

Potential effects of environmental enrichment on seabass behavior: from group to individual level

Samira Nuñez Velazquez

Master's Thesis

Master's degree in Marine Ecology

at the

UNIVERSITAT DE LES ILLES BALEARS

2018-2019

UIB Master's Thesis Supervisor: Pablo Arechavala Lopez

UIB Master's Thesis Co-Supervisor: Carlos Diaz Gil

Abstract	3
1. Introduction	4
2. Material and methods	6
2.1. Experimental design	6
2.2. Effects of EE at Group level	6
2.3. Effects of EE at Individual level	9
Ethical statement	
3.Results	
3.1 Effects of EE at Group level: First experimental period	
3.2 Effects of EE at Individual level: Second experiment	14
4. Discussion	
Acknowledgments	
References	

Potential effects of environmental enrichment on seabass behavior: from group to individual level

Abstract

A function-based approach of appropriate welfare in aquaculture consists in the ability of the fish to adapt to its captive environment and keep its biological functions correctly working. Environmental enrichment (EE) has been considered as a highly recommended tool to guarantee or improve the welfare of laboratory or captive fish over the last years. As the fish are reared in large numbers at high densities, most farmers usually use behavioral welfare indicators to assess the condition of the whole group. However, behavioral responses are dependent also on the coping style that characterizes an individual. The European seabass (Dicentrarchus labrax) is one of the most important fish species in terms of aquaculture production in Europe. Given that it is a very sensitive species to stressful conditions, the aim of this work was to analyze the effects of EE on seabass behavior, as a proxy of its welfare status. The spatial distribution and the growth performance were tested at group level, while the exploratory behaviour and learning process were assessed individually by risk-taking test. Fish were maintained during 29 days with structural EE showed no differences in growth performance and spatial distribution. Despite of that, the behavioural responses were different in seabass during feeding period. Regarding individual behavioral measurements Stress Coping Style (SCS) the fish reared under control condition showed higher exploratory activity. However, one of the control tanks presented an abnormal behaviour compare to the rest possibly due to its spatial location within the hatchery. Moreover, without including this tank, the treatment had no effect on the risk-taking test until the last week. Furthermore, the enriched fish showed a tendency of learning trough the weeks. These differences are indicative that enrichment could promote behavioural plasticity and proactive behaviour which might be advantageous for the aquaculture. This study evidences positive effects of structural enrichment on seabass behavior, but also that the aquaculture industry needs to further research this topic considering species-specific biology and behavioral characteristics before implementing environmental enrichment protocols.

Keywords: aquaculture- welfare- behavior- structural environmental enrichment- European seabass- individual stress coping style

1. Introduction

The welfare of farmed animals is a key requirement of animal-rearing systems. The EU Council Directive 98/58/EC provides minimum protection and welfare standards for animals kept for farming purpose, irrespective of the species and including fishes (Fraser et al, 2012). However, the definition of welfare can be different depending on the context and the approach given. Function-based approaches link welfare with the biological and physiological condition of the animal, while a feeling-based definition includes the emotional state and balance between positive and negative subjective experiences (Huntingford & Kadri, 2008). In fish farming (aquaculture) a function-based approach of appropriate welfare consists in the ability of the fish to adapt to its captive environment and keep its biological functions correctly working (Ashley, 2007). Increasing the survival, quality, growth, even to improve the appearance of the fish are issues that contribute positively to the economic benefits of the fish farmers, besides to be in concomitance with the law (Näslund & Johnsson, 2016). Therefore, in this context, there is a big concern about how to improve the welfare of captive fish. The captivity environment is considerably different from that experienced in nature (Huntingford, 2004). For example, space is restricted, migration is not possible, the food is easily attainable, and the risk of predator does not exist (Saraiva et al, 2019). In addition, other characteristics of the routine of husbandry could potentially affect welfare, such as handling, transportation, high densities and confinement (Huntingford 2004; Ashley, 2007; Huntingford et al, 2012; Näslund & Johnsson, 2016). It has been proved that this kind of stressors can induce changes in the behaviour and physiology of the fish. For example, a reduction in the food intake levels or in feed conversion efficiency can result in a reduced or negative growth performance (Leal et al, 2011). Since behaviour is the best-known indicator of the biological and mental state of an animal, it can be an useful tool to assess the welfare in captive fish (Saraiva et al, 2019). Many studies have been focused on describing behavioural indicators of poor welfare, while information about promoting good welfare and strategies to improve it needs further research (Martins et al, 2012).

Environmental enrichment (EE) has been considered as a highly recommended tool to guarantee or improve the welfare of laboratory or captive fish over the last years (Brydges &Braithwaite, 2009). It refers to improving the environment of captive animals, by increasing the environmental complexity in its physical, temporal and/or social dimensions, and consequently can be a good approach to promote positive welfare of captive fish. Indeed, structural EE that is, a deliberate addition of physical complexity to the rearing environment, has been confirmed to be beneficial in several biological aspects, such as growth performance, behavior and cognitive abilities (e.g. Näslund & Johnsson, 2016; Sullivan et al, 2016). Depending on the objective, the enrichment can be (1) physical if it is an added structure or any modification; (2) sensorial in which the sensory organs are stimulated; (3) occupational when the possibilities for exercise or psychological challenge are given; (4) dietary which includes changes in the type or the delivery of food; and (5) social if any type of contact with conspecifics and/or other species is allowed. All these types of enrichment are often missing in an aquaculture context; and moreover, the farms are kept under standardized conditions, for example using tanks of the same color and shape (Näslund & Johnsson, 2016). The natural environment cannot be exactly recreated in the hatcheries, so the objective when designing enrichment is to modify elements of the artificial environments in order to provide welfare benefits without compromising the biosecurity of the farms (Lee et al, 2019). However, the effect of the EE on fish welfare is not always clear because the reaction can be different depending on the species, the life-stage, the number of fish affected, the husbandry system and the type of the enrichment (Sullivan et al,

2016; Toni et al, 2019). Therefore, before designing and using any structure as physical EE, we must take into account 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). Therefore, it is necessary to expand the knowledge of the effects of EE and its applicability in the aquaculture industry in a wider extent, adapting EE solutions to the biology of the species as well as the farming systems and development (Arechavala-Lopez et al, 2019).

Farmed fishes can show changes in foraging behaviour, ventilatory activity, aggressiveness, and swimming behaviour during the rearing period. These can be linked with acute stress and therefore, act as indicator of poor welfare. Other measurements as exploratory behaviour, feed anticipatory activity and reward-related operant behaviour can be used as indicator of good welfare and positive emotions (Martins et al, 2012). As the fish are reared in large numbers at high densities, most farmers usually use behavioural welfare indicators to assess the condition of the whole group. However, individual variations exist and can be the key to understand group behaviour. For example, group swimming behaviour consists in the assessment of the horizontal and vertical distribution, swimming speed and direction of the whole group. Nevertheless, it has observed in model studies that one fish with different swimming parameters can affect the behaviour of the whole group (Romey, 1996). Moreover, the behavioural differences of this single fish could be due to a different ability to deal with stressful conditions, which can result in a different state of welfare. Therefore, the assessment of welfare in a whole group can be not totally representative of the welfare of each individual and vice versa (Martins et al, 2012).

For that reason, it should be noted that behavioural responses are dependent also on the coping style that characterizes an individual (Martins et al, 2012). It also means that most behavioural variation could be due to intra-individual variation and not to measurement errors or uncontrolled variation in environmental conditions as it is often supposed (Jolles et al, 2019). On the other hand, different responses to stress can be linked with the two major types of coping style: individuals that are shy and adaptive to stress conditions could be considered as reactive; while individuals more aggressive and bolder are the proactive (Koolhaas et al, 1999). However, Stress Coping Style (SCS) is not a rigid characteristic, the individuals can adjust their behaviour to changing environmental conditions. That means the same individual can be reactive under specifics conditions and proactive under others, this ability is called behavioural plasticity. Furthermore, as these individual differences can be consistent in time, some individuals can be much more predictable in a given context than others (Biro & Adriaenssens, 2013).

To date, most of the studies on EE have been focused on species of ornamentation (aquaria) or experimentation (e.g. zebrafish *Danio rerio*), and especially on tilapia (*Oreochromis niloticus*), salmonids (*Salmo salar, Oncorhynchus* spp.) or cod (*Gadus morhua*) as species of aquaculture interest (Näslund & Johnsson, 2016). Regarding farmed fish species of interest in the Mediterranean basin, such as Gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) there is still a lack of knowledge about the effects of EE on these species in rearing conditions. For example, it has been shown that the alteration of the color of the walls and the substrate of the tanks increased growth and reduced aggression of sea bream, suggesting lower stress levels in the enriched environment (Batzina & Karakatsouli, 2012, 2014; Batzina et al, 2014a,b,c,d), and the presence of structures in experimental conditions modify the spatial distribution of the shoal inside the cage (Arechavala-Lopez et al, 2019). Nevertheless, studies concerning European seabass are still absent. The seabass is identified as one of the most

important fishes in terms of aquaculture potential in Europe (Leal et al, 2011). This fish is well known for being very sensitive to the stressful conditions; for instance, the reduction of its feeding activity due human presence is documented (Rubio et al, 2010). Moreover, husbandry stress during early life stages affects the health status of juvenile seabass increasing mortality rates and disease responsiveness (Varsamos et al, 2006).

Therefore, the aim of this work was to analyze the effect of EE on seabass behaviour, as a proxy of welfare status. In order to provide an overall approach, the individuals were tested at both group and individual levels. The spatial distribution and the growth performance were tested at group level, while the risk-taking test was assessed using a group-approach but acquiring behavioural parameters individually.

2. Material and methods

2.1. Experimental design

Four hundred and twenty seabass (mean body mass 16.52 ± 5.6 g) were obtained from a commercial hatchery (Aqüicultura Balear S.A.- Culmarex; Mallorca, Spain) and acclimated to the laboratory conditions for one week at the Laboratory of Marine Research and Aquaculture (LIMIA) in Port d'Andratx, Mallorca, Spain. They were randomly distributed in 6 circular tanks (water volume 150 L) in groups of 70, and maintained at a temperature of 20 ± 1°C and on a light: dark (12 h:12 h) photoperiod (Fig 1). Salinity was 38 PSU and dissolved oxygen was kept close to saturation by aeration through diffusion stones. The tanks were provided with mechanical filters, with a semi-open flow seawater system, UV sterilization, and compressed air supply. Three tanks were enriched with 3 plant-fiber ropes hanging from one edge of the tank to the other, two parallel (130 cm) and one perpendicular larger (170 cm), all of them at different depths and similar distances among them (Fig 2a). The other three tanks were the control treatment (Fig 2b). The choice of this type of enrichment was made in regards of the swimming behaviour of the species, given that seabass made vertical movements in the water column and the horizontal ropes might represent an obstacle/challenge. They were daily fed by hand at 13.00 p.m a commercial pelleted diet (sinking pellets; 2% of their body mass) specific for seabass (Skretting[®] 106 Perla MP). All tanks were thoroughly cleaned daily by siphoning faeces and uneaten pellets, and about 2/3 of water were removed once a week. The seabass juveniles were maintained under experimental conditions for 84 days (12/03/2019 - 03/06/2019), during which they were exposed to two different experimental periods to investigate effects at the group and individual levels respectively. During the entire experiment nine individuals were found dead.

2.2. Effects of EE at Group level

2.2.1. Growth parameters

The first experiment lasted 29 days. In order to assess the effect of EE on seabass growth, seabass juveniles were anesthetized (Tricaine methanesulfonate, MS-222; 0.1 g L⁻¹) at the beginning (T₀) and at the end (T₂₉) of the experiment, and the body length (SL: standard length, cm) and weight (TW, gr) were measured. The Condition factor (C.F. = 100 x TW x SL³) was calculated for each individual. Variations in SL, TW, and CF were estimated as the differences of mean values of each tank between the beginning (T₀) and the end (T₂₉) of the experimental period. The following growth performance parameters were calculated for the whole fish group in each tank: Specific Growth Rate [SGR = 100 x [(In TW₂₉) x (In TW₀)] x D⁴, TW₂₉: mean final

body mass, g; TW₀: mean initial body mass, g; D₋₁: days of rearing], Daily Growth Index [DGI = $100 \times [(TW_{29})_{1/3} - (TW_0)_{1/3}] \times D_{-1}]$; Daily Feed Intake [DFI = $100 \times F_D \times TW_M^{-1}$, F_D: average dry feed potentially consumed per fish per day, g; TW_M: mean fish weight per tank obtained as an average of the initial and final weight, g]; and Feed Conversion Rate [FCR = F_T x (TW₂₉- TW₀)-₁, F_T: total average dry feed available per fish over the entire rearing period, g].

Data regarding body measurements (SL, TL, CF) and growth parameters (SGR, DGI, DFI, FCR) were analyzed by univariate General linear model (GLM, Type III, α =0.095; SPSS statistical package) with treatment (enriched and control) as a fixed factor. In every analysis the tank was considered as a random factor nested within treatments to account for the possible effects. Levene's test was applied to analyze data heterocedasticity.



Fig 1. Experimental tanks at LIMIA (1-4-5 Enriched, 2-3-6 No enriched)





Fig. 2 a) Tank with enviromental enrichment. b) Control treatment

2.2.2. Horizontal distribution

During the first 29 days (12/03/2019- 09/04/2019) behaviour was recorded for 2 hours every four days from the top of each tank. First and last period of 30 min were excluded to eliminate any possible disquiet caused to the fish by the setting of the cameras. At the middle of the recording, the fish were fed in order to have the activity influenced or not by the food stimulus (PreFeeding: before feeding, DurFeeding: during/after feeding). Spatial distribution was analyzed converting one hour of video into 30 frames using VirtualDub (v1.10.4; Lee, 2013) every 2 minutes. The position of every fish in each of the frames was referenced to an XY plane using ImageJ software (Schneider et al, 2012). The first frame of each video was used to determine the position of the enrichment objects and the arena exterior polygon (using 10 points around the arena in the control frames, and 18 points in the frames with enrichment)

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 analyzed 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 tanks, where fish spent the 90% (Usage 90%) and 50% (Usage 50%) of the total amount of time (see Models 1 and 2); ii) mean distance among individuals in centimeters (Mean dist cm) (see Model 3). Since the fish were not marked it was not possible to identify which or how many individuals were related to above mentioned parameters, so data refer to the whole fish group in each tank.

The data were analysed using generalized linear mixed models (GLMM, 'Imer' function in R, 'Ime4' library, Bates et al, 2015) with treatment (Treat) and feeding period (Feeding) as fixed factors (see Models below). The tank was considered as random factor nested within week to account for the possible effects of both variables.

Model 1. Usage 50% ~ Treat*Feeding + (1 | Tank/Week) Model 2. Usage 90% ~ Treat*Feeding + (1 | Tank/Week) Model 3. Mean dist cm ~ Treat*Feeding + (1 | Tank/Week)

2.3. Effects of EE at Individual level

2.3.1. Risk-taking test

Once the first period of the experiment finished, fish were individually PIT-tagged (TrovanH, Netherlands) and maintained in the same conditions for another month before to start the second part of the experiment, where fish were exposed to a risk-taking test (or exploratory test) in order to assess individual behavioural traits or stress coping styles (SCS) (Castanheira et al. 2013). The risk-taking test is a group-based test that consists of testing the ability of the fishes rearing in different conditions to explore a new risky area. Two cages connected by a tunnel was settled inside a bigger tank were the environmental conditions were similar to the previous experimental period (Fig 3 a,b). One cage was provided with unattainable food to encourage the passage and it was considered as the risky area. A PIT-tag detection antenna (diameter 100/125 x 620mm, Trovan[®], Netherlands) was located around the opening of the tunnel, which allowed monitoring individual passages from one cage to the other. Each group of fish from each tank of the previous experiments was left in the safe area (empty cage)

for 1 hour and 15 minutes. They were acclimated during the first period of 15 minutes while they were not allowed to pass through the tunnel. The number of movements between cages was determined through antenna detections. The test was repeated four times (every four days for 16 days: 08/05/2019-24/05/2019) and it was recorded with a Sony[®] camera to verify the correct functioning of the antenna if it was necessary. According to previous studies, proactive fishes are behaviourally characterized by being faster to explore unknown environments and high risk-taking conduct (Castanheira et al, 2017).





Fig 3. a) Experimental cages at Limia b) Schematic of the experimental cages in risk taking test.

a)

2.3.2. Stress coping style assessment

First, the passes in which the time between the previous pass and the next one of the same fish was less than 19.45 seconds were removed from the entire data set. The reason behind this was to automatically determine "fake passes" in which the fish remained motionless inside the tunnel and hence the antenna was continuously detecting it. This was achieved using a segmented regression with an unknown breakpoint precisely to identify the time period which could be considered as new "real passes" along the tunnel (19.45 seconds; Muggeo, 2003). After this, a Bayesian approach was followed to fit generalized linear mixed effects models (GLMMs, R library "MCMCgImm" (Hadfield 2010; Dingemanse & Dochtermann 2013; Harrison et al, 2014; Alos et al, 2017; Sbragaglia et al, 2019)) that were used to test for differences in the number of fish passes through the tunnel among tanks, weeks and between treatments. Zero inflation Poisson structure was considered accounting for the type of data that was being fitted. The GLMM included week and treatment as fixed effects and the identity of the fish and the tank as random intercept terms. In this model, we used the entire data set without considering differences in size of the fish because it was previous tested and no size effect was found on the number of passing through the antenna. The parameters, 97.5% credibility intervals, and Pvalues were estimated using a Bayesian Markov chain-Monte Carlo approach with uninformative priors. We set up the initial iterations to 500000, after discarded the initial 1000 iterations (burn-in period); 1 out of 100 of the remaining iterations were kept to prevent autocorrelation (thinning strategy) to obtain 4990 posterior samples. The convergence of the MCMC chains was assessed by visual inspection of the chains. The adjusted repeteability (Adjusted-R) was estimated as the quotient of the between-individual variance (the variance across random intercepts attributed to the individuals: Vind) and the sum of Vind and the withinindividual or residual variance (the variance associated with the tank and measurement error) for a given behavioural trait in accordance with previous studies (Nakagawa & Schielzeth 2010).

Ethical statement

All the procedures with fish were approved by the Ethical Committee of Animal Experimentation (CEEA Ref. 85/02/18) and carried out strictly by trained and competent personnel, 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.

3. Results

3.1 Effects of EE at Group level: First experimental period

After 29 days under experimental conditions, fish body measurements and growth parameters were estimated and compared between EE and NE conditions (Table 1). No statistical differences (GLM) were found between EE and NE fish regarding growth in length (SL:

p=0.189) and weight (TW: p= 0.287), condition factor (CF: p=0.417), specific growth rate (SGR: p= 0.134), daily growth index (DGI: p=0.164), daily feed intake (DFI: p=0.132) and food conversion rate (FCR: p=0.474).

Table 1. Average values (\pm SE) of increments on body length (Δ SL; cm), body weight (Δ TW; g) and condition factor (Δ CF), as well as estimated specific growth rates (SGR), daily growth indices (DGI), daily feed intake (DFI) and food conversion rates (FCR) of juvenile seabass kept under experimental enriched (EE) and non-enriched (NE) conditions during 29 days

	ΔT.W	ΔS.L	ΔC.F	S.G.R	D.G.I	D.F.I	F.C.R
Enriched	4.52±0.10	0.27±0.03	0.28±0.02	28.85±0.68	264.96±2.69	1.57±0.05	1.83±0.04
Non-enriched	5.03±0.88	0.43±0.27	0.21±0.15	30.27±1.12	271.64±6.23	1.46±0.08	1.68±0.32

Regarding the horizontal distribution of juvenile seabass inside experimental tanks no difference was observed in terms of percentage of the space used during the 90% of the total amount of time between treatments (p=0.431) and feeding periods (p=0.438) (Tables 2 and 3; Fig 4). Similarly, no significant differences were observed in terms of the 50% of the total amount of time between treatments (p=0.194) and between feeding periods (p=0.87) (Tables 2 and 3; Fig 4). Additionally, the mean distance among individuals did not differ between treatments (p=0.892) and between feeding periods (p=0.820). However, significant differences (p<0.05) were found between feeding periods for all variables regardless treatment applied.

	NE			EE	All tanks		
	PreFeed	DurFeed	PreFeed	DurFeed	PreFeed	DurFeed	
Usage 90%	36.85±3.29	40.18±5.05	34.19±3.39	38.71±6.29	35.52±3.57	39.45±6.03	
Usage 50%	11.69±0.96	12.47±0.94	10.91±1.06	11.74±1.60	11.30±1.08	12.11±1.35	
Meandist. cm	16.10±0.79	12.47±0.94	16.10±0.95	17.13±1.50	16.01±0.86	17.10±1.33	

Table 2. Percentages of the space used during a certain amount of the total of time (50% and 90%) and the mean of the distance between fish (cm.) in the tanks with and without enrichment, and in all tanks.

Table 3. Results of the linear mixed-effect models for the percentage of the space used where fish spent the 90% (Usage 90%) and 50% (Usage 50%) of the total amount of time and the mean distance among individuals in centimeters. Interaction model fitted using treatment (Control/Treatment) and Feeding (pre and during) as fixed effects. Estimates: estimate value; std. Error: Standard error of the estimate; p-values via Kenward-Roger approximation. The six different tanks and the weeks were used as random effects of the model. τ 00, tank is the between tanks variance and σ 2 is the within each tank variance.

	Usage 50%			Usage 90%			Mean dist (cm)		
Predictors	Estimates	std. Error	р	Estimates	std. Error	p	Estimates	std. Error	р
(Intercept)	11.74	0.34	<0.001	38.71	1.24	<0.001	17.13	0.33	<0.001
NE	0.73	0.49	0.194	1.48	1.75	0.431	0.07	0.47	0.892
PreFeeding	-0.83	0.23	0.001	-4.52	1.08	<0.001	-1.04	0.22	<0.001
TreatNE:PreFeeding	0.05	0.32	0.870	1.19	1.52	0.438	0.07	0.31	0.820
Random Effects									
s2	0.62			13.88			0.58		
t00	0.61 Week:Tank			8.81Week:Tank			0.58 Week:Tank		
	0.20 Tank			1.74 Tank			0.18 Tank		
ICC	0.57			0.43			0.57		
N	8 Week 6 Tank			8 Week 6 Tank			8 Week 6 Tank		
Observations	96			96			96		
Marginal R2 / Conditional R2	0.178 / 0.646			0.172 / 0.529			0.159 / 0.637		



Fig 4. Horizontal distribution of juvenile seabass reared under enriched (EE) and non-enriched (NE) conditions before feeding and after feeding in experimental tanks. Colour gradient shows the percentage of fish occupancy throughout the whole experimental period. Dashed line represents the perimeter of the water inside the tanks.

3.2 Effects of EE at Individual level: Second experiment

The fish size had no effect on the number of passes through the tunnel (Fig 5). The number of passes was different between treatments (p=0.0257, p<0.05, Fig 6). The fish reared in bared tanks showed a higher number of passes (449 ± 749) compared to fish from enriched tanks (326 ± 181). Additionally, as the weeks went by, the number of passes increased in every tank (p<0.001, Fig 7). However, these differences between treatments were biased. We observed that the tank 2 showed an abnormal behaviour compared to the rest of the tanks, and consequently, we considered this control tank as "no valid" and rerun the analysis excluding it. After removing it the treatment did not show a significant effect on the behaviour (p=0.6325, p>0.05), however the week did have a significant effect on the number of passes (p<0.001, Fig 8). Moreover, the interaction between treatment and week showed significant differences (p=0.0377, p<0.05, Fig 9).



Fig 5. Scatter plot of the fish size (S.L: standard length in cm) vs. the number of passes through the antenna. Lines represented a lineal regression for each group with slopes almost equal to 0.



Tanks

Fig 6. Density estimation of number of passes through the tunnel during the 4 weeks and in the 6 tanks. (EE: 1-4-5, NE:2-3-6) (including tank 2). Colour gradient shows the probability of the passes.



Fig 7. Total density of passes through the tunnel during the 4 weeks between treatments (including tank 2). Colour gradient shows the probability of the passes.



Fig 8. Total density of passes through the tunnel during the 4 weeks between treatments (excluding tank 2). Colour gradient shows the probability of the passes.



Fig 9. Number of passes in the different weeks of experiment (excluding tank 2). Each line is an individual fish, some of the individuals presented a higher number of passes even in the first weeks.

4. Discussion

Enriched environments did not affect the growth performance of seabass at group level. Different studies indicate that the effects on growth can be positive, negative or no effects. In agreement with this study, Arechavala-Lopez et al (2019) found that the vertical enrichment did not have an effect on the growth of juvenile gilthead seabream. Similarly, in other aquaculture species reared under enrichment conditions, the growth was no different than the control (Brockmark et al, 2010; Roberts et al, 2011; Ren et al, 2019). For instance, White et al (2018) did not find any difference on the effect of vertical enrichment on various species of salmonids (Salmo trutta, Oncorhynchus tshawytscha and Salmo salar). However, positive effects of enriched environments have been also reported. For instance, the addition of a color substrate on reared seabram enhanced the growth performance, even reduced the aggressiveness (Batzina & Karakatsouli, 2012, 2014; Batzina et al, 2014a,b,c,d). In addition, a previous study on rainbow trout showed that the total tank weight gain was significantly higher in the structurally complex tanks than in the control (Kientz & Barnes, 2016). In this case the authors explained that the obstacles reduce the swimming velocity, therefore the energetic demand decreases, and this could be a factor contributing to gain in weight and size. Moreover, the addition of structural enrichment could promote territorial behaviour rising aggressive interactions among fish, which could compromise the welfare of the whole group, even with negative effects on growth (Barreto et al, 2011; Woodward et al, 2019). This inconsistency among studies, however, confirms the fact that the enrichment effect can be considerably different between species, life stages, rearing systems and moreover, it strongly depends on the type or design of the structural enrichment (Näslund & Johnsson, 2016).

Similarly, the horizontal distribution did not show any significant change between treatments. Arechavala-Lopez et al (2019) observed no difference in terms of percentage of the space used by seabream reared in an enriched environment. In the long term, as the space used and the distance between fish increase, the encounter among individuals might decrease, and consequently, the aggressiveness and fin damage might be also reduced. Despite the fact that horizontal distribution did not differ between treatments in this study, the seabass juveniles showed a wider distribution during the feeding period. Since the videos were recorded from the top of circular tanks, the wide distribution could be understood as a visual effect due to high densities of fish in the upper water column. Andrew et al (2002) reported that seabass and seabream remained near the surface during feeding in sea cages. Both species arise in the water column to catch the food before it reaches the bottom and therefore, they are more scattered through the entire tank/cage (Oikonomidou et al, 2019). Both seabass and seabream are species which show higher aggressiveness during feeding (Andrew et al, 2002; Aimon et al, 2019; Oikonomidou et al, 2019). In this sense, our work provided experimental evidence that the seabass behavioural responses change during feeding; and aspect that must be taken into account for further fish welfare management and productivity (Aimon et al, 2019; Oikonomidou et al, 2019).

Concerning effects of the enriched environments at individual level, the fish reared without enrichment showed a significative higher number of passes (p<0.05). However, control tank number 2 (non-enriched) presented an abnormal behaviour compare to the rest of the tanks. A surprisingly bigger number of passes were detected when this tank was tested (passes tank 2: 1314, mean of passes rest of the no enriched tanks: 16.5, mean of passes enriched tanks: 245), and we could graphically observe that the differences between treatments are mainly due to this tank (Fig 6). Consequently, we decided to rerun the analysis without including tank number 2 and we observed that the passes of the fish reared in enriched environments were higher compared to enriched ones. Moreover an increased learning capacity in individuals reared under enriched conditions was evidenced. Thes abnormal behaviour could due probably to the physical position in the laboratory, since it was close to a gate that communicates with the exterior being disturbed by daily routines in the facility (see Fig 2). Sometimes the effect of an external stimuli can change the behaviour or alter the normal functioning of the group. Seabass is indeed very sensitive to stressful conditions and the minimal presence of human can produce a change in its behaviour (Rubio et al, 2010). Therefore, after excluding the data from tank 2, results revealed behavioural differences attributable to the enriched treatment, regardeless external stimuli. An increment of the number of passes was observed in all the tanks throughout the time, being more evident in seabass with enriched conditions.

We can observe different behavioural responses and plasticity at individual level throughout the time, which indicate that structural environmental enrichment promotes the exploratory behaviour and learning capabilities of seabass. Millot et al (2014) demonstrated that this temporal tendency is a relevant indicator of fish learning process and habituation. Other previous studies showed that more complex/enriched environments improve behavioural flexibility and cognitive ability of fish by either stimulation cell proliferation in diverse brain regions or by enhancing the adaptability to novel situations and learning (e.g. Braithwaite and Salvanes 2005; Salvanes and Braithwaite 2005; Lee and Berejikian 2008; Strand et al. 2010; Salvanes et al. 2013). The fact that the exploratory activity is higher in fish reared in enriched conditions indicate that structural enrichment could promote not only cognitives habilities but also proactive fish individuals. Commonly, proactive fishes have several characteristics that are

advantageous in farm industry such as (1) fast growth (2) fast response and low sensitiveness to stressful conditions, (3) be dominant during aggressive encounters, (3) low social influences, (4) less behavioural flexibility, (5) high immunity, (6) high feed efficiency, (7) high exploratory behaviour and feeding motivation in new environments and in some species (8) high metabolic rates (Castanheria et al, 2017). Regarding plasticity, only few studies have addressed if proactive and reactive coping styles are plastic or not. Frost et al (2006) found that the boldrainbow trout were more plastic in their responsiveness than shy individuals and thus made them more adaptable to changing conditions in natural environments. In contrast, studies in other species demonstrated that bold or proactive individuals are relatively 'fixed' in their behaviour compared with shy or reactive individuals (Koolhaas et al, 1999; Ruiz-Gomez et al, 2011).

The experiment duration in the present work, however, might be too short to observe higher effects on environmental enrichment on seabass. In several previous experiments the time the fish is exposed to enriched environments ranged between 60 to 365 days to demonstrate a significative effect of the enrichment (Strand et al, 2010; Roberts et al, 2011; Näslund et al, 2013; Ullah et al, 2017). In addition, the experiment of Brockmarck et al (2007) did not show any effects on Atlantic salmon after 123 days under enriched conditions, but they did observe improvements after 311 days. Moreover, the density of juveniles reared in each tank of the present work could have affected the results. Even though the common stocking density for seabass on commercial farms is higher (d: 30–35 kg/m³) (Di Marco et al, 2008), experimentally, too many fish could make more difficult the assessment by visual analysis according to the dimensions of the experimental tanks, which have to be taken into account for proper structure designs. In this sense, we assessed only one type of enrichment structures and this might not the most suitable for this species. Further research must include other kinds of structures and substrates. Concerning the assessment of welfare, it is necessary to consider a wider picture and consider physiological parameters. Besides the behavioural responses, the physiological status of farmed fishes reflects the stress-level or the feeling of wellbeing living in a captivity environment (Papoutsoglou et al, 2008). In particular, brain neurotransmissor monoamines play an important role in behavioural processes as they are involved in social status, aggression and feeding behaviour. Also, they are sensitive to a wide range of stressors (Zhdanova & Reebs, 2006). On Gilthead seabream has been reported that the alteration of the color of the walls and the substrate of the tanks modifies the monoamines and cortisol level (Batzina & Karakatsouli, 2012, 2014; Batzina et al, 2014a,b,c,d). Therefore, in order to have an overall approach of the effect of the enrichment on European seabass, further research should include physiological analysis together with behavioural assessment, as fish welfare indicators.

The assessment of the effects of environmental enrichment on fish behaviour at individual and group level must be taken into account in the designing of farming environments that promotes optimum welfare and, consequently, contributes positively to the economic benefits of the fish farmers. This study provided yet another evidence that structural enrichment might improve welfare conditions of farmed fish, but also that the farming industries need to consider the species-specific biology and behaviour before any standard enrichment protocols are implemented. The challenge remains open: design suitable structures without compromise the biosecurity and the economic resources of the farm.

Acknowledgments

This work is a contribution of the Joint Associated Unit IMEDEA-LIMIA. I would like to thank the staff at LIMIA for their help with maintenance and taking care of fish and tanks during the experimentation process, as well as Aqüicultura Balear S.A.U for their support and interest in this project. Also, the University of Balearic Island and the Santander Bank made possible my enrollment in the master program and consequently this work. Many people were important in the development of this study, particularly I would like to thank Guillermo Follana Berná and Maria Guadalupe Gil for their diary help and support.

References

Aimon, C., Le Bayon, N., Le Floch, S., & Claireaux, G. (2019). Food deprivation reduces social interest in the European seabass *Dicentrarchus labrax*. Journal of Experimental Biology, 222:1-9.

Alos, J., Martorell-Barcelo M., & Campos. A. C. (2017). Repeatability of circadian behavioural variation revealed in free-ranging marine fish. Royal Society Open Science 4(2):160791

Andrew, J. E., Noble, C., Kadri, S., Jewell, H., & Huntingford, F. A. (2002). The effect of demand feeding on swimming speed and feeding responses in Atlantic salmon *Salmo salar L.*, gilthead sea bream *Sparus aurata L*. and European seabass *Dicentrarchus labrax L*. in sea cages. Aquaculture Research, 33:501-507.

Arechavala-Lopez, P., Diaz-Gil, C., Saraiva, J.L., Moranta, D., Castanheira, M.F., Nuñez-Velazquez, S., Ledesma-Corvi, S., Mora-Ruiz, M.R., Grau, A. (2019) Effects of structural environmental enrichment on juvenile seabream (*Sparus aurata*) under feed deficit conditions. Applied Animal Behaviour Science, submitted.

Ashley, P. J. (2007). Fish welfare: current issues in aquaculture. Applied Animal Behaviour Science, 104:199-235.

Barreto, R. E., Carvalho, G. G. A., & Volpato, G. L. (2011). The aggressive behaviour of Nile tilapia introduced into novel environments with variation in enrichment. Zoology, 114: 53-57.

Bates, D., Maechler, M., Bolker, & Walker B. S. (2015). Fitting Linear Mixed-Effects Models Using Ime4. Journal of Statistical Software, 67:1-48

Batzina, A., Dalla, C., Papadopoulou-Daifoti, Z., & Karakatsouli, N. (2014a). Effects of environmental enrichment on growth, aggressive behaviour and brain monoamines of gilthead seabream *Sparus aurata* reared under different social conditions. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 169:25-32.

Batzina, A., Dalla, C., Tsopelakos, A., Papadopoulou-Daifoti, Z., & Karakatsouli, N. (2014b). Environmental enrichment induces changes in brain monoamine levels in gilthead seabream *Sparus aurata*. Physiology & Behaviour, 130:85-90.

Batzina, A., Kalogiannis, D., Dalla, C., Papadopoulou-Daifoti, Z., Chadio, S., & Karakatsouli, N. (2014c). Blue substrate modifies the time course of stress response in gilthead seabream *Sparus aurata*. Aquaculture, 420:247-253.

Batzina, A., & Karakatsouli, N. (2012). The presence of substrate as a means of environmental enrichment in intensively reared gilthead seabream *Sparus aurata*: growth and behavioural effects. Aquaculture, 370:54-60.

Batzina, A., & Karakatsouli, N. (2014). Is it the blue gravel substrate or only its blue color that improves growth and reduces aggressive behaviour of gilthead seabream *Sparus aurata*?. Aquacultural Engineering, 62:49-53.

Batzina, A., Sotirakoglou, K., & Karakatsouli, N. (2014d). The preference of 0+ and 2+ gilthead seabream *Sparus aurata* for colored substrates or no-substrate. Applied Animal Behaviour Science, 151:110-116.

Biro, P. A., & Adriaenssens, B. (2013). Predictability as a personality trait: consistent differences in intraindividual behavioural variation. The American Naturalist, 182:621-629.

Braithwaite, V. A., & Salvanes, A. G. (2005). Environmental variability in the early rearing environment generates behaviourally flexible cod: implications for rehabilitating wild populations. Proceedings of the Royal Society B: Biological Sciences, 272:1107-1113.

Brydges, N. M., & Braithwaite, V. A. (2009). Does environmental enrichment affect the behaviour of fish commonly used in laboratory work?. Applied Animal Behaviour Science, 118: 137–143.

Brockmark, S., Neregård, L., Bohlin, T., Björnsson, B. T., & Johnsson, J. I. (2007). Effects of rearing density and structural complexity on the pre-and post-release performance of Atlantic salmon. Transactions of the American Fisheries Society, 136:1453-1462.

Brockmark, S., Adriaenssens, B., & Johnsson, J. I. (2010). Less is more: Density influences the development of behavioural life skills in trout. Proceedings of the Royal Society B: Biological Sciences, 277:3035–3043.

Calenge, C. (2006). The package "adehabitat" for the R software: a tool for the analysis of space and habitat use by animals. Ecological Modelling, 197:516–519.

Castanheira, M. F., Herrera, M., Costas, B., Conceição, L. E., & Martins, C. I. (2013). Can we predict personality in fish? Searching for consistency over time and across contexts. PLoS One, 8, e62037.

Castanheira, M. F., Conceição, L. E., Millot, S., Rey, S., Bégout, M. L., Damsgård, B., Kristiansen T., Hoglund, E., Oyvind, O. & Martins, C. I. (2017). Coping styles in farmed fish: consequences for aquaculture. Reviews in Aquaculture, 9:23-41.

Di Marco, P., Priori, A., Finoia, M. G., Massari, A., Mandich, A., & Marino, G. (2008). Physiological responses of European sea bass *Dicentrarchus labrax* to different stocking densities and acute stress challenge. Aquaculture, 275:319–328.

Dingemanse, N. J., & Dochtermann, N. A. (2013). Quantifying individual variation in behaviour: Mixed-effect modeling approaches. Journal of Animal Ecology, 82:39–54.

Fraser, T. W., Fjelldal, P. G., Hansen, T., & Mayer, I. (2012). Welfare considerations of triploid fish. Reviews in Fisheries Science, 20:192-211.

Frost, A. J., Winrow-Giffen, A., Ashley, P. J., & Sneddon, L. U. (2007). Plasticity in animal personality traits: Does prior experience alter the degree of boldness?. Proceedings of the Royal Society B: Biological Sciences, 274:333–339.

Hadfield, J. D. (2010). MCMCglmm: MCMC Methods for Multi-Response GLMMs in R. Journal of Statistical Software, 33:1–22.

Harrison, P. M., Gutowsky, L. F. G., Martins, E. G., Patterson, D. A., Cooke, S. J. & Power M. (2014). Personality-dependent spatial ecology occurs independently from dispersal in wild Burbot (*Lota lota*). Behavioural Ecology, 26:483–492.

Huntingford, F. A. (2004). Implications of domestication and rearing conditions for the behaviour of cultivated fishes. Journal of Fish Biology, 65:122-142.

Huntingford, F. A., & Kadri, S. (2008). Welfare and fish. Fish welfare, 1:19-32.

Huntingford, F., Kadri, S., & Jobling, M. (2012). Introduction: aquaculture and behaviour. Aquaculture and behaviour, 1-35.

Jolles, J. W., Briggs, H. D., Araya-Ajoy, Y. G., & Boogert, N. J. (2019). Personality, plasticity, and predictability in sticklebacks: bold fish are less plastic and more predictable than shy fish. Animal Behaviour, 154:193-202.

Kientz, J. L., & Barnes, M. E. (2016). Structural complexity improves the rearing performance of rainbow trout in circular tanks. North American Journal of aquaculture, 78:203-207.

Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., De Jong I. C., Ruisb, M. A. W., & Blokhuisb, H. J. (1999). Coping styles in animals: current status in behaviour and stress-physiology. Neuroscience & Biobehavioural Reviews, 23: 925-935.

Leal, E., Fernández-Durán, B., Guillot, R., Ríos, D., & Cerdá-Reverter, J. M. (2011). Stress-induced effects on feeding behaviour and growth performance of the seabass (*Dicentrarchus labrax*): a self-feeding approach. Journal of Comparative Physiology B, 181:1035-1044.

Lee, A. (2013). VirtualDub video processing software. Retrieved from 537. http://www.virtualdub.org

Lee, J. S. F., & Berejikian, B. A. (2008). Effects of the rearing environment on average behaviour and behavioural variation in steelhead. Journal of Fish Biology, 72:1736-1749.

Lee, C. J., Paull, G. C., & Tyler, C. R. (2019). Effects of environmental enrichment on survivorship, growth, sex ratio and behaviour in laboratory maintained zebrafish *Danio rerio*. Journal of fish biology, 94:86-95.

Martins, C. I., Galhardo, L., Noble, C., Damsgård, B., Spedicato, M. T., Zupa, W., Beauchaud, M., Kulczykowska, E., Massabuau, J., Car ter, T., Rey Planellas, S., Kristiansen, T. & Planellas, S. R. (2012). Behavioural indicators of welfare in farmed fish. Fish Physiology and Biochemistry, 38:17-41.

Millot, S., Bégout, M. L., & Chatain, B. (2009). Risk-taking behaviour variation over time in sea bass *Dicentrarchus labrax*: Effects of day-night alternation, fish phenotypic characteristics and selection for growth. Journal of Fish Biology, 75:1733–1749.

Muggeo, V. M. (2003). Estimating regression models with unknown break-points. Statistics in medicine, 22:3055-3071.

Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. Biological Reviews, 85:935–956.

Näslund, J., & Johnsson, J. I. (2016). Environmental enrichment for fish in captive environments: effects of physical structures and substrates. Fish and Fisheries, 17:1-30.

Näslund, J., Rosengren, M., Del Villar, D., Gansel, L., Norrgård, J. R., Persson, L., Winkowski, J. J. & Kvingedal, E. (2013). Hatchery tank enrichment affects cortisol levels and shelter-seeking in Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences, 70: 585-590.

Oikonomidou, E., Batzina, A., & Karakatsouli, N. (2019). Effects of food quantity and distribution on aggressive behaviour of gilthead seabream and European seabass. Applied Animal Behaviour Science, 213:124-130.

Papoutsoglou, S. E., Karakatsouli, N., Batzina, A., Papoutsoglou, E. S., & Tsopelakos, A. (2008). Effect of music stimulus on gilthead seabream *Sparus aurata* physiology under different light intensity in a re-circulating water system. Journal of fish biology, 73:980-1004.

Ren, Y., Xiong, M., Yu, J., Li, W., Li, B., Liu, J., & Zhang, T. (2019). Effects of artificial submersed vegetation on consumption and growth of mandarin fish *Siniperca chuatsi* (Basilewsky) foraging on live prey . Journal of Freshwater Ecology, 34:433–444.

Romey, W. L. (1996). Individual differences make a difference in the trajectories of simulated schools of fish. Ecological Modelling, 92:65-77.

Roberts, L. J., Taylor, J., & de Leaniz, C. G. (2011). Environmental enrichment reduces maladaptive risk-taking behaviour in salmon reared for conservation. Biological Conservation, 144: 1972-1979.

Rubio, V. C., Sánchez, E., & Cerdá-Reverter, J. M. (2010). Compensatory feeding in the seabass after fasting and physical stress. Aquaculture, 298: 332-337.

Ruiz-gomez, M. D. L., Huntingford, F. A., Øverli, Ø., Thörnqvist, P., & Höglund, E. (2011). Response to environmental change in rainbow trout selected for divergent stress coping styles. *Physiology & Behaviour*, *102*: 317–322.

Salvanes, A. G. V., & Braithwaite, V. A. (2005). Exposure to variable spatial information in the early rearing environment generates asymmetries in social interactions in cod (*Gadus morhua*). Behavioural Ecology and Sociobiology, 59:250.

Salvanes, A. G. V., Moberg, O., Ebbesson, L. O., Nilsen, T. O., Jensen, K. H., & Braithwaite, V. A. (2013). Environmental enrichment promotes neural plasticity and cognitive ability in fish. Proceedings of the Royal Society B: Biological Sciences, 280:20131331.

Saraiva, J. L., Arechavala-Lopez, P., Castanheira, M. F., Volstorf, J., & Heinzpeter Studer, B. (2019). A Global Assessment of Welfare in Farmed Fishes: The FishEthoBase. Fishes, 4: 30.

Sbragaglia, V., Alós, J., Fromm, K., Monk, C. T., Díaz-Gil, C., Uusi-Heikkilä, S., Honsey, E. A., Wilson, D. M. A. & Arlinghaus, R. (2019). Experimental Size-Selective Harvesting Affects Behavioural Types of a Social Fish. Transactions of the American Fisheries Society, 148:552–568.

Schneider, C.A., Rasband, W.S., & Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9, 671e675.

Strand, D. A., Utne-palm, A. C., Jakobsen, P. J., Braithwaite, V. A., Jensen, K. H., & Salvanes, A. G. V. (2010). Enrichment promotes learning in fish. Marine Ecology Progress Series, 412:273–282.

Sullivan, M., Lawrence, C., & Blache, D. (2016). Why did the fish cross the tank? Objectively measuring the value of enrichment for captive fish. Applied Animal Behaviour Science, 174:181-188.

Toni, M., Manciocco, A., Angiulli, E., Alleva, E., Cioni, C., & Malavasi, S. (2019). Assessing fish welfare in research and aquaculture, with a focus on European directives. Animal, 13: 161-170.

Ullah, I., Zuberi, A., Khan, K. U., Ahmad, S., Thörnqvist, P. O., & Winberg, S. (2017). Effects of enrichment on the development of behaviour in an endangered fish mahseer (*Tor putitora*). Applied animal behaviour science, 186:93-100.

Varsamos, S., Flik, G., Pepin, J. F., Bonga, S. W., & Breuil, G. (2006). Husbandry stress during early life stages affects the stress response and health status of juvenile seabass, *Dicentrarchus labrax*. Fish & shellfish immunology, 20: 83-96.

White, S. C., Barnes, M. E., Krebs, E., Huysman, N., & Voorhees, J. M. (2018). Addition of vertical enrichment structures does not improve growth of three salmonid species during hatchery rearing. Journal of Marine Biology and Aquaculture, 4:48-52.

Woodward, M. A., Winder, L. A., & Watt, P. J. (2019). Enrichment Increases Aggression in Zebrafish. Fishes, 1–13.

Zhdanova, I., & Reebs, S. (2006). Circadian Rhythms in Fish. 10.1016/S1546-5098(05)24006-2.