Effects of hypoxia on growth and metabolism of juvenile turbot

K. Pichavant [a,*], J. Person-Le-Ruyet [a], N. Le Bayon [a],
A. Sévère [a], A. Le Roux [a], L Quéméner [a], V. Maxime [b],
G. Nonnotte [b], G Boeuf [a]

[a] *Laboratoire de Physiologie des Poissons, IFREMER, Centre de Brest, BP 70, 29280 Plouzané, France*
[b] *Laboratoire de Physiologie des Systèmes Intégrés, Université de Bretagne Occidentale, 6 avenue le Gorgeu, BP 809, 29285 Brest cédex, France*

Abstract

The effects of hypoxia on growth, feed efficiency, nitrogen excretion, oxygen consumption and metabolism of juvenile turbot (120 g) were studied in a 45-day experiment carried out in sea water at $17.0 \pm 0.5°C$ and 34.5 ppt salinity. Fish were fed to satiation at O_2-concentrations of 3.5 ± 0.3, 5.0 ± 0.3 mg l^{-1} (hypoxia) and 7.2 ± 0.3 mg l^{-1} (normoxia). Both feed intake (FI) and growth were significantly lower under hypoxia than under normoxia, with no significant differences being observed between 3.5 and 5.0 mg O_2 l^{-1}. During the first 2 weeks of the experiment, FI was halved under hypoxic conditions, and there were large differences among treatments in feed conversion ratio (FCR), i.e., it was 3.2, 1.5, and 0.9 in turbot exposed to 3.5, 5.0, and 7.2 mg O_2 l^{-1}, respectively. Thereafter, FCR was not significantly affected by O_2-concentration. Nitrogen excretion and oxygen consumption of feeding fish were significantly higher under normoxia than under hypoxia, but following 7 days of feed deprivation oxygen consumption was similar under normoxia and hypoxia. Plasma osmolarity, ionic balance, and acid-base status were not affected by the two hypoxic conditions tested. Overall, our results indicate that turbot have some capacity to adapt to relatively low ambient O_2-concentrations. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Turbot; Hypoxia; Growth; Feed utilization; Oxygen consumption; Nitrogen excretion; Ion-regulation; Acid-base status

* Corresponding author. Tel.: +33-0298-2248-57; fax: +33-0298-2243-66.
E-mail address: kpichava@ifremer.fr (K. Pichavant).

1. Introduction

Low O_2-concentrations are known to modify growth rate, feed efficiency, and metabolism of fish (Brett, 1979; Jobling, 1994), but the long-term effects of reduced environmental O_2-concentrations have been elucidated for only a few fish species (Brett and Blackburn, 1981; Cech et al., 1984; Pedersen, 1987; Van Dam and Pauly, 1995).

Some data relating to the environmental requirements of juvenile turbot (*Scophthalmus maximus*) are available. The species is relatively euryhaline, and growth and osmolarity disturbances are only observed below 6 ppt (Waller, 1992; Gaumet et al., 1995). The optimal temperature for growth of juveniles is 16–20°C (Burel et al., 1996), and this is not markedly affected by photoperiod in the period prior to sexual maturity (Imsland et al., 1995, 1997; Pichavant et al., 1998). Turbot are relatively tolerant to ammonia: no growth reduction is observed below 0.1 mg l^{-1} UIA-N (unionised form of ammonia), but above 0.8 mg l^{-1} UIA-N growth ceases (Person-Le-Ruyet et al., 1997). Little is known about the effects of hypoxia, with data mainly relating to acute survival (Person-Le-Ruyet, 1993) or rates of oxygen consumption measured under different environmental conditions (Brown et al., 1984; Waller, 1992; Gaumet et al., 1995; Burel et al., 1996).

The present study was undertaken to examine the long-term effects of O_2-concentrations on feeding, growth, feed utilization and nitrogen excretion in juvenile turbot. The study was carried out over 45 days in an attempt to highlight the strategies used by the fish to adapt to hypoxia: oxygen consumption was measured at different O_2-concentrations, and blood was sampled for monitoring of the physiological status of the fish.

2. Materials and methods

2.1. Fish and rearing conditions

The experiment was carried out on 480 6-month-old hatchery-reared juvenile turbot. Fish had been reared at IFREMER, Brest (48°N) according to methods described by Person-Le-Ruyet et al. (1991).

The experiment was performed in 1 m^2 Swedish-type tanks, with an effective water volume of 450 l. Tanks were supplied with a continuous flow of water at 17.0 ± 0.5°C and 34–34.5 ppt salinity. The photoperiod was maintained at 16L:8D, and light intensity at the water surface was 2 W m^{-2}. Ambient pH and ammonia concentration were monitored at 4-day intervals.

The fish, divided into groups of 80, were randomly distributed among six tanks and were then allowed to adapt to the rearing and feeding conditions for 4 weeks under normoxia (O_2-concentration = 7.2 mg l^{-1}). They were fed by hand to apparent satiation (feed intake, FI was assumed to equal to feed provision) twice a day with a commercial dry pellet (Le Gouessant, 4.5 mm diameter; total protein and crude fat 59.1% and 19.8% of dry matter, respectively).

2.2. Experimental design

After the adaptation period, the fish were exposed to one of the following O_2-concentrations for 45 days, i.e. either 7.2 mg l^{-1} (95% of O_2-saturation, control tanks, normoxia), or 5.0 mg l^{-1} (65% of O_2-saturation), or 3.5 mg l^{-1} (45% of O_2-saturation). The experiment was conducted on replicated groups of fish (initial weight: 120 ± 5 g, mean $\pm$ SE) for each O_2-concentration. Water flow rates were 10 and 15 1 min^{-1} under hypoxic and normoxic conditions, respectively.

At day 0, the start of the experiment, O_2-concentrations in the inflowing water were decreased, and the desired levels were achieved within 4 h. These levels were then maintained until the end of the experiment. Decreases in O_2-concentration were obtained using an oxygen depletion system (Fig. 1) adapted from Bennett and Beitinger (1995). Sea water first flowed through an open aeration column packed with polypropylene spheres, and then through a column where nitrogen was injected. Oxygen removal was controlled by the nitrogen flow, and total gas pressure was measured with a tensionometer (300C Novatech®). Surface gas exchange in the rearing tanks was limited by setting the water inflow under the water surface. The O_2-concentration in each tank was checked manually twice a day, and adjusted when necessary. It was also monitored semi-continuously (two 24 h-cycles per week, minimum) as described by Gaumet et al. (1995).

2.3. Studied parameters

All fish in each tank were individually weighed to the nearest 0.1 g at 15 days intervals. Prior to weighing fish had been fasted for 18 h, without anesthesia. Feed utilization parameters were calculated as follows:

- apparent feed conversion ratio (FCR): feed provided per unit biomass gain;
- daily feed intake FI: $100 \times$ (feed provided per day per unit biomass);
- protein efficiency ratio (PER): biomass gain per unit feed protein provided; and
- protein utilization coefficient (PUC): $100 \times$ (protein gain per unit feed protein provided).

Fish body composition was determined using freeze-dried samples of 3×10 fish from the initial population and 3×8 fish per tank (six samples per O_2-concentrations tested) at the end of the experiment. Chemical analyses of food and fish were performed in triplicate for each sample according to AOAC (1984) methods, i.e. dry matter (24 h at 105°C), ash (7 h at 550°C), crude lipid (dichloromethane extraction with an automatic Soxtec system Ht®, Perstorp Analytical) and crude protein determined from nitrogen concentration ($N \times 6.25$) by Dumas method with an Elementary NA 2000®, Thermo Separation Products.

Total ammonia nitrogen (TAN) and urea-N concentrations of the inlet and outlet water were measured according to the procedures described by Dosdat et al. (1994). Measurements were undertaken over 3 consecutive days (32–35), using sequential sampling for 24 h by a peristaltic pump. These data were used to estimate average daily excretion rates, expressed in mg TAN or urea-N kg body weight $(bw)^{-1}$ day^{-1}, and body weight was estimated by the extrapolation of growth curve between days 30 and
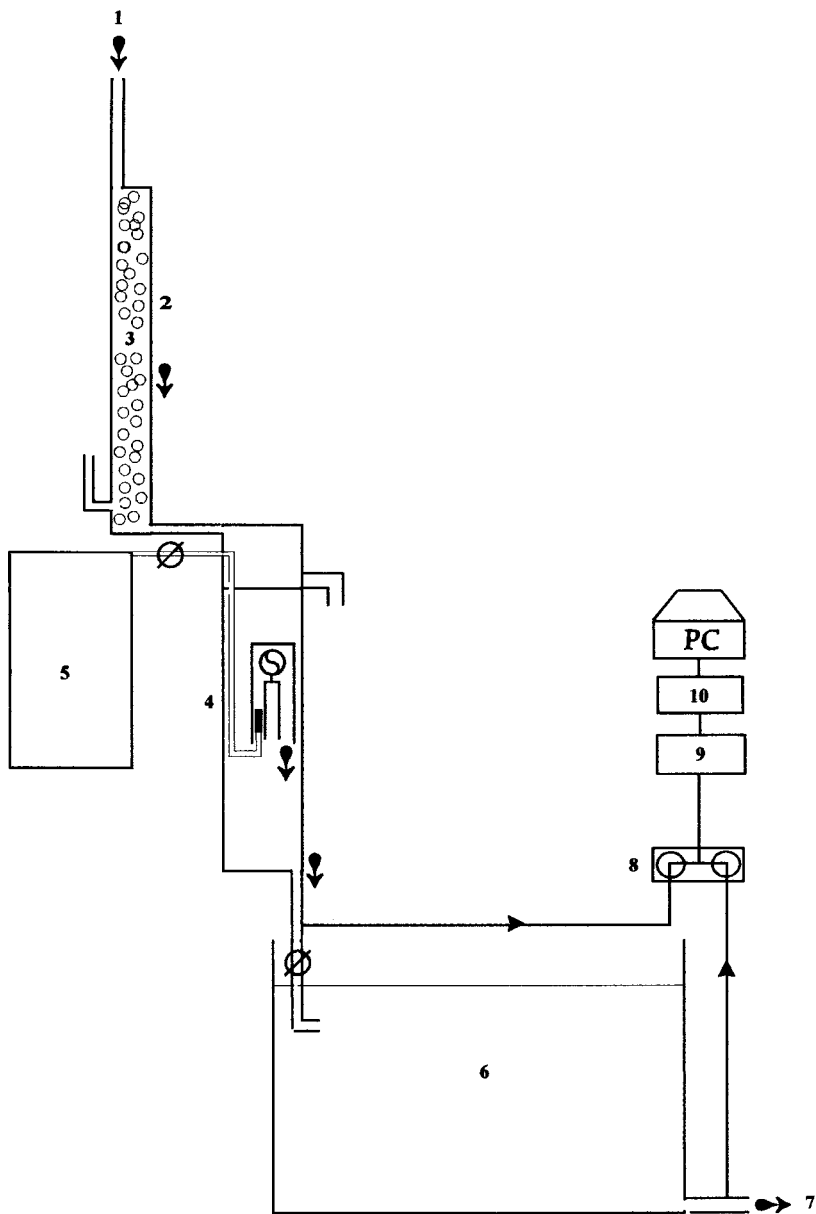
Fig. 1. Experimental set-up showing rearing tanks, oxygen depletion system, and environmental monitoring. (1) water inflow, (2) aeration column, (3) polypropylene spheres, (4) nitrogen injection system, (5) nitrogen tank, (6) rearing tank, (7) water outflow, (8) solenoid valves, (9) O_2 measuring cell, (10) oxymeter, PC: computer, ➡ : direction of sea water flow, ⊘ : pump, ∅: solenoid valves, ➤ : inlet and outlet sea water sample.
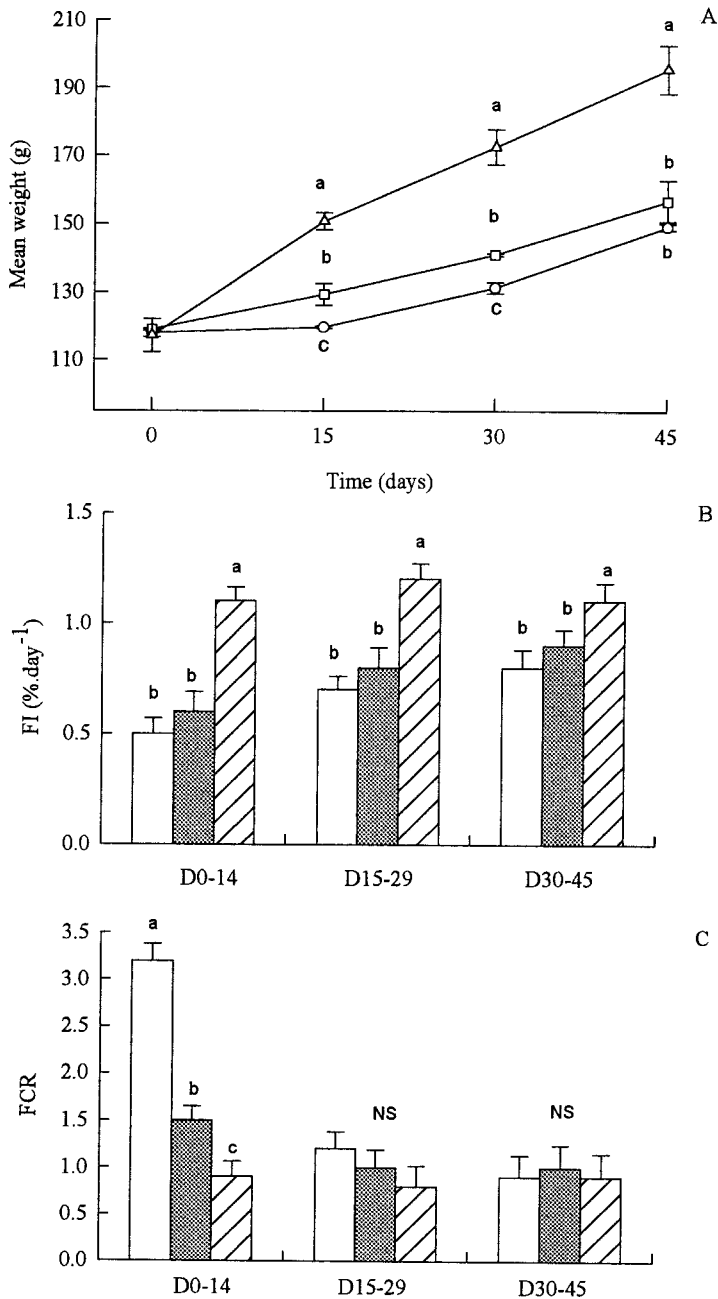
Fig. 2. Change of mean weight over time in turbot (A) in relation to O_2-concentration: 3.5 ($\bigcirc$), 5.0 ($\square$) and 7.2 ($\triangle$) mg O_2 1^{-1}. Means are given with standard errors ($n = 2$ replicates). Change of feed provisioning over time (FI, B) and feed conversion ratio (FCR, 2C) in relation to O_2-concentration: 3.5 ($\square$), 5.0 ($\blacksquare$) and 7.2 (open rectangle with two diagonal lines). Letters indicate inter-group statistical differences with means not sharing a common letter being significantly different ($P < 0.05$). NS = no significant difference ($P > 0.05$).

45. Concomitantly, the daily excretion patterns were examined by manual water sampling each hour, and hourly excretion rates were expressed in mg TAN or urea-N kg bw^{-1} h^{-1}.

Oxygen consumption (MO_2) was determined for both fed fish and fish deprived of food for 7 days (monitored in darkness) according to the method described by Gaumet et al. (1995). MO_2 was monitored each 15 min using the difference in O_2-concentration between the water inflow (O_2 inlet) and outflow (O_2 outlet) of each tank. After correction for O_2 variations measured in a similar tank without fish, MO_2 was calculated as follows: $MO_2 = (O_2$ inlet $- O_2$ outlet) Qw bw^{-1} where Qw is the water flow through the tank, and bw is the fish body weight. MO_2 was expressed in μmol O_2 g bw^{-1} h^{-1}.

At intervals (day 0, 2, 7, 15, 30, 45), blood samples were obtained by puncture of caudal vessels from fish fasted for 18 h (three fish per tank on day 0 and six fish per tank from day 2 to day 45). Haematocrit and blood pH (Metrohm® pHmeter fitted with a microflow pH sensor) were determined immediately after blood sampling. Blood plasma total CO_2-concentrations were measured using a Sigma Diagnostics enzymatic kit (132-UV) within 15 min. All other blood analyses were performed on plasma samples that had been stored frozen: osmolarity, chloride, sodium and potassium concentrations as described by Gaumet et al. (1995) and TAN, urea-N, and lactate concentrations using Sigma Diagnostics enzymatic kits (171-UV, Urea Nitrogen-535, and 735-UV). Plasma cortisol concentrations were measured by a specific radioimmunoassay adapted from Lamers et al. (1992).

2.4. Data analysis

Statistical analyses were conducted using STATISTICA for Windows. All results are expressed as mean $\pm$ SE. Differences in weight, blood parameters concentrations, daily nitrogen excretion and O_2-consumption were tested by a nested-ANOVA, and tanks were considered as nested factor. One-way ANOVA was used for FI, FCR, PER and PUC. 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 multiple comparison test), data expressed in %, were arcsinus square-root transformed.

Table 1
Day 0 and day 45 body composition (% of bw) of turbot in relation to O_2-concentration. Means are given with SEs ($n = 2$ replicates). Letters indicate inter-group statistical differences with means not sharing a common letter being significantly different ($P < 0.05$). NS = no significant difference ($P > 0.05$)

	$[O_2]$ (mg l^{-1})	Water (%)	Protein (%)	Fat (%)	Ash (%)
Day 0	7.2 ± 0.3	78.2 ± 0.3	15.1 ± 0.5	2.0 ± 0.2	4.0 ± 0.3
Day 45	3.5 ± 0.3	77.5 ± 1.5	16.3 ± 0.9[a]	2.2 ± 0.1	4.1 ± 0.3
	5.0 ± 0.3	78.2 ± 1.5	15.1 ± 0.6[b]	2.4 ± 0.7	3.8 ± 0.1
	7.2 ± 0.3	77.7 ± 0.3	14.9 ± 0.4[b]	2.6 ± 0.2	3.5 ± 0.2
		NS		NS	NS

3. Results

3.1. Effects of hypoxia on growth performance, FI, and feed efficiency

O_2-concentrations were maintained close to predetermined levels throughout the 45-day experiment. Fish appeared healthy throughout all treatments, and there was no mortality in any group.
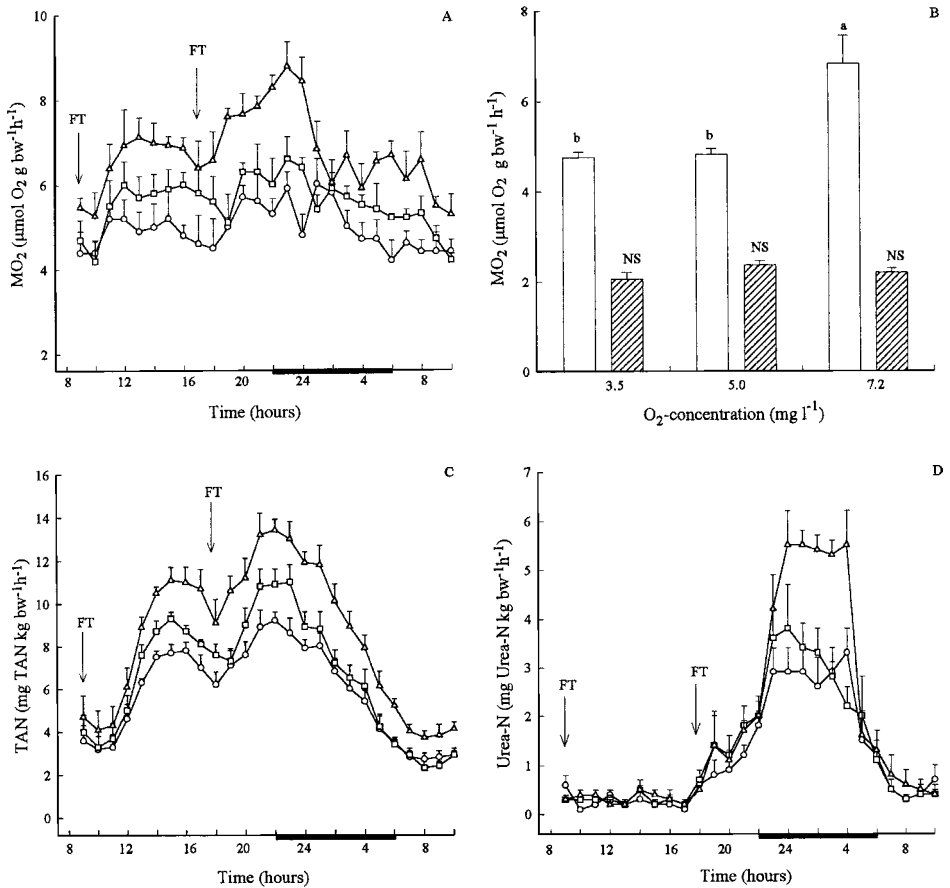

Fig. 3. Daily patterns of oxygen consumption (MO_2) of turbot (A) held at different O_2-concentrations: 3.5 (○), 5.0 (□) and 7.2 (Δ) mg O_2 l^{-1}. Data are means with standard errors ($n = 2$ replicates). Average MO_2 of fed (□) turbot and of resting (▨) turbot (deprived of food for 7 days and in darkness) in relation to O_2-concentration (B). Means are given with standard errors ($n = 2$ replicates). Letters indicate intergroup statistical differences with means not sharing a common letter being significantly different ($P < 0.05$). NS = no significant difference ($P > 0.05$). Daily patterns of total ammonia nitrogen, TAN (C) and urea-N excretion (3D) in turbot in relation to O_2-concentration: 3.5 (○), 5.0 (□) and 7.2 (Δ) mg O_2 l^{-1}. Data are means with standard errors ($n = 2$ replicates). The horizontal bar on x-axis indicates the dark phase of photoperiod, and → is feeding time.

Exposure to hypoxia markedly depressed the growth of the turbot (Fig. 2A). From day 0 to day 15, the fish exposed to 3.5 mg O_2 l^{-1} gained very little weight, and at 5.0 mg O_2 l^{-1} weight gain was much reduced than in the normoxic control. Thereafter, hypoxic groups displayed some improvement in growth, but the final mean weight was 25% lower under the two hypoxic conditions than under normoxia (without any significant differences between the two hypoxic conditions after day 30).

There were also differences among groups in FI and efficiency (Fig. 2B,C). FI was positively correlated with ambient O_2-concentration. During the first 2 weeks of the experiment, FI in fish exposed to 3.5 and 5.0 mg l^{-1} was only half that of fish under normoxia, and apparent FCRs were high. Thereafter, differences in FI between the three groups were somewhat reduced, and FCR was similar among all groups. No significant differences were observed in final body composition of fish except for relative protein content under 3.5 mg O_2 l^{-1} (Table 1). PERs were similar (23–24%) at 5.0 and 3.5 mg O_2 l^{-1}, and significantly higher at 7.2 mg O_2 l^{-1} (27%). In the same way, PUC was related to O_2-concentration: it was 1.2–1.3 at 5.0 and 3.5 mg O_2 l^{-1} and 1.9 at 7.2 mg O_2 l^{-1}.

3.2. Oxygen consumption

Oxygen consumption (MO_2) in fed fish started to increase 2 h after each meal, and reached a maximum 4 to 5 h later (Fig. 3A). In these fish, MO_2 was lower under hypoxia than under normoxia, as seen by the examination of the average oxygen consumption rate of fed turbot (Fig. 3B). Following a 7-day period of feed deprivation in darkness, the MO_2 was two- to three-fold lower than that of fed fish, and no significant differences were noticed between groups (Fig. 3B).

3.3. Nitrogen excretion

The average daily TAN excretion rate was significantly affected by O_2-concentration, i.e. 151.3 ± 6.5, 170.6 ± 4.6, and 219.6 ± 9.1 mg TAN kg bw^{-1} day^{-1} at 3.5, 5.0, and

Table 2
Blood parameters at day 0 and 45 of turbot in relation to O_2-concentration. Means are given with SEs ($n = 2$ replicates). NS = no significant difference ($P > 0.05$)

	Day 0	Day 45	Day 45	Day 45	
$[O_2]$ (mg l^{-1})	7.2 ± 0.3	3.5 ± 0.3	5.0 ± 0.3	7.2 ± 0.3	
Osmolarity (mosM l^{-1})	308 ± 3	310 ± 2	309 ± 3	310 ± 2	NS
Cl^- (mmol l^{-1})	144 ± 2	146 ± 2	146 ± 2	145 ± 1	NS
Na^+ (mmol l^{-1})	155.3 ± 2.4	154.1 ± 1.8	156.6 ± 2.1	156.5 ± 2.0	NS
K^+ (mmol l^{-1})	3.6 ± 0.2	3.5 ± 0.1	3.4 ± 0.2	3.4 ± 0.1	NS
TAN (mg l^{-1})	3.0 ± 0.3	2.7 ± 0.4	2.9 ± 0.3	3.2 ± 0.2	NS
Urea-N (mg dl^{-1})	13.7 ± 0.8	12.4 ± 0.5	13.1 ± 0.4	14.1 ± 0.6	NS
Haematocrit (%)	20 ± 2	19 ± 2	18 ± 3	21 ± 3	NS
Cortisol (ng ml^{-1})	2.9 ± 0.5	3.0 ± 0.8	3.0 ± 0.7	3.1 ± 0.7	NS

Table 3
Change of blood acid-base status over time of turbot in relation to O_2-concentration. Means are given with SEs ($n = 2$ replicates). NS = no significant difference ($P > 0.05$)

$[O_2]$ (mg l^{-1})		Day 0	Day 2	Day 7	Day 15	Day 30	Day 45	
3.5 ± 0.3			7.74 ± 0.31	7.63 ± 0.21	7.80 ± 0.24	7.77 ± 0.23	7.82 ± 0.28	NS
5.0 ± 0.3	pH	7.68 ± 0.22	7.65 ± 0.17	7.72 ± 0.28	7.75 ± 0.19	7.61 ± 0.26	7.79 ± 0.24	NS
7.2 ± 0.3			7.75 ± 0.26	7.63 ± 0.16	7.72 ± 0.14	7.72 ± 0.19	7.80 ± 0.21	NS
3.5 ± 0.3			7.7 ± 0.4	7.8 ± 0.4	8.1 ± 0.3	8.2 ± 0.2	8.0 ± 0.1	NS
5.0 ± 0.3	Total CO_2 (mmol l^{-1})	8.4 ± 0.2	8.5 ± 0.3	8.1 ± 0.1	8.2 ± 0.2	8.5 ± 0.3	8.1 ± 0.5	NS
7.2 ± 0.3			8.5 ± 0.3	8.4 ± 0.4	8.2 ± 0.3	8.1 ± 0.2	8.6 ± 0.3	NS
3.5 ± 0.3			0.04 ± 0.02	0.02 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.07 ± 0.04	NS
5.0 ± 0.3	Lactate (mmol l^{-1})	0.04 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	NS
7.2 ± 0.3			0.03 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	NS

7.2 mg O_2 l^{-1}, respectively. The average daily urea-N excretion rate was also dependent on O_2-concentration and was equal to 29.4 ± 4.1, 33.6 ± 5.2, and 43.2 ± 2.8 mg Urea-N kg bw^{-1} day^{-1} at 3.5, 5.0, and 7.2 mg O_2 l^{-1}, respectively. Some differences in the magnitude of daily patterns of TAN and urea-N excretion were observed between groups although general trends tended to be similar. TAN excretion increased 3 h after each meal (Fig. 3C), and a delayed single urea-N excretion peak occurred during the dark phase (Fig. 3D).

3.4. Fish physiological status

No significant differences among any of the physiological indicators tested were noticed (Tables 2 and 3).

4. Discussion

During the 45-day experiment, in juveniles turbot, there was a marked reduction of FI, decrease in growth, and a temporary reduction of feed conversion efficiency with a decrease in environmental O_2-concentrations. Similar responses had been previously observed in some other fish species (Brett, 1979; Brett and Blackburn, 1981; Pedersen, 1987; Pouliot and De La Noüe, 1989; Van Dam and Pauly, 1995). Fish exposed to reduced O_2-concentrations respond both with energy-saving strategies and with responses to maintain the supply of oxygen to the tissues. In hypoxia, the reduction of FI may be interpreted as a way to reduce energy demand and thereby, decrease O_2 requirements (Van Dam and Pauly, 1995). Below an O_2-concentration threshold, FI may be suppressed due to the fact that reduced oxygen availability would be unable to sustain the high energy demands of a well-fed fish. A preliminary test showed us that turbot ceased feeding below 2–2.5 mg O_2 l^{-1}. The reduction of FI at 3.5 and 5.0 mg O_2 l^{-1}

had consequences for growth (25% decrease in 45 days, this study). Comparisons with other studies are difficult due to differences in hypoxia duration, fish size, nature of diet or temperature applied. Nevertheless, it is generally agreed that oxygen acts as a limiting factor for growth, and in some species (salmonids, *Oncorhynchus kisutch*, socheye, *O. nerka*, largemouth bass, *Micropterus salmoides*, carp, *Cyprinus carpio* and European sea bass, *Dicentrarchus labrax*) growth has been reported to become dependent on O_2-concentration below 4 to 5 mg l^{-1} (Brett, 1979; Brett and Blackburn, 1981; Thetmeyer et al., 1999) whereas in rainbow trout, *O. mykiss*, the threshold O_2-concentration for growth seems higher, 7 mg l^{-1} (Pedersen, 1987).

In the study reported here, the daily O_2-consumption of the fed turbot was strongly dependent on O_2-concentration. It is possible that, to save energy, turbot submitted to hypoxia were less active than those under normoxia, and this may have contributed to the decreased rate of O_2-consumption. However, Jobling and Davies (1980) showed that the increase in rate of O_2-consumption after feeding was strongly correlated with meal size and duration, the post-prandial increase in MO_2 of fed fish resulting from the energy requirements for digestion and absorption, biosynthesis and storage of nutrients (Jobling, 1994). Thus, the FI reduction among turbot exposed to hypoxia may explain much of the decrease in post-prandial O_2-consumption rate observed in our experiment: the FI of fish exposed to hypoxia was twice less than the one of fish submitted to normoxia.

Within the range of O_2-concentrations tested, hypoxia did not affect MO_2 of unfed turbot. Most teleosts are oxygen regulators, and are able to regulate rates of O_2-consumption independently of environmental oxygen over a wide range (Fritsche and Nilsson, 1993). Indeed, O_2-consumption rates cannot be maintained independent of environmental O_2-levels indefinitely, and at some critical O_2-concentration oxygen uptake will be limited by, and become dependent upon down to the ambient level. In unfed turbot, MO_2 can be maintained down to 1 mg O_2 l^{-1}, the concentration that is close to the lethal O_2-concentration for this species (Person-Le-Ruyet, 1993; Maxime et al., in press).

TAN excretion was also dependent on O_2-concentration, and closely follows the FI pattern under hypoxia; TAN excretion is strongly correlated with meal size and duration (Kaushik, 1980; Dosdat et al., 1995). In turbot, urea excretion occurred during a single pulse of 4–5 h duration, this pulsatile excretion being related to the change from light to darkness (Pichavant et al., 1998).

The physiological responses of fish to hypoxia are dependent on species, intensity, and exposure duration (Jensen et al., 1993). The results reported here showed that, in turbot, an exposure to moderate hypoxia (45% of O_2-saturation) had no long-term effect on hydromineral balance or acid-base status; moreover, no sign of stress was noticed. These data were in agreement with those reported on another flatfish, the sole, *Solea solea* in which no major change in the physiological status was observed after a 12-h exposure to a hypoxic challenge of 40% O_2-saturation (Dalla Via et al., 1994; van den Thillart et al., 1994). This suggests that flatfish can adapt rapidly and effectively to O_2-concentrations as low as 3.2–3.5 mg l^{-1}. This adaptation is attributable to several changes that may occur during long-term exposure to non-lethal hypoxia: increases in the oxygen-transporting capacity (increase of O_2-haemoglobin affinity, blood volume or

haemoglobin concentration) or increase in the tissue glycogen reserves (Jobling, 1994). Further investigations are required to elucidate the mechanisms involved in this adaptation in turbot.

Acknowledgements

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