Effects of structural environmental enrichment on welfare of juvenile seabream (*Sparus aurata*)

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A B S T R A C T

Current production systems of finfish aquaculture, and in particular intensive farming systems, can cause welfare problems leading not only to poor condition of the fish but also to a decrease in product quality. Adding structural environmental enrichment (EE) to bare rearing environments may improve the welfare of certain cultured fish. In this study we experimentally demonstrate the positive effects of adding structural EE on rearing environments of juvenile seabream (*Sparus aurata*). Fish maintained for 35 days with EE showed less aggression and interactions with the net pen, and lower erosion of pectoral and caudal fins, compared to fish kept in bare conditions (non-enriched, NE). In addition, EE modified the horizontal distribution of fish in the experimental cage, increasing the use of the inner areas. Non-significant effects of EE were observed on fish body condition and growth, and on brain monoamines levels and mortality. Nevertheless, this work highlights the potential use of structural EE to improve welfare of juvenile seabream, which might be feasible to apply at larger-commercial scale.

1. Introduction

Gilthead seabream (*Sparus aurata*) is one of the most important marine species in the Mediterranean aquaculture sector. Currently, the largest productions of seabream come from intensive farming and can be located both on land and in floating cages at sea (Pavlidis and Mylonas, 2011; Trujillo et al., 2012). Since hatching, each seabream takes almost 2 years to reach 400 g, and the commercial size ranges from 250 g to more than 1500 g. The farming facilities are generally designed and constructed to optimize growth and health (Føre et al., 2018). Nevertheless, the production of fin-fish aquaculture, and in particular intensive farming systems, can cause welfare problems such as stress, health problems, and even mortality at any stage of the production process (Conte, 2004). Moreover, psychological stress is now regarded as a phenomenon impairing the welfare of farmed fish, which may have consequences on production traits (Galhardo and Oliveira,

2009). Stressors in aquaculture, such as handling, transportation, stocking densities and feeding are unavoidable, and reducing both stress and its harmful effects is a fundamental goal for successful growth, production and welfare (Ashley, 2007).

Environmental enrichment (EE), that is, a deliberate addition of physical complexity to the rearing environment, has been considered as a highly recommended tool to guarantee or improve the welfare of laboratory or captive fish (Brydges and Braithwaite, 2009). Structural EE allows the animals a greater control over their environment and experience new situations, approaching the behaviours typical of their species in wild life. Its objective is to improve the psychological and physiological wellbeing of animals in captivity, providing sensorial and motor stimulation in order to help meet their behavioural and psychological needs, increase the behavioural and animal skills options, while reducing the frequency of abnormal behaviours (e.g. vacuum activities, erratic swimming, stereotypic behaviours, high ventilatory

activity) (Brydges and Braithwaite, 2009; Näslund and Johnsson, 2016). It has been shown that EE influences several aspects of the biology and behaviour of fish in captivity. For example, structurally complex habitats offer shelter from aggressive conspecifics, avoid injuries and decrease susceptibility to diseases, modify swimming activity and energy expenditure, increase cognitive capacity and exploratory behaviour, increase appetite and growth rate or physical condition, and in early stages (larvae) can increase the survival rate and decrease the rate of deformity (Näslund and Johnsson, 2016). Enrichment may therefore be an effective way to offer choice, encourage exploration, facilitate curiosity and overall promote positive welfare (Fife-Cook and Franks, 2019).

It is important to note that these effects often vary in direction and magnitude, and that each species and stage of life needs special consideration with respect to its natural history and preferences. It is of special concern for fish farmers that the application of structural EE in tanks or sea cages can be associated with some problems if we compare them with bare environments commonly used. For example, some structures or objects can accumulate food particles and faeces (e.g. enrichment with substrates), making cleaning and disinfection tasks difficult, and thus compromising the health and well-being of fish (Näslund and Johnsson, 2016). In these cases, it is advisable to increase the periodicity of the cleanings or to use internal filters that reduce the accumulation of particles in the structures. It can also happen that the structures used leak potentially hazardous chemicals to the environment (e.g. PVC phthalates), or that their design is inadequate and causes physical disturbances or damage to the fish (e.g. small holes, cracks, protrusions) increasing the risk of infections or the mortality rate and generally reducing their well-being. Another aspect to consider is that the introduction of new objects in the environment can cause negative mental states ("neophobia") in some fish, or that the number of structures is scarce and there is an increase of territoriality and consequently aggressive interactions (Castanheira et al., 2013, 2017; Woodward et al., 2019). Proxies of aggression such as skin and fins lesions and other injuries are usually used as indicators of welfare, considering the time and resources implied in direct or video recorded observations (Martins et al., 2012). The direct relation between aggression and injuries has been demonstrated for a variety of species (e.g. Almazán-Rueda et al., 2004; Jones et al., 2010). Finally, some shelters can lead to a decrease in diet and growth or even to low levels of oxygen if there are a large number of shelters occupied together. Before designing and using any structure as EE, therefore, we must take into account all these aspects, as well as the species-specific biological and behavioural needs and the characteristics of the environment in which enrichment is intended, without forgetting the methods to observe and quantify the parameters that allow a correct evaluation of the welfare of the fish (Martins et al., 2012). Thus, it is necessary to expand the knowledge of the effects of EE and its applicability in the aquaculture industry in a wider context, adapting EE solutions to the biology of the species and the farming systems.

Group swimming behaviour and the use of space in the containment structure (tank, pond, cage, etc) may be good indicators of welfare in fish (Martins et al., 2012). Farming usually implies high densities and individual fish have to respond to the behaviour of others. Adopting polarised schooling is an effective way to minimise the risk of collisions (Føre et al., 2009). In fact, polarity, group velocity and inter-individual distances are characteristics of fish shoals that strongly affect individual members (Pitcher and Parrish, 1993; Parrish et al., 2002). Many behavioral processes, including aggression, stress effects and social behavior seem to be regulated by brain serotonergic and dopaminergic systems, mediators of neuronal plasticity and cognitive functions (Winberg and Nilsson, 1993; Lillesaar, 2011). Serotonine (5-HT) is believed to be involved in the regulation of agonistic behaviour among diverse animal groups, where increased serotonergic activity appears to have an inhibitory effect on aggressive behaviour (Huntingford and Turner, 1987; Miczek et al., 1994). Conversely, dopamine (DA) appears

to have a stimulating effect on aggression, with dominant individuals presenting higher DA levels in the brain (Winberg and Nilsson, 1993). Noradrenaline (NA) seems to be positively correlated with stress, while its effects on aggression are not clear (Winberg and Nilsson, 1993). Therefore, these monoamines and their precursors may be reliable indicators of aggression and stress, and therefore, of fish welfare.

To date, most of the studies on structural EE have been focused on species of ornamentation (e.g. aquaria) or experimentation (e.g. zebrafish *Danio rerio*), and on tilapia (*Oreochromis niloticus*), salmonids (*Salmo* spp*., Oncorhynchus* spp.) or gadids (*Gadus morhua*) as species of aquaculture interest (Näslund and Johnsson, 2016). Regarding farmed fish species of interest in the Mediterranean basin, such as gilthead seabream, the knowledge about the effects of structural EE on these species under rearing conditions is still sparse. It has just been shown that the alteration of the colour of the walls and the substrate of the tanks increased growth and reduced aggression, affecting social interactions and suggesting lower stress levels in the enriched environment (Batzina and Karakatsouli, 2012, 2014; Batzina et al., 2014a, b,c,d). The present study aimed to investigate experimentally whether the presence of structural EE in experimental conditions may have effects on welfare indicators such as growth, fin condition, aggressiveness, horizontal distribution, and brain monoamines of hatchery-reared gilthead seabream juveniles.

2. Material and methods

2.1. Fish, experimental design and holding conditions

Gilthead sea bream juveniles were obtained from a commercial hatchery (Aqüicultura Balear S.A.- Culmarex; Mallorca, Spain) and acclimated to the laboratory conditions for 15 days, before distribution to experimental cages, at the Laboratory of Marine Research and Aquaculture (LIMIA) in Port d´Andratx, Mallorca, Spain. The influence of EE was evaluated experimentally in 80 gilthead seabream juveniles of mean initial body mass (mean ± S.E.) 3.8 ± 0.1 g (age 135 days after hatch; DAH) which were randomly distributed in eight experimental floating cages (foam ring and plastic net, circular: 32 cm diameter × 25 cm depth), with initial rearing densities of about $2 \, kg \, m^{-3}$ (ten fish per cage). Four cages were enriched with three plant-fibre ropes attached equally-distant to the bottom of each cage with two nots alongside and a little buoy on top to keep them in vertical position. Both structural enriched and non-enriched cages (hereafter EE and NE cages respectively) were located inside an indoor bigger tank (10,000 L), at 30 cm distance from each other, with a semi-open flow seawater system, provided with mechanical filters, UV sterilisation and compressed air supply. The photoperiod was operated on a 10:14 h light/dark cycle and water quality was checked daily: mean temperature (± SE) was $20 \pm 1 \, °C$ degrees throughout all the experiment, salinity was 38 ppm and dissolved oxygen was kept close to saturation by aeration through diffusion stones. The tank was thoroughly cleaned daily by siphoning waste settlement (faeces and uneaten pellets) underneath experimental cages. Fish were maintained under experimental conditions for 35 days (22/03/2018–26/04/2018). They were daily fed by hand at 9:00 a.m. a commercial pelleted diet (sinking pellets; 2% of their body mass) specific for gilthead seabream (Skretting® Perla MP).

2.2. Body condition, growth parameters and fins erosion

Both at the beginning (T_0) and at the end (T_{35}) of the experiment, fish were anaesthetized (Tricaine methanesulfonate, MS-222; $0.1 \, g \, L^{-1}$), individually photographed (standardized picture for all the individuals), measured (standard length, SL; cm) and weighted (total weight, TW; g). Condition factor (C.F. $= 100 \times TW \times SL^{-3}$) was calculated for each individual. Variations in SL, TL and CF were estimated as the differences of mean values of each cage between the beginning (T_0) and at the end (T_{35}) of the experimental period. In addition,

growth performance parameters were assessed at cage level as follows: Specific Growth Rate (SGR) = $100 \times [(\ln TW_{35}) \times (\ln TW_0)] \times D^{-1}$, where TW_0 and TW_{35} are the initial and final mean weights at each cage respectively, and D are the rearing days (D = 35 days); Daily Growth Index (DGI) = $100 \times [(TW_{35})^{1/3} - (TW_0)^{1/3}] \times D^{-1}$, where TW_0, TW_{35} and D are as before; and Feed Conversion Rate (FCR) = $F_T \times (TW_{35} - TW_0)^{-1}$, where F_T is the total average dry feed available per fish over the entire rearing period, and TW_0 and TW_{35} are as before. Potential damages on caudal and pectoral (left) fins were visually assessed at the beginning (T_0) and at the end (T_{35}) of the experiment regarding changes in total fin area compared to a perfect fin. Accordingly, Fin Erosion Index (FEI) was calculated for caudal and pectoral (left) fins, which consists in a numerical system based on a visual assessment of fin condition on a scale from 0 (perfect fin) to 4 (short and dysfunctional fin) (Person-Le Ruyet and Le Bayon, 2009; Arechavala-Lopez et al., 2013). Results were expressed as mean erosion levels of a specific fin of all fish per cage examined per experimental condition and sampling period (T_0, T_{35}). The reproducibility and objectivity of these methods were assessed by conducting inter- and intra-operator tests on the same individuals. Two independent assessments were made by the same two trained operators.

2.3. Brain monoamine levels

At the end of the experimental period (T_{35}) all living fish from experimental cages were euthanized with an overdose of anaesthetic (Tricaine methanesulfonate, MS-222; $0.1\,g\,L^{-1}$) and they were subjected to brain sampling by decapitation (including the medulla oblongata). Brain samples were immediately stored at $-80\,°C$ until analysed for brain monoamine levels. Whole brains were weighted and homogenized with an Ultra-Turrax homogenizer (Type Tp 18/10, Janke and Kunkel, Germany) in 1 mL of cold 0.4 M $HClO_4$, 0.01% K2EDTA and 0.1% $Na_2S_2O_5$, and the homogenate was centrifuged at 40,000 g for 15 min at 4 °C. The resulting supernatant was filtered (0.45 μm pore diameter, Millex LH, Millipore) and aliquots were injected into the HPLC system on a reversed-phase column (Spherisorb S3 ODS1 C18; 3-μm particle size range) coupled to a Tracer ODS2 C18 pre-column (2–5 μm particle size range, Teknokroma, Barcelona, Spain); maintained at 35 °C. The mobile phase consisted of 0.1 M KH_2PO_4; 2.1 mM octane sulfonic acid; 0.1 mM K2EDTA; 2 mM KCl and 12% methanol (pH 2.7–2.8, adjusted with 85% H_3PO_4), which was pumped at a flow rate of $0.8\,ml\,min^{-1}$ with a Waters M-600 controller solvent delivery system (Waters, Spain). The compounds were detected electrochemically by means of a cell with a glassy working carbon electrode with an applied oxidation potential of $+0.75$ V against an in situ Ag/AgCl reference electrode (Waters M-2465 Electrochemical Detector). The current produced was monitored by use of an interphase (Waters busSAT/IN Module) connected to a PC. The concentrations of the compounds in a given sample were calculated by interpolating the corresponding peak height into a parallel standard curve using the Waters Breeze software. This method enables to quantify in only one chromatographic run the brain pool of serotonine (5-HT), dopamine (DA) and noradrenaline (NA) primarily stored within neurons; levels of 5-hydroxytryptophan (5-HTP, serotonin precursor) and dihydroxyphenylalanine (DOPA, dopamine precursor), and some monoaminergic metabolites: homovanillic acid (HVA), 5-hydroxy-indolacetic acid (5HIAA) and dihydroxy-phenylacetic acid (DOPAC); which can reveal recent use of these neurotransmitters (Sarubbo et al., 2015).

2.4. Behaviour and horizontal distribution

During the experimental period, behaviour was recorded once a week from the top of each cage. Recordings (120 min duration) took place from 10:00 to 12:00, all cages at the same time, using cameras Sony HDR AS50®. First period of 30 min was excluded to eliminate any possible disquiet caused to the fish by the setting of the cameras.

Behavioural parameters were assessed by visually examining a total of 7 frames, of 15 s each, at 5 min. intervals between frames, using VirtualDub (v1.10.4; Lee, 2013) software. Aggressive-related behaviours were estimated by counting the number of aggressive acts (i.e. chasing, nipping and occasionally biting among fish) in each cage. Results were shown as aggressive acts per individual and minute (N $fish^{-1}\,min^{-1}$). In addition, fish interactions with cage structures, such as net (all cages) and enrichment structures (only EE cages) were counted, and shown as number of interactions per individual and minute (N $fish^{-1}\,min^{-1}$). The number of fish individuals present at each cage was also recorded every day, given that some individuals died by cannibalisms, which was reported as percentage of total alive individuals per day in each treatment.

Horizontal distribution of fish within the cage was also examined once a week from video recordings. A total interval of 35 min from the middle of the video recording was converted into 104 still frames using VirtualDub (v1.10.4; Lee, 2013) every 20 s to avoid autocorrelation between the occupied positions. Afterwards the position of every individual in each of the frames of each video was referenced to a xy plane using ImageJ software (Schneider et al., 2012). The position of the enrichment objects and the arena exterior polygon (using at least 6 points around the arena) was also recorded from each video sequence. A customized R script (www.r-project.org) was developed to: 1) obtain the central point of the arena; 2) derive the margins of the arena; 3) rescale all the videos to the known size of the arena (to avoid small differences in camera positioning); and 4) reference the positions of each individual at each frame. Fish positions were analysed with "adehabitatHR" package to estimate several parameters of use of space and visualize the 2D kernel density plots within the arena borders (Calenge, 2006). The following parameters were estimated: i) percentage of the space used within the experimental cage, where fish spent the 95% (SP_{95}) and 50% (SP_{50}) of the total amount of time; ii) mean distance (DIS) among individuals (in cm); and iii) percentage of the space used at the inner part (SP_{IN}) of the experimental cage (50% centre of total area). Since the fish were not marked it was not possible to identify which or how many individuals showed the above mentioned behaviours, so data refer to the whole fish group in each cage.

2.5. Data analysis

Data regarding body measurements (Sl, TL, CF), growth parameters (SGR, DGI, FCR) and fin erosion indices (FEI caudal and pectoral) were analysed by univariate general lineal model (General Linear Model, Type III, α = 0.095; SPSS statistical package) with treatment (EE and NE) as fixed factor. In the case of brain monoamine levels and distribution parameters (SP_{95}, SP_{50}, DI, SP_{IN}) cage was considered as a random factor nested within treatments to account for cage effects. A multi-way repeated measures ANOVA (General Linear Model, Type III, α = 0.95; SPSS statistical package) was applied to study the effect of time, cage and treatment (EE, NE) on behavioural (aggressiveness and net-interactions). Levene´s test was applied to assess homogeneity of variance, and the data were log-transformed in order to obtain normal distribution when necessary. All values presented in the text and figures are untransformed means (bars-plot) or medians (box-plot) $\pm$ S.E.

2.6. Ethical statement

All the procedures with fish were approved by the Ethical Committee of Animal Experimentation (CEEA-UIB, Spain; Ref. 85/02/18) and carried out strictly by trained and competent personal, in accordance with the European Directive (2010/63/UE) and Spanish Royal Decree (RD53/2013) to ensure good practices for animal care, health, and welfare.

Table 1

Average values ($\pm$ SE) of initial values (T_0), final values (T_{35}) and increments (Δ) on body length (SL; cm), body weight (TW; g) and condition factor (CF), as well as estimated specific growth rates (SGR), daily growth indices (DGI), and food conversion rates (FCR) of juvenile seabream kept under experimental enriched (EE) and non-enriched (NE) conditions.

	Enriched (EE)	Non-enriched (NE)
SL_0	5.21 ± 0.05	5.17 ± 0.05
SL_{35}	6.68 ± 0.08	6.50 ± 0.07
ΔSL	1.43 ± 0.04	1.32 ± 0.05
TW_0	3.86 ± 0.10	3.73 ± 0.08
TW_{35}	7.04 ± 0.27	6.58 ± 0.19
ΔTW	3.12 ± 0.15	2.86 ± 0.10
CF_0	2.71 ± 0.04	2.69 ± 0.03
CF_{35}	2.33 ± 0.03	2.38 ± 0.03
ΔCF	-0.37 ± 0.03	-0.29 ± 0.08
SGR	1.67 ± 0.07	1.62 ± 0.03
DGI	2.97 ± 0.14	2.73 ± 0.10
FCR	1.61 ± 0.08	1.75 ± 0.07

3. Results

3.1. Body condition, growth parameters and fins erosion

After 35 days under experimental conditions, fish body measurements and growth parameters estimated did not show any significant difference between EE and NE conditions (Table 1). At the end of the rearing experiment, no statistical differences were found between EE and NE fish regarding growth in length (SL: ($F_{(1,6)}$ = 2.242; p = 0.185) and weight (TW: $F_{(1,6)}$ = 1.924; p = 0.215), condition factor (CF: $F_{(1,6)}$ = 0.624; p = 0.459), specific growth rate (SGR: $F_{(1,6)}$ = 0.342; p = 0.580), daily growth index (DGI: $F_{(1,6)}$ = 1.924; p = 0.215) and food conversion rate (FCR: $F_{(1,6)}$ = 1.850; p = 0.223) (Table 1). However, the presence of EE significantly improved the fins condition, reducing erosion of pectoral and caudal fins (Fig. 1). Values of FEI on pectoral fins on EE fish showed a significant decreased during the experiment ($F_{(1,45)}$ = 21.816, p = 0.001) while no significant difference was observed on NE fish ($F_{(1,43)}$ = 0.002; p = 0.961); resulting in different pectoral fins conditions between treatments at the end of the experiment ($F_{(1,53)}$ = 10.434, p = 0.002) (Fig. 1a). Regarding caudal fin condition, a significant increase in fin erosion values was observed in both treatments (EE: $F_{(1,45)}$ = 15.725, p = 0.001; NE: $F_{(1,43)}$ = 63.267, p = 0.001); and values of fin erosion on caudal fins were higher in NE fish compared to EE fish ($F_{(1,53)}$ = 5.695, p = 0.021) (Fig. 1b).

3.2. Brain monoamine levels

The presence of EE in experimental cages did not show any effect on brain monoamine levels of juvenile seabream. Average ($\pm$ SE) levels of brain 5-HT (EE: 1510.16 $\pm$ 93.76 pg g^{-1}; NE: 1694.85 $\pm$ 79.6 pg g^{-1}) did not show significant differences between

treatments ($F_{(1,47)}$ = 1.906, p = 0.174) and among cages ($F_{(7,47)}$ = 1.640, p = 0.148). Brain NA levels (EE: 364.79 $\pm$ 17.42 pg g^{-1}; NE: 390.47 $\pm$ 14.34 pg g^{-1}) did not differ between treatments ($F_{(1,47)}$ = 0.830, p = 0.367) and among cages ($F_{(7,47)}$ = 2.029, p = 0.072). DA levels (EE: 100.87 $\pm$ 5.07 pg g^{-1}; NE: 100.47 $\pm$ 4.04 pg g^{-1}) did not show a significant difference between treatments ($F_{(1,47)}$ = 0.247, p = 0.622) but significantly differed among cages ($F_{(7,47)}$ = 3.165, p = 0.008). Similarly, brain levels of serotonine precursor 5-HTP (EE: 548.22 $\pm$ 36.55 pg g^{-1}; NE: 537.65 $\pm$ 27.41 pg g^{-1}) did not show any difference between treatments ($F_{(1,47)}$ = 0.058, p = 0.811) but significantly differed among cages ($F_{(7,47)}$ = 3.250, p = 0.007). Dopamine precursor DOPA levels (EE: 340.51 $\pm$ 27.01 pg g^{-1}; NE: 415.09 $\pm$ 29.31 pg g^{-1}) did not show any difference between treatments ($F_{(1,47)}$ = 2.728, p = 0.105) and among cages ($F_{(7,47)}$ = 1.278, p = 0.282). Likewise, monoaminergic metabolite HVA levels (EE: 36.38 $\pm$ 5.94 pg g^{-1}; NE: 32.68 $\pm$ 4.71 pg g^{-1}) did not show any difference between treatments ($F_{(1,47)}$ = 0.085, p = 0.772) and among cages ($F_{(7,47)}$ = 0.487, p = 0.839). Brain 5-HIAA levels (EE: 82.21 $\pm$ 10.37 pg g^{-1}; NE: 69.18 $\pm$ 3.58 pg g^{-1}) did not differ between treatments ($F_{(1,47)}$ = 0.836, p = 0.365) and among cages ($F_{(7,47)}$ = 1.699, p = 0.132). Brain levels of metabolite DOPAC (EE: 17.78 $\pm$ 1.57 pg g^{-1}; NE: 18.13 $\pm$ 1.71 pg g^{-1}) did not show any difference between treatments ($F_{(1,47)}$ = 0.138, p = 0.712) and among cages ($F_{(7,47)}$ = 2.053, p = 0.068).

3.3. Behaviour and horizontal distribution

The number of total aggressive acts of juvenile seabream reared in EE (2.95 $\pm$ 0.10 acts min^{-1} fish^{-1}) were significantly lower ($F_{(1,280)}$ = 7.052, p = 0.033) than fish reared in NE condition (3.15 $\pm$ 0.12 acts min^{-1} fish^{-1}), and did not show any difference among cages ($F_{(7,280)}$ = 3.292, p = 0.117) and throughout the 5-weeks experimental period ($F_{(4,280)}$ = 2.291, p = 0.085) (Fig. 2a). The number of interactions with the net of the experimental cage was significantly lower ($F_{(1,280)}$ = 79.693, p = 0.001) in EE fish (2.22 $\pm$ 0.11 acts min^{-1} fish^{-1}) compare to NE (3.37 $\pm$ 0.17 acts min^{-1} fish^{-1}), which also significantly differed among periods ($F_{(4,280)}$ = 29.554, p = 0.003) but not among cages ($F_{(7,280)}$ = 5.290, p = 0.052) (Fig. 2b). Regarding fish interactions with EE structures, an average of 0.175 $\pm$ 0.04 acts min^{-1} fish^{-1} were observed during the total experimental period. Additionally, mortality events by cannibalism were observed; both treatments showed similar decrease in percentages of live fish throughout the experimental period (Fig. 3).

According to the horizontal distribution of juvenile seabream inside experimental cages (Fig. 4), no significant difference was observed between treatments in terms of percentage of the space used during the 95% (SP_{95}) of the total amount of time (EE: 19.38 $\pm$ 0.95%, NE: 19.67 $\pm$ 1.03%; $F_{(1,30)}$ = 0.933, p = 0.342); and no significant difference was detected throughout the experimental period ($F_{(4,30)}$ = 1.945, p = 0.129). Similarly, the percentage of the space used the 50% of the total amount of time (SP_{50}) did not differed between treatments (EE:
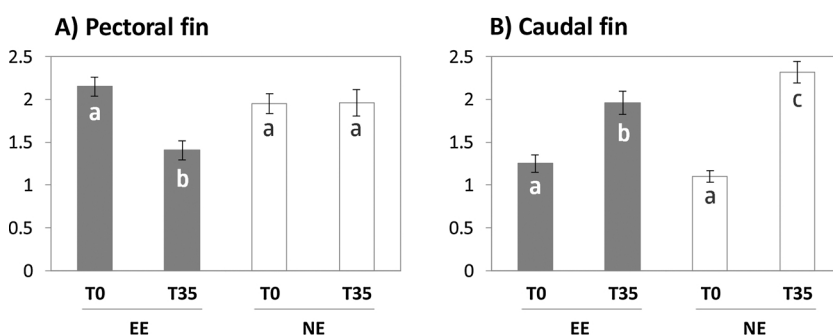

A) Pectoral fin

B) Caudal fin

Fig. 1. Bar-plot showing the average ($\pm$ SE) of fin erosion indices (FEI) estimated for pectoral and caudal fins of juvenile seabream reared under enriched (EE, grey bars) and non-enriched (NE, white bars) conditions at the beginning (T0) and at end of the experiment (T35). Significant differences among treatments and times are marked with dissimilar letters (p < 0.05).

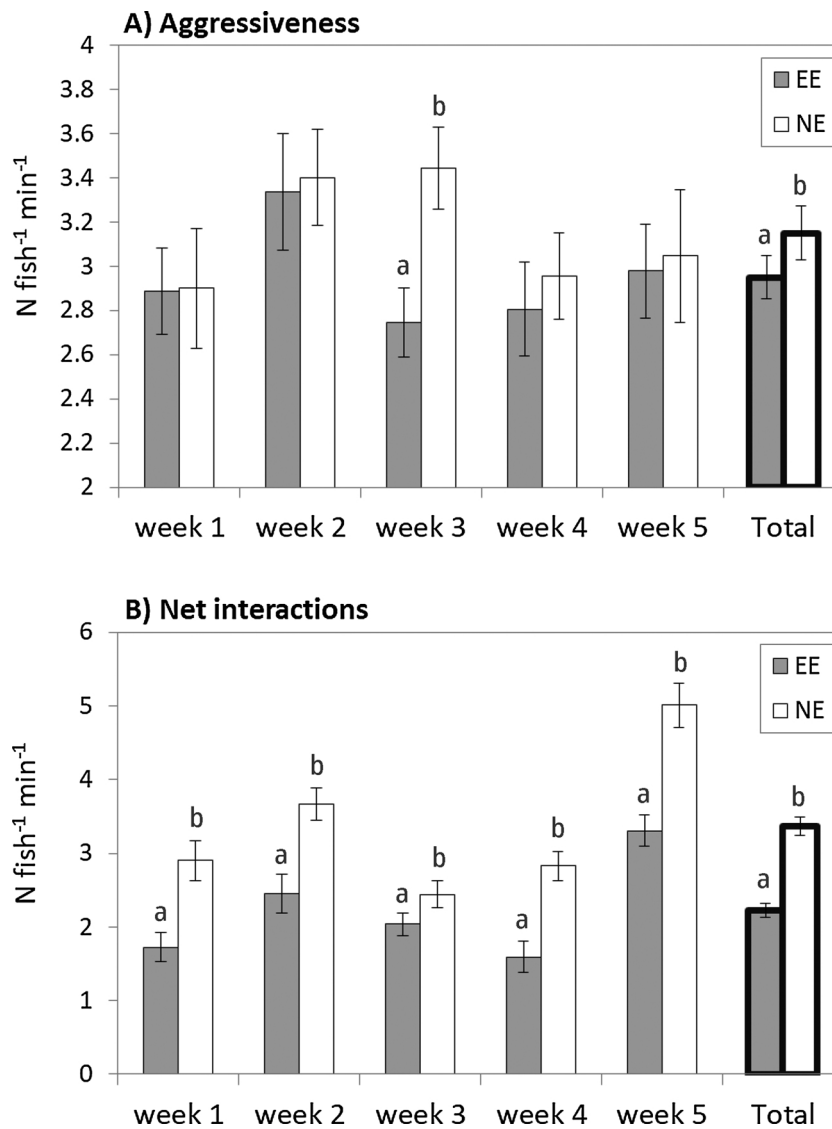**Fig. 2.** Bar-plots showing the mean (± SE) number of (A) aggressive acts (N min-1 fish-1) and (B) interactions with the net of the experimental cage (N min^{-1} fish^{-1}) of juvenile seabream reared under enriched (EE, grey bars) and non-enriched (NE, white bars) conditions throughout the 5-weeks experimental period. Significant differences among treatments at each experimental period are marked with dissimilar letters ($p < 0.05$).
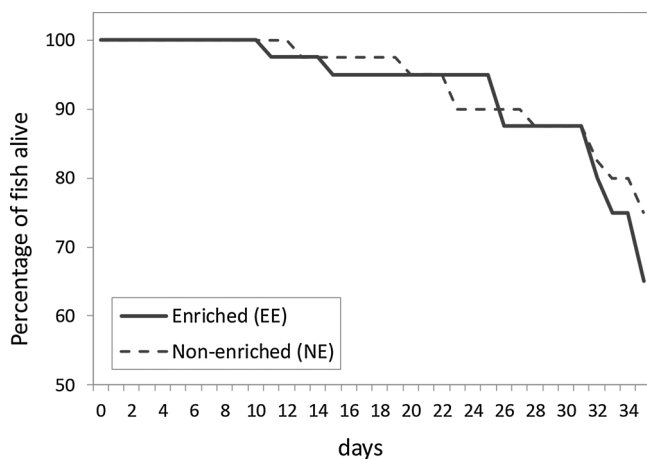

Fig. 3. Percentage of juvenile seabream alive at enriched cages (EE, black line) and non-enriched cages (NE, dashed line) throughout the experimental period.

5.60 ± 0.37%, NE: 6.38 ± 0.35%; $F_{(1,30)} = 2.431$, $p = 0.129$) nor through the experimental period ($F_{(4,30)} = 1.934$, $p = 0.131$). Mean distance among individuals (DI) did not differ between treatments (EE: 10.09 ± 0.14 cm, NE: 10.45 ± 0.12 cm; $F_{(1,30)} = 1,122$, $p = 0.298$) and through the experimental period ($F_{(4,30)} = 2.569$, $p = 0.058$). However, fish reared in EE cages presented significantly higher proportion of the use of the inner part (SP$_{IN}$) of the experimental cage (EE: 45.17 2.10%) compared to NE fish (NE: 37.16 1.79)($F_{(1,30)} = 9.808$, $p = 0.006$), but did not differ through the experimental period ($F_{(4,30)} = 0.220$, $p = 0.925$).

4. Discussion

The present results show that EE modified distribution of gilthead seabream inside the experimental cage, entailing a higher use of the inner area of the cage. In addition, a lower interaction with the net and lower aggressiveness among individuals was shown, which consequently, improved pectoral and caudal fins conditions. This agrees with previous works in territorial fish assessing the functions of structural EE of reducing aggression and territory size, and allowing more individuals per unit area (Eason and Stamps, 1992; Dolinsek et al., 2007;
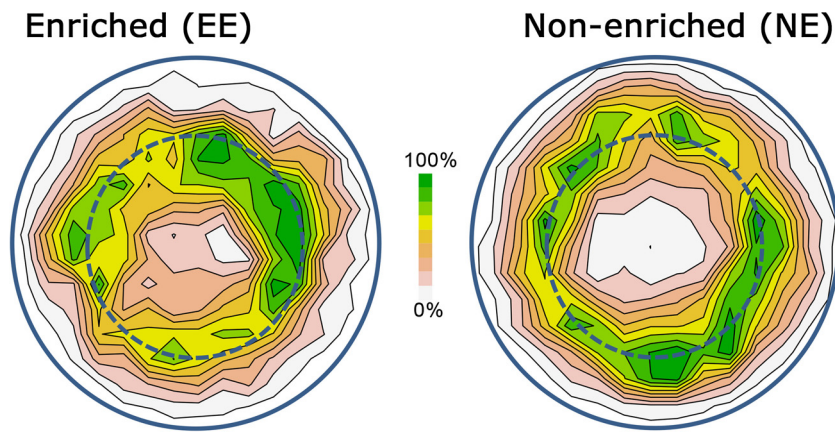
Enriched (EE)

Non-enriched (NE)

Fig. 4. Horizontal distribution of juvenile seabream reared under enriched (EE) and non-enriched (NE) conditions in experimental cages. Colour gradient shows the percentage of fish occupancy throughout the whole experimental period. External line represents the perimeter of the experimental cage and dashed line represents the inner part of the cage (SP_{IN}).

Gustafsson et al., 2012). In bare environments, like rearing circular tanks or cages, fish like seabream shoal in polarised schools swimming in circles next to the net walls and apparently do not use the centre of the cage frequently. Providing physical complexity promotes the spatial exploration, changing the distribution of the fish (increasing the use of the inner part of the cage) and giving more space between the net and the fish shoal. Fish interactions with the net-walls decreased in EE conditions, reducing the probability of escape from the cage, which might lead to negative environmental and economic consequences (Arechavala-Lopez et al., 2018). In agreement, previous studies on Atlantic cod (*Gadus morhua*) reared with EE structures showed a reduced swimming activity, as well as a more context-dependent variability in shoaling, and spent less time interacting with the net cage (Salvanes and Braithwaite, 2005; Salvanes et al., 2007; Moberg et al., 2011; Zimmermann et al., 2012).

A decrease of net-interactions and aggressiveness in EE-reared environments also contributes to better pectoral and dorsal fins conditions of sea bream juveniles. Reduced fin damage in enriched environments has been observed in salmonid species (Bosakowski and Wagner, 1995; Wagner et al., 1995; Berejikian et al., 2000, 2001; Arndt et al., 2001; Berejikian and Tezak, 2005; Näslund et al., 2013). Some enriching structures lead to increased sheltering opportunities and/or visual isolation for subordinates, reducing the levels of aggression. The structures used to enrich the environment in the present study (i.e. plant-fibre ropes) however did not provide a proper shelter. Therefore, reductions in fin deterioration seem to mostly depend on increased inter-individual space and reduced abrasion with the cage structure, though other synergic effects due to fish aggressiveness, activity and/or territoriality cannot be ruled out. Indeed, increasing environment complexity with physical structures like plant-fibre ropes, entangled plastic strips or net structures, inhibit fish manoeuvrability, restrict visual contact and reduce general activity levels, which consequently, reduce aggressive behaviour and cannibalism (Näslund and Johnsson, 2016). Shelter structures used in aquaculture (i.e. covers made by entangled plastic strips or mesh) have been shown to improve growth and survival in catfish species (Hossain et al., 1998; Coulibaly et al., 2007). However, other studies using similar covers (i.e. half PCV cylinders and plastic mesh) reported that cannibalism was not reduced (Baras et al., 1999; Rahmah et al., 2014). The differing results could depend on the fact that shelter structures were different between the studies, as well as number of fish, experimental time and rearing environment. Similarly, contradictory results can also be found in terms of growth, which seem to depend mainly on fish species and size, reflecting the ecology of the species (Näslund and Johnsson, 2016). In our study, growth and survival of sea bream juveniles were not affected by the presence of structural EE. Juvenile sea bream is highly aggressive at this life-stage, especially during feeding competition (Andrew et al., 2004). It is possible that growth would eventually differ among treatments in a longer experiment: in a similar study, Batzina et al (2014a) showed size

differences in sea bream reared with different substrates during 98 days, while our experiment lasted 35. Other non-exclusive explanations might include the influence of dominance hierarchies of this species and the associated agonistic behaviours, which mainly occur during feeding (e.g. Goldan et al., 2003; Andrew et al., 2004; Montero et al., 2009; Arechavala-Lopez et al., 2019) or due to territoriality (Eason and Stamps, 1992; Baras and Jobling, 2002). Food is a valuable resource for fish and any factor that may favour food monopolisation may also increase competition for food and intensify aggression, even in the case of intensive aquaculture where food is usually offered abundantly (Oikonomidou et al., 2019). However, territoriality and related aggression (including cannibalism) seem to be dependent on fish species, life-stage and food availability too; but also on fish abundances, activity and competitor densities, among other factors (Eason and Stamps, 1992; Baras and Jobling, 2002).

Behavioural and physiological responses to stress have common control mechanisms in the brain, and the monoamine neurotransmitters play a vital role (Winberg and Nilsson, 1993; Ashley, 2007). Lower activity of brain monoamines, lower basal cortisol levels and reductions in agonistic behaviours among fish were previously reported on seabream reared with gravel substrate where fish can search for food, suggesting lower stress levels in the enriched environment, though effects were only apparent with certain colours (Batzina and Karakatsouli, 2012; Batzina et al., 2014a,b,c). Our findings did not show significant differences between treatments regarding brain monoamines concentrations. Similar to the lack of growth effect or survival rates, the lack of differences in brain monoamines might be a result of shorter duration of the experiment or confounding effects of social hierarchies; but the possibility of chemical communication among fish from different cages cannot be ruled out. Indeed, the experimental cages were all situated in one tank with same water, and this setting could have a buffer effect on the influence of the enrichment. It is known that gilthead seabream present a high olfactory sensitivity to conspecific body-fluids, mainly to compounds present in the intestinal fluid (Hubbard et al., 2003). These signals are very dependent on the physiological state of the fish and particularly on their level of stress (Olivotto et al., 2002; Saraiva et al., 2015). Nevertheless, any positive effect in welfare indicators would have to override putative stress signals in the water; and therefore, even though we have a conservative approach where control fish not experiencing enhanced conditions may be emitting stress signals, other aspects of the welfare of treated individuals were improved via structural EE. Therefore, further research is needed to assess the potential positive and negative effects of enriched environments on fish behaviour and physiology under optimum rearing conditions.

The effects of adding physical structures in rearing environments are likely to be species specific and dependent on the fish life-stage, but also on the type of structure, level of complexity added and characteristics of the captivity environment or farming system. It is

therefore essential to adapt EE solutions carefully to biological needs of the species, as well as to control that the intended effects really are achieved (Näslund and Johnsson, 2016), in order to ensure positive effects on fish welfare in cultured conditions or avoid undesirable ones. Importantly, the construction of natural-like environments is often hampered by our biased and often inappropriate perception of fish sensory worlds (Saraiva et al., 2018). Prior to implementing EE structures on the farming industry, preference tests can be used to gain knowledge about which environment an animal desire (Dawkins, 2006; Volpato et al., 2007), and consequently, helping to design appropriate EE. Many captive species do indeed show preferences for structural enrichment when given a choice, at least in some contexts, but may differ among species, populations and age classes, and often time-dependent (Näslund and Johnsson, 2016). Other species do not always prefer structural EE, thus, better to keep the rearing environment simple rather than provoke undesirable effects. There is however a lack of knowledge on the effect of EE on many other fish species of farming interest, as well as on many types of EE structures and farming systems. Concerning seabream, there are still many questions to be solved before implementing any kind of structural EE at an industrial scale, but we hope this study can help to move towards this end. Fin-fish aquaculture industry might show special interest if implementing structural EE helps to reach higher yield through increasing survival, growth or quality, and therefore, producing economic benefits (Olesen et al., 2010; Grimsrud et al., 2013). However, such economic gains can be uncertain due to gaps between ethical attitudes and actual consumer behaviour (Carrigan and Attalla, 2001). Implementing proper and feasible EE structures might be a good complement in fish welfare for the industry, benefiting from a better public perception, commercialization and acceptance of products, but also in terms of efficiency, quality and quantity of production (Ashley, 2007).

Declaration of Competing Interest

No conflict of interest.

Acknowledgements

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