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Acute stress response of European sea bass *Dicentrarchus labrax* under blue and white light

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ABSTRACT

Recent data suggest that specific light wavelength can alleviate fish acute stress response by counteracting or reducing the stress-induced cortisol increase. The European sea bass *Dicentrarchus labrax*, widely reared in the Mediterranean, is very sensitive to handling stress, so that typical rearing procedures during on-growing (e.g. grading) are avoided. The present study aimed at investigating whether exposure to blue (480 nm) or white light (BL or WL, respectively) could alleviate European sea bass acute stress response. Fish (initial weight 130.9 ± 0.4 g) were reared (seawater recirculating system) for 63 days under BL or WL and then subjected to 1 hour confinement or left undisturbed (control). Confinement of fish under BL resulted in a higher cortisol increase, no dopaminergic activation and lower brain serotonergic activity than under WL. In contrast, WL confined fish showed a lower cortisol increase coupled with higher brain serotonergic activity and increased levels of brain dopamine. Stress-induced hematocrit increase was lower when fish were confined under BL and triacylglycerides increase was only observed for WL reared fish. Differences in some parameters between unstressed BL and WL fish suggest that light wavelength had an effect on fish physiological status irrespective of stress. Although present results are not conclusive on which fish groups were more or less stressed, they do confirm that light wavelength can differentiate European sea bass response to acute stressors. Further studies to elucidate biological mechanisms of light spectrum effects will reinforce its efficacy as a tool to manipulate intensively reared fish stress response.

1. Introduction

The European sea bass *Dicentrarchus labrax* is one of the most important commercial fish widely reared in the Mediterranean. Although sea bass is a species that can endure adverse environmental conditions (Petochi et al., 2011; Pichavant et al., 2003), it is quite susceptible to handling procedures commonly applied during its rearing (Morales-Nin et al., 2011). The increased sensitivity of sea bass has been related to its strong and prolonged stress-induced cortisol increase and increased biosynthetic capacity of interrenal tissue involving a high turnover in the corticosteroid production and release when compared with other species (Rotllant et al., 2003; Varsamos et al., 2006). The net result of this sensitivity is the fact that in sea bass farming, grading procedures which would result in more homogeneous fish populations are avoided especially during the on-growing phase (EFSA, 2008).

Due to the species great economic value, it is of importance to investigate ways to alleviate sea bass acute stress response. Efforts applied to other species have shown that dietary manipulations may be a promising means towards attenuating stress response (Jalali et al., 2010; Tahmasebi-Kohyani et al., 2011). In the case of rainbow trout *Oncorhynchus mykiss* it has been possible, through selective breeding, to establish lines of high and low stress responsiveness (Øverli et al., 2005; Pottinger and Carrick, 1999). It has also been suggested that exposure of several species to specific light spectrum (Barcellos et al., 2006; Heydarnejad et al., 2011; Karakatsouli et al., 2008; Volpato and Barreto, 2001) can counteract or reduce the stress-induced cortisol increase after the application of an acute stressor, such as confinement or chasing.

Apart from its effects on fish acute stress response, light spectrum may also affect growth performance, behavior and physiological status (Karakatsouli et al., 2007, 2008, 2010; Luchiari and Freire, 2009; Marchesan et al., 2005; Owen et al., 2010). The observed effects have been suggested to be related to species natural habitat lighting conditions and adaptation of visual abilities. Interestingly, in two species of different living ethology (gilthead sea bream *Sparus aurata* and rainbow trout *O. mykiss*) growth reduction after exposure to specific light wavelength was accompanied by increased brain monoamine activity, but no change in plasma cortisol levels (Karakatsouli et al., 2007).

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Physiological responses of fish to stressors are described as primary (increases in stress hormones and brain neurotransmitter activity), secondary (initiated by primary response, involving changes at blood and tissue level e.g. increases in glucose, red blood cells, decreases in tissue energy reserves, osmoregulatory disturbances etc., aiming to allow fish to cope with the stressor and maintain homeostasis) and tertiary (failure to maintain homeostasis, depressed whole-animal performance) (Barton, 2002; Ellis et al., 2012; Winberg and Nilsson, 1993). Brain neurotransmitters emerge as sensitive indicators of stress since both serotonergic and dopaminergic systems are rapidly activated in several cases of acute and chronic stress (Gesto et al., 2008; Lepage et al., 2002; Schjolden et al., 2006; Winberg and Nilsson, 1993) and have also been implicated in hypothalamus-pituitary-interrenal (HPI) axis activity and cortisol regulation in fish during stress (Barton, 2002; Øverli et al., 2005; Winberg and Nilsson, 1993).

Light spectrum can be easily and with little cost manipulated in land-based aquaculture systems and also in commercial sea cages (Leclercq et al., 2011; Migaud et al., 2007) rendering this rearing condition a potential tool for controlling stress. Although visual function has not yet been determined for sea bass, variations of plasma, ocular or pineal melatonin levels due to different light spectra (Bayarri et al., 2002; Vera et al., 2010), behavioral observations during progressive light color shift from shorter to longer wavelengths or the opposite (Marchesan et al., 2005), as well as positive blue light effects on sea bass larvae performance (Villamizar et al., 2009) indicate that sea bass is able to respond to light spectrum, especially to blue light.

The purpose of the present study was to investigate whether exposure to blue or white light could alleviate European sea bass acute stress response. To this end, fish were reared under blue or white light and then subjected to confinement stress. Stress response was evaluated using typical stress response parameters, as well as brain neurotransmitters.

2. Materials and methods

2.1. Experimental design

Eighty specimens of European sea bass *D. labrax*, previously acclimated to laboratory conditions, of mean initial body weight (±SEM) 130.9±0.4 g and total length 24.7±0.1 cm were randomly distributed (10 fish per group) in eight tanks (glass, length×height×width: 88×36×68 cm, volume capacity 215.4 L, rearing density 6 kg/m^3). Experimental tanks were part of an indoor recirculating seawater system provided with mechanical and biological filters, UV-sterilization, compressed air supply and cooling water apparatus. Water flow rate was 1.8 L/min and all tanks were thoroughly cleaned once a week. Water physiochemical properties were monitored daily and water quality was maintained as follows (mean±SEM): salinity, 35.3±0.1 g/kg; temperature, 23.0±0.06 °C; dissolved oxygen, 6.4±0.01 mg/L (93.0±0.07% saturation); pH, 7.30±0.002; total ammonia nitrogen, 0.276±0.0065 mg/L; unionized ammonia nitrogen, 0.0017±0.00052 mg/L; nitrite nitrogen, 0.044±0.0010 mg/L.

Fish were reared under white and blue light (WL and BL groups respectively) for 63 days (four tanks per light color). Light color was achieved by covering light source (Cool White fluorescence lamps, OSRAM DULUX D/E 26W/840 G24Q-3) with colored filters (blue #165; LEE Filters, Andover, Hampshire, England, UK) while no filter was used for white light color (full spectrum). Light spectrum was specified using a constant slit Krüss spectroscope equipped with a graduated scale that was wavelength calibrated. Blue filter had peak transmission at 480 nm (83% relative transmission). In order to avoid complications due to room lighting, all tank sides were covered with opaque covers and light source was placed, through appropriate opening in top cover, above each tank, at approximately 10 cm from water surface. All experimental populations were subjected to photoperiod 12 L-12D (with half hour dawn and dusk simulation) and light intensity, in all treatments, was adjusted to 350 lx. Light manipulation was controlled with winDim 4.0e PC software and light intensity measured by means of digital light meter (RS 180–7133, RS Components Ltd., Corby, Northants, UK).

Fish were fed by hand a commercial pelleted diet (moisture, 7.1%; crude protein, 45.7%; crude lipid, 21.6%; ash, 6.3%; nitrogen-free extract + crude fiber, 19.3%) twice daily from Monday to Friday, once on Saturday, while no food was given on Sunday. Fish were fed 1% of their body weight per day throughout the experiment and were individually weighed every three weeks for food quantity adjustment.

During the last three days of the experimental period (i.e. prior to confinement), fish were food deprived. At the end of the experimental period, half fish populations (two tanks from each light color) were subjected for 1 h to confinement stress by lowering water level at 5 cm height (dorsal fin out of the water, rearing density 55 kg/m^3). Special care was taken to maintain high water oxygenation (above 90% saturation) as water was not exchanged during confinement. The other half fish populations (two tanks from each light color) remained undisturbed and used as controls (rearing density 7.6 kg/m^3). All fish were anesthetized, in their tanks, using 2-phenoxyethanol at a dose of 0.019 mL/g/L (or 360 mg/L).

2.2. Sampling and analytical methods

After complete anesthetization (within 1 min) fish were individually measured (weight and total length) and bled from the ventral aorta. About 0.2 mL of each blood sample was immediately used for measuring hematocrit (12,000 rpm for 5 min). The remaining blood was centrifuged (12,000 rpm for 10 min) and obtained plasma was frozen at −25 °C in different aliquots, so that for each analysis samples were thawed only once. Plasma was used for the determination of glucose, triacylglycerides, albumin (enzymatic colorimetric methods, Elitech diagnostics, Sees, France), osmolality (cryoscopic osmometer, Gonotec Osmomat 010) and cortisol, which was measured by radioimmunoassay, using a commercially available kit (Coat-A-Count Cortisol, DPC, Los Angeles, CA, USA) that has been previously validated for fish (Ainsworth et al., 1985). In the present study, the sensitivity of the assay was 0.2 μg/dL and intra- and inter-assay coefficient of variation was 3.2 and 6.5% respectively.

After blood sampling, for half the fish of each population the whole brain was removed by decapitation, weighed (precision 0.1 mg), frozen in dry ice and stored at −80 °C until analysis of brain neurotransmitters. The whole procedure (weighing, blood and brain sampling) lasted approximately 3 ½ min for each fish.

Frozen brains were homogenized and deproteinized 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 14,000 rpm for 30 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 described by Papadopoulou-Daifotis et al. (1995) with some minor modifications. A reverse-phase ion pair chromatography was used in all analyses of noradrenaline (NA), 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). The mobile phase consisted of an acetonitrile-50 mM phosphate buffer (10.5:89.5) pH 3.0, containing 300 mg L^{-1} 5-octylsulfate sodium salt as the ion-pair reagent and 20 mg L^{-1} Na_2EDTA. Reference standards were prepared in 0.2 N perchloric acid solution containing 7.9 mM $Na_2S_2O_5$ and 1.3 mM Na_2EDTA. The sensitivity of the assays was always tested using external standards and a HPLC system BAS-LC4B with an amperometric detector. The working electrode was glassy carbon; the columns were Thermo Hypersil-Keystone, 150×2.1 mm 5 μ Hypersil, Elite C18 and the HPLC system was connected to a computer. Samples were quantified by comparison of