Comparative Biochemistry and Physiology, Part A 169 (2014) 25–32

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Effects of environmental enrichment on growth, aggressive behaviour and brain monoamines of gilthead seabream *Sparus aurata* reared under different social conditions

Alkisti Batzina [a], Christina Dalla [b], Zeta Papadopoulou-Daifoti [b], Nafsika Karakatsouli [a,*]

[a] Department of Applied Hydrobiology, Faculty of Animal Science and Aquaculture, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece
[b] Department of Pharmacology, Medical School, University of Athens, Mikras Asias 75, 115 27 Goudi, Athens, Greece

ARTICLE INFO

Article history:
Received 5 September 2013
Received in revised form 2 December 2013
Accepted 4 December 2013
Available online 8 December 2013

Keywords:
Aggressive behaviour
Brain monoamines
Density
Dopamine
Growth
Serotonin
Sparus aurata
Substrate

ABSTRACT

The presence of blue or red-brown substrate on the tank bottom has been previously reported as an efficient means of environmental enrichment for gilthead seabream. The present study aimed to investigate whether this enrichment is still beneficial when gilthead seabream is reared under different social conditions (i.e. a lower 4.9 kg m^{-3} and a higher 9.7 kg m^{-3} density). Water exchange was adjusted according to fish biomass to exclude density effects on water quality. In the enriched tanks single-colour glass gravel was used as substrate (blue and red-brown substrate, or BS and RBS respectively), while control tanks had no gravel. Growth, aggressive behaviour and size distribution results indicated that the lower density created a less favourable social environment. In both densities studied, BS enhanced growth, suppressed aggression and reduced brain serotonergic activity. In the condition of intense social interactions (i.e. the lower density) BS also reduced brain dopaminergic activity. These results along with the negative correlations observed between brain monoamines and fish body mass, indicated that substrate and density effects are socially-induced. However, there may be several biotic and/or abiotic factors interfering with substrate effects that should be investigated before the practical use of a substrate in land-based intensive aquaculture.

1. Introduction

Manipulation of housing conditions within the scope of environmental enrichment has been considered beneficial for the welfare of captive animals since it provides for their behavioural and psychological needs (Shepherdson et al., 1998). Increased structural complexity resulted in improved growth (Arndt et al., 2001; Ottesen et al., 2007), survival (Coulibaly et al., 2007a) and foraging behaviour (Strand et al., 2010) for several reared fish species. It is also known that environmental enrichment influences fish aggressive behaviour (Kadry and Barreto, 2010; Nijman and Heuts, 2011; Torrezani et al., 2013) and cognitive abilities (Brown et al., 2003; Strand et al., 2010), which have been suggested to be mediated by modifications in brain size and cell proliferation (Lema et al., 2005; Kihslinger and Nevitt, 2006; Gonda et al., 2009; von Krogh et al., 2010). Moreover, brain neurotransmitters are considered to be mediators of cognitive function and behaviour (Hsu et al., 2011). Besides, brain monoamines have been shown to be modified in mice and rats (Rasmuson et al., 1998; Naka et al., 2002; Brenes et al., 2008) and in fish (Höglund et al., 2005) when reared in enriched environment.

In intensive aquaculture, fish are usually kept in high densities to provide for high productivity needs. It is well reported that density affects survival, feeding, nutritional status, growth performance, health, behaviour, etc. (e.g. North et al., 2006; Coulibaly et al., 2007b; Kaspersson et al., 2010; Laiz-Carrión et al., 2012). Density is a multifaceted issue since the number/size of specimens in a limited space defines, above all, water quality and social interactions, which are strongly related to fish welfare (for reviews see Ellis et al., 2002; Ashley, 2007). There is extensive literature reporting the involvement of fish brain monoamine neurotransmitters in social interactions, especially aggressive behaviour and social status (for reviews see Winberg and Nilsson, 1993a; Johnsson et al., 2006). Despite the obvious relation of density and social conditions, studies investigating density effects on brain monoamines are limited. In particular, increased density resulted in increased serotonergic activity in rainbow trout *Oncorhynchus mykiss* (Laursen et al., 2013a, 2013b) and increased dopaminergic activity in white seabream *Diplodus sargus* (Papoutsoglou et al., 2006) which were associated with high social stress.

Given the emerging scientific interest for environmental enrichment as a means to improve welfare, especially in intensively farmed fish, it is essential to investigate its efficiency in combination with other rearing parameters important for aquaculture, such as density. Although studies investigating the combined effects of density and enrichment are limited, previous data showed that the effect of enrichment on fish

* Corresponding author.
E-mail address: nafsika@aua.gr (N. Karakatsouli).

1095-6433/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cbpa.2013.12.001

may or may not be modified by density (aggressive behaviour; Kelley et al., 2006; survival; Coulibaly et al., 2007a).

Gilthead seabream *Sparus aurata* is one of the most important Mediterranean aquaculture species. It has been previously reported that the addition of substrate in the tank bottom can be an efficient means of environmental enrichment. In particular, fish reared with blue or red-brown substrate showed enhanced growth performance and suppressed aggressive behaviour compared to fish reared with green or without substrate (Batzina and Karakatsouli, 2012). The present study aimed to investigate whether the beneficial effects of blue or red-brown substrate can be apparent when gilthead seabream is reared under different social conditions. To provide for a multi-parametric approach, substrate and density effects were evaluated through growth performance, aggressive behaviour and brain monoamine neurotransmitters.

2. Materials and methods

2.1. Ethical note

The study was carried out in accordance with the EU Directive 2010/63/EU, national laws (PD 160/91) for animal experiments and the Uniform Requirements for manuscripts submitted to Biomedical journals.

2.2. Experimental design

Gilthead seabream *S. aurata* juveniles were obtained from a Greek commercial hatchery and acclimated to laboratory conditions in glass tanks for approximately six months. Three hundred and six fish of mean initial body mass (mean ± S.E.) 25.2 ± 0.16 g (age 0+) were randomly distributed in 12 tanks (glass, rectangular 41 × 49 × 44 cm; all sides, apart from the front and top ones, externally covered with light blue styrofoam; water volume 88.4 L), in six duplicated treatments according to a 3 × 2 factorial design. Fish were reared in tanks with blue or red-brown substrate and no substrate-Control (BS, RBS and C respectively) under two densities, D: 17 fish tank^{-1} (192.3 fish m^{-3} or 4.9 kg m^{-3}) and 2D: 34 fish tank^{-1} (384.6 fish m^{-3} or 9.7 kg m^{-3}). The initial fish groups were homogeneous with a coefficient of mass variation ranging from 10.88% to 11.65% ($P > 0.05$). In the enriched tanks, a uniform layer (2.5 cm height) of single colour (blue or red-brown) glass gravel (size: 6–12 mm; Hermes S.A. Decorative Materials, Koropi, Greece) was used as substrate. Control tanks had no gravel on the bottom (glass bottom). This substrate does not chemically interact with water, while it is compatible with gilthead seabream natural habitat structure and colouration (Basurco et al., 2011).

Fish were maintained under experimental conditions for 98 days. They were fed, by hand, a commercial pelleted diet (sinking pellets) for gilthead seabream (moisture, 5.33%; crude protein, 46.73%; crude lipid, 23.11%; ash, 5.91%; nitrogen-free extract + crude fibre, 18.92%) 3% of their body mass – that gradually decreased to 1.5% according to body mass and water temperature (Lupatsch and Kissil, 1998) – three times daily (8:30, 11:30 and 14:30) from Monday to Friday, once on Saturday (11:00), while no food was offered on Sundays. In the enriched tanks, pellets settled on substrate surface and each meal was consumed within five minutes in all treatments. Fish were individually weighed every 2 weeks and food quantity was adjusted accordingly. No mortality was observed during the experimental period.

The experimental tanks were part of the same indoor recirculating seawater system (total water volume capacity 11 m^3; renewal 3% make-up water), provided with mechanical (polyester filter pad) and biological filters (submerged gravel biofilter), UV sterilisation and compressed air supply. The water flow rate in each tank was adjusted according to body mass at a level of 1.8 L min^{-1} kg^{-1} in order to avoid density effects on water quality. All tanks were thoroughly cleaned once a week as described in Batzina and Karakatsouli (2012). Water physicochemical characteristics were monitored daily.

The fish were subjected to a photoperiod of 12-h light:12-h dark and light intensity, in all treatments, was adjusted to 220 lx at water surface. Light source (cool white fluorescence lamps) was placed 1 m above and at a distance of 5 cm from the front side of each tank.

2.3. Aggressive behaviour observations

Aggressive behaviour was recorded in weeks 11, 12 and 13 from the front side of the tank. Video recordings were thus limited since in Batzina and Karakatsouli (2012) aggression results for gilthead seabream were similar throughout the rearing period and were not affected by the observation week. Recordings (10 minute duration) took place between meals, namely from 8:45 to 10:15 and from 11:45 to 13:15, at 5 minute intervals from Tuesday to Friday. Mondays were not included since fish were not fed on Sundays and their behaviour might have been modified. Taking into account the possible effect of observation time on fish behaviour, a recording schedule was followed to obtain video recordings of each tank at each observation time. This resulted in one video tank^{-1} or two video treatment^{-1} for all observation times. A total of 12 video tank^{-1} (or 24 video treatment^{-1}) were obtained. Nine minutes of each video recording were analysed. Although experimental fish had been accustomed to and not disturbed by the presence of the experimenter, the first minute of each video was excluded to eliminate possible disquiet caused to the fish by the setting of the camera. Aggressive behaviour was estimated by counting the number of aggressive acts. Behavioural patterns observed and counted as one aggressive act were: a) chasing without nipping or biting, b) nipping without prior chasing, c) biting without prior chasing, d) chasing that ended up as nipping and e) chasing that ended up as biting. Data refer to the whole fish group since fish were not marked and it was impossible to identify which one performed or received an attack.

2.4. Sampling and analytical methods

At the end of the experimental period 5 fish from each D group and 10 fish from each 2D group (i.e. 29.4% of each fish group) were euthanised with an overdose of anaesthetic (2-phenoxy-ethanol; 1.5 mL L^{-1}) and they were subjected to individual weighing (precision 0.01 g), body measurements (vernier caliper, precision 0.1 mm) and brain sampling within 3 min. The whole brain was removed by decapitation (including the medulla oblongata) and once isolated it was weighed (precision 0.1 mg), frozen in dry ice and stored at −80 °C until analysed for brain neurotransmitters. Remaining fish of each group were anaesthetised (2-phenoxy-ethanol; 0.4 mL L^{-1}) and subjected to individual weighing and body measurements.

Frozen brains were homogenised and deproteinised in 500 μL of 0.2 N perchloric acid solution containing 7.9 mM $Na_2S_2O_5$ and 1.3 mM Na_2EDTA. The homogenate was centrifuged at 15,000 *g* for 45 min in 4 °C and the supernatant was again stored at −80 °C until analysis of neurotransmitters was performed by high-performance liquid chromatography (HPLC) with an electrochemical detector (ECD), as previously described (Papoutsoglou et al., 2006). Brains were analysed for dopamine (DA) and its metabolites 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA). Additionally, the ratios of DOPAC/DA, HVA/DA, (DOPAC + HVA)/DA and 5-HIAA/5-HT were calculated as an index of dopamine and serotonin turnover rate, in order to have a better evaluation with respect to the serotonergic and dopaminergic activity.

2.5. Calculations and data analysis

Specific Growth Rate [$SGR = (\ln M_{fn} - \ln M_{in}) \times 100 \times t^{-1}$, M_{fn}: mean final body mass, g; M_{in}: mean initial body mass, g; t :days of rearing], Coefficient of mass Variation [CV = 100 × (standard deviation) × (mean body mass, g)$^{-1}$], Food Conversion Ratio [FCR = (food consumed,