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Acid–base balance in sea bass (*Dicentrarchus labrax* L.) in relation to water oxygen concentration

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Abstract

The influence of water oxygen concentration on the acid–base balance of sea bass was evaluated. Fish weighing 200–250 g were cultured under different dissolved oxygen concentrations of 64%, 97%, 150% and 250% saturation (92.7, 140.5, 217.5 and 362.7 mmHg respectively) under mild hypoxia, normoxia, mild hyperoxia and high hyperoxia conditions. The results showed that high hyperoxia and mild hypoxia conditions modified some blood parameters significantly when compared with fish held under the normoxia condition, while no differences were shown with respect to the acid–base balance of fish cultured under normoxia and mild hyperoxia conditions. This testifies that the mild hyperoxia condition does not produce physiological disturbances in the acid–base status of sea bass and it could be considered a favourable condition in sea bass land-based farming, mainly in comparison with the mild hypoxia condition, responsible for other physiological problems.

Keywords: acid–base balance, sea bass, water oxygenation

Introduction

In fish, unlike terrestrial animals, the breathing rate is primarily based on oxygen availability (Gilmour 1998). This typical response is due to the unsettled oxygen partial pressure in water. With respect to acid–base equilibrium, it is obtained by fish exchanging, with the surrounding environment HCO_3^-/Cl^- and H^+/Na^+ in equal amounts in order to maintain electroneutrality (Heisler 1986). With respect to the acid–base balance of fish cultured under different dissolved oxygen (DO) concentrations, more information is available for fish held under deep short-term hypoxia (Thomas & Hughes 1982a, b; Thomas, Fievet & Motais 1986; Maxime, Pichavant, Boeuf & Nonnotte 2000) and severe short-term hyperoxia (Wood & Jackson 1980; Goss & Wood 1990; Takeda 1990) conditions. According to Thomas & Hughes (1982a), a deep hypoxia condition (40 mmHg) causes respiratory and metabolic acidosis in rainbow trout (*Oncorhynchus mykiss* Walbaum), with an increase in lactate levels, while a moderate hypoxia condition (60 mmHg) only provokes an increase in the ventilatory frequency, with respiratory alkalosis during the following 24 h. Severe environmental hyperoxia induces respiratory acidosis due to hypoventilation and hypoperfusion of the respiratory apparatus (Goss & Wood 1990), causing acid–base balance disturbances similar to those observed for short- and long-term environmental hypercapnia (Claiborne & Heisler 1986; Perry, Malone & Ewing 1987; Fivestal, Olsen, Kloften, Ski & Stefansson 1999; Cecchini, Saroglia, Caricato, Terova & Sileo 2001).

Nevertheless, major information being available on the effects of severe hypoxia and/or elevated hyperoxia, little information may be found regarding the effects of long-term exposure of fish to DO concentrations simulating conditions in which most aquaculture farms are operative.

In Mediterranean land-based farming, DO availability represents one of the more important limiting factors, mainly during the summer season, when the high temperature reduces the oxygen solubility. In land-based rearing conditions where paddling aeration is applied, DO concentrations are usually 60–80% of the saturation value, whereas on farms where pure oxygen is applied, DO concentrations are usually maintained in a mild hypersaturation condition. The mild hyperoxia condition leads to morphological adaptations of the respiratory apparatus in sea bass (*Dicentrarchus labrax* L.) (Cecchini, Saroglia, Terova, Caricato & De Stradis 1999; Saroglia, Cecchini, Terova, Caputo & De Stradis 2000), improves growth and feed conversion (Saroglia, Terova-Saroglia, Knight & Cecchini 1995), and increases serum immunoglobulin concentration (Scapigliati Scalia, Marras, Meloni & Mazzini 1999) and specific antibody response (Cecchini & Saroglia 2002). Moreover, it is known that low oxygen availability produces a general stress status in fish (Kakuta 1998; Perry & Gilmour 1999), increasing the susceptibility to infective agents in the case of both natural and experimental infections (Mqolomba & Plumb 1992; Candan, Kucker & Karatas 1996; Bunch & Bejerano 1997).

The aim of this research was to study the effect of different DO concentrations, ranging between 'mild' hypoxia and 'mild' hyperoxia (92.7 and 217.5 mmHg), on the acid–base balance of sea bass, in order to explain the large performance differences among fish farms where pure oxygen is applied instead of simple paddling aeration. Moreover, a high hyperoxia condition (250% of the saturation value, 363.7 mmHg) was included in order to show possible observed differences in acid–base status.

Materials and methods

Fish, experimental conditions and blood analysis

Four groups of 20 fish each, 12 months old and ranging from 200 to 250 g in body weight, were randomly placed in four 500-L square tanks connected to a common water recirculation system. Initially, the temperature was 23 ± 0.2 °C, salinity $14\,g\,L^{-1}$, $[CO_2]$ $4\,mg\,L^{-1}$, and DO was maintained around 100% saturation (144.9 mmHg) with a photoperiod of 10L : 14D. Fish were fed to apparent satiation twice a day using commercial extruded feed (Nutreco®, Skretting, Mozzecane, Verona, Italy).

Water inflows were set in order to avoid total ammonia nitrogen concentrations above $0.1\,mg\,L^{-1}$ and unionized ammonia concentrations above $0.01\,mg\,L^{-1}$. After 1 week of acclimatization, the DO concentration was progressively changed in each tank and stabilized at four concentrations: mild hypoxia (64% saturation), normoxia (97%), mild hyperoxia (150%) and high hyperoxia (250%), 92.7, 140.5, 217.5 and 362.7 mmHg, respectively. In order to maintain the different DO concentrations, pure oxygen was injected directly into the tanks of the two hyperoxia groups using a diffuser tube, continuous aeration was provided in the tank of the normoxia group, while neither oxygenation nor aeration was provided in the tank of the hypoxia group. DO concentrations were constantly monitored by four dataloggers (mod. Bobcat 2010 meter/datalogger, Seametrix, Aberdeen, Scotland), collecting data every 15 min.

After 5 weeks of acclimatization under the experimental conditions, five fish from each experimental group were anaesthetized (MS-222, $200\,mg\,L^{-1}$) and submitted to blood sampling from the branchial artery, using heparinized glass microtubes (microsampler, AVL®, Scientific Corporation, Roswell, GA, USA). Blood samples (about 150 µL) were analysed immediately for pH, PCO_2, PO_2 and some plasma ions (HCO_3^-, Na^+), using a blood gas analyser (AVL®, mod. OPTI 2), as described by Cecchini *et al.* (2001).

Statistical analysis

Statistical evaluation was performed using a commercial program (Systat, Evanston, IL, USA). Experimental data were processed by one-way analysis of variance (ANOVA) in order to evaluate if the blood parameters were affected by the different experimental conditions. Differences between means were determined by Tukey's pairwise comparisons of means, and significance was accepted at $P \leq 0.05$.

Results

Exposure to different DO concentrations affected blood pH ($P \leq 0.01$) (Fig. 1), PCO_2 ($P \leq 0.01$) (Fig. 2), plasma bicarbonate concentration ($P \leq 0.001$) (Fig. 4), but not PO_2 ($P = 0.113$) (Fig. 3) and natriaemia (plasma sodium concentration) ($P = 0.352$) (Fig. 5).

Even if natriaemia was never statistically different, it was lower in fish held under a high hyperoxia condition ($150.9 \pm 4.8\,mmol\,L^{-1}$) than in fish held under the other conditions, where it ranged between 157.7 and $158.0\,mmol\,L^{-1}$.

The high hyperoxia condition (250% saturation, 362.7 mmHg) caused a significant increase in blood PCO_2 (Fig. 2) and plasma bicarbonate concentration (Fig. 4). The blood pH (Fig. 1) of fish held under a high hyperoxia condition was higher than that pH of fish cultured under the other DO condition, even if it was

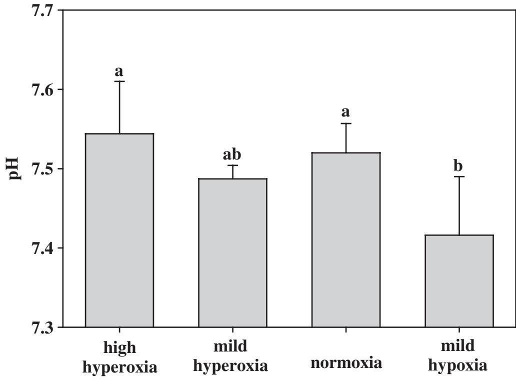

Figure 1 Blood pH in sea bass (*Dicentrarchus labrax* L.) cultured under different dissolved oxygen conditions. Data are expressed as mean and standard deviation. Different letters mean significant differences at $P \leq 0.05$ (small letter) or $P \leq 0.01$ (capital letter). No significant differences appear between groups marked with the same letter.

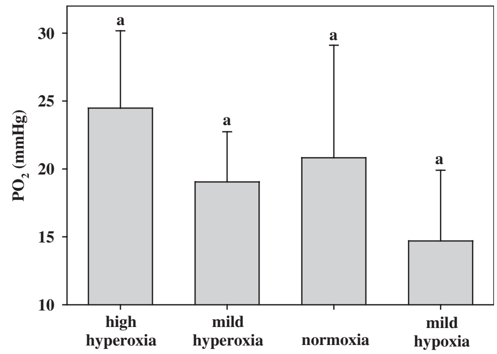

Figure 3 PO_2 in sea bass (*Dicentrarchus labrax* L.) cultured under different dissolved oxygen conditions. Data are expressed as mean and standard deviation. Different letters indicate significant differences at $P \leq 0.05$ (small letter) or $P \leq 0.01$ (capital letter). No significant differences appear between groups marked with the same letter.

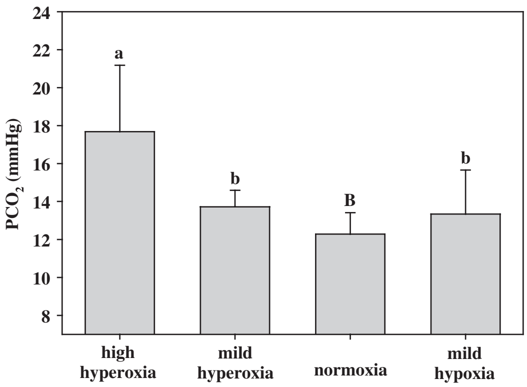

Figure 2 PCO_2 in sea bass (*Dicentrarchus labrax* L.) cultured under different dissolved oxygen conditions. Data are expressed as mean and standard deviation. Different letters indicate significant differences at $P \leq 0.05$ (small letter) or $P \leq 0.01$ (capital letter). No significant differences appear between groups marked with the same letter.

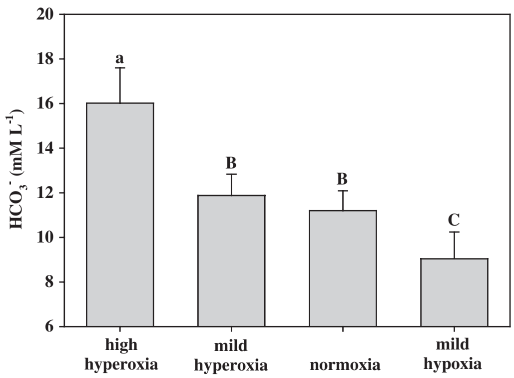

Figure 4 Plasma bicarbonate concentration in sea bass (*Dicentrarchus labrax* L.) cultured under different dissolved oxygen conditions. Data are expressed as mean and standard deviation. Different letters indicate significant differences at $P \leq 0.05$ (small letter) or $P \leq 0.01$ (capital letter). No significant differences appear between groups marked with the same letter.

significantly different only versus the blood pH of fish held under a mild hypoxia condition.

The mild hypoxia condition caused a reduction in both blood pH and plasma bicarbonate concentrations when compared with all or a part of the other conditions.

No significant differences appeared between the data obtained from fish cultured under normoxia and mild hyperoxia conditions. In fact, pairwise comparisons of the means obtained from these two groups showed that neither blood pH ($P = 0.764$), PCO_2 ($P = 0.737$) or plasma bicarbonate concentration ($P = 0.802$) were different.

Discussion

Our results showed that chronic exposure to different DO concentrations can affect the acid-base balance of sea bass. Long-term high hyperoxia (362.7 mmHg) produced compensatory alkalosis in sea bass, demonstrated by an increase in blood pH and bicarbonate concentration, owing to blood PCO_2 rise. This brought about acid–base disturbances similar to those observed in sea bass during chronic environmental hypercapnia (Cecchini *et al.* 2001). In fact, during environmental hyperoxia fish are exposed to respiratory acidosis due to hypoventilation

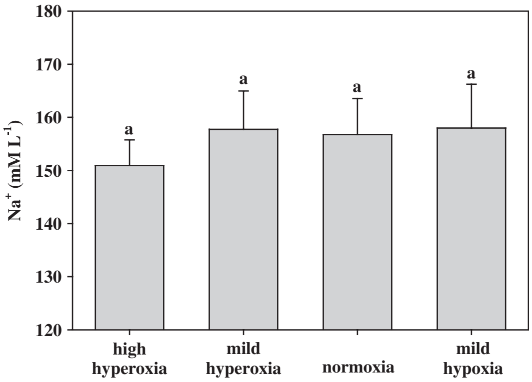

Figure 5 Natriaemia in sea bass (*Dicentrarchus labrax* L.) cultured under different dissolved oxygen conditions. Data are expressed as mean and standard deviation. Different letters indicate significant differences at $P \leq 0.05$ (small letter) or $P \leq 0.01$ (capital letter). No significant differences appear between groups marked with the same letter.

and hypoperfusion of the respiratory apparatus (Wood & Jackson 1980; Wood, Wheatly & Höbe 1984; Takeda 1990; Claiborne 1998). Thus, the uptake of HCO_3^- from the surrounding environment, in order to compensate the respiratory acidosis, causes an increase in blood pH, similar to what Cecchini *et al.* (2001) showed in sea bass exposed to chronic environmental hypercapnia. In fact, blood pH was higher in the high hyperoxia group than in other experimental groups (Fig. 1), although significant differences occurred among fish held under the high hyperoxia condition and fish held under normoxia and mild hypoxia conditions ($P \leq 0.05$).

On the contrary, the mild hypoxia condition caused a significant reduction in plasma bicarbonate concentration in our experiment, probably owing to an increase in the amplitude and frequency of gill ventilation, as shown in similar conditions in other fish species (Thomas & Hughes 1982a; Kinkead & Perry 1991; Maxime *et al.* 2000).

No differences were observed between fish held under mild hyperoxia and normoxia conditions. This testifies that mild hyperoxia does not produce physiological disturbances in the acid–base status of sea bass when compared with normoxia. Thus, mild hyperoxia should be considered the fitter DO condition in sea bass land-based farming, mainly because in previous papers it was shown as being able to improve growth and feed conversion (Saroglia *et al.* 1995) and to modulate some immunological parameters of sea bass, as observed by Scapigliati *et al.* (1999) with respect to the serum immunoglobulin levels, and by Cecchini & Saroglia (2002) with respect to the specific antibody response.

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