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An experimental study of some haematological parameters of cage aquacultured sea bass *Dicentrarchus labrax* L. under extreme environmental conditions : preliminary results

Étude expérimentale de certains paramètres hématologiques du loup Dicentrarchus labrax *L. élevé en cage dans des conditions d'environnement extrêmes : résultats préliminaires*

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ABSTRACT

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1 - The aim of this investigation is to evaluate possible changes in some haematological (Haematocrit, Haemoglobin) and biochemical (total serum proteins) parameters of a population of cage aquacultured European Sea bass (Dicentrarchus labrax *L.*) *under extreme environmental conditions (sea-water temperature T, dissolved oxygen concentration DO) in Argostoli bay, Kephalonia (Greece).*
2 - All haematological and biochemical parameters measured were higher in February (T = 12.5°C, DO = 8.4 mg/l, feeding rate = 0.38 kg food/100 kg fish biomass) and lower in August (T = 24.5°C, DO = 6.4 mg/l, feeding rate = 1.8 kg food/100 kg fish biomass).
3 - No Sea bass mortality was observed in this population under these extreme environmental conditions.

RÉSUMÉ

Castritsi-Catharios J., S. Kavadias, 1993 [Étude expérimentale de certains paramètres hématologiques du loup (Dicentrarchus labrax L.*) élevé en cage dans des conditions d'environnement extrêmes : résultats préliminaires]. Mar. Life,* ***3*** *(1-2) : 95 - 98.*

1 - L'objectif de cette étude est la vérification de changements évuentuels des paramètres hématologiques (hématocrite, hémaglobine) et biochimiques (protéines totales du plasma) d'une population européenne de bars élevés en cage dans des conditions environnementales extrêmes (température de l'eau de mer, concentration d'oxygène dissous dans le golfe d'Argostoli, Céphalonie (Grèce).
2 - Toutes les valeurs des paramètres hématologiques et biochimiques calculées, étaient plus élevées en février (T = 12,5°C, DO = 8,4 mg/l, débit alimentaire = 0,38 kg de nourriture / 100 kg de masse ichtyologique) et plus basses en août (T = 24,5°C, DO = 6,4 mg/l, débit alimentaire = 1,8 kg de nourriture / 100 kg de masse ichtyologique).

INTRODUCTION

Clinical methods for monitoring the physiological condition of Teleosteans exposed to a variety of environmental conditions have been used widely in the past. Haematology of fishes is affected by water temperature and dissolved oxygen concentration (Courtois 1975, Cech *et al.*, 1978 ; Esch and Hazen 1980 ; Carmichael *et al.*, 1984 ; Zanuy and Carrillo 1984 ; Hadjkacem *et al.*, 1987 ; Lochmiller *et al.*, 1989). The haematology of fishes is also affected by starvation, feeding rates and quantity of

food consumed (Sakamoto and Yone 1978 ; Santulli and D'Amellio 1986 ; Sakamoto and Yone 1979). Osmoregulation of teleosteans is affected by water temperature (Stanley and Colby 1971 ; Maetz and Evans 1972 ; Courtois 1975). Routine haematological methods are used in fish haematology (Blaxhall and Daisley 1973).

In this work we have evaluated some haematological (Haematocrit (HCT), Haemoglobin (Hb) and biochemical (Total serum proteins (TSP)) parameters of a cage aquacultured population of European Sea bass under extreme environmental conditions. We did not investigate other parameters such as Total serum lipid (TSL) concentration because this was a preliminar attempt and more experiments are planned for the future. We expect different TSL concentration between summer and winter because of different feeding rates and growth rates (G). Sea bass growth rates and feeding rates are positively correlated with water temperature ((Zanuy and Carrillo 1985 ; Hidalgo *et al.*, 1987 ; Kephalonian Fisheries, pers. comm).

MATERIALS AND METHODS

1 - Study area and sample collection

This study was conducted on fishes cultured in cages by the Kephalonian Fisheries in Argostoli bay (salinity 38 ‰) (Figure 1). Sampling took place in February and August 1991. Sea bass fry (mean weight 1 g) was transfered from the hatchery to the cages (kames type, 7 x 7 m, mesh size 6 mm, density 120 fishes/m^3, and stocked untill they reached commercial weight (.350 g). Fishes (average weight 280 g) were collected from Kames type cages (7 x 7 m, mesh size 13 mm, density 50 fishes/m^3) with dip nets. Blood samples were taken from at least 10 fishes in each case. Fishes were not anaesthetized. Blood sampling was accomplished within 6 minutes of fish collection : each fish was immobilized by holding it by the head and the tail. A 2 ml sample of whole blood was obtained from the caudal vein using 2.5 ml plastic syringes. Handling was the same for all the fishes. The 6 minute intervals are short and are not expected to have resulted in stress and abnormalities in the measured parameters of the experiments. Blood samples were stocked in 3 ml vacuum tubes containing potassium EDTA as anticoagulant agent. Tubes were placed in ice and quickly transfered to the laboratory.

Figure 1 - Location of Argostoli Bay (Kefalonia) in the Ionian Sea./*Situation de la baie d'Argostoli (Céphalonie), en mer Ionienne.*

2 - Water temperature and oxygen measurements

In Argostoli bay, water temperature was highest and dissolved oxygen concentration lowest in August (T = 24.5°C, DO = 6.4 mg/l). The opposite was the case in February (T = 12.5°C, DO = 8.5 mg / l). Temperature measurements were made with an ordinary mercury thermometer. Seasonal temperature changes appear in Figure 2. Variations between day and night have not been recorded as systematically as seasonal variations. Dissolved oxygen concentration was measured in the morning (10 a.m.) with a Phox type oxygonometer.

3 - Nutrition - Growth rates

The size of pellets increased as the size of fishes increased. The daily feeding rate (Kg food/100 Kg of fish biomass) increased with the weight of fish and with water temperature. Sea bass growth rates (G), are positively correlated with sea water temperature.

Our experimental fishes were fed on a diet of dry pellets (Aqualim Hellenic S.A.) size 4.5 mm. The mean analysis of the pellets is : crude protein 46 %, crude fat 12 %, crude ash 13 %, crude fiber 3 %, moisture 11 %, Vit A 25000 U1, Vit D3 2500 U1, Vit E 100 mg, Antioxidant = Ethoxyquin.

Feeding was regularly distributed through the whole day period. A small quantity of food was spread by automatic feeders (Tess aquaculture type) at predetermined short intervals. All the food was spread and consumed within 24 hours.

4 - Haematology assays

We determined the haematocrit (Hct) by the microhaematocrit capillary tube (heparinized) method. Blood samples were centrifuged at 10500 rpm for 5 min using a Hawksley and Sons Ltd centrifuge.

Haemoglobin concentration (Hb) was determined by spectrophotometry, following the cyanmethemoglobin procedure (Boehringer Mannheim kit, 124729). The rest of the blood was transfered to the Ependorf and centrifuged at 10500 rpm for

Table 1 - Haematological analysis of European Sea bass acclimated to different environmental conditions in Argostoli bay./*Analyse hématologique du loup européen, acclimaté à différentes conditions de l'environnement dans la baie d'Argostoli.*

	February 1991	August 1991
T(°C) 12.5	12.5	24.5
DO (mg/l)	8.4	6.5
Feeding rate (kg food/100 kg fish)	0.38	1.8
G (pair month)	1.5 %	17.0 %
Hct (%)	49.16±3.95	46.36±2.91**
Hb (g/100 ml)	9.08±0.65	8.56±0,76**
TSP (g/100 ml)	6.61±0,19	5.81±0,25**

** Difference between two months statistically significant (Student's t-test) at P<0.05

$$G \text{ (pair month)} = \frac{\text{final weight (g) - initial weight (g)}}{\text{initial weight (g)}} \times 100$$

5 min. Plasma aliquots were stocked at -20°C for further analysis.

Total serum protein concentration was determined with the Biuret colorimetric method (Boehringer Mannheim kit, 124281).

5 - Statistical analysis

The statistical significance of differences in haematological and biochemical parameters was tested using Student's t-test (at P = 0.05).

RESULTS AND DISCUSSION

There was a statistically significant difference in all haematological parameters measured (P<0.05) between February and August. Haematocrit, haemoglobin and total serum protein concentration were lower by 6 % in August than in February (Table 1).

Within the annual cycle, sea water temperature and feeding rates are highest, and dissolved oxygen concentration lowest in August.

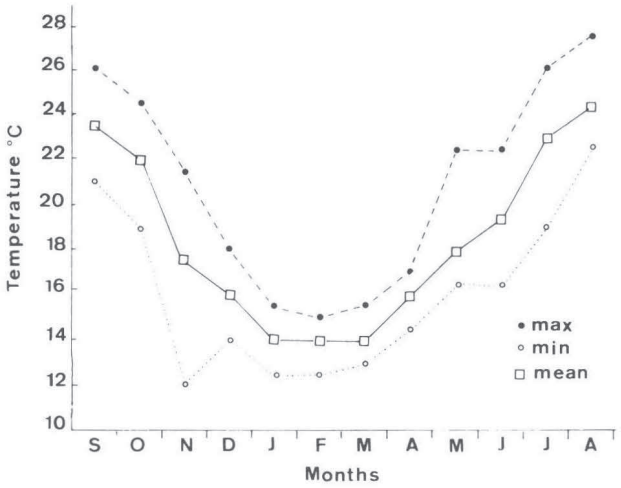

Figure 2 - Temperature variation of the water in Argostoli Bay (Ionian Sea) during the periods 1988-1989, 1989-1990, 1990-1991./*Variations de la température de l'eau en baie d'Argostoli (mer Ionienne) pendant les périodes 1988-1989, 1989-1990, 1990-1991).*

Erythropoietic stimulation of red blood cell production is commonly associated with changes in tissue oxygenation such as hypoxia (Widman 1983 ; Hall *et al.*, 1926) noted significant elevations of Hb in marine fish subjected to acute oxygen stress. Similarly, Dheer (1988) reported significant increases in Hb and Hct in *Channa punctatus* maintained at temperatures above 30°C for 6 weeks. Cameron (1970) also noted increases in Hb and decreases in Hct in *Langoodon rhomboids* subjected to high temperatures (see Discussion in Lochmiller *et al.*, 1989).

There are several possible explanations of the observed changes in Sea bass haematology. The winter to summer decrease in Hct and Hb may reflect a normal seasonal rhythm that is a response to changing photoperiod (Lochmiller *et al.*, 1989)

Lochmiller *et al.* (1989) also suggested that a further reduction in red blood cell parameters of *Morone saxatilis* during the summer could have occurred as a result of prolonged thermal and oxygen stress resulting in secondary nutritional problems that are known to adversely influence erythropoietic mechanisms (Lochmiller *et al.*, 1982). In our case, no Sea bass nutritional problems were observed because of standardized food. Hct and Hb mean values are in agreement with values suggested by Doimi (1985) for aquacultured Sea bass. We do not have data from liver spleen or digestive tract.

Cech *et al.*, (1979) showed a close relationship between oxygen stress of inspired water and arterial blood in *Micropterous salmoides.* In *M. salmoides,* haemoglobin has a high affinity with oxygen in arterial blood. Thus, the oxygen content of arterial blood would be little affected by decreases in the oxygen stress of arteriel blood over a broad range of environmental stress. The relationship between environmental temperature and oxidative

metabolic rate in aquatic ectotherms is well documented. Beamish (1970) estimated the standard metabolic rate of Largemouth bass (150 g body weight) to be between about 105 (at 20°C) and 160 mg O_2/kg, hour at 30°C (Cech *et al.*, 1979, Discussion). In Sea Bass there is a positive correlation between sea water temperature and growth rates (Zanuy and Carrillo 1985 ; Kephalonian Fisheries, pers. comm.).

Total serum proteins are one of the blood components that influence blood osmotic pressure (Courtois 1975). No significant salinity changes are observed in Argostoli bay during the year. Sakamoto and Yone (1978) suggested that total protein in Red Sea Bream serum could decrease due to deficiency of amino acids required for protein synthesis. In our case no nutritional problems were observed in either February or August.

Therefore it appears that the 12 % decrease of total serum protein concentration in August was not caused by changes in salinity by nutritional deficiencies. In Sea bass, Hadjkacem *et al.* (1987) noted that the total serum protein concentration remained constant after acute exposure to higher temperatures (20 to 26°C, 16 to 26°C). In sea water adapted *Platicthys flesus* Maetz and Evans (1972) observed reduced sulphate in the extracellular space of the parietal muscle at the lower adaptation temperature (6°C), which is indicative of an increased cellular water content. At the 16°C adaptation temperature, sulphate space was increased.

Alter experiments with acute exposure to cold of *Alosa pseudoharengus* in fresh water, Stanley and Colby (1971) observed a decrease in the extracellular space and an increase in the cellular space. The response was similar in sea water. These shifts suggest a movement of water into the cell, perhaps due to a decrease in the activity of water within the cell. Acute exposure of fish to heat gives the opposite response and these changes suggest a shift of water out of the cell, which might be due to an increase in the water activity within the cell. Movement of water into the cells in August can explain the 6 % decrease of Hct in our case.

In summary Hb, Hct and TSP of the fishes in Argostoli bay seem to be affected by parameters such as sea-water temperature, dissolved oxygen concentration and feeding rates. They may follow a seasonal rhythm. Our experiments are the first in aquacultured Sea bass in Argostoli bay, Kephalonian Fisheries. More investigations on other haematological and biochemical characters will take place in the near future as part of a continuing scientific programme.

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