Low stocking density negatively affects growth, metabolism and stress pathways in juvenile specimens of meagre (*Argyrosomus regius*, Asso 1801)

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Abstract

The influence of culture density on growth, stress system and metabolism has been assessed in *A. regius* juveniles submitted to different initial stocking densities (3, 7, 10 and 13 g L⁻¹) for 40 days. On days 0, 12, 27 and 40 of the experiment, biometric parameters were measured. In addition, plasma, liver and muscle samples were taken for biochemical analysis at the end of experimental time. Our results showed better growth rates and feed utilization in those specimens held at high stocking densities when compared to those under low stocking densities. In addition, higher plasma cortisol, glucose and triglycerides levels found in the lowest stocking density tested suggest an activation of the stress system in fish held at this condition. Moreover, no changes in hepatic metabolites were observed, while values of muscle triglycerides, amino acids and lactate enhanced when stocking density increased. In conclusion, the culture of juvenile *A. regius* at higher initial densities (at least 13 g L⁻¹) ensures better growth and the optimization of the existing facilities, improving welfare of this species in aquaculture systems.

Key words: *Argyrosomus regius*, growth, meagre, metabolism, stocking density, stress.

1. INTRODUCTION

The meagre (*Argyrosomus regius*, Asso 1801) is a gregarious species, which shifts in groups. It is very voracious, with feeding habits based on smaller fish species (Cupleidae and Mugilidae families) and crustaceans (Jiménez et al., 2005). Thus, fishing pressure, environmental pollution and degradation of spawning areas (Sadovy and Cheung, 2003) have led to a decline in the wild Mediterranean Sea populations (Quero, 1989).

Aquaculture of this species is fairly recent and can be cultured in different types of installations (Cárdenas, 2010; Jiménez et al., 2005). According to FAO (Monfort, 2010) and other studies, most of the harvest that comes from intensive production is carried out in onshore tanks, cages and earthen ponds (Ortega and De la Gándara, 2007; Rodríguez-Rúa et al., 2007; Vargas-Chacoff et al., 2014), although higher growth rates have been reported when its culture is carried out in cages (Pastor et al., 2002). There are a number of features that make *A. regius* promising and attractive to producers, such as its growth potential, high fecundity and good acceptance by consumers (Cárdenas, 2010; Jiménez et al., 2005). In addition, several authors have noted the importance of developing the aquaculture activity of meagre for conservation purposes. In this context, *A. regius* became a suitable candidate for aquaculture diversification (Cárdenas, 2010) for several reasons: i) easy adaptability to captivity (Quémener, 2002); ii) eurythermic and euryhaline capacities (Lavié et al., 2008; Ruiz-Jarabo et al., submitted); iii) high growth rate, reaching around 2 kg in ten months of culture (Calderón et al., 1997; Jiménez et al., 2005); and iv) high nutritional value and good acceptance by consumers (Giogios et al., 2013; Grigorakis et al., 2011; Poli et al., 2003).

In this sense, the good adaptive responses of animals to farming conditions will enable to the improvement of growth and reproduction processes of this species. Furthermore, actual culture processes tend to minimize the occurrence of stressors, improving animal welfare and thus obtaining a profitable production due to reduced incidence of physiological and

behavioural disorders (Ashley, 2007; Conte, 2004; Pickering, 1998; Van Weerd and Komen, 1998). One strategy to increase the benefits is the optimization of space, increasing the stocking density culture in the harvest. However, a situation of overcrowding activates stress system, increasing plasma catecholamines and cortisol as a first consequence. Cortisol enhances metabolic rate, glycogenolysis, lipolysis, and is associated with degradation of muscle proteins (Laiz-Carrión et al., 2003; Mommsen et al., 1999). In fact, high cortisol levels caused by stressful situations induce an enhancement in metabolic processes to produce an extra energy supply (Mommsen et al., 1999; Wendelaar Bonga, 1997). All of these internal disorders could negatively interfere with other physiological processes such as growth, reproduction and immune system (Barton and Iwama, 1991; Pickering, 1998). Hence, it is commonly accepted that high stocking densities generally leads to increased cortisol levels in different fish species (Ashley, 2007; Wendelaar Bonga, 1997). However, a negative effect on growth rates related to low stocking densities has been reported in Japanese meagre (*Argyrosomus japonicus*). This fact could be attributed to the gregarious nature of this species, which requires the grouping in shoals to avoid stressful situations (Pirozzi et al., 2009).

Thus, the main objective of this study was to analyse the physiological modifications related to growth, feed utilization, stress and metabolic parameters in specimens of juvenile meagre (*Argyrosomus regius*) held under different stocking densities during their fattening in tanks.

2. MATERIAL AND METHODS

2.1. Animals and experimental procedures

Juvenile specimens of *A. regius* ($n = 200$) (82.96 ± 3.12 g body mass, and 20.2 ± 1.9 cm standard length) came from a stock population of the *IFAPA Centro “El Toruño”* (El Puerto de Santa María, Cádiz, Spain). Fish acclimated to our facility conditions (S%, pH, temperature, water hardness, etc.) were randomly distributed, in duplicate, in eight 250 L-fibreglass tanks continuously aerated in a flow through system. In each tank, the number of specimens was adjusted in order to get the four initial different experimental densities assessed: i) low stocking density (LSD, 3 g L⁻¹; 10 individuals·tank⁻¹), ii) medium stocking density (MSD, 7 g L⁻¹; 20 individuals·tank⁻¹), iii) high stocking density (HSD, 10 g L⁻¹; 30 individuals·tank⁻¹), and iv) extra high stocking density (EHSD, 13 g L⁻¹; 40 individuals·tank⁻¹). During the 40 days (April-May, 2010) of the experiment, oxygen concentration was always above the recommended values for the culture of this species (> 5 mg L⁻¹). Thus, it was achieved through control of the flow input to the different tanks set at 2, 4, 6 and 8 L min⁻¹ for LSD, MSD, HSD and EHSD conditions, respectively. Both stocking densities and experimental period were achieved according with previous studies performed in this species and others, where important changes in growth rates, metabolism and endocrine parameters have been reported (de las Heras et al., 2015; Laiz-Carrión et al., 2012; Menezes et al., 2015; Pirozzi et al., 2009; Roo et al., 2010; among others). Parameters such as nitrites, nitrates and ammonium (measured weekly), as well as pH and turbidity (daily measured) were also analysed to ensure the water quality required. Fish were fed 4 times a day (distributed throughout the daylight) with a total of 2 % of their body mass (Skretting, Burgos, Spain) using Eheim 3581 “Feed-Air” digital automatic feeders (EHEIM GmbH & Co KG, German). No food remains were observed on the bottom of the tank after feeding procedures.

2.2. Sampling

At 0, 12, 27 and 40 days after starting the experiment, 10 fish from each experimental tank ($n = 20$ per density) were anesthetized with a dose of 0.4 mL L^{-1} (40 ppm) of clove oil previously dissolved in ethanol at a ratio of 1:9, and sampled for body mass (BM) and standard body length (BL). These sampling points were used to adjust food ration to maintain the initial 2 % of their body mass during the experiment. In addition, five specimens of each tank ($n = 10$ per density) were anaesthetized with a lethal dose of clove oil (0.7 mL L^{-1}), killed by decapitation and sampled at the end of the experiment (40 days after the start of the experiment). Blood was collected from the caudal peduncle with ammonium-heparinized syringes (Sigma, H-6279, 25000 units / 3 mL of saline 0.9 % NaCl). Plasma was obtained by centrifugation of whole blood (3 min, 10850 rpm, 4 °C) and stored at -80 °C until analysis. Then, the liver was removed from the visceral package and weighed separately to calculate hepatosomatic index (HSI), whereas white muscle samples were obtained from the back of the fish. Both samples were immediately snap-frozen in liquid nitrogen and stored at -80 °C for further analysis of metabolites. Animals were fasted 24 hours before sampling. The experiment was performed following the Guidelines of the European Union (2010/63/UE) and the Spanish legislation /RD 1201/2005 and law 32/2007) for the use of laboratory animals.

2.3. Feed data

Weight increase and feed consumption were used to calculate the following nutritional and biometrical indexes: weight gain (WG, g) weight gain (WG, %), feed conversion ratio (FCR), condition factor (K), feed efficiency rate (FER), specific growth rate (SGR) and specific feed rate (SFR).

137 $WG\ (g) = BM_f\ (g) - BM_i\ (g)$

138 $WG\ (\%) = [(BM_f\ (g) - BM_i\ (g)) / BM_i\ (g)] * 100$

139 $FCR = FI_T\ (g) / WG_T\ (g)$

140 $K\ (\%) = 100 * (BM\ (g) / BL^3\ (cm))$

141 $FER\ (\%) = 100 * (\Delta BM\ (g) / FS_t\ (g))$

142 $SGR\ (\% \cdot day^{-1}) = 100 * [(\ln BM_f - \ln BM_i) / t\ (days)]$

143 $SFR\ (\% \cdot day^{-1}) = 100 * (FS_d\ (g) / BM_T\ (g)) / t\ (days)$

144 Where BL = fish body length; BM = fish body mass; BM_f = final body mass; BM_i = initial body
 145 mass; BM_T = total body mass; ΔBM = body mass increase; FI_T = total food intake; FS_d = daily
 146 food supplied; FS_t = total food supplied; t = time; WG_T = total weight gain.

147

148 **2.4. Plasma and tissue parameters**

149 For the assessment of tissue metabolite levels, samples from liver and muscle were finely
 150 minced on an ice cold Petri dish, and subsequently homogenized by mechanical disruption
 151 (Ultra-Turrax, T25basic, IKA ®-WERKE) with 7.5 vol. (w/v) of ice-cool 0.6 N perchloric
 152 acid and neutralized after the addition of the same volume of 1 M $KHCO_3$. After that, the
 153 homogenates were centrifuged (30 min, 13000 rpm, 4 °C) and the supernatants were
 154 recovered in different aliquots, which were stored at -80 °C until used in metabolite assays.
 155 Metabolite concentrations in plasma (glucose, lactate, triglycerides), liver (glucose and
 156 triglycerides) and muscle (glucose, lactate and triglycerides) were obtained using commercial
 157 kits from Spinreact (Barcelona, Spain) (Glucose-HK Ref. 1001200; Lactate Ref. 1001330;
 158 Triglycerides ref. 1001311) with reactions adapted to 96-well microplates. Liver and muscle
 159 glycogen levels were assessed using the method from **Keppler and Decker (1974)**, in which
 160 the glucose obtained after glycogen breakdown (after subtracting free glucose levels) was
 161 determined using the commercial kit described above for glucose. Plasma total proteins

analysis was carried out using the bicinchoninic acid method and a commercial kit (Pierce BCA Protein Assay Kit, ref. 23225) using bovine serum albumin as standard, whereas total α -amino acids in plasma, liver and muscle were determined by colorimetric analysis with ninhydrin (Sigma N7285) dissolved in acetate cyanide, as described by **Moore (1968)**. All the assays were run on an Automated Microplate Reader (PowerWave 340, BioTek Instrument Inc., Winooski, USA) controlled by KCjunior™ software. Standards and samples were measured in duplicate.

Plasma cortisol levels were measured by Enzyme Immune-Assay (EIA) using microtiter plates (MaxiSorp™, Nunc, Roskilde, Denmark) as previously described by **Martos-Sitcha et al. (2014)** for other teleost species.

2.5. Data analysis and statistical methods

Results are presented as mean $\pm$ SEM. Normality and homogeneity of variances were checked by the Kolmogorov-Smirnov's and Levene's tests, respectively. Comparisons between groups were analysed using one-way analysis of variance (ANOVA) taking stocking density as the main factor, followed by a Tukey's post-hoc test. Significance was taken at $P < 0.05$. All tests performed were carried out using the *Statistics* software for Windows.

3. RESULTS

3.1. Evolution of stocking density and growth

At the end of the experiment (40 days post-transfer to the four different stocking densities) a clear increase in body mass and weight gain was observed in all groups tested when compared with the initial values registered, reaching 3.62 g L⁻¹ (LSD group), 7.32 g L⁻¹ (MSD group), 11.64 g L⁻¹ (HSD group) and 16.45 g L⁻¹ (EHSD group) (Figure 1, and Tables 1 and 2). Thus, a direct pattern of changes in body mass was observed respect to stocking density from the first sampling point at day 12 post-transfer (LSD < MSD < HSD < EHSD), which was maintained during the time that experiment lasted (Figure 1). In addition, weigh gain (WG) confirms greater growth rates in those specimens held at the highest stocking density (EHSD) when compared to LSD and MSD groups (Table 2), while no differences in condition factor (K) were observed during the time that experiment lasted (Table 2). According to changes observed in both growth rates and feeding conditions, different index were calculated (Table 2). While no variations were detected in SFR, an inverse pattern of changes in FCR was observed respect to stocking density (LSD ≥ MSD > HSD > EHSD), whereas the opposite situation (LSD < MSD < HSD < EHSD) in FER and SGR was noted (Table 2).

3.2. Plasma

Plasma cortisol showed an inverse relationship respect to stocking density (LSD > MSD > HSD ≥ EHSD), presenting those specimens held at LSD the maximum values (Figure 2). In addition, plasma glucose and triglycerides showed a similar trend that cortisol, but only significant higher levels in the LSD group were found for both parameters (Figure 3). Moreover, no differences in lactate, total proteins and α-amino acids values were observed between all groups analysed (Table 2).

204

205 **3.3. Liver**

206 All the hepatic metabolites analysed (glucose, glycogen, total α -amino acids and
207 triglycerides), together with hepatosomatic index (HSI), did not present statistically
208 significant differences between the experimental densities tested (Table 4).

209

210 **3.4. Muscle**

211 Triglycerides presented the highest levels in both HSD and EHSD groups (Figure 4A),
212 while α -amino acids and lactate values showed the statistically highest values in the EHSD
213 group (Figure 4B and 4C, respectively). Finally, no significant differences between groups for
214 glucose and glycogen values were observed (Table 3).

4. DISCUSSION

In aquaculture activity, space optimization by increasing stocking density is necessary to enhance profits. Several studies have examined the effect of stocking densities on growth and metabolism of different commercial species, such as gilthead sea bream *Sparus aurata* (Montero et al., 1999), red porgy *Pagrus pagrus* (Laiz-Carrión et al., 2012), Senegalese sole *Solea senegalensis* (Andrade et al., 2015; Salas-Leiton et al., 2008), Atlantic salmon *Salmo salar* (Hosfeld et al., 2009), or European sea bass *Dicentrarchus labrax* (Lupatsch et al., 2010). These studies have demonstrated that high stocking densities induce negative consequences on several physiological processes due to activation of stress pathways (Barton, 2002; Wendelaar Bonga, 1997).

Our study reveals that stocking density affects growth rates in juvenile *A. regius* specimens. Thus, the higher growth, both in body mass and standard body length, observed in fish held at higher stocking densities (HSD and EHSD) just after 12 days of experiment, is consistent with those results obtained in the same stages (~120 days post-hatching, dph) of *A. japonicus* maintained at different densities (Pirozzi et al., 2009). Similar results obtained in both species suggested that chronic activation of stress system in those fish held at lower stocking densities produce a negative impact on growth (see below). In addition, previous studies performed in *A. regius* larvae from 0 till 30 days post-hatching revealed that lower larval stocking density (50 larvae L⁻¹) promoted better growth rates just after 14 days of experiment in terms of standard body length, body mass and dry weight when compared with higher larvae densities (100 larvae L⁻¹) (Roo et al., 2010). Thus, these results together with those found in the present study suggest that effects of stocking density could be observed at least until juvenile stages, and also that this factor is clearly size- and/or age-dependent in this species. Even so, the opposite response has been previously described in other fish species, in which higher growth rates are promoted in specimens held at lower stocking densities in

different stages of development (de las Heras et al., 2015; Irwin et al., 1999; Pankhurst and Van der Kraak, 1997; Pickering, 1993). In this regard, it can be concluded that growth processes in teleost fish are influenced by stocking density depending not only on the age of the specimens, but also in the species.

Studies on different species (*Oncorhynchus mykiss*, *Scophthalmus maximus*, *D. labrax*) have proved that the highest growth rates are obtained in those conditions where the animals present low feed conversion rates (FCR) (Eroldogan et al., 2004; Van Ham et al., 2003; Zoccarato et al., 1994), since higher body mass increases are produced attending to the feeding ration provided. Thus, our results concerning the extra high stocking density (EHSD) confirmed that higher growth rates and optimization of the digestive processes are carried out when compared to those fish held at lower stocking densities (Eroldogan et al., 2004; Van Ham et al., 2003). In addition, it is important to remark that these higher growth rates observed are produced i) by no differences in food supply respect to the biomass held in each experimental condition, as shown by the absence of variations in the specific feed rate (SFR), as well as ii) by the highest feed efficiency rate (FER) described. In the present study the SGR (0.40 and 0.53 % day⁻¹), FCR (1.25 and 0.86) and FER (80.96 and 116.64 %) in the HSD and EHSD treatments, respectively, are higher than those observed by other authors (Ortega and De la Gándara, 2007). This suggests that, at least for *A. regius* juvenile specimens, specimens cultured at higher densities grew faster than those held at lower densities (LSD and MSD).

It is well established that inappropriate culture densities induce an activation of stress system, resulting in elevated plasma cortisol levels and also disorders in the metabolism (Barton, 2002; Barton and Iwama, 1991). However, the present results obtained in juvenile *A. regius* cultured at different densities are not consistent with the classic pattern described for other teleosts species. Herein, cortisol levels showed a negative relationship respect to

stocking density, and their high plasma concentrations (described as a primary stress response) detected in LSD and MSD groups suggest an activation of the stress system. Under these culture conditions a metabolic reorganization (considered as a secondary stress response) is necessary to cope with the stocking density challenge and a lower growth rate (categorized as a tertiary stress response) is thus observed (**Barton, 2002; Wendelaar Bonga, 1997**). These results agree with our personal observations as a better shoal cohesion, behaviour and feeding responses during the daily maintenance and care of the animals occurred in those fish held at higher densities (HSD and EHSD) when compared to those maintained at LSD and MSD. These reports are in accordance with the gregarious nature of the juveniles of this species, as they are observed gathering in large groups in brackish waters (**Sobrino et al., 2005**). Similar observations were also described for the close-related species *A. japonicus* (**Pirozzi et al., 2009**). On the other hand, plasma glucose is a common secondary response to stress, and it is associated to the metabolites mobilization related to plasma cortisol enhancement, thus facing the extra energy expenditure caused by this situation (**Wendelaar Bonga, 1997**). In several teleost species, it has been described that excessed stocking densities raise both plasma cortisol and glucose levels (**Sangiao-Alvarellos et al., 2005; Vijayan et al., 1990**), while the same effects were shown in other species at low stocking densities (**de las Heras et al., 2015; Menezes et al., 2015**). Thus, *A. regius* juveniles maintained under LSD conditions (present study) show high plasma glucose levels associated to elevated cortisol concentrations.

Triglycerides are also an important source of energy during acclimation to different stocking densities (**Herrera et al., 2009; Laiz-Carrión et al., 2012**) as our results confirmed. Thus, the high plasma triglycerides levels observed in specimens held under LSD and MSD conditions could be related to the low values of this metabolite in muscle, suggesting lipid mobilization maybe due to the lipolytic action of cortisol (**Mommsen et al., 1999**).

Proteins are the major dietary component of the carnivorous teleosts, being also protein catabolism the main source of energy for this taxon. In addition, free amino acids mainly come from the intestinal absorption of hydrolysed proteins ingested in the diet, and its main functions are related to i) protein synthesis, ii) synthesis of glucose *via* gluconeogenesis, and iii) their use for energy supply (Cowey and Walton, 1989; Mommsen and Walsh, 1992). Our results related to nitrogen metabolism in plasma did not show significant differences for the groups tested. Even so, the highest levels of muscle free amino acids in the EHSD group suggest that this metabolite is been used for somatic growth increase, while in LSD and MSD groups are used as a source of energy to cope with an stressful situation (Barton, 2002; Barton and Iwama, 2001; Wendelaar Bonga, 1997). In addition, the highest lactate levels found in muscle at EHSD suggest a metabolic mobilization to this tissue using this metabolite as a substrate for gluconeogenesis, thus increasing metabolic stores and body growth (Cowey and Walton, 1998). For this reason, and from an energy point of view, the use of lactate in muscle and other tissues is very successful (Brooks, 2000; Hamann et al., 2001; Kelley et al., 2002), suggesting the absence of variations in plasma as a consequence of this metabolite conversion into an energy fuel supply, or even due to its use as metabolic reserves.

Liver is considered as an important store of metabolic energy mostly in the form of glycogen and triglycerides, so their quantification in this tissue represents a good indicator of the nutritional status of the fish (Soengas et al., 2007). However, our results showed no statistically significant differences not only in any of the hepatic parameters studied, but also in the hepatosomatic index (HSI), even when somatic growth is affected. These results may suggest that hepatic metabolism is not severely affected by stocking density at this life stage. Thereby, the described differences in growth between treatments are a result of changes in the metabolism balance of peripheral tissues, like the muscle.

In conclusion, this study has confirmed that stocking density is a factor of paramount importance in the culture of *A. regius* juveniles. We sustain that low stocking densities could be stressful for the fish, with detrimental effects on growth. Notwithstanding of the high stocking densities tested in this study (up to 13 g L⁻¹), the optimal for this species has not been yet described, unless relevant information for the aquaculture industry is disclosed hereby. Thus, further studies should be focused in searching a higher density where the growth is maximized without jeopardizing the health of the animals.

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FIGURE LEGENDS

Figure 1. Evolution of body mass in *A. regius* specimens cultured at different initial stocking densities (LSD, MSD, HSD and EHSD) during 40 days. Data are presented as mean $\pm$ SEM for each density tested ($n = 20$). Different letters indicated significantly differences between experimental groups at the same time ($P < 0.05$).

Figure 2. Plasma cortisol levels in *A. regius* specimens cultured at different initial stocking densities (LSD, MSD, HSD and EHSD) during 40 days. Data are presented as mean $\pm$ SEM ($n = 10$). Different letters indicated significantly differences between experimental groups ($P < 0.05$).

Figure 3. Plasma glucose (A) and triglycerides (B) levels in *A. regius* specimens cultured at different initial stocking densities (LSD, MSD, HSD, and EHSD) during 40 days. Data are presented as mean $\pm$ SEM ($n = 10$). Different letters indicated significantly differences between experimental groups ($P < 0.05$).

Figure 4. Muscle triglycerides (A), amino acids (B) and lactate (C) levels in *A. regius* specimens cultured at different initial stocking densities (LSD, MSD, HSD and EHSD) during 40 days. Data are presented as mean $\pm$ SEM ($n = 10$). Different letters indicated significantly differences between experimental groups ($P < 0.05$).

TABLES

Table 1. Evolution of stocking density, growth and survival in *A. regius* specimens cultured at different initial stocking densities (LSD, MSD, HSD and EHSD) during 40 days. Data are presented as mean $\pm$ SEM ($n = 20$) for each density tested. Different letters indicated significantly differences between experimental groups ($P < 0.05$).

	<i>LSD</i>	<i>MSD</i>	<i>HSD</i>	<i>EHSD</i>
Initial density (g L⁻¹)	3.32 $\pm$ 0.03	6.64 $\pm$ 0.04	9.99 $\pm$ 0.06	13.23 $\pm$ 0.02
Final density (g L⁻¹)	3.62 $\pm$ 0.14	7.32 $\pm$ 0.09	11.64 $\pm$ 0.10	16.45 $\pm$ 0.88
Final body mass (g)	90.45 $\pm$ 3.61 ^a	91.50 $\pm$ 1.13 ^a	98.65 $\pm$ 1.48 ^b	102.82 $\pm$ 5.52 ^b
Final total length (cm)	20.90 $\pm$ 0.14	21.10 $\pm$ 0.10	21.50 $\pm$ 0.28	21.45 $\pm$ 0.21
Survival (%)	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	99.40 $\pm$ 0.98	100.00 $\pm$ 0.00

Table 2. Growth and feeding index in *A. regius* specimens cultured at different initial stocking densities (LSD, MSD, HSD and EHSD,) during 40 days. Data are presented as mean $\pm$ SEM ($n = 20$). Different letters indicated significantly differences between experimental groups ($P < 0.05$). K: condition factor; WG: weight gain; SGR: specific growth rate; SFR: specific feed rate; FCR: feed conversion rate; FER: feed efficiency rate.

	<i>LSD</i>	<i>MSD</i>	<i>HSD</i>	<i>EHSD</i>
K (%)	0.99 $\pm$ 0.02	0.97 $\pm$ 0.01	0.99 $\pm$ 0.02	1.04 $\pm$ 0.02
WG (g)	7.40 $\pm$ 2.49 ^a	8.45 $\pm$ 0.51 ^a	15.40 $\pm$ 0.52 ^{ab}	20.12 $\pm$ 0.56 ^b
WG (%)	8.90 $\pm$ 2.56 ^a	10.17 $\pm$ 0.51 ^a	18.50 $\pm$ 0.49 ^{ab}	24.32 $\pm$ 0.53 ^b
SGR (% day⁻¹)	0.19 $\pm$ 0.04 ^a	0.23 $\pm$ 0.01 ^a	0.40 $\pm$ 0.03 ^b	0.53 $\pm$ 0.02 ^c
SFR (%)	0.68 $\pm$ 0.01	0.66 $\pm$ 0.01	0.68 $\pm$ 0.01	0.67 $\pm$ 0.01
FCR	2.35 $\pm$ 0.67 ^a	1.87 $\pm$ 0.13 ^a	1.25 $\pm$ 0.11 ^{ab}	0.86 $\pm$ 0.03 ^b
FER (%)	46.00 $\pm$ 13.00 ^a	53.50 $\pm$ 3.50 ^a	80.96 $\pm$ 7.01 ^{ab}	116.64 $\pm$ 3.50 ^b

Table 3. Plasma metabolites in *A. regius* specimens cultured at different initial stocking densities (LSD, MSD, HSD and EHSD) during 40 days. Data are presented as mean $\pm$ SEM ($n = 10$).

	<i>LSD</i>	<i>MSD</i>	<i>HSD</i>	<i>EHSD</i>
Lactate (mM)	2.08 $\pm$ 0.21	2.44 $\pm$ 0.21	2.18 $\pm$ 0.20	2.51 $\pm$ 0.13
Total proteins (g dL⁻¹)	28.19 $\pm$ 1.04	28.14 $\pm$ 0.83	29.62 $\pm$ 0.64	26.43 $\pm$ 1.04
Aminoacids (mM)	20.49 $\pm$ 0.57	20.89 $\pm$ 1.14	18.71 $\pm$ 0.73	20.79 $\pm$ 0.99

Table 4. Hepatosomatic index (HSI) and liver metabolites in *A. regius* specimens cultured at different initial stocking densities (LSD, MSD, HSD and EHSD) during 40 days. Data are presented as mean $\pm$ SEM ($n = 10$). Values are expressed as mg of each specific metabolite per g of wet weight of tissue.

	<i>LSD</i>	<i>MSD</i>	<i>HSD</i>	<i>EHSD</i>
HSI	1.50 $\pm$ 0.11	1.45 $\pm$ 0.09	1.70 $\pm$ 0.07	1.44 $\pm$ 0.11
Glucose (mg glc g ww⁻¹)	2.93 $\pm$ 0.26	2.47 $\pm$ 0.19	3.38 $\pm$ 0.33	2.34 $\pm$ 0.33
Glycogen (mg glc g ww⁻¹)	26.88 $\pm$ 0.33	26.15 $\pm$ 0.52	26.87 $\pm$ 0.47	27.31 $\pm$ 0.61
Aminoacids (mg aa g ww⁻¹)	14.31 $\pm$ 3.56	14.51 $\pm$ 3.95	15.89 $\pm$ 3.32	15.91 $\pm$ 5.93
TAG (mg TAG g ww⁻¹)	4.13 $\pm$ 0.47	3.99 $\pm$ 0.37	4.26 $\pm$ 0.50	4.69 $\pm$ 0.49

Table 5. Muscle metabolites in *A. regius* specimens cultured at different initial stocking densities (LSD, MSD, HSD and EHSD) during 40 days. Data are presented as mean $\pm$ SEM ($n = 10$). Values are expressed as mg of glucose per g of wet weight of tissue.

	<i>LSD</i>	<i>MSD</i>	<i>HSD</i>	<i>EHSD</i>
Glucose (mg glc g ww⁻¹)	1.46 $\pm$ 0.09	1.17 $\pm$ 0.14	1.31 $\pm$ 0.13	1.15 $\pm$ 0.13
Glycogen (mg glc g ww⁻¹)	0.10 $\pm$ 0.02	0.10 $\pm$ 0.05	0.16 $\pm$ 0.05	0.21 $\pm$ 0.13

Figure 1. *Millán-Cubillo et al.*

Figure 2. *Millán-Cubillo et al.*

Figure 3. *Millán-Cubillo et al.*

Figure 4. Millán-Cubillo *et al.*