Aquaculture 448 (2015) 29–37

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Influence of stocking density on growth, metabolism and stress of thick-lipped grey mullet (*Chelon labrosus*) juveniles

V. de las Heras [a,1,2], J.A. Martos-Sitcha [a,b,*,2], M. Yúfera [a], J.M. Mancera [b], G. Martínez-Rodríguez [a]

[a] Instituto de Ciencias Marinas de Andalucía, Consejo Superior de Investigaciones Científicas (ICMAN-CSIC), E-11510 Puerto Real, Cádiz, Spain
[b] Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Campus de Excelencia Internacional del Mar (CEI-MAR), University of Cádiz, E-11510 Puerto Real, Cádiz, Spain

ARTICLE INFO

Article history:
Received 12 December 2014
Received in revised form 7 April 2015
Accepted 21 May 2015
Available online xxxx

Keywords:
Chelon labrosus
Growth hormone
IGF-I
Metabolites
Stocking density
Stress

ABSTRACT

Physiological responses to different stocking densities and their subsequent effects on growth rate, energy metabolism and endocrine system were assessed in thick-lipped grey mullet (*Chelon labrosus*) juveniles. Three different groups of 150 fish (0.383 ± 0.020 g body mass, 101 days post-hatching (dph)) were maintained in triplicate under three different stocking densities: 0.7, 2.0 and 6.7 kg·m^{-3}. All individuals were sampled at days 0, 20, 45 and 75 to obtain biometric parameters, while 25–30 specimens from each treatment were sampled for the plasma, liver and pituitary collection at the end of the experiment (75 days). The lowest growth increase, both in body mass and total length, was shown in the group held at the highest stocking density (6.7 kg·m^{-3}), just by the end of the experiment (176 dph). Moreover, higher plasma cortisol and glucose values were obtained in the group stocked at 0.7 kg·m^{-3}, whereas individuals maintained under the maximum density (6.7 kg·m^{-3}) had the highest hepatic glycogen and lowest glucose content. In addition, growth hormone (GH) and insulin-like growth factor (IGF-I) gene expression increased in the group maintained under the highest stocking condition. Our results indicate that *C. labrosus* juveniles activated both Hypothalamus–Pituitary–Interrenal (cortisol) and somatotropic (GH/IGF-I) axes to modulate metabolic and stress pathways in specimens held at different stocking densities to compensate their growth rates.

1. Introduction

Stocking density is an important factor to be considered in fish aquaculture. Several studies have evaluated the effects of rearing densities on growth and metabolism in cultured fish species (Herrera et al., 2009; Li et al., 2012; Montero et al., 1999; Sangiao-Alvarellos et al., 2005). High biomass could activate stress response affecting negatively different metabolic pathways related to lipid, carbohydrate and protein metabolism (Costas et al., 2008; Laiz-Carrión et al., 2012), while low stocking densities could suppose, due to an inadequate use of space, higher production costs and lower profitability for the industry. Moreover, under intensive fish culture systems, high stocking densities together with insufficient water renovation in the tanks could decrease water quality (i.e., increase in ammonium or nitrite concentrations) compromising the growth of specimens (Deane and Woo, 2007; Dosdat et al., 2003; Ferreira et al., 2013; Foss et al., 2009; Le Ruyet et al., 1997; Sinha et al., 2012; Wajsbrot et al., 1993). However, in some species from family Sciaenidae (*Argyrosomus japonicus* and *Argyrosomus regius*), a positive relationship between growth rates and stocking densities has been reported (Millán-Cubillo et al., 2011; Pirozzi et al., 2009). This fact could be related to the gregarious nature of these species, requiring shoaling to avoid stressful situations.

Activation of different metabolic pathways involved in stress response is species dependent as well as related to several factors (e.g., size, age and metabolic status) (McCue, 2010; Méndez and Wieser, 1993; Shimeno et al., 1990). Thus, preservation of glycemia and other metabolites for fuelling energy required by different tissues is necessary, being the liver the main organ of glycogen/glucose turnover, ammoniagenesis, fatty acid synthesis, and gluconeogenesis (Peragón et al., 1998). Moreover, exposure to stressors activates several endocrine systems, where cortisol becomes a primary mediator of stress mechanisms. This hormone is the main corticosteroid secreted by teleosts and its function has been described as an important player acting in both glucocorticoid and mineralocorticoid functions (McCormick, 2001). In fact, chronic high plasma cortisol values could compromise the energy available for several physiological processes as growth, reproduction, immune response, osmoregulation and metabolism, because of an increase in energy consumption (Laiz-Carrión et al., 2009; Mommsen et al., 1999; Pickering, 1993; Wendelaar Bonga, 1997).

* Corresponding author at: Instituto de Ciencias Marinas de Andalucía, Consejo Superior de Investigaciones Científicas, Avda. República Saharaui 2, E-11510 Puerto Real, Cádiz, Spain.
E-mail address: juanantonio.sitcha@icman.csic.es (J.A. Martos-Sitcha).
[1] Present address: Futuna Blue España S.L., Dársena Comercial Pesquera s/n, 11500 El Puerto de Santa María, Cádiz, Spain.
[2] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.aquaculture.2015.05.033
0044-8486/

The role of growth hormone/insulin-like growth factor-I (GH/IGF-I) axis for promoting growth is widely established in teleosts (Björnsson, 1997; Butler and Le Roith, 2001; Pérez-Sánchez, 2000). In addition, a negative influence of stress situation, mediated by high chronic plasma cortisol values, has been also demonstrated. Thus, cortisol decreases pituitary GH and hepatic IGF expression, as well as plasma values of both hormones inducing growth inhibition in specimens under stress situation (Laiz-Carrión et al., 2009; Pickering, 1993; Rotllant et al., 2000; Saera-Vila et al., 2009; Wendelaar Bonga, 1997).

Thick-lipped grey mullet (*Chelon labrosus*), a fish species from the family Mugilidae, is a marine teleost cultured in natural earthen ponds under extensive or semi-intensive regimes and often associated with other fish species with higher economical value as Senegalese sole (*Solea senegalensis*), gilthead sea bream (*Sparus aurata*) or European sea bass (*Dicentrarchus labrax*). In addition, *C. labrosus* has been described as an easily cultivable species and could constitute a new candidate for aquaculture diversification (Ben Khemis et al., 2006, 2013; Boglione et al., 1992; Zouiten et al., 2008). Mullet species are considered as low trophic level feeders, obtaining their energy directly from the first trophic level (Brusle, 1981). These fish, including *C. labrosus*, have been described as omnivorous species in the early stages of development, tending to become herbivorous over time (de las Heras et al., 2013; Pujante et al., 2011; Wassef et al., 2001). The utilization of carbohydrates in the diet could help achieve the development of feeds with minimized cost, as it is an inexpensive source of energy (Zouiten et al., 2008), and reduce the pressure in the fishing of species used for the production of fishmeal and oil for feed formulation.

Therefore, this study aimed to evaluate the effect of different stocking densities, a common stressor in the aquaculture activity, on i) growth, ii) metabolism, as well as on iii) stress and GH/IGF-I axes, in juveniles of *C. labrosus*.

2. Material and methods

2.1. Experimental procedures

Eggs of thick-lipped grey mullet (*C. labrosus*) were obtained from natural spawning in captivity from the I.E.*S. Els Alfacs* (Sant Carles de la Rápita, Tarragona, Spain) and transferred to the *Instituto de Ciencias Marinas de Andalucía* (ICMAN-CSIC) facilities (Puerto Real, Cádiz, Spain; Experimental animal facility registry number ES110280000311). Larvae were reared in 150 L conical bottom tanks from hatching (0 day post-hatching (dph)) to 3 dph, after which they were transferred to 250 L flat bottom tanks until 101 dph. Larvae were maintained under a photoperiod of 12 h light and 12 h darkness. The oxygen concentration ranged between 7.5 and 8.5 $mg \cdot L^{-1}$ and pH between 7.6 and 7.9, with constant water temperature (18–19 °C) and salinity (35 ppt). After mouth opening (4 dph), larvae were fed rotifers (*Brachionus plicatilis*) at a density of 5 $prey \cdot mL^{-1}$. *Artemia* sp. nauplii (0.3–0.5 $prey \cdot mL^{-1}$; Ben Khemis et al., 2006) were supplied from 6 dph, which were gradually replaced with enriched meta-nauplii between 13 and 25 dph (1 $prey \cdot mL^{-1}$) and 0.1, 0.2 and 0.5 mm commercial diets ad libitum (Skretting, Burgos, Spain) becoming the only food offered to larvae from 25 dph onwards.

For the experiment, juveniles of 101 dph (day 0 of experiment) (n = 450, 0.383 ± 0.020 g [mean ± SEM] body mass and 3.270 ± 0.051 cm [mean ± SEM] total length) were transferred and randomly distributed in triplicate in 50, 15 and 9 L-tanks continuously aerated in a flow through system (~1 $renovation \cdot h^{-1}$). In each tank, water volume was adjusted in order to get three different experimental densities: i) low stocking density (LD, 0.7 $kg \cdot m^{-3}$; ~2 $individuals \cdot L^{-1}$), ii) medium stocking density (MD, 2.0 $kg \cdot m^{-3}$; ~4 $individuals \cdot L^{-1}$) and iii) high stocking density (HD, 6.7 $kg \cdot m^{-3}$; ~8 $individuals \cdot L^{-1}$). Due to the scarce information related to rearing conditions in this species, in which a mesocosm system for larval rearing was used with initial densities of ~1–2.5 $larvae \cdot L^{-1}$ (Ben Khemis et al., 2006, 2013), the stocking densities were chosen according to previous studies in larvae and fry of other teleost species, which have demonstrated to induce changes in the physiology of the animals (Houde, 1975; Irwin et al., 1999). Moreover, after each sampling point (see below) the tank water volume was modified in order to maintain selected stocking densities during the rest of the experimental time. Attending to a previous test performed during 6 days before the beginning of the experiment to set the optimal daily amount of food required, a range from 4 to 9% total body mass was offered to the main tanks. Fish were finally fed daily four times with a total of 8% of their body mass with a commercial diet (Dibaq-Diproteg S.A., Segovia, Spain) and maintained for 75 days (July–September, 2012) under a photoperiod of 12 h light and 12 h darkness, and constant salinity (35 ppt) and temperature (18–19 °C). The oxygen concentration ranged between 7.5 and 8.5 $mg \cdot L^{-1}$, pH between 7.6 and 7.9 and nitrites between 1.0 and 1.5 $mg \cdot L^{-1}$.

At 0, 20, 45 and 75 days from the start of the experiment, all individuals from each experimental stocking density (LD, MD and HD) were anesthetized with 2-phenoxyethanol (0.5 $mL \cdot L^{-1}$), weighed and measured. These sampling points were used to adjust both the food ration and the volume of water to maintain the initial stocking densities between samplings. At the end of the experiment (75 days) 25–30 specimens (8–10 per tank) of each experimental treatment were anesthetized with 2-phenoxyethanol (1 $mL \cdot L^{-1}$), killed by decapitation and sampled. Blood was collected from caudal vein cutting the caudal fin and using heparinized capillaries. After that, the plasma was obtained by centrifugation of whole blood (3 min, 10,000 *g*, 4 °C) and stored at −80 °C until analysis. Then, the liver was removed, weighed separately to calculate the hepatosomatic index (HSI), and divided into two portions. One of the pieces was immediately snap-frozen in liquid nitrogen and stored at −80 °C for analysis of metabolites. The other portion, as well as pituitary glands, was placed in Eppendorf tubes containing the appropriate volume (1/10 w/v) of RNA*later*© (Life Technologies), incubated for 24 h at 4 °C and stored at −20 °C afterwards. No mortality was observed in any experimental groups. 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.

2.2. Growth, feed conversion rates and somatic index

Weight increase and feed consumption were used to calculate the following nutritional indexes: K, condition factor; HSI, hepatosomatic index; FCR, feed conversion rate; and SGR, specific growth rate. Each index was calculated as follows:

$$K(\%) = 100 * \left(\text{fish weight(g)} / \text{fish length}^3\text{(cm)}\right)$$

$$HSI = 100 * (\text{liver weight} / \text{body weight})$$

$$FCR = \text{total food intake} / \text{total weight gain}$$

$$SGR\left(\%\ \text{day}^{-1}\right) = 100 * [(\ln \text{final weight} - \ln \text{initial weight}) / \text{experimental period in days}].$$

2.3. Plasma and liver parameters

For the assessment of metabolite levels, the livers were finely minced on an ice cold petri dish, and subsequently homogenized by mechanical disruption (Ultra-Turrax, T 25 basic, IKA®-WERKE) with 7.5 vol. (w/v) of ice-cooled 0.6 N perchloric acid and neutralized after the addition of the same volume of 1 M $KHCO_3$. Previous to centrifugation, an aliquot of each homogenate was separated for triacylglyceride (TAG) measurements. After that, the homogenates were centrifuged (30 min, 13,000 *g*, 4 °C) and the supernatants were recovered in