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Metabolic Response to Monthly Variations of *Sparus aurata* Reared in Mediterranean On-Shore Tanks

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Abstract

The aim of this study was to investigate metabolic adaptation to monthly variations in sea bream reared in Mediterranean off-shore tanks. For this purpose, each month (from October 2010 until June 2011), on 25 *Sparus aurata* always randomly captured by the same batches in Sicilian farm, fork length, body weight and visceral weight were measured and blood samples were collected. Using biometric data condition factor, viscero-somatic index were calculated and on blood samples biochemical profile was assessed. The application of one way ANOVA showed a significant effect of seasons on all biometric and biochemical parameters studied ($P<0.0001$). Seasonality do not compromise the welfare status of sea bream that have a good acclimation capacity to environmental variations. Our results could represent a further support to better understand the effect of seasonality on the growth and metabolic profile in fish. Such information could be important in aquaculture in order to optimize husbandry practices carrying out them when physiological status of fish is seasonally less efficient.

Keywords: Months, metabolic adaptation, biometric data, sea bream

Introduction

The gilthead sea bream (*Sparus aurata*) is fish species predominantly located in the Mediterranean Sea. In the last 10-15 years, the commercial farming of these species has become a common practice along the Mediterranean coastline.

As with other fish species, one of the main problems associated with sea bream culture is stress due both to the fish being confined in cages, which prevents them from migrating, and handling (Flos *et al.*, 1990). Several studies have shown immunosuppression to be a direct result of stress in this species (Page´s *et al.*, 1995; Tort *et al.*, 1998), increasing their susceptibility to opportunistic pathogens and decreasing their ability to fight infections, as clearly demonstrated in other fish species (Pickering and Pottinger, 1985). In particular cultured sea bream is affected by a pathology called ''winter syndrome'' or ''winter disease'' that causes chronic mortality during the coldest months and acute mortality episodes when the temperature rises (Gallardo *et al.*, 2003).

Being cold-blooded animal, fish is affected by the temperature of the surrounding water which influences the body temperature, growth rate, food consumption, feed conversion and other body functions (Britz *et al.*, 1997; Azevedo *et al.*, 1998). Therefore, water temperature is a driving force in the fish life because its effects are more than any other single factor. Growth and livability in fish are optimum within a defined temperature range (Gadowaski and Caddell, 1991). Although short-term changes, such as weather conditions, may influence a fish for a day or two, but temperature has more predictable and seasonal effect. Each fish species has an ideal temperature range within which it grows quickly. However, fish move into more favourable areas of a stream to regulate their body temperatures. In warmer environments fish have a longer growing season and faster growth rate but tend to have a shorter life span than in cool water. High water temperatures increase the metabolic rates, resulting in increased food demand. Although, fish can generally function in a wide range of temperatures, but they do have an optimum range, as well as lower and upper lethal temperatures, for various activities (Kausar and Salim, 2006). Wild gilthead sea bream normally live in an environment whose temperature ranges from 11 °C in winter to 23 °C in summer, without any

apparent problems related to the temperature changes.

To achieve good growth of fish in aquaculture, fish must be healthy, well-fed and the water must be of the required quality. An important tool in the development of aquaculture system, particularly in regard to the use in detection of healthy from diseased or stressed animal (Rainza-Paiva *et al.*, 2000; O'Neal and Weirich, 2001) is the study of the physiological and haematological characteristics of cultured fish species. Early detection of changes in glucose, lactate, lipid metabolism, and also fluctuations in the catalytic concentrations of enzymes are required in order to identify the cause of disorders in fish. So, analysis of blood indices had proven to be a valuable approach for analysing the health status of animals as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting (Bahmani *et al.*, 2001). *S. aurata* is economically important specie distributed in Europe and its culture requires a good correlation between the environment and organism to avoid the influence of environmental stressful factors on health, growth rate and survival of fish. In view of this, the aim of this study was to assess, in sea bream, the changes in biochemical profile, a good indicator of metabolic adjustment, during acclimation to seasonal variations on sea bream.

Materials and Methods

The study was conducted on *Sparus aurata* provided from a Sicilian fish farm (Acquazzurra, Pachino, Sicily, Italy). The experimental protocol was started in October 2010 and lasted until June 2011. Fish, reared in outside tanks and subjected to natural photoperiod, were fed twice (9:00; 18:00) every day with a commercial diet specific for this specie. In Table 1 were reported the mean weight of fish and the tank density recorded in farm during the experimental protocol. Each month, blood samples were collected from 25 *S. aurata* always randomly captured by the same batches (using 2-Phenoxyethanol at the concentration of 400 ppm/l) by confinement and netting from tanks. Table 2 shows monthly recorded water parameters of tanks used for fish collection.

The time needed to transport the fish from the cage to the laboratory was approximately 10min. In the laboratory, the tanks were equipped with aerators. On all animals, blood samples were collected by caudal vein using a 2.5 ml sterile plastic syringe with a 22G X 1 1/2" needle. The time elapsing from capture to blood withdrawal was less than 5 min. After blood sampling, the fish were individually weighted to the nearest 0.01g (Mark 2200, BEL Engineering Srl, Monza) and their fork length (*L*) were recorded. Moreover visceral weight (*Wv*) was recorded. Growth performance was assessed using condition factor (CF), viscero-somatic index (VSI). For the assessment of glucose and lactate on whole blood, a portable blood glucose analyzer (ACCU-Chek Active, Roche Diagnostics GmbH, Mannheim, Germany) a portable blood lactate analyzer (Accusport, Boehringer Mannheim, Germany).

Blood samples were placed in non-heparinised tubes to test protein profile, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea,

Table 1. Mean weight of *S.* and tanks density recorded in farm each month

Month	Mean Weight (g)	Density (Kg/m^3)
October 2010	83.00±10.25	35
November 2010	107.00±16.23	44
December 2010	120.00±18.70	33
January 2011	137.00±16.20	37
February 2011	149.00±19.41	39
March 2011	162.50±21.60	44
April 2011	167.00±23.13	26
May 2011	199.00±28.20	31
June 2011	239.00±19.24	37

Table 2. Water parameters recorded in the tanks contained the batches using during experimental period

Month	Water Parameters		
	Temperature (°C)	Salinity (‰)	Dissolved oxygen (ppm)
October 2010	23.4	40	5
November 2010	20.1	40	5
December 2010	15.7	40	5
January 2011	14.2	40	5
February 2011	13.6	40	5
March 2011	13.7	40	5
April 2011	15.9	40	5
May 2011	17.9	40	5
June 2011	21.7	40	5

calcium, magnesium, cholesterol and triglycerides.

Following standing at room temperature for 20 min, the Falcon tubes were centrifuged at room temperature at 1300g for 10 min and the obtained serum stored at -20°C until analysed. The concentration of serum total proteins was determined by biuret method using an automated analyzer UV Spectrophotometer (SEAC, Slim, Florence, Italy). The protein fractions were performed using an automated system (Sel Vet 24, SELEO Engineering, Naples, Italy) according to the procedures described by the manufacturer. In particular for each sample, 25 μL of serum were applied to numbered sample wells. Each holder accommodate up to 24 samples. Films were electrophoresis for 28 minutes at 450V. After electrophoresis, films were simultaneously fixed using an automated system, stained in red stain acid solution for 10 minutes, and then dried at 37 ° C. After destaining in acetic acid and drying completely for 15 minutes films were scanned on a densitometer, electrophoretic curves plus related quantitative specific protein concentrations for each sample were displayed. Relative protein concentrations within each fraction were determined as the optical absorbance percentage, and absolute concentration (g/dL) was calculated using the total protein concentration. The major protein fractions were divided, according to the recommendation by the manufacturer from cathode to anode as albumin, alpha, beta 1, beta 2, and gamma-globulins, respectively. ALT, AST, urea, calcium, magnesium, cholesterol and triglycerides were determined by means of commercial kits (SEAC, Florence, Italy) using on UV spectrophotometer (Slim, SEAC, Florence, Italy). Protocols of fish and experimentation were reviewed and approved in accordance with the standards recommended by the *Guide for the Care and Use of Laboratory Animals* and Directive 86/609 CEE.

Statistical Analysis

Data obtained for biometric data and different blood parameters were tested for normality using Kolmogorov-Smirnov test. $P<0.05$ was considered statistically significant. Each month for each fish, condition factor was calculated as: $W \times 100\ L^{-3}$, where W is the wet weight of animal and L is length; at the same way viscero-somatic index was calculated as: $Wv \times 100/W$, where Wv is the visceral weight and W wet weight. One-way analysis of variance (ANOVA) was used to determine a statistically significant effect of season on all biometric and serum parameters measured. Bonferroni's multiple comparisons test was used for post hoc comparisons.

Data were analyzed using statistical software Prism v. 5.00 (Graphpad Software Ldt., USA, 2003).

Results

Biometric Data

Seasonal variations (Means ± SD) of data relative to body weight, visceral weight and fork length are showed in Table 3. The application of one-way ANOVA showed a significant effect of different seasons on all biometric parameters assessed ($P<0.0001$).

From the start to the study until June, *S. aurata* showed a constant increase in *W*. The higher increase there was between October and November ($\Delta=28.83$). At the turn of March and April there was a lower difference in weight gain ($\Delta=5.91$). *Wv* showed the similar trend with high increase ($\Delta=2.02$) straddling October and November even if the higher weight gain was between April and May ($\Delta=2.77$). A higher decrease of *Wv* ($\Delta= -0.22$) at the turn of March and April. *L* gain showed a higher increase between May and June ($\Delta=3.48$) and higher decrease ($\Delta= -1.92$) at the turn of April and May. A significant effect on CF in *S. aurata* was found by months. In Table 4 are showed CF and VSI recorded during the study.

Biochemical Parameters

The application of one way ANOVA showed a high significant effect of seasons on all biochemical parameters studied ($P<0.0001$). Figure 1 and 2 reported the trends of biochemical parameters and proteins profile recorded during experimental period together with the results of Bonferroni's multiple comparisons test.

Table 3. Mean ± SD of biometric data recorded in *S. aurata* during experimental period

		Sparus aurata	
Month	Weight (g)	Visceral weight (g)	Fork length (cm)
October 2010	82.42 ± 10.25^{a}	6.08 ± 0.75^{a}	16.69 ± 0.88^{a}
November 2010	111.25 ± 6.88^{b}	8.11 ± 0.82^{ab}	18.26 ± 0.66^{b}
December 2010	125.16 ± 18.70^{bc}	8.96 ± 1.83^{bc}	18.48 ± 0.90^{b}
January 2011	139.13 ± 16.70^{cd}	10.94 ± 2.31^{cd}	19.70 ± 0.79^{c}
February 2011	148.68 ± 19.41^{de}	11.26 ± 2.10^{e}	19.92 ± 0.77^{c}
March 2011	159.14 ± 21.62^{e}	11.59 ± 2.24^{d}	20.98 ± 1.15^{d}
April 2011	165.05 ± 23.13^{e}	11.37 ± 2.96^{d}	21.22 ± 0.87^{d}
May 2011	185.90 ± 28.87^{f}	14.14 ± 3.37^{e}	19.30 ± 1.09^{c}
June 2011	212.55 ± 19.24^{g}	15.16 ± 2.60^{e}	22.78 ± 0.56^{e}

Means without the same alphabetic characters at different months within the same parameter represent statistical differences ($P<0.0001$).

Table 4. Parameters useful to assess growth performance of *S. aurata*: condition factor (CF) and viscero-somatic index (VSI)

Month	CF	VSI
October 2010	1.78±0.21	7.45±1.07
November 2010	1.83±0.18	7.28±0.49
December 2010	1.98±0.17	7.12±0.61
January 2011	1.82±0.15	7.83±1.12
February 2011	1.88±0.16	7.56±1.02
March 2011	1.74±0.29	7.31±1.25
April 2011	1.73±0.20	6.89±1.64
May 2011	2.59±0.33	7.61±1.31
June 2011	1.80±0.14	7.18±0.89

The results are expressed as Mean ± SD

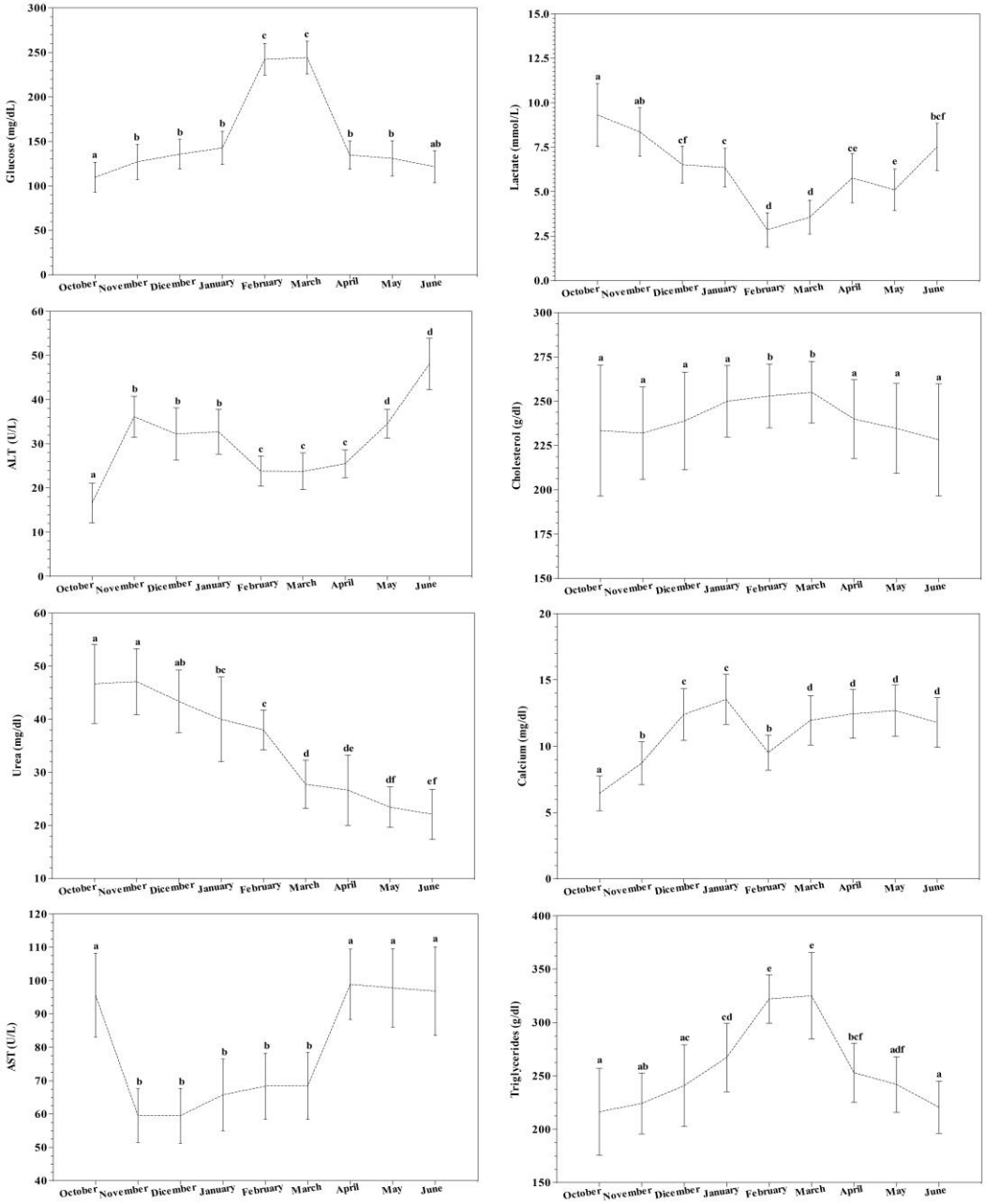

Figure 1. The trends (Mean ± SD) of the biochemical parameters recorded during experimental periods in sea bream. Means without the same alphabetic characters at different months within the same parameter represent statistical differences ($P<0.0001$).

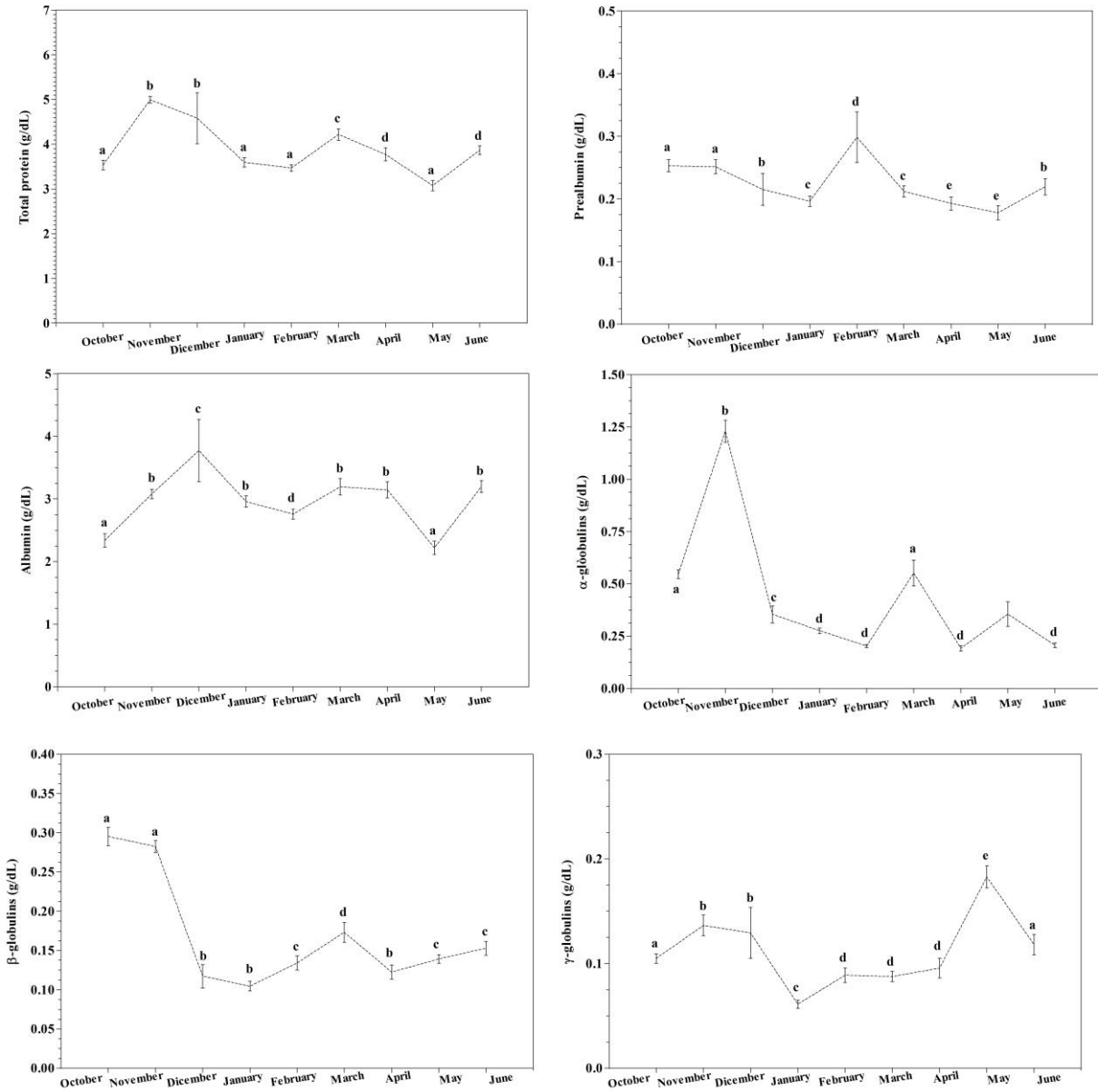

Figure 2. The trends (Mean ± SD) of the protein profile recorded during experimental periods in sea bream. Means without the same alphabetic characters at different months within the same parameter represent statistical differences (P<0.0001).

Discussion

Biometric Data

In warmer environments fish have a longer growing season and faster growth rate but tend to have a shorter life span than in cool water. High water temperatures increase the metabolic rates, resulting in increased food demand. Although, fish can generally dwell in a wide range of temperatures, but they do have an optimum range, as well as lower and upper lethal temperatures, for various activities.

Our results showed that sea bream exhibited a significant slowdown in growth during the colder months (from January to April). This trend is possibly related to water temperature that can affect diet and metabolism (Person-Le Ruyet *et al.*, 2004) and regulates the feed intake. This specie stop growing at 10 °C and below 13°C food intake ceases (Ibarz *et al.*, 2010). The higher increase in *W* was found at the turn of October and November when water temperature is higher than other months. Confirming our results, Buentello *et al.* (2000) demonstrated that an increase in temperature increases the activity of digestive enzyme, which may accelerate the digestion of the nutrients, thus resulting in better growth.

Another environmental factor which affected growth of fish was sunlight. In sea bream even if the higher increase in *W* was observed in October, the higher increase in *L* was observed straddling May and June. We can claim that there is uncouple growth-promoting effect of light-temperature cycling as has been demonstrates in different studies (Imsland *et al.*, 1995).

Regarding to CF, it is a measure of the condition of fish. If CF<1.0 a fish is in poor condition and if CF>1.4 a fish is in good to excellent conditions. Our results showed that, even if in some months CF values are next to 1, this factor is always >1. This confirms that seasonal variation do not compromise the welfare status of fish object of our study.

Biochemical Parameters

In ectothermic organisms, physiological rates can be adjusted to compensate for some changes in temperature. In fish, thermal acclimation is generally determined by metabolic changes, during which an initial period of thermal stress is followed by a gradual compensation. When a stable metabolic level that is consistent between the old and new thermal state is reached, the animal is considered to be fully acclimated (Maricondi- Massari *et al.*,1998).

It has been reported that weakening of the population when exposed to cold is caused by reduced aerobic scope at the low limits of the species' thermal niche (Pörtner *et al.*, 2005; 2009; Pörtner and Farrell, 2008). The obtained data seem to be in line with a reduction in aerobic capacity in the *S. aurata* when exposed to cold. The accumulation of lactate in the blood during cold months is probably due to the increase in L-LDH activity in the aerobic tissues as red muscle and heart that indicates an activation of anaerobic component of metabolism during exposure to cold as reported by Kyprianou *et al.* (2010).

Fish are generally thought to have a limited ability to utilize carbohydrate and possess low metabolic rates, especially when compared with mammals. The clearance of glucose from plasma is sluggish, leading a number of authors to regard fish in general as "glucoseintolerant" (Mommsen and Plisetskaya, 1991; Wilson, 1994; Gallego *et al.*, 1995; Wright *et al.*, 1998). In this study we found an increase in glucose values during colder moths (February and March). In according to our results, Sun *et al.* (1992, 1995) observed a significant hyperglycemia in tilapia *Oreochromis niloticus* L. subjected to a 14-16 °C temperature, while Ottolenghi *et al.* (1995) found that blood glucose level, in *Ictalurus melas,* did not changes in relation to seasonal periods (spring and autumn) although its decreased with increasing water temperature.

Some authors consider that fish utilize dietary carbohydrate poorly (Walton and Cowey, 1982), thus protein is an important energy fuel in fish (Peragón *et al.*, 1999; Kikuchi, 1999). A decrease in total protein that we found in sea bream during colder months is probably due both to the its use as energetic resource and to the effect of starvation. Temperature, in fact regulates also the feed intake, as this specie stop growing at 10 °C and below 13 °C food intake ceases (Pastoureaud, 1991; Ibarz *et al.*, 2010). Moreover total protein is used as an indicator of liver impairment increased concentration can be caused by structural liver alterations reducing aminotransferase activity with a concurrent reduction in the deamination capacity (Bernet, 2001). So, in fish AST and ALT mainly find in hepatocytes and cardiomyocytes, respectively, playing important roles in protein metabolism and can be used to monitor the health status of fish (Wang *et al.*, 2005). When liver and myocardial cells are damaged or their permeability increases, AST and ALT will be released into the blood, resulting in elevated blood transaminase. Some studies have shown that various forms of stress can cause the increase of the plasma ALT and AST activities in the fish (Cho *et al.*, 1994) and that these enzyme are responsive to temperature change in fish (Jürss, 1979). This confirms changes in transaminases levels during different months of monitoring.

As total protein also urea, used as indicator of gill and kidney dysfunctions, showed a decrease from February, probably in response to starvation.

Cholesterol is one of the components of cell membranes and an important raw material for synthesis of steroid hormones, while triglycerides can store energy (Chang *et al.*, 2006). Our results showed that cholesterol slight change in colder months while underline a transient increase in the triglycerides levels during the colder months and this has been interpretated as a mobilitation of the lipid deposits to use as fuels.

One of the divalent ions, calcium (Ca^{2+}), serves a number of functions in fish. It combines with phosphorus (P) for the deposition of bone. It is possible that bone serves as a reservoir of calcium for plasma and tissues. Additionally, Ca^{2+} appears to be important in the reproduction and mitochondrial functions. It is generally recognized that Ca^{2+} has an important role in osmoregulation (Wurst and Stickney, 1989). Another divalent ion, magnesium (Mg^{2+}), acts as a cofactor of phosphohydrolases and phosphotransferases. In addition to being a component of bone, Mg^{2+} occurs in many metalloenzymes and during Mg^{2+} deficiency many metabolic functions are affected Thus, the levels of serum electrolytes offer important knowledge concerning the health status of diseases of and impact of stress on fish (Wurst and Stickney, 1989; Evans 1993). Changes in calcium and magnesium levels we found were probably due to the effect of photoperiod on the daily rhythmicity of electrolytes. This is in according to Pavlidis *et al.* (1999) who showed that among different blood parameters also electrolytes display daily rhythms in fish

Aquaculture fish production has increased significantly over the past few decades and with it the incidence of disease outbreaks, often associated with an intensification of the culture conditions. Disease, in turn, has caused substantial economic loss to the aquaculture industry. In order to mitigate disease outbreaks in aquaculture, it is necessary to develop disease control strategies based on a better

understanding of the effects of husbandry methods and environmental stressors on the health status of farmed fish. Our results could represent a further support to better understand the effect of seasonality on the growth and metabolic profile in fish. Such information could be important in aquaculture in order to optimize husbandry practices carrying out them when physiological status of fish is seasonally less efficient.

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