 99.9% purity was added to the water from a pressurized bottle in a mixing tank, then the water was distributed to the experimental tanks.

The water flow was 8 l/min in all the experimental tanks. Seawater was added to the tanks in order to neutralize the acidity of the water. This is common practice in many Norwegian commercial hatcheries to reduce aluminum toxicity. Seawater addition increased with fish size in order to adjust salinities from 0.5‰ for 10 g fish to 5‰ for 30 g fish and larger. The light regime was in accordance to that used for O+ smolts production recommended by Handeland (1998). The fish were exposed to a 12 h light and 12 h darkness regime for 6 weeks, then continued with a 24 h light regime for

Table 1
Schedule of the trial and targeted CO_2 levels (mg/l) in the high CO_2 group during the FW stage

Period	Day	Duration (days)	Targeted CO_2 level (mg/l)
1	1–27	27	10
2	28–92	64	20
3	93–135	41	35