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Effects of O2 supersaturation on metabolism and growth in juvenile turbot (Scophthalmus maximus L.)

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Abstract: Effects of O2 supersaturation on metabolism and growth were studied in juvenile turbot (Scophthalmus maximus L.). When fish were reared for 30 days in water containing O2 at 147% or 223% air saturation, there were no significant differences in food intake, growth, food conversion or protein utilization compared to fish exposed to normoxia (100% air saturation in water outlet). Exposure to hyperoxia resulted in increased body fat deposition. Daily rates of O2 consumption of resting fish were not affected by O2-concentrations, and there were no significant differences in rates of nitrogenous excretion among fish exposed to the different O2-concentrations. Turbot tolerated severe hyperoxia, 350% air saturation, for 10 days.

There were changes in acid–base balance that compensated for the respiratory acidosis resulting from O2 supersaturation. Blood pH was regulated within 24 h (it averaged 7.69 over the 30-day experiment) by significant increases in plasma CO2 content and pCO2. Plasma CO2 was dose dependent averaging 11.3 and 18.9 mmol l−l under 147% and 224% O2 saturation, respectively, compared to 6.7 mmol l−l under normoxia. Over the 30-day experiment, the only change in hydromineral balance was a slight, but non-significant decrease in plasma chloride content in fish exposed to hyperoxia (137 mmol l−l compared to 139 under normoxia). There were no changes in haematocrit, haemoglobin and red blood cell counts (they averaged 18.3%, 3.7 g dl−1 and 1.37×106 mm−3, respectively) and no signs of stress (plasma cortisol averaged 3.8 ng ml−1) related to exposure to O2-supersaturation for 30 days.

Keywords: Turbot; Hyperoxia; Growth; Metabolism; Acid–base; Ion-regulation

1. Introduction

In many modern aquaculture facilities fish are reared at high densities, which requires water treatment to limit the accumulation of metabolic wastes and reduce the risk of oxygen depletion. Oxygen supplementation can increase the carrying capacity of a fish farm and O_2 supplementation systems are installed in many land-based freshwater and marine fish farms. However, very little is known about the long-term biological effects that occur when fish are exposed to O_2-supersaturated waters.

The short-term physiological responses of fish to environmental hyperoxia have been described (Wood, 1991; Truchot, 1994). Hyperoxia induces respiratory acidosis because the fish hypoventilate, but the acidosis is usually rapidly and effectively compensated by ionoregulatory adjustments that lead to a rise in plasma HCO_3^- concentration. Blood O_2 transport and delivery to tissues is easier, because arterial and venous O_2 tensions are higher than under normoxia (Barrett and Taylor, 1984). There may also be changes in gill structure (Goss et al., 1994) including changes involving chloride cells. The latter may explain why short-term exposure (6 h to 1 week) to severe hyperoxia (364% air saturation) may impair the hypo-osmoregulatory ability of coho salmon (*Oncorhynchus kisutch*) smolts in sea water (Brauner, 1999). There are few studies on the long-term effects of O_2 supersaturation on fish growth, but there is no evidence of any substantial growth and food conversion improvement when salmonids are reared in O_2-supersaturated waters (Edsall and Smith, 1990, 1991; Caldwell and Hinshaw, 1994). Nevertheless, many fish farmers believe that higher than normal oxygen levels lead to increased growth and improve food conversion.

The main aims of this study were to determine to what extent juvenile turbot (*Scophthalmus maximus* L., also called *Psetta maxima* R.) can adapt to hyperoxia (350% air saturation for 10 days) and to examine whether health status and growth might be affected by a longer exposure (30 days) to low (150%) or moderate (220% air saturation) hyperoxic conditions.

2. Materials and methods

2.1. Fish and rearing conditions

Two experiments were carried out on hatchery-reared juvenile turbot (7-8 months after hatching) held in 1-m^2 Swedish-type tanks with an effective volume of 430 l. Each tank was supplied with running sea water (34-34.5‰ salinity; 17-17.1 °C) at an exchange rate of 110% h^{-1}. The photoperiod was maintained constant on a 18 L:6 D cycle and light intensity was 2 W m^{-2} at the water surface.

Fish were randomly distributed among the tanks (initial biomass, 11 kg m^{-3}) and, except in the preliminary test, were then allowed to adapt to the rearing and feeding conditions for 20 days under an O_2-concentration of 7.2 mg l^{-1} (95% air saturation). They were hand-fed twice a day to apparent satiety on a commercial extruded dry pellet (Le Gouessant®, protein 49.1% and crude fat 11.5%).

2.2. Experimental design

In a preliminary test, 3 groups of 60 turbot (mean weight 80 g) were exposed to O_2-concentrations of 100, 200 and 350% air saturation for 10 days. Exposure to hyperoxia commenced immediately after fish were transferred to tanks. At the end of the test, 8 fish per treatment were euthanized and blood sampled for cortisol analysis and 8 other fish were slaughtered and blood sampled for analysis of blood acid-base status and ionic balance (blood sampling procedures are described further on).

A 30-day experiment was carried out using duplicate groups of 90 turbot (initial mean weight, 53-56 g) per O_2-treatment. Following the 20-day adaptation period, conditions were changed within 4 h to the nominal constant O_2-concentrations tested: 100, 150 and 230% air saturation. The O_2-concentrations selected represent a reference (normoxia) and O_2-concentrations encountered in land-based marine fish farms that use O_2 supplementation.

Each group of two tanks was fitted with a polyvinylchloride column (130 cm long and 11 cm in diameter) open to the atmosphere at the top. Sea water first flowed over polypropylene spheres in the upper part of the column, and then through the lower part of the column where pure oxygen was injected. Water was then distributed to the two tanks, 15 cm below the water surface. The O_2-concentration in each tank (outlet water concentrations) was checked manually twice a day and adjusted when necessary by changing the O_2 injection rate. Total gas pressure was checked with a tensionometer (300 C Novatech®) at the start and the end of the experiment.

2.3. Studied parameters

All fish were counted at the start and the end of the experiment and they were bulk weighed to estimate total initial biomass in each tank. Fish growth was estimated by individual weighing of 40 fish per tank on day 0, day 15 and day 30 that represents 44% of the group at the start of the experiment (initial number was 90 per tank) and 60% at the end of the experiment (66 fish left prior to the last sampling). Turbot do not flight from netting, as a result there is no sampling problems as in pelagic fish. In this study, we take advantage of this specific behavior of turbot in weighing only a part of the population (from our experience in turbot culture, when using weight-sorted turbot, sampling size can be restricted to 30 fish). Prior to weighing, fish were fasted for 18 h and no anesthesia was used during measurements. The specific growth rate (SGR) was calculated as: $100 (\ln w_f - \ln w_i) day^{-1}$, where w_i and w_f are the initial and final mean wet body weight, respectively.

As some fish were regularly removed by sampling, food conversion ratio (FC) and daily food intake (FI) were calculated taking into account the estimated biomass per sampling interval (day1-7, 8-15 and 16-30) using the following expressions:

. FC: total food intake per unit total fish bw gain;

. FI: 100 × (amount of food intake per day per unit average total bw);

where food intake = total food provided; body weight (bw) gain = (final mean body wet weight - initial mean wet body weight) × fish number and, average total bw = $(day_f\ bw + day_i\ bw)^{-2}$.

Day 0-30 protein efficiency ratio (PER) and protein retention were calculated as follows:

. PER: total fish bw gain per unit food protein intake;

. Protein retention : 100 × (fish protein gain per unit food protein intake);

where food protein intake and fish protein gain are expressed as dry weight.

Fish body composition was determined using freeze-dried samples of 3 × 4 fish at the start (day 0), and 3 × 4 fish per tank at the end (day 30) of the experiment (six samples per O_2-concentration tested). Proximate analyses of food and fish were performed in triplicate for each sample according to AOAC (Association of Official Analytical Chemists, 1984) methods: i.e. dry matter (24 h at 105°C), ash (7 h at 550°C), crude fat (dichloromethane extraction with a Sostec System Ht®), and crude protein (Dumas method with an Elementary NA 2000®, N × 6.25).

The average daily excretion rates of total ammonia nitrogen (TA-N) and urea-N were determined from TA-N and urea-N concentrations in the inlet and outlet water measured by automatic colorimetry as described in Dosdat et al. (1994). Measurements were undertaken over three consecutive days (day 21-23) by sequential sampling over 24 h using a peristaltic pump. Excretion rates were calculated as follows: daily TA-N or urea-N excretion rate (mg kg^{-1} bw day^{-1}) = (outlet concentration - inlet concentration) × Q_w bw^{-1}, where Q_w = flow rate (l day^{-1}) and bw was estimated by inference from the growth curve.

Oxygen consumption (MO_2) of fed fish was measured at the same time as nitrogen excretion, and that of resting fish was measured at the end of the experiment using fish that had been deprived of food for 7 days and held in darkness. MO_2 was estimated from the difference in O_2-concentration between the water inflow (O_2 inlet) and outflow (O_2 outlet) of each tank, using procedures described in Gaumet et al. (1995). Following correction for O_2 variations measured in a tank without fish, MO_2 was calculated as follows:

MO_2 (µmol O_2 g^{-1} bw h^{-1}) = (O_2 inlet - O_2 outlet) × Q_w bw^{-1}, where Q_w is water flow through the tank and bw is fish body weight.

On days 1, 7, 15 and 30, blood samples were obtained by cardiac puncture (to collect venous blood) of fish fasted for 18 h (6 slaughtered fish per tank without replacement). Haematocrit (10000 rpm, 5 min) and blood pH (Metrohm® pHmeter fitted with a Broadley® micro flow-through pH electrode) were determined immediately after blood sampling. On day 1 and day 30, blood samples were also taken for total haemoglobin concentration (Sigma Diagnostics enzymatic Kit 525) and red blood cell counts (Thoma's haemacytometer). On all sampling days, blood was centrifuged (3500 rpm, 5 min), samples for plasma total-CO_2 concentrations were taken within 15 min, and then stored at 4 °C until measurements were made within 6 h, using Sigma Diagnostics enzymatic Kit (132-UV). CO_2 partial pressure (PCO_2) was calculated from blood pH and total CO_2; physiological parameters given by Boutilier et al. (1985) for rainbow trout (*Oncorhynchus mykiss*) at 15 °C, were used for calculations: (αCO_2 = 0.0535 mmol l^{-1} . $Torr^{-1}$, pK_1 = 6.7811-0.0893 pH, and pK_2 = 9.80). All other blood analyses were performed on plasma samples that had been stored at -20°C: osmolarity (Advanced microosmoter®), chloride (Chloridometer Radiometer®), sodium and potassium concentrations (Electrolyte Beckman analyser®). Radio-immunoassay was used to measure plasma tri-iodothyronine, T_3, (Boeuf et al., 1989).

On days 1 and 30, blood samples were taken from an additional 6 fish per tank for plasma cortisol analysis. Fish were euthanized using ethylene-glycol-monophenyl-ether (1.3 ml l^{-1} water) and caudal vessel puncture used to collect blood (heparin-syringe). Blood was centrifuged (3500 rpm, 5 min) and plasma samples taken for cortisol analysis and stored at -20°C. Plasma cortisol concentrations were measured using a specific radio-immunoassay adapted from Fostier et al., 1982.

2.4. Data analysis

All results are expressed as mean ± SE. Statistical analyses were conducted using Statistica for Windows. Differences in weight and blood parameters concentrations versus O_2-concentration and time were tested by a two-way nested ANOVA, and tanks were considered as nested factor. One-way ANOVA was used for FI, FCR, PER, protein retention, daily nitrogenous excretion rate and MO_2. Significant ANOVA were followed by a *post hoc* multiple comparison test (Newman-Keuls test). Differences were considered significant at $P<0.05$. Prior to analysis (ANOVA and post-hoc multicomparison test), data expressed in %, were arcsinus square-root transformed.

3. Results

Throughout the two experiments, O_2-concentrations remained stable, the fish looked healthy, and there were no mortalities. In the 10-day preliminary test, subjective observations gave the impression that the turbot responded to a change from 91% O_2 saturation (control) to extreme hyperoxia (350% air saturation) by increasing swimming activity and developing a paler body color. Food intake increased as O_2-saturation was increased (Table 1). Plasma CO_2 concentration was ca 2.5 times higher in fish held in O_2-supersaturated water, and chloride concentrations decreased significantly as O_2-concentrations increased (Table 1). Blood pH, plasma cortisol concentrations and osmolarity were not significantly affected by exposure to elevated water O_2-concentrations for 10 days.

Table 1. Food intake and blood parameters in turbot exposed to 3 O_2-concentrations for 10 days.
Data are means ± SE: O_2-concentrations, *n* = 14, physiological indicators, *n* = 8. Letters following means indicate intergroup statistical differences between ambient O_2-concentrations (one-way ANOVA and Newman-Keuls test). Means not sharing a common letter are significantly different ($P<0.05$). No letter, no significant differences.

O_2-saturation (% air saturation)	90.7 ± 0.9	217.4 ± 2.8	338.6 ± 4.5
O_2-concentration (mg l^{-1})	6.9	15.2	26.6
Food intake (% bw day^{-1})	0.98	1.29	1.49
Haematocrit (%)	22.7 ± 0.7	20.0 ± 0.9	19.0 ± 0.5
Blood pH	7.64 ± 0.04	7.70 ± 0.05	7.69 ± 0.06
Plasma CO_2 (mmol l^{-1})	5.6 ± 0.63[a]	13.65 ± 0.90[b]	13.92 ± 1.83[b]
PCO_2 (mm Hg)	2.88 ± 0.32[a]	6.07 ± 0.40[b]	6.34 ± 0.83[b]
Osmolarity (m0sm l^{-1})	312 ± 2	308 ± 1	306 ± 2
Chloride (mmol l^{-1})	144.6 ± 0.8[a]	136.2 ± 0.5[b]	129.2 ± 0.8[c]
Sodium (mmol l^{-1})	152.6 ± 0.7	153.6 ± 0.3	153.0 ± 0.4
Potassium (mmol l^{-1})	3.0 ± 0.1	3.0 ± 0.1	3.4 ± 0.2
Cortisol (ng ml^{-1})	3.5 ± 1.6	2.2 ± 0.8	2.9 ± 0.60

In the main, 30-day experiment, there was no significant difference in final weight between turbot reared for 30 days in O_2-supersaturated water (147 and 223% air saturation) and those reared in O_2-saturated water (100%), although there was a trend towards a higher specific growth rate in fish held in O_2-supersaturated water (Table 2). Supplemental oxygen had no significant effect on the average food intake, food conversion or protein utilization parameters (PER, protein retention, body protein concentration). A significantly higher body fat deposition was observed in the turbot held under oxygen supplementation conditions. Daily average nitrogen excretion rates of turbot fed to satiation or urea to total nitrogen excretion ratios (17-19%) were not markedly affected by O_2-concentration, although there were some differences in food intake during the measurement period (15% higher in the 147% air saturation group than in the two other groups). The food intake differences probably explain

the higher MO_2 of the fish in the 147% air saturation group. Following a 7 day-period of food deprivation in darkness, MO_2 did not differ among the groups of turbot.

Table 2. Growth, food utilisation, N-excretion and oxygen consumption rate in turbot exposed to 3 O_2-concentrations for 30 days.
Data are means ± SE: O_2-concentrations, n = 46, weight data and food utilisation parameters, n = 2 (replicates), excretion and MO_2 data, n = 3, body composition, n = 9. Nitrogenous excretion and MO_2 of fed fish were measured day 21-23 and MO_2 in resting fish (deprived of food for 7 days and held in darkness) at the end of the experiment. Letters following means indicate intergroup statistical differences between ambient O_2-concentrations (nested ANOVA and Newman-Keuls test). Means not sharing a common letter are significantly different ($P<0.05$). No letter, no significant differences.

O_2-saturation (% air saturation)	100.1 ± 1.1	147.4 ± 0.8	223.6 ± 1.6
O_2-concentration (mg l^{-1})	7.6	11.4	17.5
Day 0 mean weight (g)	56.5 ± 1.1	53.6 ± 1.2	53.3 ± 1.0
Day 30 mean weight (g)	95.5 ± 2.2	97.9 ± 2.6	97.8 ± 2.6
Specific growth rate	1.75 ± 0.11	2.0 ± 0.05	2.02 ± 0.12
Food intake (% bw day^{-1})	1.20 ± 0.00	1.32 ± 0.05	1.32 ± 0.40
Food conversion	0.70 ± 0.01	0.70 ± 0.01	0.69 ± 0.02
Protein efficiency ratio	3.2 ± 0.2	3.3 ± 0.1	3.3 ± 0.1
Protein retention (%)	44.7 ± 2.9	44.8 ± 1.0	44.7 ± 1.2
Day 30 water body content (% bw)	79.7 ± 0.3	79.5 ± 0.3	79.8 ± 0.1
Day 30 protein body content (% bw)	13.9 ± 0.5	13.6 ± 0.1	13.4 ± 0.2
Day 30 lipid body content (% bw)	2.0 ± 0.1[a]	2.8 ± 0. 1[b]	2.9 ± 0.1[b]
Day 30 ash body content (% bw)	3.9 ± 0.1[a]	3.7 ± 0.1[b]	3.6 ± 0.1[b]
TA-N excreted (mg kg^{-1} bw day^{-1})	317 ± 1	321 ± 2	297 ± 1
Urea-N excreted (mg kg^{-1} bw day^{-1})	75.9 ± 6.1	73.7 ± 5.0	61.2 ± 5.6
Fed fish MO_2 (µmol g^{-1} bw h^{-1})	7.2 ± 0.1[a]	8.7 ± 0.1[b]	7.6 ± 0.1[a]
Resting fish MO_2 (µmol g^{-1} bw h^{-1})	2.9 ± 0.1	2.4 ± 0.1	2.6 ± 0.3

With the exception of plasma CO_2 concentrations and PCO_2, there were no significant changes in the physiological parameters in relation to O_2-saturation (100, 147 and 224 % air saturation) or measured duration of exposure (days 1, 7, 15 and 30), (Table 3 and Table 4). Dose dependent increases in plasma CO_2 concentrations and PCO_2 were observed as water O_2-saturation increased (Table 3). Plasma CO_2s were significantly higher in fish exposed to

O_2-supersaturated waters, but large differences between groups exposed to 147 and 224% air saturation were only observed during the second half of the experiment. Although there seemed to be some decrease in plasma chloride concentration as water O_2-concentrations increased, the values obtained were not significantly different from those of fish exposed to normoxia.

Table 3. Changes with time of blood acid-base and hydromineral balance in turbot exposed to 3 O_2-concentrations for 30 days.
Data are means ± SE: physiological data, n = 12 (2 replicates) per sampling date. Upper case letters following means indicate, per sampling date, intergroup statistical differences between ambient O_2-concentrations (nested ANOVA and Newman-Keuls test). Lower case letters following means indicate statistical differences between exposure durations. Means not sharing a common letter are significantly different ($P<0.05$). No letter, no significant differences.

O_2-saturation (% air saturation)		Day 1	Day 7	Day 15	Day 30
100.1 ± 1.1		7.62 ± 0.03	7.72 ± 0.03	7.75 ± 0.04	7.68 ± 0.03
147.4 ± 0.8	Blood pH	7.64 ± 0.04	7.72 ± 0.03	7.74 ± 0.04	7.69 ± 0.04
223.6 ± 1.6		7.66 ± 0.03	7.72± 0.02	7.75 ± 0.03	7.70 ± 0.02
100.1 ± 1.1		7.4 ± 0.5^{A}	11.5 ± 0.4^{A}	10.0 ± 0.9^{A}	8.7 ± 0.5^{A}
147.4 ± 0.8	Plasma CO_2 (mmol l^{-1})	9.9 ± 0.7^{B}	14.0 ± 0.3^{B}	11.8 ± 0.4^{A}	11.3 ± 0.4^{B}
223.6 ± 1.6		15.4 ± 0.4$^{C, a}$	18.3 ± 0.4$^{C, b}$	19.2 ± 1.0$^{B, b}$	18.9 ± 0.3$^{C, b}$
100.1 ± 1.1		3.8 ± 0.3^{A}	4.5 ± 0.2^{A}	3.7 ± 0.4^{A}	3.8 ± 0.4^{A}
147.4 ± 0.8	PCO_2 (mm Hg)	5.2 ± 0.4^{B}	6.3 ± 0.5^{B}	5.0 ± 0.5^{A}	5.4 ± 0.5^{B}
223.6 ± 1.6		7.9 ± 0.7^{B}	7.9 ± 0.5^{B}	7.8 ± 0.8^{B}	8.4 ± 0.4^{C}
100.1 ± 1.1		307 ± 1	302 ± 1	305 ± 1	307 ± 2
147.4 ± 0.8	Osmolarity (mmol l^{-1}	307 ± 1	305 ± 1	304 ± 1	307 ± 2
223.6 ± 1.6		307 ± 2	303 ± 2	305 ± 2	307 ± 1
100.1 ± 1.1		137 ± 1	136 ± 1	141 ± 1	139 ± 2
147.4 ± 0.8	Chloride (mmol l^{-1})	134 ± 1	135 ± 1	139 ± 1	137 ± 1
223.6 ± 1.6		132 ± 1^{a}	132 ± 1^{a}	136 ± 1^{b}	137 ± 1^{b}
100.1 ± 1.1		157.6 ± 0.9	157.2 ± 0.4	155.6 ± 1.6	158.8 ± 1.3
147.4 ± 0.8	Sodium (mmol l^{-1})	157.1 ± 1.0	158.5 ± 0.8	155.0 ± 1.1	157.5 ± 1.6
223.6 ± 1.6		157.6 ± 1.0	157.4 ± 1.0	154.6 ± 1.6	158.0 ± 1.2
100.1 ± 1.1		3.2 ± 0.1	3.6 ± 0.1	3.3 ± 0.1	3.1 ± 0.2
147.4 ± 0.8	Potassium (mmol l^{-1})	3.2 ± 0.1	3.6 ± 0.1	3.3 ± 0.1	2.9 ± 0.1
223.6 ± 1.6		3.1 ± 0.1	3.6 ± 0.1	3.5 ± 0.1	3.5 ± 0.2

Table 4. Changes with time of some blood parameters in turbot exposed to 3 O_2-concentrations for 30 days. Data are means ± SE: physiological data, *n* = 12 (2 replicates) per sampling date. T3 = tri-iodothyronine. The absence of letters following means indicate no statistical differences (P>0.05) between ambient O_2-concentrations (upper case letters) or exposure duration (lower case letters).

O_2-saturation (% air saturation)		Day 1	Day 7	Day 15	Day 30
100.1 ± 1.1		19.4 ± 0.9	18.1 ± 0.5	18.6 ± 0.9	19.0 ± 1.0
147.4 ± 0.8	Haematocrit (%)	17.5 ± 0.5	16.4 ± 0.7	19.3 ± 0.8	21.4 ± 1.1
223.6 ± 1.6		17.0 ± 0.5	17.3 ± 0.9	17.5 ± 0.9	18.7 ± 0.6
100.1 ± 1.1		3.8 ± 0.2			3.7 ± 0.2
147.4 ± 0.8	Haemoglobin (g dl^{-1})	3.8 ± 0.1			3.6 ± 0.2
223.6 ± 1.6		3.6 ± 0.1			3.6 ± 0.2
100.1 ± 1.1		1.39 ± 0.04			1.46 ± 0.05
147.4 ± 0.8	Erythrocyte (10^6 mm^{-3})	1.32 ± 0.04			1.45 ± 0.05
223.6 ± 1.6		1.28 ± 0.04			1.36 ± 0.05
100.1 ± 1.1		1.2 ± 0.1	1.1 ± 0.9	1.3 ± 0.1	1.3 ± 0.2
147.4 ± 0.8	T3 (ng ml l^{-1})	1.1 ± 0.1	1.2 ± 0.1	1.4 ± 0.1	1.4 ± 0.1
223.6 ± 1.6		1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
100.1 ± 1.1		3.2 ± 1.0			3.0 ± 1.3
147.4 ± 0.8	Cortisol (ng ml l^{-1})	3.3 ± 1.1			4.0 ± 1.5
223.6 ± 1.6		4.5 ± 1.4			5.2 ± 1.3

4. Discussion

Rearing turbot in O_2-supersaturated water for 30 days resulted in no substantial increase in growth when compared to rearing in O_2-saturated water (100% saturation). The subsampling method used in this study to estimate fish growth may have made difficult to detect growth nuances. Nevertheless, the apparent trend towards higher growth rates in O_2-supersaturated water was in agreement with studies on rainbow trout or cutthroat trout, *O. clarki*, (Edsall and Smith, 1990, 1991). When rainbow trout (~ 100 g) were fed to satiation (using demand feeder), mean weight gain was 34% greater when fish were reared in 187% air saturation for 100 days than when they were reared at 95% saturation, but the difference was not statistically significant due to within-treatment variability. In the same species and size range,

in comparison with normoxic conditions, no marked differences in growth performances have been observed when fish were reared under 130% O_2-saturation for 3 months (Caldwell and Hinshaw, 1994) or 147-220% saturation for 2 months (Jewett et al., 1991). When fishes are reared in O_2-supersaturated water, it cannot be stated with confidence that fish eat more food and there is no improvement in food efficiency (Edsall and Smith, 1991). In this study, there was a higher fat deposition in whole fish when held under O_2-supersaturated conditions while food intake increase was not significant. Further experiments of long duration (6-12 months) will be needed to elucidate the effects of O_2-supersaturated water on turbot growth (using tagged fish) and body composition. It will be of high interest to determine if O_2-supersaturated water lead to subcutaneous fat accumulation, with low muscle and viscera lipids levels, as observed when high dietary lipids levels are used (Regost et al., 2001).

There seems to be no major survival risk for juvenile turbot under conditions of O_2-supersaturation up to at least 220 %, and they survived exposure to 350% air saturation for 10 days. This findings are in agreement with those for rainbow trout and dogfish, (*Scyliorhinus canicula*), (Barrett and Taylor, 1984; Hobe et al., 1984). However, rainbow trout and eel (*Anguilla anguilla*) cannot survive hyperbaric oxygenation for more than a few hours (5-15 h at 2 ATA, absolute atmosphere of oxygen), because the combination of hyperoxia and high pressure causes severe gill damage (Barthelemy et al., 1981; Sebert et al., 1984).

Some changes in acid-base balance were observed amongst turbot exposed to O_2 supersaturation. These changes, which were probably induced to compensate for respiratory acidosis, were similar to those described in other fish species following short-term exposure to hyperoxia (Bornancin et al., 1977; Hobe et al., 1984; Wood et al., 1984; Wood, 1991; Wood and LeMoigne, 1991; Gilmour and Perry, 1994). Exposure to hyperoxic conditions

results in an increase in plasma PCO_2 and a decrease in blood pH as a consequence of a reduction in gill ventilation. In turbot, any decrease of blood pH was compensated within one day, probably as a result of HCO_3^- accumulation. This is inferred from the observation of increased plasma total CO_2 content and PCO_2. Plasma CO_2 accumulation stabilized within one or 7 days depending on the water O_2-saturation to which the fish were exposed. Branchial acid-base flux (H^+ excretion and HCO_3^- uptake) is usually coupled to ion exchange (Cl^- excretion and Na^+ uptake). In our study, a significant decrease in plasma Cl^- was only seen in the 10 day-preliminary test, in fish exposed to 220-340% O_2 saturation and there were only minor changes in plasma Na^+ contents irrespective of exposure conditions. The relatively steady hydromineral and acid-base balance may be explained by the fact that turbot can adapt rapidly and effectively to variations in several environmental factors (temperature, salinity, ammonia, hypoxia), (Boeuf et al., 1999; Pichavant et al., 2000). Tolerance of hyperoxic conditions may be species-specific related and may also depend upon life history stage. In coho salmon, for example, exposure of smolts to hyperoxia in fresh water may affect their ability to osmoregulate in sea water and thereby give rise to mortality following transfer to sea water (Brauner, 1999).

The metabolic and other physiological measurements indicate that turbot can adapt to O_2-supersaturated water. There were no influences on nitrogenous excretion, no signs of stress and no disturbances to thyroid hormone regulation. The MO_2 of resting turbot was not influenced by exposure to O_2-supersaturated water, which is in agreement with observations made on other fish species (Dejours et al., 1977; Wilkes et al., 1981; Barrett and Taylor, 1984; Berschick et al., 1987). Exposure to hyperoxic conditions did not elicit moderate anaemia in turbot, although this has been observed in rainbow trout (Jewett et al., 1991; Caldwell and Hishaw, 1994). In the 10 day-experiment, there was a trend towards a lower

haematocrit in fish subjected to hyperoxic conditions. Further studies should focus on the O_2-carrying properties of blood at different O_2-concentrations to enable interspecific comparisons of respiratory adjustments to hyperoxia.

To conclude, turbot can adapt readily to moderate hyperoxic conditions. The lack of significant improvement in growth performances of turbot reared in O_2-supersaturated waters suggests that the main interest of O_2 supplementation is to maintain water O_2-concentration near air saturation level.

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