Aquaculture 280 (2008) 146–153

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Long-term separate and combined effects of environmental hypercapnia and hyperoxia in Atlantic salmon (*Salmo salar* L.) smolts

Camilla Diesen Hosfeld [a,*], Annhild Engevik [a], Ted Mollan [a], Torleif Markussen Lunde [a], Rune Waagbø [b], Anne Berit Olsen [c], Olav Breck [b,e], Sigurd Stefansson [d], Sveinung Fivelstad [a]

[a] Department of Environment and Aquaculture Technology, Faculty of Engineering, Bergen College University, Nygårdsgaten 112, P.O. Box 7030, N-5020 Bergen, Norway
[b] The National Institute of Nutrition and Seafood Research, P.O. Box 2029 Nordnes, N-5817 Bergen, Norway
[c] National Veterinary Institute Bergen, P.O. Box 1263 Sentrum, N-5811 Bergen, Norway
[d] Department of Biology, University of Bergen, Bergen High Technology Centre, N-5020 Bergen, Norway
[e] Marine Harvest Norway, P.O. Box 4102 Dreggen, N-5035 Bergen, Norway

ARTICLE INFO

Article history:
Received 23 June 2006
Received in revised form 6 May 2008
Accepted 8 May 2008

Keywords:
Hyperoxia
Hypercapnia
Oxygen
Carbon dioxide
Haematology
Salmo salar L.

ABSTRACT

Atlantic salmon (*Salmo salar* L.) parr (mean start weight 50 g) were reared in freshwater (FW) and exposed to three levels of oxygen saturation measured in effluent water; control group (93% O_2, LO_2), medium (111% O_2, MO_2) and high (123% O_2, HO_2). Further three groups were exposed to similar water oxygen levels in combination with elevated carbon dioxide levels (17–18 mg L^{-1} CO_2), named LO_2–CO_2, MO_2–CO_2 and HO_2–CO_2, respectively. The experiment was run in duplicate tanks for 42 days, and the fish were subsequently transferred to the same seawater (SW) regime for 45 days for an assessment of post-smolt growth. As a consequence of the CO_2 addition, tank pH levels in the FW period were reduced from 6.7 to 5.9 for the hypercapnia groups compared to for the normcapnia groups. Water temperature in FW ranged between 6.4 and 9.0 °C. Citrate was added to the water to complex labile aluminium.

In the CO_2 groups observed ventilation frequencies were significantly increased compared to the control ($p<0.05$). This difference declined towards the end of the FW period, suggesting acclimation to elevated CO_2. The degree of oxygenation appeared to contribute to the acclimation as the lowest mean ventilation frequency on day 36 was found in the HO_2–CO_2 group and the highest in the LO_2–CO_2 group. Lower plasma chloride and sodium levels were observed in the CO_2 groups relative to the respective oxygenation groups during the FW period, while plasma chloride and sodium levels were normalised to equal levels for all groups after 44 days in SW. No significant differences were found among treatments for blood concentrations of red blood cells, haemoglobin, potassium and glucose during the experiment.

By termination of the FW period, the HO_2 group had significantly higher body weight than all other groups ($p<0.05$), with specific growth rate significantly higher than the CO_2 groups ($p<0.05$). Further, the condition factor was significantly lower in all the CO_2 groups at the end of the FW period compared to the control and normcapnia groups ($p<0.05$). Although variable among replicates, occurrence of nephrocalcinosis was 10 times higher in the hypercapnia groups than in the control and normcapnia groups. Mortality was negligible (<2.0%) during the trial, and most of the mortality occurred following SW transfer.

1. Introduction

With increasing intensification in Atlantic salmon (*Salmo salar* L.) farming, the demands for knowledge on limiting production related factors such as water flow, oxygen requirements, and tolerance levels for CO_2 are of increasing importance. Intensive farming is often equivalent to reduced water supply, compensated by water oxygenation. In a commercial production situation there are variations between tanks, in biomass, fish size, oxygen consumption, water flow and temperature. When oxygen is introduced at one central point in the system this may cause uneven oxygen conditions, like supersaturation in some tanks and not in others. Water qualities that may appear under intensive fresh water (FW) production with oxygenated water, with elevated O_2 and CO_2, reduced pH and elevated concentrations of metal ions, represent considerable challenges to gill function and ventilation. Hypercapnia is characterized by the presence of an abnormally high level of CO_2 in the blood (P_{CO2}), often as a result of elevated CO_2-concentrations in the water (Heisler, 1984). The rise in blood partial CO_2 pressure can cause a "respiratory" acidosis, which may be compensated for over time (Goss et al., 1994). Physiological effects of acidosis include reduced oxygen transport to tissues, by reducing the haemoglobin oxygen affinity (Bohr effect) and oxygen binding capacity (Root effect) (Pelster and Decker, 2004), and

* Corresponding author. Tel.: +47 986 79 759, +47 55 58 77 74; fax: +47 55 58 77 90.
E-mail address: camilla.hosfeld@hib.no (C.D. Hosfeld).

0044-8486/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2008.05.009

increased ventilation frequency. In a farming situation, the latter may appear either directly caused by gill irritation or indirectly through tissue oxygen depletion (Staurnes et al., 1984). As regulation of gill ventilation in fish is primarily oxygen-oriented, supersaturation of water oxygen may result in reduction of gill ventilation, increased arterial P_{CO2} (hyperoxia-induced hypercapnia) and subsequently acidosis (Wood, 1991; Heisler, 1984; Person-Le Ruyet et al., 2002). Previous research has shown reduced food intake (Smart, 1981) and reduced growth (Fivelstad et al., 1999, 2003a) as a consequence of water induced hypercapnia. Long-term hypercapnic conditions have consistently been shown to reduce the condition factor in salmon smolts (Fivelstad et al., 1998, 1999, 2003a,b), probably related to stress, increased energy spenditure and reduced feed intake. Nephrocalsinosis, caused by deposits of calcium phosphates in the kidneys has been associated with elevated CO_2 levels (Smart et al., 1979; Gebauer et al., 1992; Fivelstad et al., 1999, 2003a,b).

Due to a lack of information about the potential long-term challenges of oxygen supersaturated water, there has been an extensive debate in Norway regarding hyperoxia exposure and its potential risks and benefits. Few long-term experiments have been done where the total gas pressure has been monitored and findings from hyperoxia experiments vary. Both positive and negative growth effects have been found (Stefansson et al., 2007). Brauner (1999) found reduced hypoosmoregulatory ability in coho salmon (*Oncorhynchus kisutch*) after a short time exposure to hyperoxia (364%). A study on spotted wolfish (*Anarhichas minor* Olafsen) showed no impact on body weight in fish reared under hyperoxic (14.5 mg L^{-1} at 8 °C, 122%) conditions relative to a control group (Foss et al., 2003). Person-Le Ruyet et al. (2002) found no significant growth effects or signs of stress in turbot (*Scophthalmus maximus* L.) exposed to hyperoxic conditions (water oxygen at 147% and 224% air saturation) for 30 days. The study found only a slight decrease in plasma chloride and no changes in haematological status as a result of the treatment. Anadromous fish, such as salmon, are especially dependent on the water quality in the freshwater phase as this may directly affect the survival and performance in SW (Kroglund and Finstad, 2003). In order to maintain a sound industrial production of salmon it is of great importance to establish safe and optimum levels of various freshwater quality parameters. Despite lack of evidence, there is a belief amongst many fish farmers that elevated oxygen levels leads to increased growth and improved food conversion (Person-Le Ruyet et al., 2002).

A series of experiments have been performed at Bergen University College over the years, focusing on the effects of freshwater CO_2 exposure in the range 15–20 mg L^{-1} at the presmolt stage (Fivelstad et al., 1999, 2003a,b). The aim of present study was to investigate the effects of exposure to environmental hyperoxia (up to 125% saturation) both separate and in combination with environmental hypercapnia (18 mg L^{-1}) on Atlantic salmon smolt performance in FW and on the recovery capacity in SW. A carbon dioxide concentration of 18 mg L^{-1} was chosen in the present experiment both for comparative reasons and because its relevance to practical farming situations. In addition, the pH for the carbon dioxide groups was planned to be in the same range as reported by Fivelstad et al., 2003a, 2004. Fivelstad et al. (2003a) found that a drop in water pH induced by environmental hypercapnia can cause labile aluminium to become toxic to smolts. In the present experiment, citrate was added to the water to eliminate aluminium as a confounding factor.

2. Materials and methods

2.1. Experimental set-up

The study was performed at Bergen University College. The freshwater source used in this experiment was Lake Svartediket, Bergen's water reservoir. The water is soft (conductivity 24–32 μS cm^{-1}, calcium 0.5–0.7 mg L^{-1}), acidic (pH ca 5.7) and with a relatively high aluminium content (Fivelstad et al., 2003a,b, 2004). Before entering the header tank the water was heated, led through a column aerator removing excess N_2 and then filtered through a mechanical UNIK filter with fibre-cloth (Unik Filtersystem AS, Norway). In the header tank (450 L) the pH was regulated by an automatic pH control system (ProMinent Dulcotest PHD 1201, ProMinent Dosiertechnik GmbH, Germany) by the addition of NaOH to the water (pH 6.5–6.8). Using a peristaltic pump (Alitea C6XV, Watson–Marlow Alitea AB, Sweden) $NaHCO_3$ was added from stock solution to increase the alkalinity (buffer capacity) of the water, and $Na_2S_2O_3*5H_2O$ was added in the same way to remove chlorine. Citrate (tri-sodium citrate-dihydrate, $C_6H_5Na_3O_2*2H_2O$) was added to the header tank, using two peristaltic pumps in a redundancy system (Alitea C6XV, Watson–Marlow Alitea AB, Sweden), in order to complex labile aluminium. Pressurised air was applied to mix the chemicals thoroughly into the water.

From the header tank one part of the water flow passed through an EWOX low-pressure column aerator (Ewos AS, Norway) where pure oxygen was added from a pressurised gas bottle before entering the mixing tanks, while the other part was led directly to the mixing tanks. Oxygen levels were regulated in the different mixing tanks by adjusting the mix of these two water qualities. Carbon dioxide was added to three of the groups through control valves attached to a porous limestone placed in the mixing tanks (pH 5.9). The laboratory was equipped with six mixing tanks, each of them supplying water to two circular tanks (120 L). This allowed for a flow through system with six replicated experimental groups (12 tanks in total).

The study included a FW exposure period lasting 42 days: LO_2 (control, 93% O_2), MO_2 (111% O_2), HO_2 (123% O_2), LO_2–CO_2 (97% O_2+18.2 mg L^{-1} CO_2), MO_2–CO_2 (111% O_2+18.0 mg L^{-1} CO_2) and HO_2–CO_2 (127% O_2+17.2 mg L^{-1} CO_2). Bo-Laks-strain Atlantic salmon presmolts were obtained from Lindås Fiskeopdrett AS during February 2004 (not vaccinated), and acclimated to control conditions (pH 6.5–6.8, ca 2 mg L^{-1} CO_2) for five weeks (Table 1). The experimental groups were randomly allocated to tanks which were each randomly stocked with ca 60 smolts (mean start weight: 50 g, density: 25 kg m^{-3}) and specific water flow was adjusted to 1 L kg^{-1} min^{-1}. The fish were exposed to simulated natural photoperiod (60° N), and were fed a commercial dry diet (Ewos micro 15, 50% protein, 22% lipid, Ewos AS, Norway) by use of automatic feeders, in 15 s intervals during light hours. Manufacturers' tables were consulted to ensure that the fish was fed slightly in excess based on an estimated ration relative to tank biomass and

Table 1
Mean water oxygen saturation [% and mg L^{-1}], CO_2 [mg L^{-1}], ambient P_{CO2} [mm Hg], pH and temperature [°C] during the freshwater period (days 0 to 42)

	LO_2	n	MO_2	n	HO_2	n	LO_2–CO_2	n	MO_2–CO_2	n	HO_2–CO_2	n
O_2 [%]	93±0.5	(84)	111±0.6	(84)	123±0.7	(84)	97±0.3	(84)	111±0.6	(84)	127±0.6	(84)
O_2 [mg L^{-1}]	11.3±0.09	(84)	13.3±0.07	(84)	14.6±0.11	(84)	11.7±0.06	(84)	13.4±0.06	(84)	15.3±0.06	(84)
CO_2 [mg L^{-1}]	2.2±0.15	(12)	2.1±0.12	(12)	2.5±0.28	(12)	18.2±1.13	(26)	18.0±0.53	(26)	17.2±0.51	(26)
P_{CO2} [mm Hg]	0.7±0.05	(12)	0.6±0.04	(12)	0.8±0.09	(12)	5.4±0.32	(26)	5.3±0.15	(26)	5.1(±0.14)	(26)
pH	6.7±0.01	(84)	6.7±0.01	(84)	6.5±0.01	(84)	5.9±0.01	(84)	5.9±0.01	(84)	5.9±0.01	(84)
Temperature [°C]	7.7±0.02	(84)	7.9±0.01	(84)	8.0±0.01	(84)	7.8±0.03	(84)	7.8±0.06	(84)	7.9±0.02	(84)

The table show mean values±SEM, where *n* represent the number of samples regularly taken from the replicated tanks.