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Yearly growth and metabolic changes in earthen pond-cultured meagre *Argyrosomus regius*

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Summary: Metabolic modifications associated with natural environmental conditions were assessed in the meagre *Argyrosomus regius* cultured in earthen ponds under natural photoperiod and temperature. Juvenile specimens (90-100 g initial weight) were sampled (plasma, liver and muscle) every two months for 18 months (between December 2004 and May 2006). Specimens showed seasonal variations in growth rate, with the highest values in spring and summer. Plasmatic, hepatic and muscular metabolite levels and hepatic and muscular metabolic enzymes also showed significant variations throughout the year. Enzymatic activity related to carbohydrate metabolism in the liver (HK, FBPase and G6PDH) showed great modifications in summer, increasing glycogenogenic pathways, while amino acid metabolism (GDH and GOT activity) was enhanced in spring and summer. However lipid-related (G3PDH activity) metabolic enzymes did not show a clear seasonal pattern. In muscle, enzymatic activity related to amino acid, lipid and lactate metabolism (LDH-O activity), but not carbohydrate metabolism, showed seasonal changes in parallel with changes in growth rate. Thus *A. regius* specimens showed a trend to grow in summer months and mobilize their energy reserves in winter. Differences in the hepatic level were observed between the first and the second year of the study, suggesting the possible existence of metabolic changes related to specimen age or size. Our results indicate that growth and metabolic responses in *A. regius* are environmentally dependent and that this species is a very good candidate for diversification in aquaculture.

Keywords: *Argyrosomus regius*; energy; growth; metabolic parameters; ponds; seasons.

Crecimiento y cambios metabólicos anuales en corvina *Argyrosomus regius* cultivada en esteros

Resumen: Modificaciones metabólicas asociadas a condiciones ambientales temporales fueron evaluadas en la corvina *Argyrosomus regius*, cultivadas en esteros con fotoperiodo y temperatura natural. Ejemplares juveniles (90-100 g de peso inicial) fueron muestreados (plasma, hígado y músculo) cada dos meses durante 18 meses (entre diciembre de 2004 y mayo de 2006). Las muestras mostraron variaciones estacionales en la tasa de crecimiento, con valores más altos durante la primavera y el verano. Niveles de metabolitos plasmáticos, hepáticos y musculares, así como las actividades de enzimas metabólicas hepáticas y musculares también presentaron variaciones significativas a lo largo del año. La actividad enzimática relacionada con el metabolismo de carbohidratos en el hígado (HK, FBPasa y G6PDH) mostró altas modificaciones en el verano, el aumento de las vías glucogenogénicas, mientras el metabolismo de aminoácidos (actividades de GDH y GOT) se incrementó en temporadas de primavera y verano. Sin embargo la actividad de G3PDH (enzima metabólica relacionada con los lípidos) no mostró un claro patrón estacional. En el músculo, la actividad enzimática respecto a los aminoácidos, lípidos y el metabolismo del lactato (LDH-O) presentó cambios estacionales en paralelo con los cambios en la tasa de crecimiento, pero el metabolismo de los carbohidratos no presentó cambios estacionales. Así especímenes de *A. regius* mostraron una tendencia a crecer en los meses de verano y movilizar sus reservas de energía en invierno. Se observaron diferencias en el nivel hepático entre el primer y el segundo año de estudio, lo que sugiere la posible existencia de cambios metabólicos relacionados con la edad o el tamaño del espécimen. Nuestros resultados indican que el crecimiento y las respuestas metabólicas en *A. regius* dependen del medio ambiente, pero también indican que esta especie es una muy buena candidata para la diversificación de la acuicultura.

Palabras clave: *Argyrosomus regius*; energía; crecimiento; parámetros metabólicos; esteros; estaciones del año.

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INTRODUCTION

The meagre *Argyrosomus regius* (Asso 1801) is a widely distributed sciaenid along the Atlantic coast (northward to southern Norway and southward to the Congo) and in the entire Mediterranean Sea (Chao 1986). In recent years stagnant markets in the Mediterranean have stimulated the diversification of aquaculture to novel species such as flatfish (*Scophthalmus rhombus, Dicologoglossa cuneata, Solea senegalensis*), sea bream (*Pagrus pagrus*), and croakers (*Umbrina cirrosa*). In this context, *A. regius* became a suitable candidate species for aquaculture diversification (Cárdenas 2010) for several reasons: i) its easy adaptability to captivity (Quéméner 2002); ii) its high capacity to tolerate a wide range of temperatures and salinities (Lavié et al. 2008, Márquez et al. 2010); iii) its high growth rate, reaching 1 kg in ten months of culture (Calderón et al. 1997 Jiménez et al. 2005); and iv) its excellent flesh quality, with high nutritional value and good acceptance by consumers (Quéméner 2002, Poli et al. 2003, Roo et al. 2010, Grigorakis et al. 2011, Giogios et al. 2013).

Several studies have been conducted recently on meagre, mainly focusing on the reproduction of this species using hormone stimulation (Poli et al. 2003, Grau et al. 2007, Duncan et al. 2012, Gil et al. 2013). Other aspects such as larval culture have been clarified by a series of studies focusing on larval growth in captivity (Cárdenas et al. 2008, Vallés and Estevez 2013, Suzer et al. 2013, Papadakis et al. 2013) and studies of food composition have optimized the diet for this species (Piccolo et al. 2008, Chatzifotis et al. 2010, 2012, Estévez et al. 2011, Sáenz de Rodrigáñez et al. 2013). Finally, studies of the growth of *A. regius* in tanks (Pastor et al. 2002, Cárdenas 2010, Estévez et al. 2011, Chatzifotis et al. 2012) and cages (Pastor et al. 2002, García-Mesa et al. 2009) have shown high growth rates in both systems. However, only a few studies have tested the maintenance of this species in earthen ponds (Jiménez et al. 2005), a culture system used mainly for aquaculture activity in Mediterranean countries such as Spain, Portugal (Dinis et al. 1999) and even Egypt (El-Shebly et al. 2007). In a preliminary study, Muñoz et al. (2008) analysed the growth of *A. regius* juveniles (22-30 g weight) maintained in these earthen ponds (November 2006 to December 2007) under two different environmental salinities (sea water 35.7±2.0‰ and brackish water 13.1±2.8‰), finding better responses in specimens grown under isosmotic environments.

These earthen ponds are quite shallow (Arias 1976) and large variations in abiotic variables (such as salinity, temperature and photoperiod) have a great influence on the physiology of cultured species. Seasonal changes in those abiotic factors affect and modulate the physiological responses, which force fish to vary osmoregulatory, metabolic and growth rates for efficient environmental adaptation (Boeuf and Payan 2001, Vargas-Chacoff et al. 2009a). Low water temperatures could induce fasting in cultured species, modifying their metabolic status and triggering an allostatic overload (Polakof et al. 2006, Estévez et al. 2011), as occurs in the gilthead seabream (*Sparus aurata*) (Ibarz et al. 2010). On the other hand, short but intensive rainfall in autumn/winter (usual in southwestern Spain) can decrease water salinity in earthen ponds, inducing osmotic and consequently metabolic challenges in cultured fish (Vargas-Chacoff et al. 2009a). To the best of our knowledge, metabolic approaches have not been performed in *A. regius*.

The aim of this study was to provide key information on the metabolic changes in *A. regius* specimens cultured in earthen ponds under natural environmental conditions during an eighteen-month period (this period was chosen because it is the time expected commercial growth of this species). This information can be useful for optimizing its culture, as it may indicate seasonal metabolic behaviour that can be used to determine the best maintenance conditions as an alternative to aquaculture practice in tanks and cages.

MATERIALS AND METHODS

Abbreviations

BSA: Bovine serum albumin
FBPase: Fructose 1,6-bisphosphatase (EC 3.1.3.11)
G6PDH: Glucose 6-phosphate dehydrogenase (EC 1.1.1.49)
GDH: Glutamate dehydrogenase (EC 1.4.1.2)
G3PDH: Glycerol 3-phosphate dehydrogenase (EC 1.1.1.8)
GOT: Aspartate aminotransferase (EC 2.6.1.1)
HK: Hexokinase (EC 2.7.1.1)
LDH-O: Lactate dehydrogenase-oxidase (EC 1.1.1.27)

Animals and maintenance

Juveniles of meagre (*A. regius,* n=90 total, n=10 for each sampled period) were supplied by a local fish farm (ACUINOVA, San Fernando Cádiz, Spain). According to the fish farm procedure, specimens (n=6500, 12.0±0.5 g weight) were placed in natural earthen ponds (88 m length × 15 m width × 1.5 m depth, total volume: 1980 m^3) in September 2004 at an initial density of 0.039 kg m^{-3}. Specimens were sampled approximately every two months for 18 months (2/12/2004, 21/2/2005, 13/4/2005, 30/5/2005, 27/7/2005, 26/9/2005, 22/11/2005, 8/3/2006 and 23/5/2006). In the first sample (2/12/2004) the fish weighed 90-100 g, while the final weight was 1100-1400 g at a final density of 4 kg m^{-3}. The mortality during the experimental period was 10%. Fish were maintained in earthen ponds under natural photoperiod, temperature and salinity regimens. Salinity was measured with an ATAGO S/MILL refractometer and temperature was measured with a digital thermometer. Both parameters were measured each day at the same time (12:00); Figure 1 shows the average variations of temperature and salinity over time (months). Fish were fed daily according to fish farm procedures (2% of body weight) with commercial dry pellets for sea bream (Dibaq-Diproteg SA, Segovia, Spain) (see Table 1 for nominal food composition). They were fasted 24

Table 1. – Proximate composition of the diet used during the experiment (% dry weight).

Pellet	Protein	Fat	Carbohydrates	Fibre	Ash	Total P	Digestible energy (Mj/Kg)
Sea bream (60-200g)	46	21	11.07	1.4	9.1	1.2	19.7
Sea bream (190-600g)	44	22	11.47	1.5	8.5	1.1	19.8
Sea bream (>600g)	42	22	14.20	1.7	8.0	1.05	19.9

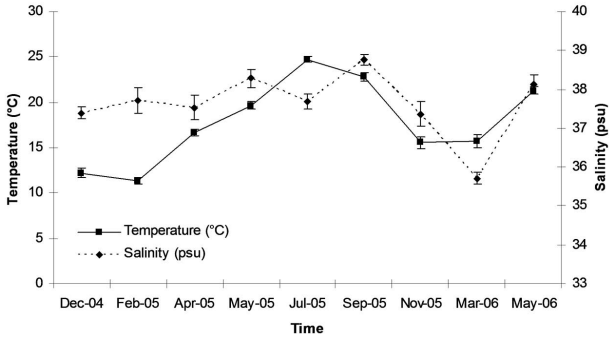

Fig. 1. – Average values for water temperature (°C) and salinity (‰) during the experimental period. Data are shown as mean±SEM. Each factor was measured daily; the average is for number of days between sampling points.

h before sampling. All experimental procedures described complied with the Guidelines of the European Union Council (86/609/EU) for the use of animals in research.

Sampling procedure

Fish were netted, submitted to lethal doses of 2-phenoxyethanol (1 mL L^{-1}) and completely euthanized by spinal section before tissues were removed, weighed and sampled (n=10 per time-point). The time between capture and sampling was always less than 3 minutes). Blood was collected from the caudal peduncle into 1-mL syringes rinsed with a solution containing 25000 units of ammonium heparin per 3 mL 0.6% NaCl. Plasma was separated from cells by centrifugation of whole blood (3 min, 10000 × g, 4°C), snap-frozen in liquid N_2 and stored at –80°C until analysis. Liver and muscle were removed from each fish, the liver was weighed and both were freeze-clamped in liquid nitrogen and stored at –80°C until further assay.

To evaluate seasonal effects on fish performance, several biometric indices were calculated:

- Fulton's condition factor (K) was calculated as $K=100 \cdot W/L^3$, where W= fish weight (g) and L= total length (cm).

- Hepatosomatic index (HSI) = (liver weight/total weight)*100

- Specific growth rate (% daily SGR) was calculated as $SGR=100(\text{Ln } W_f - \text{Ln } W_i)/T$, where W_f = final body weight (g), W_i = initial body weight (g) and T is number of days between weighings.

Plasma determinations

Plasma glucose, triglycerides and lactate levels were measured using commercial kits from Spinreact (Glucose-HK Ref. 1001200; Triglycerides Ref. 1001311; Lactate Ref. 1001330) adapted for 96-well microplates. Plasma total proteins were determined in 1:50 (v/v) diluted plasma samples using the bicinchoninic acid BCA Protein Assay Kit (Pierce #23225). All assays were performed with a Bio Kinetics EL-340i Automated Microplate Reader (Bio-Tek Instruments) using DeltaSoft3 software for Macintosh (BioMetallics Inc.).

Tissue metabolites and enzymatic activities

Frozen liver and muscle were finely minced in an ice-cooled Petri dish, vigorously mixed and divided into two aliquots to assess enzyme activities and metabolite levels. The frozen tissue used for the assessment of metabolite concentration was homogenized by ultrasonic disruption with 7.5 volumes of ice-cooled 0.6 N perchloric acid, neutralized (using 1 mol L^{-1} potassium bicarbonate) and centrifuged (30 min, 13000 g, 4°C, Eppendorf 5415R), and the supernatant was used to assay tissue metabolite levels. Tissue lactate and triglyceride levels were determined spectrophotometrically using commercial kits (Spinreact, see before). Tissue glycogen concentrations were assessed using the method of Keppler and Decker (1974). Glucose obtained after glycogen breakdown (after subtracting free glucose levels) was determined with a commercial kit (Spinreact, see above). Total α-amino acid (Total aa) levels were assessed colorimetrically using the ninhydrin method of Moore (1968) adapted to a microplate assay. The aliquots of tissues used for the assessment of enzyme activities were homogenized by ultrasonic disruption in 10 volumes of ice-cold stopping-buffer containing 50 mM imidazole-HCl (pH 8.5), 1 mM 2-mercaptoethanol, 50 mM NaF, 4 mM EDTA, 250 mM sucrose, and 0.5 mM p-methyl-sulphonylfluoride (Sigma Chemical Co., St. Louis, MO, USA), the last added as dry crystals immediately before homogenization. The homogenates were centrifuged (30 min, 13000 g, 4°C) and the supernatants were stored in different aliquots at –80°C until use in enzymatic assays. Enzyme activities were determined using a PowerWave™ 340 microplate spectrophotometer (BioTek Instruments, Winooski, VT, USA) with KCjunior Data Analysis Software for Microsoft® Windows XP. Reaction rates of enzymes were determined by changes in absorbance of NAD(P)H at 340 nm. The reactions were started by the addition of homogenates (15 µL) at a pre-established protein concentration, omitting the substrate in control wells (final volume of 275-295 µL) and allowing the reactions to proceed at 37°C (5-15 min). Protein levels were assayed in triplicate as in plasma samples. Enzymatic analyses were carried out at conditions meeting requirements for optimal velocities. The specific conditions for the assay of HK, FBPase, G6PDH, GDH, GOT, G3PDH, and LDH-O have been described previously (Laiz-Carrión et al. 2003, Sangiao-Alvarellos et al. 2003, Sangiao-Alvarellos et al. 2005, Polakof et al. 2006, Vargas-Chacoff et al. 2009b).

Statistics

Data were checked for normality, independence and homogeneity of variance before one-way analysis of variance was conducted using months (time) as a factor. Tukey's a posteriori test was used to identify significantly different groups. Logarithmic transformations of the data were performed when necessary to fulfil the conditions of the parametric analysis of variance. Statistical significance was accepted at $P<0.05$.

RESULTS

Water conditions

The abiotic water parameters temperature and salinity varied throughout the year, showing relevant temperature differences (maximum of 25°C in summer and minimum of 11°C in winter), while salinity showed sudden fluctuations between 36% and 39% (Fig. 1).

Biometrics

Length and weight showed a similar trend, with a strong linear relationship ($R^2=0.94$) between these parameters (Fig. 2). Condition factor (K), hepatosomatic index (HSI) and specific growth rate (% daily SGR) of specimens in each sampling point are shown in Table 2.

Plasma

Plasma metabolite levels are given in Table 3. All parameters showed significant modifications due to seasonal effects. Glucose concentrations showed the lowest values in summer ($P<0.05$) and lactate during winter ($P<0.05$). However, proteins showed no clear pattern of change, but were at maximum levels in late winter and spring. Maximum levels of triglycerides were observed in late winter and early spring ($P<0.05$).

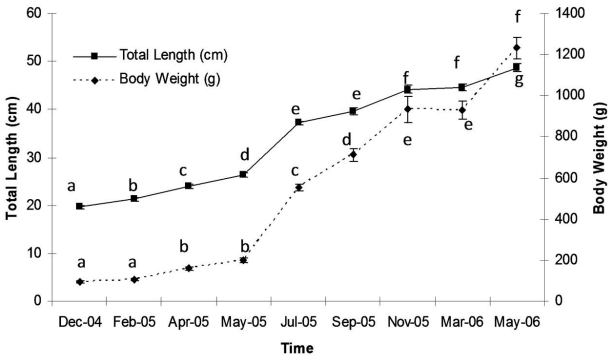

Fig. 2. – Changes in body weight (A) and total length (B) over 18 months in *A. regius* specimens cultured in an earthen pond. Data points represents mean±SEM (n=10 per group at each sampling time). Different letters indicate significant differences among sampling points ($P<0.05$, one-way ANOVA, Tukey test).

Liver

Time-related metabolic changes are shown in Table 4 (metabolite levels) and Figure 3 (enzymatic activities). Liver glycogen and glucose levels showed an inverse relationship, with glycogen stores increasing while free glucose levels decreased. Liver proteins and amino acid levels showed different patterns of change, with the highest protein values in spring and the highest

Table 2. – Seasonal growth performance indices in *A. regius* specimens cultured in earthen ponds. Further details as in legend of Figure 2.

	Dec-04	Feb-05	Apr-05	May-05	Jul-05	Sep-05	Nov-05	Mar-06	May-06
Hepatosomatic index (%)	1.63±0.01a	1.39±0.02b	1.00±0.01c	1.85±0.03d	1.81±0.01d	1.01±0.01c	1.14±0.03b	1.09±0.03b	1.18±0.01b
Condition factor	1.23±0.03a	1.11±0.02b	1.16±0.02b	1.09±0.02b	1.08±0.02b	1.14±0.03b	1.08±0.03b	1.05±0.02b	1.07±0.02b
Specific growth rate (% daily)	0.00	0.18	0.78	0.40	1.76	0.41	0.48	0.00	0.37

Table 3. – Seasonal changes in plasmatic metabolite levels in *A. regius* specimens cultured in earthen ponds. Further details as in legend of Figure 2.

	Dec-04	Feb-05	Apr-05	May-05	Jul-05	Sep-05	Nov-05	Mar-06	May-06
Glucose (mM)	6.01±0.77a	5.43±0.46a	5.82±0.60a	3.80±0.27b	2.67±0.19c	5.65±0.58a	6.51±0.39a	6.38±0.35a	3.66±0.30b
Lactate (mM)	1.33±0.08a	2.74±0.23bc	3.01±0.39bc	1.77±0.17b	3.72±0.46c	3.71±0.44c	2.0±0.24b	2.63±0.18bc	3.25±0.42c
Protein (mg/mL)	35.61±1.32ab	37.10±1.44b	33.06±1.49ab	36.67±0.67b	32.54±1.23ab	33.54±1.08ab	29.94±1.58a	35.20±1.71b	35.88±0.94b
Triglycerides (mM)	3.03±0.17a	4.32±0.33b	6.93±0.17c	3.40±0.17ab	2.43±0.40a	2.89±0.10a	1.37±0.2d	3.67±0.35ab	2.97±0.25a

Table 4. – Seasonal changes in hepatic metabolite levels in *A. regius* specimens cultured in earthen ponds. Further details as in legend of Figure 2.

	Dec-04	Feb-05	Apr-05	May-05	Jul-05
Glucose (µmol/hepatic unit)	20.99±2.97a	17.78±1.25a	23.84±2.02a	23.16±2.07a	78.01±4.85b
Glycogen (µmol glucidic units/hepatic unit)	55.09±3.39ab	43.74±4.39ab	65.64±6.82a	34.09±6.48b	139.54±14.11cde
Protein (µmol/hepatic unit)	37.89±6.19ab	27.90±2.73a	47.13±4.92b	39.42±3.12b	113.13±9.11c
Total Aa (µmol/hepatic unit)	219.83±18.64a	101.43±7.71b	114.31±11.53b	83.16±14.61b	252.17±47.56a
Triglycerides (µmol/hepatic unit)	5.14±0.82a	2.29±0.33b	3.91±0.77ab	5.19±0.77a	15.98±2.68c

	Sep-05	Nov-05	Mar-06	May-06
Glucose (µmol/hepatic unit)	152.20±7.82c	196.11±16.83cd	170.10±12.02cd	206.76±14.77d
Glycogen (µmol glucidic units/hepatic unit)	144.13±16.71cde	153.12±10.47cde	110.49±15.20d	181.976±15.24e
Protein (µmol/hepatic unit)	176.80±15.26d	231.35±22.80d	173.30±13.18d	228.79±14.99d
Total Aa (µmol/hepatic unit)	240.78±27.11a	673.54±118.85c	599.54±42.28c	3 79.27±29.08d
Triglycerides (µmol/hepatic unit)	32.61±4.88d	30.01±2.51d	24.21±0.89d	15.83±2.12c

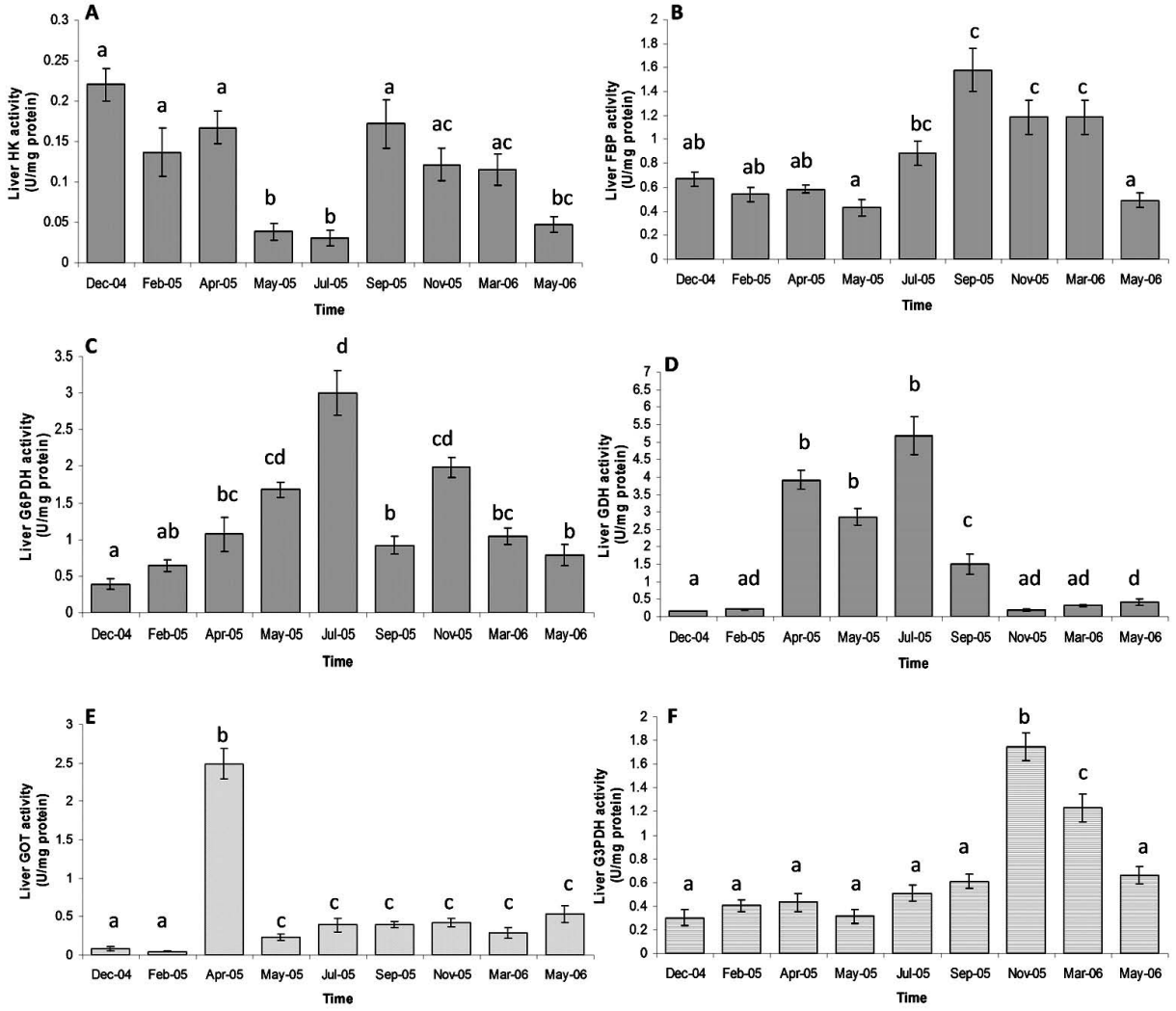

Fig. 3. – Seasonal changes in liver HK (A), FBP (B), G6PDH (C), GDH (D), GOT (E) and G3PDH (F) activities in *A. regius* specimens cultured in earthen ponds. Further details as in legend of Figure 2.

Table 5. – Seasonal changes in muscle metabolite levels in *A. regius* specimens cultured in earthen ponds. Further details as in legend of Figure 2.

	Dec-04	Feb-05	Apr-05	May-05	Jul-05
Glucose (μmol/g wet weight)	6.84±0.33a	5.18±0.20b	6.63±0.20a	6.18±0.08a	6.46±0.44a
Glycogen (μmol glucidic units /g wet weight)	0.31±0.05a	0.37±0.05ab	1.33±0.09c	1.07±0.09c	0.46±0.09b
Lactate (μmol/g wet weight)	0.91±0.05a	0.95±0.07a	0.84±0.06a	0.74±0.06a	1.36±0.07b
Protein (μmol/g wet weight)	8.17±0.32a	7.66±0.46a	7.90±0.37a	7.55±0.37a	6.47±0.21b
Total Aa (μmol/g wet weight)	34.99±3.87a	47.76±3.49ab	44.36±3.28ab	50.68±3.44b	37.22±4.02a
Triglycerides (μmol/g wet weight)	0.13±0.03	0.05±0.01	0.08±0.02	0.08±0.01	0.23±0.13
	Sep-05	Nov-05	Mar-06	May-06	
Glucose (μmol/g wet weight)	5.70±0.14b	5.45±0.11b	5.68±0.21b	5.55±0.41b	
Glycogen (μmol glucidic units /g wet weight)	0.58±0.13b	0.52±0.16b	0.58±0.16b	0.27±0.07a	
Lactate (μmol/g wet weight)	1.37±0.11b	1.24±0.06b	1.05±0.08ab	1.57±0.07b	
Protein (μmol/g wet weight)	6.03±0.25b	6.59±0.23b	6.53±0.30b	6.56±0.23b	
Total Aa (μmol/g wet weight)	34.52±3.49a	43.63±3.35ab	42.53±2.39ab	48.35±2.80b	
Triglycerides (μmol/g wet weight)	0.21±0.01	0.17±0.05	0.08±0.01	0.12±0.02	

amino acid levels in winter (P<0.05). Liver triglyceride content showed no clear seasonal pattern. Enzymatic activities related to carbohydrate metabolism (HK, FBPase and G6PDH) showed statistical differences between seasons; HK activity decreased in summer (Fig. 3A) while FBPase and G6PDH increased in summer and autumn, respectively (Fig. 3B, C). However, amino acid-related enzymes (GDH and GOT activities) were enhanced in spring and summer, while lipid-related (G3PDH activity) metabolic enzymes showed no clear seasonal pattern (P<0.05) (Fig. 3D-F).

Muscle

Seasonal changes of metabolic parameters in muscle are shown in Table 5 (metabolite levels) and Figure

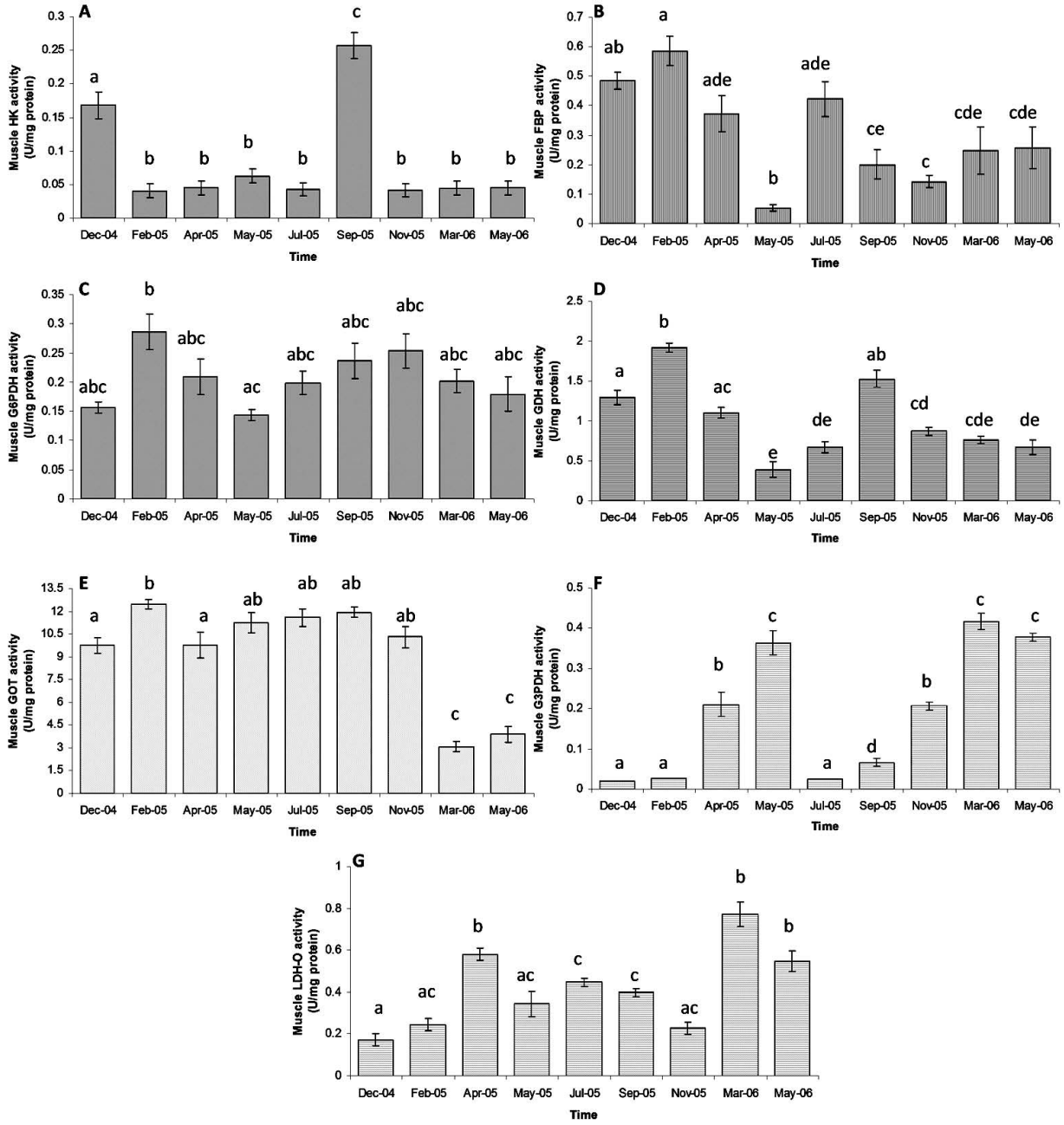

Fig. 4. – Seasonal changes in muscle HK (A), FBP (B), G6PDH (C), GDH (D), GOT (E), G3PDH (F) and LDH-O (G) activities in *A. regius* specimens cultured in earthen ponds. Further details as in legend of Figure 2.

4 (enzymatic activities). Muscle glycogen content increased in mid- to late spring (P<0.05), while glucose levels showed no clear pattern of change; lactate levels were elevated after summer. Protein content showed significant variations between seasons (P<0.05), while amino acid values increased in mid- to late spring. Triglycerides showed no statistical differences throughout the year. Carbohydrate metabolic enzymes (HK, FBPase and G6PDH) showed statistical differences between seasons: i) HK had high activity in autumn and winter, ii) FBPase decreased in spring and fall, and iii) G6PDH decreased mainly in spring but values were maintained high throughout the year (Fig. 4A-C). Amino acid–related metabolic enzyme (GDH activity) was lowest in summer (P<0.05, Fig. 4D). No pattern of variation was distinguishable for GOT activity (Fig. 4D, E). Lipid-related metabolic enzyme (G3PDH activity) showed seasonal variation, with the highest activity occurring in spring (P<0.05) (Fig. 4F). Lactic-related metabolic enzyme activity (LDH-O activity) was highest in spring and summer (P<0.05) (Fig. 4G).

DISCUSSION

The meagre (*Argyrosomus regius*) showed variations in growth rate that correlated well with the seasonal metabolic changes observed. Specimens showed the highest growth rates during spring and summer, when environmental temperatures are highest (20-25°C), while growth was lower in the cold winter months. It is

interesting that the growth enhancement was greater in the second spring (from March/06 to May/06) than in the first spring (from April/05 to May/05). This difference could be attributed to a greater temperature increase in the second spring (from 16°C to 22°C) than in the first (from 16°C to 20°C). However, this better capacity to recover from metabolic stress associated with the winter season could also be attributed to two factors: i) the greater size of specimens in the second spring, and/or ii) the possibility that the fish population contained stronger specimens, survivors from the first year of culture (Estévez et al. 2011). Many studies have been performed with *A. regius* using different culture systems (cages, tanks or earthen ponds), so the data available to compare the growth rates between them should be treated with care (Cárdenas 2010). The growth of *A. regius* appears to be higher in cages than in tanks and earthen ponds. In cages, juveniles of 110 g reached 1850 g in 8 months (a growth rate of 217.5 g per month) (Pastor et al. 2002); while in tanks, specimens of 230 g reached 380 g in 3.5 months (a growth rate of 42.8 g per month) (Chatzifotis et al. 2010). These contrasts could be attributed to many different reasons such as food and water quality and presence/absence of parasites, so no clear conclusions can be drawn from those studies. Our results showed that specimens of 90 g kept in natural earthen ponds reached 1231.3 g in 18 months (a growth rate of 63.3 g per month), indicating that *A. regius* cultured in this culture system had good growth rates and condition factor indexes (Cárdenas et al. 2008). Our results also indicate that metabolism showed temporal variation, with plasmatic, hepatic and muscular metabolites as well as hepatic and muscular metabolic enzymes showing significant variation throughout the seasons of the year.

In plasma, glucose showed its lowest values in spring and summer, while lactate showed its lowest levels in spring and autumn. These results are different from those described for other species, such as *S. aurata* (Gómez-Milán et al. 2007, Vargas-Chacoff et al. 2009a) and *Oreochromis mossambicus* (Fiess et al. 2007). In the present study, the lowest glucose or lactate concentrations in these months are associated with changes in hepatic parameters related to glucose or lactic acid metabolism (see below), and could indicate a reduction in the hepatic capacity to produce these metabolites, focusing these resources into somatic growth (Foster and Moon 1991, Sala-Rabanal et al. 2003). In addition, in spring (April) plasmatic levels of proteins, triglycerides (TAG) and liver glycogen increased, while HSI decreased (probably due to hepatic TAG mobilization), followed by a depletion in plasma glucose, proteins and TAG as well as liver glycogen (May-July), which may also be related to an acceleration in the growth of the animals (Gallardo et al. 2003, Sala-Rabanal et al. 2003, Ibarz et al. 2005, 2007, Vargas-Chacoff et al. 2009a, b). In winter (November), metabolic pathways have to confront a critical situation due to lower temperatures (Somero 2004). Plasmatic levels of lactate, TAG and proteins dropped and maintained a proper homeostasis by conversion to glucose, as indicated by the increased gluconeogenesis pathways such as liver FBPase in autumn and winter. Under these conditions there is a tendency in *A. regius* specimens to mobilize fat deposits, which may explain the observed enhancement in plasma triglyceride values. This situation agrees with previous results reported in *S. aurata* in winter (Tort et al. 1998, 2004, Gallardo et al. 2003, Vargas-Chacoff et al. 2009a). Finally, plasma proteins levels increased previous to each important growth increase period (spring), and decreased during summer months, supporting muscle (somatic) growth. A similar situation has been reported for *S. aurata* (Chaves-Pozo et al. 2008, Vargas-Chacoff et al. 2009a). Gómez-Millán et al. (2007) observed in specimens of *S. aurata* an inverse pattern of variation in plasma amino acids levels with respect to environmental temperature (with the lowest values in cool months and the highest in warm months). This pattern has been related to a higher growth rate during spring and summer months, where available plasma amino acids are necessary to synthesize structural proteins (Vargas-Chacoff et al. 2009a).

Hepatic energy metabolism processes (i.e. glycogen/glucose turnover, ammoniagenesis, fatty acid synthesis and gluconeogenesis) were affected by temporal changes, and could induce physiological changes associated with differences on growth. Variations in metabolite levels and enzymatic activities related to different abiotic (i.e. salinity, temperature) or biotic (i.e. reproduction, growth) factors have been reported in teleost species (Gómez-Millán and Sánchez-Muros 2007, Gómez-Millán et al. 2007, Arjona et al. 2009, Herrera et al. 2009, Arjona et al. 2010).

Glycogen and triglyceride content as well as HSI presented a clear pattern depending on the developmental stage of specimens and the season. The glycogen consumed in autumn is related to the increase in HK activity, glucose being its major export product, as observed in plasma glucose levels. However, in the winter moths (February/05-march/06) the lowest values of glycogen were observed, in agreement with the idea of decreased food intake due to low water temperatures and the use of this compound as an energy source (Navarro et al. 1997, Sala-Rabanal et al. 2003). This differential behaviour of hepatic glycogen stores depending on the age and/or size of the specimens deserves future research. FBPase is a key gluconeogenic enzyme that enhances liver glucose production. The results showed an increased FBPase activity at the beginning of the autumn in the second year. Taking all this evidence together, the increased total FBPase activity may indicate an upregulation of gluconeogenesis at this time, which would explain the high hepatic glucose levels observed in *A. regius* during this period. A similar FBPase activity profile has been reported in *S. aurata* (Gómez-Millán et al. 2007).

Another enzymatic activity related to carbohydrate metabolism, G6PDH, increased in level during spring months of the first year, fuelling the hepatic pentose shunt with glucose, which was enhanced in that season. An activated pentose phosphate pathway suggests an increased reducing capacity of liver, which may be related to a rise in lipid synthesis in agreement with reports for other species (Laiz-Carrión et al. 2005,

Sangiao-Alvarellos et al. 2005, Arjona et al. 2009) and with the highest plasma triglyceride levels observed in spring of the first year in specimens of *A. regius* (present results). In summer the free amino acids in the liver decreased, probably because they are transported into the muscle, where they are deposited as muscle structural proteins. As the animal grows in summer, active routes are formation (anabolism: high activity of GDH and GOT) and not degradation (catabolism), in agreement with similar studies in other species such as *S. aurata*, where high anabolic-related enzymatic activities and plasma amino acid levels were also found in summer (Gómez-Millán et al. 2007, Vargas-Chacoff et al. 2009a).

The muscle metabolism showed changes in metabolite values and enzymatic activities, but without a clear seasonal pattern. The G6PDH enzyme increased its activity in winter, when the animal does not eat or reduces its rate of intake and is supported from body reserves. Thus, in winter fish consume muscle reserves and energy is obtained via gluconeogenesis (as indicated by the high levels of FBPase in February 2004) from amino acids (Mommsen et al. 1999). This coincides with muscle HK activity, which is higher in winter months (producing glucose-6-phosphate, which would go to the pentose phosphate pathway and glycolysis), or GOT activity, which increased in this case and in February of the first year, possibly indicating a muscle catabolism that obtains basic metabolites capable of entering these energy production routes.

The TAG are used in part by the muscle to produce glycerol, and subsequently energy and reducing power via glycolysis. In this study, muscle TAG showed no variations throughout the year, but G3PDH activity increased in spring. This could be due to a better use of fats from the food. By comparison with other studies addressing the muscle levels of TAG in red porgy (*P. pagrus*) (Vargas-Chacoff et al. 2011), it can be inferred that their amount would not be related to the period of the year or even to a stressful situation.

The LDH-O in muscle showed its highest values in spring, when temperatures became warmer. This result points to an increase in lactate oxidation rates by those tissues involved in thermal accommodation that are able to use lactate as fuel (Vargas-Chacoff et al. 2009a, b). A very large fraction of the energy stored as glycogen in white muscle can be provided in the form of lactate, which besides being re-converted to glycogen in situ (Schulte et al. 1992) can also be sent to oxidative tissues via the bloodstream (Weber 1992).

In summary, the results of this study indicate that growth and metabolic responses in *A. regius* are time-dependent and position this species as a very good candidate for diversification in aquaculture. The growth of this species decreased in the coldest months, so it would be advisable to concentrate efforts on warmer growing environments or warmer months, so animals would grow faster and be more cost effective. The use of earthen ponds as a culture system for meagre appears to be a good alternative for countries that have the conditions to use them, because they are much easier to manage than offshore cultivation systems.

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