

## Seasonal biochemical changes in two aquaculture species

### *Sparus aurata* Linnaeus, 1758 and *Dicentrarchus labrax* Linnaeus, 1758

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#### Abstract

The aim of this research was to evaluate the changes of some biochemical parameters in response to environmental variation in two important fish species which have great commercial value reared in Sicily. The study, carried out on 200 fish (100 *Sparus aurata* Linnaeus, 1758 and 100 *Dicentrarchus labrax* Linnaeus, 1758), lasted 12 months. Each season, blood samples having been collected during summer solstice, autumn equinox, winter solstice and spring equinox. For each time point, 25 *S. aurata* and 25 *D. labrax* have been used and always randomly captured by the same batches from tanks. One-way analysis of variance (ANOVA), followed Bonferroni's multiple comparisons test, has used to evaluate the influence of different seasons on serum protein profile, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, calcium, magnesium, triglycerides and total cholesterol. The results showed the seasonal influence on biometric and biochemical parameters studied in *S. aurata* and *D. labrax* and that seasonal cycles can differently affects fish in several biological and physiological activities. These results could represent a valid contribution in the study of the linkage between metabolic parameters, seasonal variations and biometric indices in cultured fish to improve the management and technological support of aquaculture.

**Keywords:** Biochemical parameters, Environmental changes, European sea bass, Gilthead sea bream, Growth indices

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## Introduction

The influence of environmental factors on fish and their effects on reproduction and growth has been widely studied in the past. In aquaculture, the growth rate of fish is size-dependent and strongly influenced by environmental factors such as temperature and photoperiod (Person-Le Ruyet *et al.*, 2004). Environmental factors have negative economic impact as it impairs aquatic and aquaculture systems by affecting fish health, productivity and final product quality. Fish exhibit several responses to have the compensatory or adaptive mechanism to cope with the changing environment, maintain homeostasis and survive. These responses are very important for fish because through metabolic adjustments they ensure enough energy for the maintenance its homeostasis (Prunet *et al.*, 2008; Tort and Teles, 2012). The physiological processes in farmed species cannot escape from adverse environmental so the seasonality dominates the life cycle of fish, coordinates their reproductive activity and blood parameters, influences body weight, locomotor activity and food intake. In particular, these life events of fish are synchronized with seasonal changes of day length (photoperiod), weather (mainly temperature) and food supplies (Bromage *et al.*, 2001). The results of several studies about the effects of light intensity suggested that to manipulate physiological functions in different teleosts is required the exposure to threshold intensity levels (Taylor *et al.*, 2005; Taylor *et al.*, 2006; Migaud *et al.*, 2010). Many studies

have also been investigated the effects of several factors on body composition, energy utilization and growth in fish, but information available on seasonal patterns in metabolic resources is limited to teleost species (Faggio *et al.*, 2014a). The biochemical parameters in fish are sensitive for detecting potential adverse effects and relatively early events of environmental change. In Mediterranean countries, European sea bass and sea bream are very important economic resources, representing 46.20% of the total production of farmed fish (FAO, 2008-2011). These species, euryhaline fish with gregarious habits, are the major Mediterranean species produced by the aquaculture industry. It is known that they have evolved physiologically to live within a specific range of environmental variations and survival outside of that range can be stressful or fatal (Barton, 2002). Fish farmers want to improve fish welfare, to obtain successful farming and to mitigate diseases outbreaks in aquaculture that cause substantial economic losses.

Therefore, it is necessary to develop control strategies in order to better understand the effects of environmental stressors on the health status of cultured fish (Bowden *et al.*, 2007). It is important to provide the understanding of the compensatory or adaptive mechanism to cope with the changing environment, maintain homeostasis and survive in order to improve the precision farming. Considering that energetic metabolism and environmental factors influence the management and handling of reared

fish, the aim of this study was to evaluate the variations of biochemical parameters in different seasons into two important aquaculture species farmed in Sicily (*Sparus aurata* and *Dicentrarchus labrax*).

### Materials and methods

Two different cultured fish species *S. aurata* and *D. labrax*, obtained from a Sicilian fish farm have been used. Fish, reared in on-shore tanks (210 m<sup>3</sup>) and subjected to natural photoperiod, were fed twice a day (9:00; 18:00) with a commercial diet containing different levels of protein and lipid: 47.20% for sea bream and 45.20% for sea bass respectively (Skretting Italia SpA, Mozzecane, Verona, Italia). Fish were caught with a dip net always randomly by the same batches by confinement and netting from tanks and anesthetized using 2-phenoxyethanol at a concentration of 200 mg L<sup>-1</sup>. For each species, four samples were performed, one in each season of the year: autumnal equinox, (23 September; sunrise at 06:48, sunset at 19:55), winter solstice (21 December sunrise at 07:10, sunset at 16:45), vernal equinox (20 March; sunrise at 06:04, sunset at 18:22) and summer solstice (21 June; sunrise at 05:39, sunset at 20:24). Twenty-five *S. aurata* and twenty-five *D. labrax* in each season have been sampled (total fish sampled: 100 *S. aurata* and 100 *D. labrax*). During this

period, physic-chemical parameters of the environment were monitored (water and ambient temperature, water dissolved oxygen and salinity) and their values showed in Table 1. Fish blood samples were collected by caudal vein using a 2.5 ml sterile plastic syringe. Concentrations of lactate and glucose were immediately measured on whole blood using a portable blood glucose analyzer (ACCU-Chek Active, Roche Diagnostics GmbH, Mannheim, Germany) and 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, calcium, magnesium, triglycerides and total cholesterol. Falcon tubes have been centrifuged at 1300 g for 10 min and the obtained sera were stored at -20 °C until analysis. The concentration of serum total proteins and relative protein fractions have been assessed as described by Faggio *et al.* (2014b). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, magnesium, calcium, triglycerides and total cholesterol were determined using a commercial kits (Biosystems S.A., Barcelona) and a UV spectrophotometer (Slim, SEAC, Florence, Italy).

**Table 1: Environmental and water quality parameters of sampling site in different seasons during the study period.**

| SEASONS | Ambient Temperature (°C) | Humidity (%) | Water Temperature (°C) | Salinity (g L <sup>-1</sup> ) | Dissolved oxygen (mg dL <sup>-1</sup> ) |
|---------|--------------------------|--------------|------------------------|-------------------------------|---|
| Autumn  | 22±4                     | 73%          | 23.50                  | 0.04                          | 3.8                                     |
| Winter  | 13±4                     | 82%          | 16.00                  | 0.04                          | 4.8                                     |
| Spring  | 14±5                     | 48%          | 14.50                  | 0.04                          | 5.3                                     |
| Summer  | 27±6                     | 45%          | 22.00                  | 0.04                          | 4.0                                     |

All analyzes were performed by a single operator with a same instrument under the same conditions, and in a short period of time.

Afterward, fish were individually weighed to the nearest 0.01 g (Mark 2200, BEL Engineering Srl, Monza) and their fork length (FL) have been recorded. At last visceral weight (Wv) has registered.

Using weight (W), visceral weight (Wv) and length (L) of animals, condition factor (K) and viscerosomatic index (VSI) were calculated for each fish as follows:

$$K=W \times 100 L^{-3}; VSI=W_v \times 100/W.$$

The following biological indices were calculated:

Percentage weight (% WG):

$$\% WG=[(W_f-W_i)/W_i] \times 100$$

Where  $W_i$  is the initial weight of fish and  $W_f$  is the final weight of the fish.

Thermal growth coefficient (TGC) in according to Jobling (2003):

$$TGC (\%)=1000 \times [(final \ weight \ 1/3 - initial \ weight \ 1/3) / \sum (days \times temperature)]$$

Specific growth rate (SGR):

$$SGR (\%)=100 \times [(\ln \ final \ weight - \ln \ initial \ weight) / days]$$

The research was carried out in a Sicilian fish farm during 2014.

Protocols of animal husbandry 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 2010/63/EU for animal experiments.

#### Statistical analysis

Mean and standard deviation for all data were calculated. The data were assessed for normality (Shapiro-Wilk test) and homogeneity of variance (Bartlett test). Nonparametric tests were used when the requirements of normality and homogeneity of variance were not met. One-way ANOVA was performed for the season dependent relationships with the studied parameters using Prism v.7.00 (Graphpad Software Ltd., USA, 2003) for Windows. To compare the differences among the parameter means at  $p < 0.05$  Bonferroni's Multiple Range Tests were used.

#### Results

The values of physico-chemical parameters, measured during the study period are summarized in Table 1.

The average values, together with SD, of biometric data and biological indices recorded for each season are shown in Tables 2 and 3. All biometric data and biological indices in *S. aurata* showed a significant difference

( $p < 0.05$ ), while in *D. labrax* K and TCG (%) not showed a significant difference ( $p > 0.05$ ).

Figs. 1 and 2 show the seasonal comparisons of the biochemical parameters measured in *S. aurata* and *D. labrax*.

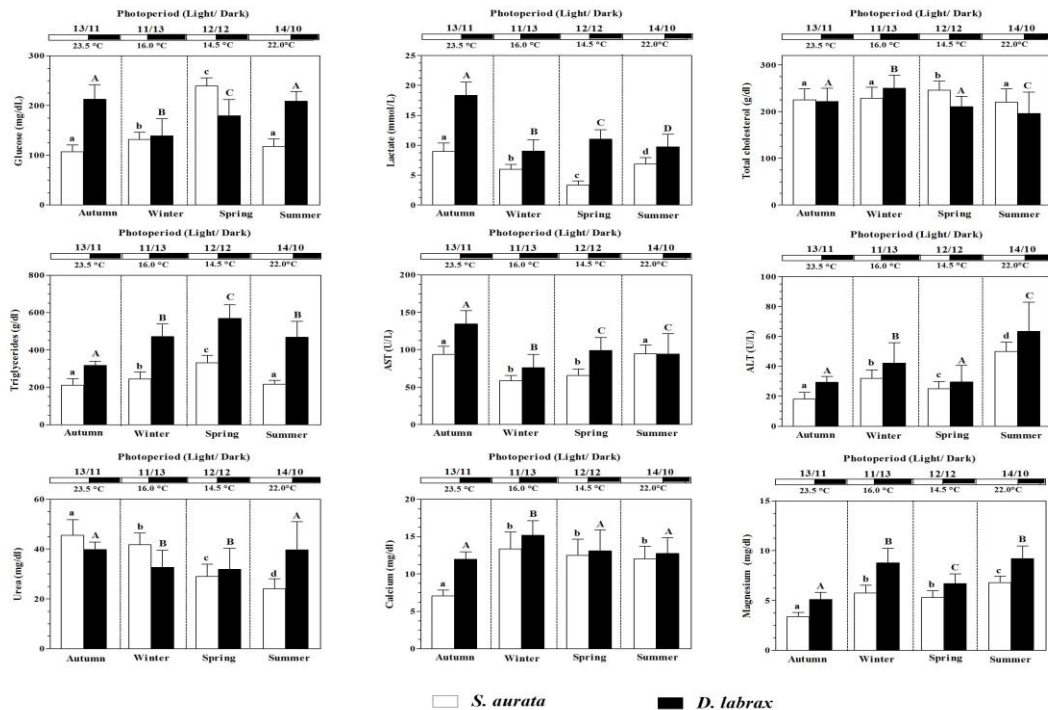


Figure 1: Mean±SD of the biochemical parameters recorded in *Sparus aurata* (n=25 each season) and *Dicentrarchus labrax* (n=25 each season) during experimental period. Means without the same alphabetic characters at different months within the same parameter represent statistical differences ( $p < 0.05$ ).

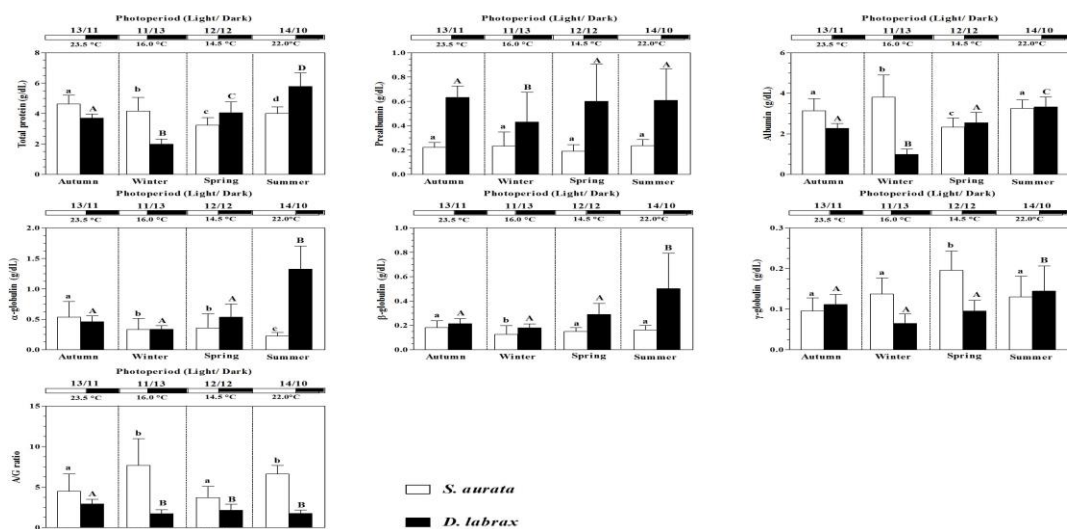


Fig. 2 Mean ± SD of the serum total protein and protein fractions recorded in *S. aurata* (n = 25 each season) and *D. labrax* (n = 25 each season) during experimental periods. Means without the same alphabetic characters at different months within the same parameter represent statistical differences ( $P = 0.05$ ).

Figure 2: Mean±SD of the serum total protein and protein fractions recorded in *Sparus aurata* (n=25 each season) and *Dicentrarchus labrax* (n=25 each season) during experimental period. Means without the same alphabetic characters at different months within the same parameter represent statistical differences ( $p < 0.05$ ).

## Discussion

All biochemical parameters exhibited a significant effect of season in both species (Tables 1 and 2). In particular, in both species lactate, AST and urea in

autumn was higher than other seasons, while triglycerides, ALT, calcium and magnesium in autumn was lower than other seasons as shown in Table 1.

**Table 2: Biometric data and biological indices recorded in *Sparus aurata* (n=25 each season) during experimental period. Values are expressed as mean±SD. Different superscript letters (a-d) in the same row indicate significant difference (ANOVA,  $p<0.001$ ).**

| <i>S. aurata</i>          | Autumn                   | Winter                    | Spring                    | Summer                    |
|---------------------------|--------------------------|---------------------------|---------------------------|---------------------------|
| Weight (g)                | 85.62±8.48 <sup>a</sup>  | 130.51±15.58 <sup>b</sup> | 151.11±17.74 <sup>c</sup> | 224.61±18.03 <sup>d</sup> |
| Visceral weight (g)       | 6.60±0.95 <sup>a</sup>   | 9.32±1.67 <sup>b</sup>    | 12.03±1.90 <sup>c</sup>   | 15.16±2.60 <sup>d</sup>   |
| Fork length (cm)          | 17.28±1.57 <sup>a</sup>  | 18.84±1.09 <sup>b</sup>   | 21.29±1.15 <sup>c</sup>   | 24.83±2.72 <sup>d</sup>   |
| K                         | 1.71±0.36 <sup>a</sup>   | 1.97±0.31 <sup>a</sup>    | 1.59±0.32 <sup>b</sup>    | 1.54±0.39 <sup>b</sup>    |
| VSI                       | 7.71±0.78 <sup>a</sup>   | 7.15±0.89 <sup>b</sup>    | 8.06±1.65 <sup>c</sup>    | 6.79±1.18 <sup>d</sup>    |
| <b>Biological indices</b> |                          |                           |                           |                           |
|                           | <b>Autumn/Winter</b>     | <b>Winter/Spring</b>      | <b>Spring/Summer</b>      |                           |
| WG (%)                    | 52.42±1.27 <sup>a</sup>  | 15.79±0.65 <sup>b</sup>   | 48.64±1.07 <sup>c</sup>   |                           |
| SGR (%)                   | 0.47±0.01 <sup>a</sup>   | 0.16±0.01 <sup>b</sup>    | 0.43±0.01 <sup>c</sup>    |                           |
| TGC (%)                   | 0.038±0.001 <sup>a</sup> | 0.019±0.001 <sup>b</sup>  | 0.045±0.002 <sup>c</sup>  |                           |

Note: K (condition factor); VSI (viscera-somatic index), WG (weight gain); SGR (specific growth rate); TGC (thermal growth rate).

In *S. aurata* serum glucose exhibited significantly higher value in winter and spring compare to autumn and summer, while in *D. labrax* it was higher in winter compare to autumn and spring, but it did not differ with summer. Cholesterol showed slight changes in colder months and a decrease in summer in both species (Table 1).

Serum total proteins showed different trends in studied species. In particular, *S. aurata* showed significantly lower values of total proteins and albumin in spring than other seasons, while  $\alpha$ ,  $\beta$  and  $\gamma$ -globulins showed significantly higher values in summer than other seasons. *D. labrax* showed significantly higher values of total proteins and all

protein fractions in summer than other seasons except for pre-albumin and A/G ratio (Table 2).

### *Biometric and biological indices*

The seasonal cycles can influence fish in various biological and physiological activities, such as nutrition, metabolism, reproduction and immunity (Bezerra *et al.*, 2013).

It is well known that water temperature is an important factor which influences growth rate of fish (Person-Le Ruyet *et al.*, 2004). The environmental temperature probably influences the growth of fish and the temperature-growth relationships are not simple. In warmer environment fish

have a longer growing season and faster growth rate but tend to have a shorter life span than in cool water.

The values of all physico-chemical parameters measured in water were considered as acceptable for *S. aurata* and *D. labrax* farming in Sicily as shown by our previous investigations (Faggio *et al.*, 2014b).

In *S. aurata*, there was a higher influence of season on biometric and biological index respect to *D. labrax*. In fact, as reported in Tables 2 and 3, during autumn/winter and spring/summer periods, *S. aurata* have not showed very different values of WG (52.42 and 48.64), SGR (0.47 and 0.43) and TCG (0.038 and 0.045) while in winter /spring period it showed a significantly lower value of WG (15.79), SGR (0.16) and TCG (0.019).

During winter /spring period the exposure of reared gilthead seabream to low temperatures might cause slower metabolic rates and disrupts feeding behavior with reduction of growth. Furthermore, the effect of photoperiod on synchronizing of an endogenous rhythm to the external environment may require more energy in shorter light periods such as in winter (11L/13D), leading to a reduction of somatic fish growth; dark period also reduced the digestive performance because of the reduced activity of fish during this period. The increase of temperature and day length is known to increase the activity of the digestive enzymes which can accelerate the digestion of the nutrients, thus resulting in better growth (Kausar and Salim, 2006).

**Table 3: Biometric data and biological indices recorded in *Dicentrarchus labrax* (n=25 each season) during experimental period. Values are expressed as mean±SD. Different superscript letters (a-e) in the same row indicate significant difference (ANOVA,  $p<0.001$ ).**

| <i>Dicentrarchus labrax</i> | Autumn                     | Winter                     | Spring                      | Summer                      |
|-----------------------------|----------------------------|----------------------------|-----------------------------|-----------------------------|
| Weight (g)                  | 88.75 ± 5.33 <sup>a</sup>  | 107.71 ± 15.6 <sup>b</sup> | 123.02 ± 24.24 <sup>b</sup> | 144.30 ± 28.47 <sup>c</sup> |
| Visceral weight (g)         | 11.20 ± 1.51 <sup>a</sup>  | 14.30 ± 3.92 <sup>bc</sup> | 14.40 ± 3.39 <sup>ce</sup>  | 15.69 ± 3.35 <sup>de</sup>  |
| Fork length (cm)            | 20.18 ± 1.55 <sup>a</sup>  | 21.45 ± 1.87 <sup>a</sup>  | 23.13 ± 2.40 <sup>ab</sup>  | 26.65 ± 10.42 <sup>b</sup>  |
| K                           | 1.10 ± 0.22 <sup>a</sup>   | 1.11 ± 0.23 <sup>a</sup>   | 1.02 ± 0.26 <sup>a</sup>    | 0.93 ± 0.27 <sup>a</sup>    |
| VSI                         | 12.66 ± 1.84 <sup>a</sup>  | 13.12 ± 2.17 <sup>a</sup>  | 12.00 ± 3.08 <sup>ab</sup>  | 10.60 ± 2.35 <sup>b</sup>   |
| <b>Biological indices</b>   |                            |                            |                             |                             |
|                             | <b>Autumn/Winter</b>       | <b>Winter/Spring</b>       | <b>Spring/Summer</b>        |                             |
| WG (%)                      | 7.70 ± 1.18 <sup>a</sup>   | 23.00 ± 0.70 <sup>b</sup>  | 44.30 ± 1.74 <sup>c</sup>   |                             |
| SGR (%)                     | 0.22 ± 0.009 <sup>a</sup>  | 0.15 ± 0.01 <sup>b</sup>   | 0.17 ± 0.03 <sup>b</sup>    |                             |
| TGC (%)                     | 0.017 ± 0.002 <sup>a</sup> | 0.016 ± 0.002 <sup>a</sup> | 0.016 ± 0.002 <sup>a</sup>  |                             |

Note: K (condition factor); VSI (viscera-somatic index), WG (weight gain); SGR (specific growth rate); TGC (thermal growth rate).

During the experimental period, *D. labrax* showed significant differences in percentage WG, SGR while TCG have

not showed a significant different among the seasons; this results probably indicate the different response

to food intake linked to adverse conditions respect to *S. aurata* (Leal *et al.*, 2011). In aquaculture, WG, SGR and TCG are models which applicable to the concave portion of the growth curve have been used. The model most widely used is the specific growth rate (SGR) based on natural logarithm of body weight, while the TGC model is a simple and flexible model that offers a simple mode of growth rate comparison for cultured fish, in fact it represents a standardized measure of growth that is unaffected by live weight, time interval and water temperature (Jobling, 2003).

K is a crude measure of the level of energy reserves (Goede and Barton, 1990) and fish health, variations of K during the year may indicate variations in nutritional status of the fish. *D. labrax* showed lower K values in all experimental periods compare to *S. aurata*, reaching a value of  $0.93 \pm 0.27$  during summer. These results agree with previous researches (Anene, 2005) in which K of other species showed seasonal variations. Other authors (Marinova *et al.*, 2011) have been found higher VSI in *D. labrax* respect to *S. aurata* confirming the results of our study. It was assumed that several fish have a genetically determined asymptotic body size and a composition of weight gain and so they are capable of adjusting their feed or energy intake to realize the genetic potential (Lupatsch *et al.*, 2003).

It is known that the growth rate under comparable conditions and feeding per demand was the highest for *S. aurata*, predicting to reach in 12 months 380 g,

compared to 325 g for *D. labrax* (Lupatsch, 2005).

#### *Biochemical parameters*

In fishes, the metabolic performance remains stable during the year because they adjust metabolic capacities to compensate for seasonal variation in water temperature (Wakeling *et al.*, 2000).

The biochemical parameters in cultured *S. aurata* and *D. labrax* are dependent on environmental conditions (seasonal variations).

In *S. aurata* glucose showed a significant increase in winter and spring compare to autumn and summer, while in *D. labrax* there is an opposite trend. Changes in basic metabolism usually reflect reductions in the energy supply source. In other fish like grass carp and milkfish, glucose levels increase rapidly upon exposure to cold treatment; they then decrease to the normal range within 2 days (Kuo and Hsieh, 2006).

Blood glucose is another parameter that is strongly influenced by environmental conditions as well as seasonal variations, nutritional status and sexual maturity (Prasad and Charles, 2010); carbohydrate utilization and glucose metabolism changes in fish depend on the species.

Reduced aerobic capacity at the short limits of the species' thermal niche causes weakening of the population when exposed to cold (Pörtner and Farrell, 2008; Pörtner *et al.*, 2009).

Our results are in accordance with a reduced aerobic capacity in both species when exposed to cold. During autumn, when there is an increase of

water temperature (23.5 °C), the accumulation of blood lactate is probably caused by the increase in L-LDH activity in the aerobic muscle cells (heart and red muscle); this indicates an activation of anaerobic metabolism during exposure to cold (Kyprianou *et al.*, 2010).

Total cholesterol is a component of cell membranes, it represents an important raw material for synthesis of steroid hormones; triglycerides can store energy (Chang *et al.*, 2006). According to Faggio *et al.* (2014a) who reported in *S. aurata* an increase of triglycerides and cholesterol in colder, underlining the mobilization of the lipid deposits as fuels, our results showed in both species similar changes of these two energetic metabolites.

Plasma proteins are responsible for vital functions such as the humoral defense, coagulation and carrying of metabolites, (Tavares-Dias *et al.*, 2009). Stress increases the amount of total serum proteins (Melo *et al.*, 2009). In fish cultured in the intensive system, a significant increase of serum proteins occurred in the cold season, while in the semi-intensive ponds, it remained unchanged (De Pedro *et al.*, 2005; Swain *et al.*, 2007). Protein metabolism is considered the most sensitive physiological responding to environmental stress. Synthesis and degradation of protein are highly regulated cellular processes and they are essential for cell nutrition. Total energy production in fish derives primarily from catabolism of proteins and amino acids. In the present investigation, *S. aurata* and *D. labrax*

showed a significant decrease in the protein levels in winter compare to other seasons that may be due to their degradation and to the possible utilization of these compounds for metabolic purposes. The lower albumin level than globulins during winter in *D. labrax* could be described as the utilization of albumin to meet the immediate energy demand hence rapid synthesis takes place in the liver. As well as serum proteins, also urea showed a decrease in colder months probably in response to starvation.

Transaminases are both plasma and non-plasma specific enzymes that are found in the tissues of fish and provide information about organ dysfunction. In most teleost fish, several biological and chemical functions are affected by enzyme activities in the body of the fish (Oruc and Uner, 2002). AST values indicate the capacity to oxidize or to mobilize amino acids for energy production or synthesis of protein of respectively (Gabriel and George, 2005). The higher scopes for growth, enhanced energy expenditure or higher protein turnover rate are represented by high AST activity observed at higher temperatures. Magnesium and calcium showed similar trend in both species studied even if in *D. labrax* they assumed highest values. Magnesium plays vital roles as an enzyme cofactor, and as an important structural component of cell membranes in the organs, muscle tissues and in extracellular fluids. Calcium plays a very important role in modulation of excitability and permeability of plasma membranes, muscular contraction,

intracellular signal or nerve signal transduction therefore in a wide range of physiological processes (Wendelaar Bonga and Pang, 1991). The effect of photoperiod on the circadian rhythmicity of electrolytes probably causes the changes in the levels of these parameters (Polakof *et al.*, 2007).

In conclusion, the obtained data indicate that some biochemical parameters in *S. aurata* and *D. labrax*, are influenced by season, temperature and photoperiod representing important variables for a correct management of aquaculture species. Even though the effects of these seasonal components in fish and the mechanisms which regulate it have not yet been fully investigated, the results of this research could represent a valid contribution in the study of the link between metabolic parameters, seasonal variations and biometric indices in cultured fish in order to improve the management in aquaculture farmed.

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