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Effect of size grading on sea bass (*Dicentrarchus labrax*) juvenile self-feeding behaviour, social structure and culture performance

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Abstract – This study aims to test the influence of size grading on self-feeding behaviour, social structure (measured by the percentage of triggering acts per individual), growth performances, and blood physiological variables of individually passive integrated transponder (PIT)-tagged sea bass juveniles, using a computerized on-demand feeding system coupled with a PIT tag monitoring device. Three consecutive periods of 27 days each were compared: a first period ($P1$) before grading (6 tanks of 100 fish; 40.2 ± 8.9 g) followed by a second period ($P2$) after grading. The protocol applied aimed to create two groups of fish of similar mean weight but with either a low or a high coefficient of variation of weight (CV_w) corresponding to an imposed difference in social disruption (T_{low}: $CV_w \sim 10\%$, 3 tanks of 60 fish each with social disruption; T_{high}: $CV_w \sim 20\%$, 3 tanks of 60 fish each, without social disruption). T_{low} and T_{high} groups were studied over $P2$, and an additional 27-day period under identical conditions ($P3$). The grading protocol used and/or time modified the social structure when comparing $P1$ and $P2$. Thereafter, during $P2$ and $P3$, no difference could be observed in growth performances, feed demand, or physiological variables between T_{low} and T_{high} groups. Feeding rhythms and social structures were similar in both groups. In conclusion, such grading practice only transiently modifies feed demand behaviour and social structure built around the self-feeder, without further improvement in individual growth performances in sea bass.

Key words: Feeding behaviour / Self feeder / Triggering activity / Heterogeneity / Growth / Grading

1 Introduction

Variation in individual growth is a common feature in many cultured fish stocks (Huntingford et al. 1990; Stefánsson et al. 2000; Smith and Fuiman 2003). Magnuson (1962) defined growth depensation as the increase in the variance of size distribution over time, due to differences in growth rates. Such variation is generally considered as a drawback in commercial fish culture (Barki et al. 2000). Indeed good aquaculture practices usually aim to minimize growth depensation to reduce food wastage, and water quality degradation. Even though growth heterogeneity has been extensively studied in both natural and cultured fish population, it remains a central problem in aquaculture.

Kestemont et al. (2003) stated that growth heterogeneity is induced by a wide range of intrinsic and environmental factors, of which the relative influence is largely unknown but could be qualified as either inherent (i.e. having a strong genetic

component that is expressed to a varying degree according to the environmental conditions) or imposed (i.e. requiring specific biotic or abiotic conditions to be manifested). Among the biotic factors, social interaction has been identified as a major cause of individual variation in growth (Jobling et al. 1993; Cutts et al. 1998) if it leads to feeding hierarchies that decrease growth of low ranked fish (Koebele 1985). It is also well known that size-related dominance determines aggressive behaviour, feeding and growth performances in fish (Abbot and Dill 1989). Some mechanisms have been proposed to explain how such hierarchies could influence growth, e.g., physiological stress (Jobling 1985; Abbot and Dill 1989; Huntingford et al. 1993; Griffiths and Armstrong, 2002), disproportional food acquisition (Koebele 1985 ; Grant 1997), activity differences (Adams et al. 1998; Sloman and Armstrong 2002), and cost of dominance (Yamagishi et al. 1974; Rubenstein 1981).

In commercial fish farming, size grading is routinely carried out to ease feeding and harvesting operations (Lee 1988; Baardvik and Jobling 1990), and to avoid reduced survival via cannibalism in piscivorous species (Goldan et al. 1997).

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This procedure is based on the assumption that grading disrupts the development of social hierarchies, and allows the smaller fish to grow better in the absence of large dominant competitors (Strand and Øiestad 1997; Liao and Chang 2002). Some experiments supported the usefulness of grading procedures based on asymmetric competition (abalone, *Haliotis tuberculata*, Mgaya and Mercer 1995), while others reported either lower growth rates in graded fish (cod, *Gadus morhua* (Lambert and Dutil 2001) or no biomass gain: Arctic charr, *Salvelinus alpinus* (Wallace and Kolbeinshavn 1988; Baardvik and Jobling 1990); Atlantic salmon, *Salmo salar* (Gunnes 1976); eel, *Anguilla anguilla* (Kamstra 1993); Dover sole, *Solea solea* L. (Overton et al. 2010). In some species, destruction of size hierarchies does improve the growth of the small fish but the growth of the large fish could be adversely affected by the stronger agonistic interaction and intraspecific aggression occurring among the graded large individuals (Baardvik and Jobling 1990; Sunde et al. 1998; Stefánsson et al. 2000). Furthermore, size grading is in itself a stressful procedure for fish (Pickering 1981) and is labour-intensive, with risks of handling damage, disease outbreak, and growth reduction (Sunde et al. 1998). This further suggests that a better understanding of size variation mechanisms would be of high interest, and that efficient feeding modes and age-for-size grading methods could be better designed (Jørgensen and Jobling 1990; Benhaïm et al. 2003).

Study of feeding behaviour may contribute to a better understanding of size variation mechanisms. Feeding is a complex behaviour encompassing several behavioural responses associated with eating, including feeding modes and habits, mechanisms of food detection, feeding frequency, food preferences (Volkoff and Peter 2006), and foraging strategies (Benhaïm et al. 2003). Self-feeders are particularly useful for the study of feeding behaviour in fish (Boujard et al. 1992) and, when coupled with a PIT tag detection antenna, have contributed to a better understanding of individual behaviour within fish groups (Alanärä and Brännäs 1993, 1996; Brännäs and Alanärä 1993; Covès et al. 1998; Rubio et al. 2004; Covès et al. 2006). This combination of techniques was successfully used in European sea bass, a commercially important species in the Mediterranean (Covès et al. 1998; Covès et al. 2006; Di-Poï et al. 2007; Millot et al. 2008, 2009). Several authors have shown that, within a group of 50 juvenile sea bass, only a few individuals defined as high-triggering fish were responsible for the majority of food demands in the group, whereas the rest of the population exhibited the defined low- or zero-triggering activity (Covès et al. 2006; Di-Poï et al. 2007; Millot et al. 2008, 2009; Millot and Bégout 2009). Lastly, self-feeding systems are also very promising for fish farming because they can improve growth and feed conversion ratios (Azzaydi et al. 1998), they are an attractive alternative to either hand-feeding or automated feeding systems due to their low labour costs (Aloisi 1994), and offer a response to the new concerns about animal welfare, even though their commercial development has been rather limited.

Surprisingly, size-grading effects have never been studied under self-feeding conditions. The aim of the present work was, therefore, to analyse the influence of a size- grading protocol on feed demand behaviour, and social structure (in terms of individual triggering activity) in sea bass. In order to mimic grading procedures used in common fish husbandry, the approach consisted of a comparison of two groups of juveniles characterized by similar mean weight but contrasting coefficient of variation on weight (CV_w = 10 vs. 20%) and initial social structure (disrupted vs. non disrupted) while held under self-feeding conditions. Growth performance and blood physiological variables were measured to complete the characterisation.

2 Materials and methods

2.1 Fish

Sea bass used in this experiment were hatched and grown in a private farm, Aquanord (France), and were never size-graded from 2 g until the beginning of the present experiment. At the mean weight of 40 g, 600 fish were transferred to the Ifremer experimental station located in L'Houmeau (France). After a two-week acclimation period, each fish was PIT-tagged by inserting a tag horizontally just behind the head to prevent any change of position after implantation. Fish were then weighed (to the nearest mg), and measured for total length (to the nearest mm). At the beginning of the experiment, fish of the experimental population had an average initial body weight (BW_i) of 40.3 ± 8.9 g and a CV_w = 22.2% (N = 600). They were randomly distributed between 6 tanks (100 fish per tank) held in a single experimental room, as described below.

2.2 Experimental set-up

The experiment was carried out in six 400 L tanks supplied with filtered seawater in a recirculating system (flow rate: 4 m³ h⁻¹ in each tank, and water renewal: 10% per day). The mean water temperature, oxygen concentration, and salinity were 21.0 ± 0.9 °C, 7.5 ± 1.5 mg L⁻¹, and 23.4 ± 1.3 g L⁻¹, respectively, throughout the experiment. Tanks were surrounded by black curtains to reduce any visual disturbance to the fish, and lit by individual 120 W lamps located about 90 cm above the water surface. Light regime was 16:8 LD (light onset at 06:00 h, UT + 1 h) with twilight transition periods of 30 min (06:00 to 06:30 and 22:00 to 22:30 h). Fish were fed a commercial sea bass diet (Neo Start 3 Le Gouessant, France; 47% crude protein, 18% lipid according to the manufacturer; 3.2 mm). Each tank was provided with a self-feeder. The device that operates these feeders is made up of a screened-type sensor (a metal rod protected in a PVC cylinder surrounded by the PIT tag detection antenna; Covès et al. 2006), and a control box. After each actuation, fish were rewarded with pellets. The feed dispensers were regulated to distribute between 0.8 g kg⁻¹ and 0.6 g kg⁻¹ of fish at the beginning and end of the experiment, respectively. The reward level was a compromise between minimizing wastage, and optimizing feed allocation to the group. Such a set up allowed us to monitor two variables of interest on a daily basis: the individual feed demand behaviour, and the group (i.e. tank) apparent feed consumption (food quantity dispensed minus waste counted on the bottom of the tank and in the sediment trap). Triggering activity was

recorded continuously for 82 days, and only stopped 48 h before and during fish handling (when no recordings were made and the fish were fasted; 10 days off in total). Fish were placed under self-feeding conditions from the very first day (D1) of the experiment, food access was possible all day (24 h) even during tank cleaning, and waste counting occurred from 10:00 to 11:00 h (UT + 1 h).

The experiment lasted 82 days in total, during which the fish were weighed, and measured (anesthetized with clove oil at 40 ppm) at fortnightly intervals: days 13 (D13), 27 (D27), 41 (D41), 54 (D54), 69 (D69), and 82 (D82).

2.3 Experimental periods

The experiment was run from 7 May to 28 July 2009, structured into three periods where the same set of variables (self-feeding behaviour, social structure, and growth performances) were measured on graded, and non-graded fish:

- The first 27-day period ($P1$) allowed variable estimation in non-graded fish. It included the self-feeder learning process, i.e., the time taken by fish in each tank to learn self-feeder triggering (6 tanks of 100 fish; $BW = 40.2 \pm 8.9$ g).
- The second 27-day period ($P2$) allowed variable estimation in graded fish. The grading was carried out at D27 by creating two groups of similar mean weight but either a high or low CV_w (T_{high}: $CV_w \sim 20\%$, 3 tanks of 60 fish; T_{low}: $CV_w \sim 10\%$, 3 tanks of 60 fish).
- The third 27-day period ($P3$) was added to $P2$, allowing the variable estimation in graded vs. non graded fish over a 54-day period ($P2 + P3$).

CV_w was calculated as: $100 \, SD \times BW^{-1}$, where SD and BW are the standard deviation and mean body weight respectively. To make the T_{high} groups, fish from both extremes of the weight distribution were eliminated in each of three randomly-selected tanks; grading was done within tanks in order to maintain the social structure previously established during $P1$. All other weight classes were retained, i.e., from 21.1 to 65.1 g. To make the T_{low} groups, fish from all three remaining tanks were pooled in a 2 m^3 tank supplied with pure oxygen, and hand-graded to select fish belonging to a weight class ranging between 33.7 and 49.7 g, thereby composing groups with a disrupted social structure compared with $P1$. Handling stress was identical for each fish since they were all weighed, and measured before being placed in their new tank. These procedures created the following group characteristics:

- T_{low} groups: $BW = 41.9$ g, $CV_w = 9.5\%$ in Tank 1; $BW = 41.7$ g, $CV_w = 9.4\%$ in Tank 2; $BW = 41.7$ g, $CV_w = 9.4\%$ in Tank 3
- T_{high} groups: $BW = 38.1$ g, $CV_w = 20.4\%$ in Tank 4; $BW = 41.1$ g, $CV_w = 22.5\%$ in Tank 5; $BW = 38.6$ g, $CV_w = 22.6\%$ in Tank 6.

2.4 Measured and calculated variables

The variables chosen to evaluate rearing performances, and feeding behaviour within periods were the following (most

were normalized against fish biomass to account for inter-tank differences): body weight, BW (g); total body length, BL (cm); specific growth rate (% body weight per day), $SGR = 100 \, (\ln BW_f - \ln BW_i)/t$, whith BW_f and BW_i being the final and initial body weight (g) respectively, and t the total number of days; Fulton condition factor ($K = 100 \, BW \times BL^{-3}$); Feed demand, FD (g kg^{-1} of fish biomass); Food wastage, FW (g); Feed conversion ratio ($FCR = FD$ / biomass gain); Feeding rhythms calculated by taking into account the feeding demand per hour;

Social structure was established according to fish individual triggering level, classifying them into 3 categories according to their proportional contribution to total number of trigger actuations within a group: high-triggering (HT) (>15% actuations), low-triggering (LT) (<15%), and zero-triggering (ZT) (<2%) individuals (based on Covès et al. 2006).

Physiological status of graded and non-graded fish was evaluated from blood samples (0.1 ml taken from the caudal vessel with a 1-ml heparinized syringe) of 4 anesthetized fish randomly taken from each tank at D82. Blood was immediately analysed using an I-Stat® Portable Clinical Analyzer (Abbott; cartridges CG8+; Heska Corporation, Fort Collins CO, USA; Harrenstien et al. 2005) to evaluate the 13 following variables:

pH; carbon dioxide partial pressure, pCO_2 (mm Hg); oxygen partial pressure, pO_2 (mm Hg); base excess, BE (mmol L^{-1}); bicarbonate, HCO_3^- (mmol L^{-1}); total carbon dioxide, total CO_2 (mmol L^{-1}); oxygen saturation, O_2 saturation (%); potassium ion, K^+ (mmol L^{-1}); sodium ion, Na^+ (mmol L^{-1}); ionized calcium, iCa (mmol L^{-1}); plasma glucose, Glu (mg dl^{-1}); hematocrit, Hct (% packed cell volume PCV), and haemoglobin, Hb (mmol L^{-1}).

2.5 Statistical analyses

All variables were compared using parametric analysis of variances (ANOVA) after verification of distribution normality, and homoscedasticity (Dagnélie 1975). When data did not fulfil these requirements, non parametric Kruskall-Wallis tests were used. Significant ANOVA were followed by a post-hoc multiple comparison test (Newman–Keuls) and significant Kruskall-Wallis tests by rank-based multiple comparisons (Zar 1984). All statistical analyses were conducted using Statistica 8 (Statsoft, USA) with the significant threshold $p < 0.05$ for all tests.

To assess for differences between tanks during $P1$, all rearing performances were compared using a two-way ANOVA with Date (three dates corresponding to 5 biometry measurement dates) and Tank as fixed factors; FD, FW, and FCR during $P1$ were compared using a two-way analysis of the variance with Date (two dates corresponding to two biometry dates) and Tank as fixed factors.

To assess the influence of size grading, mean FD, FW, and FCR during $P1$ and $P2$ were compared using a two-way ANOVA with Period as a fixed factor, and Tank as a random factor.

To assess for differences between T_{low} and T_{high} groups during $P2$ and $P3$, all rearing performance variables were compared using a 3-way ANOVA with Treatment (T_{low} vs. T_{high}),

and Date (5 dates corresponding to 5 biometry dates) as fixed factors, and the Tank as a random factor nested to Treatment.

Data on feeding demand per hour from each treatment (T_{low} and T_{high}) were submitted to Cosinor analysis to test for the existence of statistically significant daily rhythms. Cosinor analysis is based on least squares approximation of time series data with a cosine function of known period of the type $Y =$ Mesor + Amplitude $\cos((2\pi (t\text{-Acrophase})/\text{Period})$, where *Mesor* is the time series mean; amplitude is a measure of the amount of temporal variability explained by the rhythm; period (t) is the cycle length of the rhythm, i.e., 24 h for circadian rhythms; and acrophase is the time of the peak value relative to the designated time scale. Cosinor analysis also provided a statistical value for a null hypothesis of zero amplitude. Therefore, if this null hypothesis was rejected for a statistical significance of $p < 0.05$, the amplitude could be considered as differing from 0, thereby constituting evidence for the existence of a statistically significant rhythm of the period considered. In order to statistically compare T_{low} and T_{high} feeding rhythms, ANOVA was used, with Treatment and Hour as fixed factors and Tank as a random factor nested to Treatment.

For physiological blood parameters, a 2-way ANOVA was used, with Treatment as a fixed factor and Tank as a random factor nested to Treatment. Pearson coefficients (Rs) were used to test for associations between initial and final weight between biometry dates (D27-D41, D41-D54, D54-D69, D69-D82) in each tank.

To assess for differences between triggering fish categories (HT, LT and ZT), *BW*, *BL*, and *SGR* were compared between biometry dates and tanks using Kruskal–Wallis tests, while percentages of triggering fish were compared using Mann–Whitney tests.

3 Results

During the experiment, mortality rate was 2.2 ± 3.4%. On average (all tanks), biomass was 4025 ± 108 g (density 10.1 ± 0.3 kg m^{-3}) at the beginning of *P*1 and 4380 ± 517 g (10.9 ± 1.3 kg m^{-3}) at the end; 2822 ± 119 g (7.0 ± 0.3 kg m^{-3}) at the beginning of *P*2 and 3886 ± 136 g (9.7 ± 0.3 kg m^{-3}) at the end; and 3886 ± 136 g (9.7 ± 0.3 kg m^{-3}) at the beginning of *P*3 and 5144 ± 254 g (12.9 ± 0.6 kg m^{-3}) at the end.

3.1 Differences between tanks during *P*1

BW, *BL*, and K increased more from D13 (41.1 ± 8.9 g, 15.8 ± 1.1 cm, 1.0 ± 0.1%) to D26 (47.7 ± 9.9 g, 16.4 ± 1.1 cm, 1.1 ± 0.1%) than they had from D1 (40.2 ± 8.9 g, 15.5 ± 1.1 cm, 1.0 ± 0.1%) to D13. There were significant interactions between Date and Tank in all cases ($F_{(10, 1674)} = 111.4$, $p < 0.001$; $F_{(10, 1674)} = 2.8$, $p < 0.001$ and $F_{(10, 1674)} = 29.6$, $p < 0.001$ respectively) with tanks 1 and 6 being significantly higher at D26 than all other tanks at any date ($p < 0.05$ in all cases). *SGR* strongly increased from D1-D13 (0.13 ± 0.35) to D13-D26 (1.00 ± 0.26) with a significant interaction between Date and Tank ($F_{(5, 1088)} = 14.2$, $p < 0.001$). Newman–Keuls tests showed significant differences for each pairwise comparison ($p < 0.05$).

FD increased from D13 (10.9 ± 11.1 g kg^{-1}) to D26 (16.7 ± 9.7 g kg^{-1}) and there were significant Date and Tank effects ($F_{(1, 107)} = 13.5$, $p < 0.001$ and $F_{(5, 107)} = 3.2$, $p < 0.001$) respectively) values being higher in tank 4 than in tank 5 ($p < 0.05$). *FCR* decreased from D13 (1.0 ± 4.1) to D26 (0.8 ± 2.8) and there was a significant interaction between Tank and Date ($F_{(5, 107)} = 2.9$, $p < 0.05$) with Tank 3 at D13 being higher than all other tanks at any date ($p < 0.05$ for each pairwise comparison). *FW* was negligible during *P*1 (lower than 1 g per day on average).

3.2 Influence of size grading protocol

FD increased by 24% from *P*1 (14.3 ± 6.1 g kg^{-1} day^{-1}) to *P*2 (18.7 ± 10.7 g kg^{-1} day^{-1}) ($F_{(1, 180)} = 10.7$, $p = 0.02$), and neither a Tank effect nor an interaction between Tank and Period were observed. FW was 0.2 ± 0.6 g during *P*1 and 4.5 ± 4.9 g during *P*2. Differences could not be tested because an interaction existed between Period and Tank ($F_{(5, 180)} = 22.8$, $p < 0.001$) due to one tank where the amount of uneaten food pellets collected daily increased after size grading (from 0.5 ± 1.1 to 23.6 ± 21.0 g). In all other tanks, this amount remained very low (between 0–2 g per day) before and after size grading. Mean food conversion rate were 1.5 ± 2.7 and 1.5 ± 0.5 for *P*1 and *P*2 respectively but differences could not be tested for the same reason as above: an interaction between Tank and Period was observed ($F_{(5, 180)} = 2.7$, $p = 0.02$).

During *P*1, percentages of fish observed in the ZT category were between 87 and 93% (Fig. 1 *P*1). These percentages decreased during *P*2 in each tank except tank 2 (Fig. 1 *P*2) ($Z = -2.9$, $p < 0.05$). At the same time, percentages of HT fish increased ($Z = -1.89$, $p = 0.057$), except in tank 6: 9–11 vs. 15–17% in tank 1, 12–14 vs. 33–35% in tank 2, 6–8 vs. 9–11% in tank 4, 6–8 vs. 19–20% in tank 5, for *P*1 and *P*2 respectively (Fig. 1), and percentages of LT fish increased ($Z = -2.9$, $p < 0.05$) in all tanks (14.2 ± 2.5 vs. 31.5 ± 8.0%).

3.3 Comparison of T_{low} and T_{high} treatments during *P*2 and *P*3

3.3.1 Rearing performances

The evolution of mean *BW*, *BL* and K of all groups during *P*2 + *P*3 is presented in Figure 2. There was no difference in growth performances between the T_{low} and T_{high} groups i.e., no Treatment effect was recorded for any dependant variable (Table 1). This was confirmed by the stability of the CV_w (Fig. 2D). In T_{low} groups, CV_w even tended to slightly decrease from the beginning to the end of the experiment (from 20.9 ± 1.3% to 18.8 ± 1.0%) whereas it slightly increased in T_{high} groups (from 9.9 ± 0.2% to 10.8 ± 1.0%). The *SGR* decreased irregularly during the course of the experiment (Fig. 2E), being similar at D26 and D41, lower at D54, higher again at D69, and reaching the lowest value at D82 (Table 1). *SGR* was not exactly the same among tanks, as tanks 1 and 6 showed a different evolution over the experiment. Tank 1 *SGR* increased from D41 to D54 (but was at the lowest level compared with the other tanks at D41), decreased between D54 and D69 and
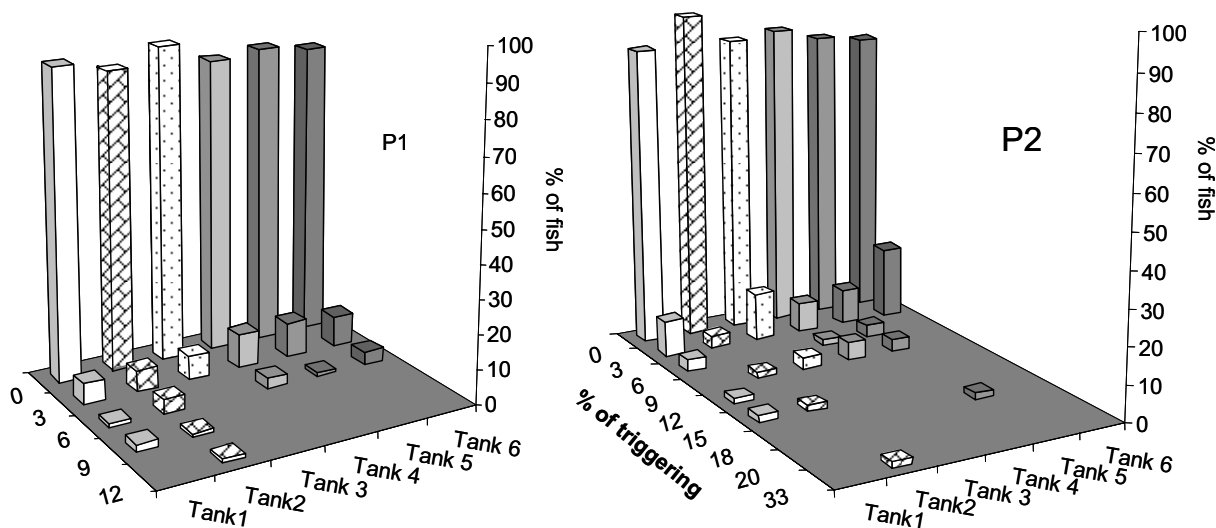
Fig. 1. Social structure measured by the percentage of triggering acts per individual before and after size grading. Percentages of fish are given in each triggering activity class: 0 refers to 0–2, 3 to 3–5 etc. *P*1: before size grading (Day 1-Day 27). *P*2: after size grading (Day 28-Day 55). In *P*2, T_{low}: tanks 1, 2 and 3; T_{high}: tanks 4, 5 and 6.
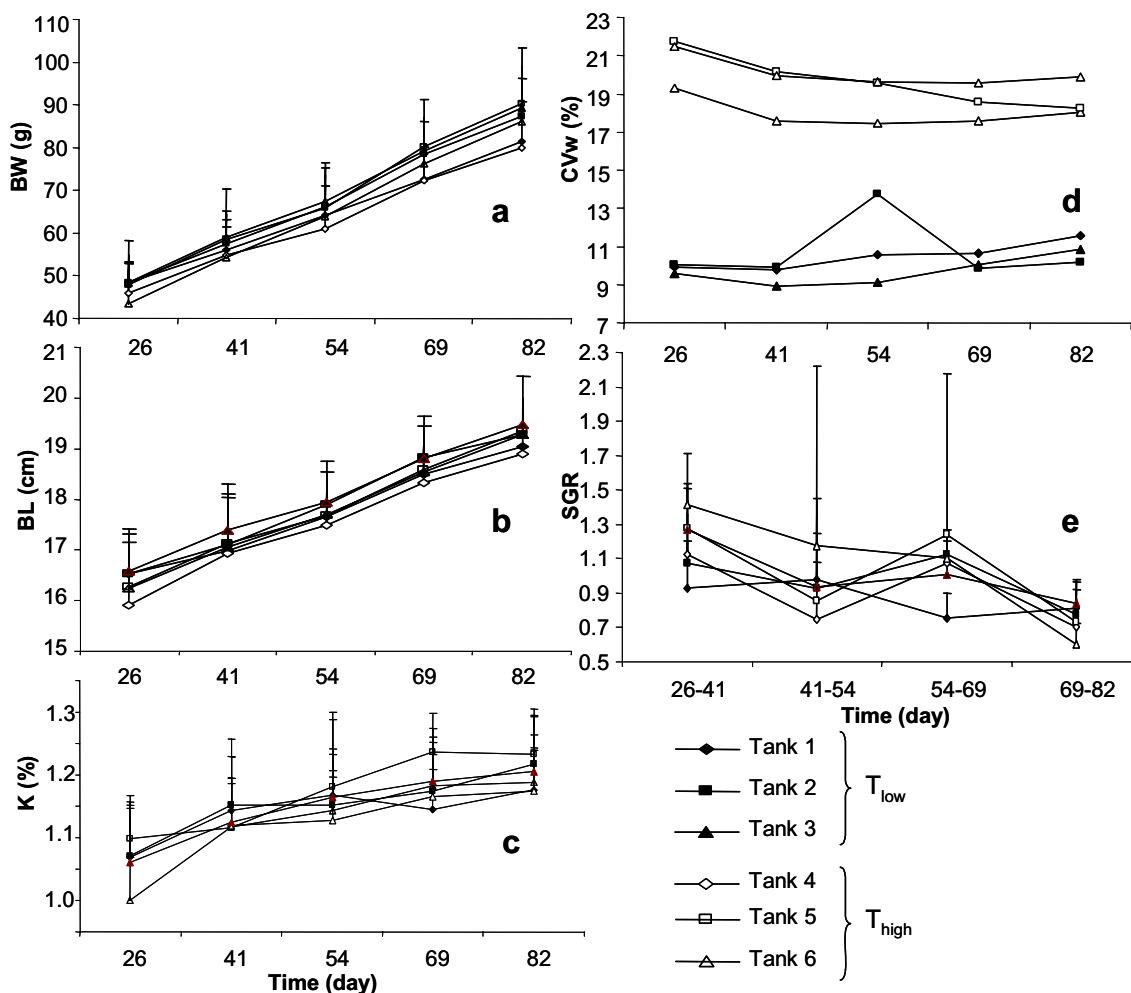
Fig. 2. Growth performances (mean ± *SD*) of two groups of sea bass graded for high (T_{high}) and low (T_{low}) coefficient of variation of weight. T_{low}: CV_w ~ 10%, and T_{high}: CV_w ~ 20%. *BW*: body weight; *BL*: total body length; K: Fulton index; CV_w: coefficient of variation of body weight. *SGR*: specific growth rate; *SD*: standard deviation.

Table 1. Results of ANOVA and Newman–Keuls tests used to analyse the mean differences between treatments (T_{low}: $CV \sim 10\%$: low CV of weight; T_{high}: $CV \sim 20\%$: high CV of weight) (fixed factor), Date (fixed factor) and Tanks (random factor nested to Treatment). BW: Body weight; BL: Total body length; SGR: specific growth rate. D1: Day 26; D2: Day 41; D3: Day 54; D4: Day 69; D5: Day 82. Significance threshold was $p < 0.05$.

	Source	df	F	p	Newman–Keuls
	Treatment	1&1771	0.5	0.519	
BW (g)	Tank (treatment)	4&1771	31.3	<0.001	
	Date	4&1771	782.6	<0.001	D5 > D4 > D3 > D2 > D1
	Treatment	1&1771	1.7	0.268	
BL (cm)	Tank (treatment)	4&1771	0.7	0.562	
	Date	4&1771	115.9	<0.001	D5 > D4 > D3 > D2 > D1
	Treatment	1&1771	0.1	0.805	
SGR	Tank (treatment)	4&1771	5.1	<0.001	
	Date	4&1771	65.3	<0.001	D5 < D1=D2 < D4 < D3

Table 2. Spearman coefficients (r) between initial and final body weight in T_{low} ($CV \sim 10\%$: low CV of weight) and T_{high} ($CV \sim 20\%$: high CV of weight). T_{low} (Tanks 1, 2 and 3) and T_{high} (Tanks 4, 5 and 6). ** $p < 0.01$, *** $p < 0.001$. Results of rank-based multiple comparisons between tanks are shown with the letters a and b. When two tanks have different letters they significantly differ at $p < 0.05$.

	D41		D54		D68		D82	
Tank	r	p	r	p	r	p	r	p
1	0.92	***	0.94	***	0.96	**	0.95	**
2[a]	0.85	***	0.90	***	0.92	***	0.97	**
3	0.88	***	0.96	**	0.95	**	0.99	**
4	0.96	***	0.99	**	0.98	**	0.98	**
5[b]	0.98	**	0.99	**	0.99	**	0.99	**
6	0.97	**	0.98	**	0.98	**	0.99	**

Table 3. Mean ± SE. Cosinor values for feeding demand per hour (FD, g kg^{-1}) in groups with high (T_{high}) and low (T_{low}) coefficient of variation in weight. T_{low}: $CV_W \sim 10\%$, and T_{high}: $CV_W \sim 20\%$. Mesor and amplitude are expressed in g kg^{-1}. The reference phase for the acrophase refers to the time of day and is expressed in hours (h:min).

Feeding demand (FD)	T_{low}	T_{high}
Acrophase (h:min)	8:17 ± 0:27	11:34 ± 0:34
Amplitude (g kg^{-1})	2.05 ± 0.11	1.40 ± 0.10
Mesor (g kg^{-1})	2.67 ± 0.08	2.01 ± 0.07
p	***	***

increased again till the end of the experiment. Tank 6 was the only one to show a regular decrease in SGR from the beginning to the end of these periods. At D82, all tanks had reached similar SGR values.

Spearman rank correlation coefficients for BW calculated between two successive dates were close to 1 in both treatment groups and significant in all cases (Table 2). Nevertheless, observed tendencies were different between the T_{low} and T_{high} groups. In T_{high} groups, coefficients remained very stable during the experiment, demonstrating a linear relationship at a value of 0.98 between initial and final body weight. In T_{low} groups, Spearman rank correlation coefficient increased (0.85 to 0.98 in average) from the beginning to the end of the experimental period. There was a significant Tank effect ($H_{(5,23)} = 13.7$, $p = 0.017$), with tank 2 having a higher coefficient than tank 5 ($p = 0.04$).

3.3.2 Feeding behaviour

FD and FW were similar in T_{low} (18.3 ± 0.7 g kg^{-1}day^{-1} and 14.0 ± 24.0 g) and T_{high} groups (17.6 ± 4.5 g kg^{-1}day^{-1} and 0.9 ± 1.3 g) throughout the whole period but Tank (Treatment) effect was significant for both variables ($F_{(4, 121)} = 7.8$, $p < 0.001$ and $F_{(4, 121)} = 33.6$, $p < 0.001$). Indeed, FW was

very low in all tanks (0.6 ± 1.6 g) except tank 3 (41.7 ± 33.7 g). Date effect was not significant for FD or for FW. No difference in mean FCR was evident between the two groups: 1.3 ± 0.1 to 2.4 ± 0.3 from the beginning to the end of the experiment for T_{low}, and 1.3 ± 0.3 to 1.9 ± 0.4 for T_{high}, but there were significant Date and Tank (Treatment) effects ($F_{(3, 121)} = 13.3$; $p < 0.001$ and $F_{(4, 121)} = 3.7$, $p < 0.01$). The Tank (Treatment) effect came from tank 4, where FCR was lower than in the other tanks (1.09 ± 0.27 vs. 1.50 ± 0.52). Newman–Keuls post- hoc tests showed that FCR increased with time ($p < 0.01$ for each pairwise comparison).

3.3.3 Feeding rhythm

A highly significant daily rhythm in FD was observed in both treatments, with the acrophase in the morning (Table 3). Mesor and Amplitude were higher in T_{low} than T_{high} groups.

Feeding demand varied between a minimum of 1.2 and a maximum of 3.8 g kg^{-1} during $P2 + P3$. Two main feeding activity peaks were observed at 06:00 and 11:00 h (Fig. 3). No difference could be observed in the mean overall demand of T_{low} and T_{high} groups but Hour effect was highly significant ($F_{(23, 2100)} = 9.1$, $p < 0.001$), with higher demands at 6:00 and 11:00 h compared to all other time slots ($p < 0.01$ for each pairwise comparison). The number of demands observed during these two peaks did not differ, but Tank (Treatment) effect was significant $F_{(4, 2100)} = 23.5$, $p < 0.001$.
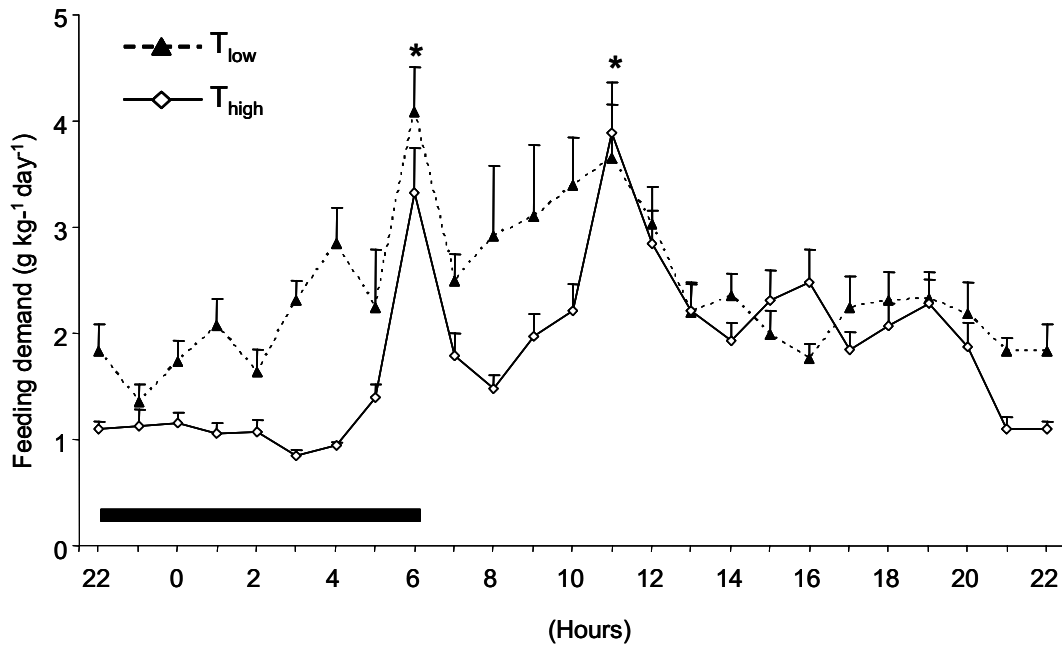
Fig. 3. Feeding rhythm difference (mean ± SE) between T_{low} ($CV \sim 10\%$: low CV of weight) and T_{high} ($CV \sim 20\%$: high CV of weight). The black mark on the X-axis represents the night period. * indicates a significant feeding peak ($p < 0.05$).

Table 4. Percentage of fish in each tank for each feed-demand behaviour category; SGR: specific growth rate (mean ± SD). Data are given for $P1$ (Day 1-Day 27), $P2$ (Day 28-Day 55) and $P3$ (Day 56-Day 82),. T_{low} (group with low coefficient of variation in weight, $CV \sim 10\%$) are in tanks 1, 2 and 3; T_{high} (group with high coefficient of variation in weight, $CV \sim 20\%$) are in tanks 4, 5 and 6. High triggering: >15% actuations, low-triggering: <15% and zero-triggering: <2%.

Tank	High-triggering						Low triggering						Zero-triggering					
	%			SGR			%			SGR			%			SGR		
	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
1	0.0	1.3	1.7		1.1 ± 0.0	0.9 ± 0.0	11.5	23.7	21.7	1.8 ± 0.6	1.1 ± 0.4	0.9 ± 0.1	88.5	75.0	76.7	1.5 ± 0.9	1.0 ± 0.2	0.8 ± 0.2
2	0.0	3.0	1.7		0.9 ± 0.0	0.8 ± 0.0	15.6	25.3	13.6	1.5 ± 1.2	1.2 ± 0.2	0.8 ± 0.1	87.5	71.7	84.7	1.7 ± 0.8	1.0 ± 1.5	0.8 ± 0.1
3	0.0	0.0	1.7			0.8 ± 0.0	11.5	30.0	18.3	1.4 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	91.7	70.0	80.0	1.4 ± 1.0	1.0 ± 0.0	0.8 ± 0.1
4	0.0	0.0	0.0				17.7	36.7	27.1	1.5 ± 0.3	0.9 ± 0.1	0.7 ± 0.1	86.5	63.3	72.9	1.2 ± 0.3	0.7 ± 0.2	0.7 ± 0.2
5	0.0	1.7	1.9		0.8 ± 0.0	0.7 ± 0.0	13.5	28.3	18.5	1.4 ± 0.4	0.9 ± 0.1	0.7 ± 0.1	88.5	70.0	79.6	1.4 ± 0.4	0.9 ± 0.2	0.7 ± 0.1
6	0.0	0.0	0.0				15.6	45.0	22.0	0.5 ± 1.1	1.2 ± 0.2	0.8 ± 0.1	88.5	55.0	78.0	0.3 ± 0.4	1.3 ± 0.3	0.9 ± 0.1

3.3.4 Social structure

The percentage of high, low or zero triggering fish is presented in Table 4. No HT fish were observed during $P1$. This category appeared during $P2$ in two of the three T_{low} groups (1 and 3%) and in one T_{high} group (2%). At the end of $P3$, there were 2% of HT fish in all T_{low} groups, and 2% in one tank out of the T_{high} groups. In T_{low}, 2 fish were responsible for 28% of the triggering-activity in tank 1, 1 fish for 32% in tank 2, and 1 fish for 18% in tank 3. In T_{high} groups, none of the fish showed a triggering activity higher than 15% except in tank 5 (1 fish responsible for 19% of the triggering activity). In tank 4, five fish were responsible for 15% of the triggering activity; and in tank 6, 10 fish were responsible for 50% of the triggering activity. At the same time, 18 and 81% of LT and ZT fish were observed respectively in T_{low} groups, vs. 23 and 77% in T_{high} groups. None of these percentages were significantly different between T_{low} and T_{high} groups.

The growth performances of HT, LT and ZT fish (Table 4) were not significantly different. For all variables, Date effect was significant ($p < 0.001$ in all cases): BW ($H_{(4,1780)} = 1203.0$); BL ($H_{(4,1780)} = 1064.0$); SGR ($H_{(4,1758)} = 546.0$); K ($H_{(4,1780)} = 430.4$). The variability was higher between tanks than between social categories i.e. Tank effect was significant in all cases ($p < 0.01$): BW ($H_{(5,1780)} = 25.9$); BL ($H_{(5,1780)} = 21.1$); SGR ($H_{(5,1758)} = 39.1$); K ($H_{(5,1780)} = 64.5$). SGR variability came from the HT category at $P1$ (Table 4), SGR was indeed lower in tanks 1 and 2 compared to LT and ZT categories, but higher in tanks 5 and 3. Conversely, SGR was similar in low and zero-triggering individuals.

3.3.5 Fish physiology

Blood plasma characteristics were similar in both T_{low} and T_{high} groups (Table 5). No Treatment effect was recorded for any variables and there was only a Tank effect for blood pO_2.

Table 5. Blood plasma parameters (mean $\pm$ *SD*) in in groups with high (T_{high}) and low (T_{low}) coefficient of variation in weight. T_{low}: $CV_w \sim 10\%$, and T_{high}: $CV_w \sim 20\%$ at the end of the experiment (Day 82). *df*: degree of freedom; * indicates a significant difference, threshold: $p < 0.05$.

			Treatment		Tank (treatment)	
	T_{low}	T_{high}	*df* 1 & 18		*df* 4 & 18	
			F	p	F	p
pH	7.2 $\pm$ 0.1	7.2 $\pm$ 0.1	0.0	0.9	1.0	0.4
pCO_2 (mm Hg)	32.2 $\pm$ 7.1	30.4 $\pm$ 4.8	0.0	0.8	0.4	0.8
pO_2 (mm Hg)	36.1 $\pm$ 13.5	37.8 $\pm$ 10.9	0.0	0.8	3.6	0.02*
BE (mmol L^{-1})	-14.6 ± 2.6	-16.2 ± 2.0	0.0	0.9	0.9	0.5
HCO_3^- (mmol L^{-1})	13.2 $\pm$ 2.3	11.8 $\pm$ 1.4	0.0	0.9	0.8	0.5
Total CO_2 (mmol L^{-1})	14.2 $\pm$ 2.5	12.6 $\pm$ 1.6	0.0	0.9	0.8	0.5
O_2 saturation (%)	57.4 $\pm$ 21.1	57.8 $\pm$ 16.9	0.0	0.9	0.7	0.6
Na$^+$ (mmol L^{-1})	156.8 $\pm$ 8.5	159.6 $\pm$ 10.2	0.5	0.5	1.1	0.4
K$^+$ (mmol L^{-1})	4.4 $\pm$ 1.1	4.3 $\pm$ 0.7	0.1	0.8	0.8	0.5
iCa (mmol L^{-1})	1.1 $\pm$ 0.2	1.2 $\pm$ 0.4	0.5	0.5	0.7	0.6
Glucose (mg dl^{-1})	137.5 $\pm$ 58.4	129.5 $\pm$ 32.8	0.9	0.4	0.1	0.9
Hct (% PCV)	25.6 $\pm$ 3.3	26.6 $\pm$ 6.4	0.6	0.5	1.1	0.4
Hb (mmol L^{-1})	8.7 $\pm$ 1.0	9.0 $\pm$ 2.2	0.9	0.4	1.0	0.4

This was due to tank 3, where blood pO_2 was lower compared with the other tanks, but only the difference with tank 1 was significant ($p < 0.05$).

4 Discussion

4.1 Effects of the size grading protocol

The main modification observed after applying the size grading protocol in this experiment was a 24% increase in feed demand. This difference cannot be totally explained by the grading protocol because the period preceding its application included a self feeder learning phase that was characterized by a low food demand but short, in accordance with the classic behaviour of this species (Rubio et al. 2003, 2004; Covès et al. 2006; Millot et al. 2008). This feed-demand increase was neither accompanied by food wastage nor feed conversion rate improvement, but showed similar and high day to day variations before and after size grading. This would confirm the need for a period of 22 days for the feed-demand to reach a suitable and stable level of feed reward for a group of European sea bass, as pointed out by Sánchez-Vázquez et al. (1994). The data also showed that the high variability of the food conversion rate between tanks at the beginning of the experiment was probably linked to the learning process, as this disappeared after size grading.

Triggering activity was also modified after size grading, with no fish displaying more than 12–14% of the total activity before size grading and conversely, almost none displaying less than 15% after size grading. According to the feed-demand behaviour classification previously described (Covès et al. 2006; Di-Poï et al. 2007, 2008; Millot et al. 2008), the present study therefore confirmed that within a group of 60–100 sea bass juveniles, only a few individuals were responsible for the majority of food demands, whereas the rest of the population exhibited low- or zero-triggering activity. It also showed, however, that high-triggering fish appeared only after size grading.

4.2 The effects of a reduction in the body weight coefficient of variation

The main result is the absence of impact on growth performances over the 54-days of the experiment, a phenomenon that has already been described in many species such as eel, turbot, *Scophthalmus maximus*, or Arctic charr (Kamstra 1993; Strand and Øiestad 1997; Sunde et al. 1998; Wallace and Kolbeinshavn 1988), although this is the first time this has been observed under self-feeding conditions. The growth was almost linear and mean *SGR* values were slightly higher than values already published for sea bass of the same size reared under similar conditions (Muller-Feuga 1998; Millot et al. 2008).

Another interesting result was in the evolution of the body weight coefficients of variation, which increased in the T_{low} groups while slightly decreasing in T_{high} groups. This finding agrees with previous work done by Carmichael (1994) in channel catfish *(Ictalurus punctatus)*, and by Sunde et al. (1998) in turbot. In the latter species, growth heterogeneity of graded groups was enhanced until it reached the value of the un-graded group. This has been called heterogeneity stabilisation or convergence. According to these authors, this phenomenon is always due to altered feeding behaviour or social hierarchical order. They hypothesized that fish competition was very high just after size grading, and decreased with time. In some species, size grading might lead to the destruction of size hierarchy, the growth of the large fish being adversely affected by the stronger agonistic interaction, and intraspecific aggression occur among the graded large individuals (Baardvik and Jobling 1990; Stefánsson et al. 2000; Sunde et al. 1998). In sea bass, no studies have reported a high level of aggression under self-feeding conditions, as the reward level after each trigger actuation usually optimizes feed allocation to the group. This was verified in the present study, where the regular growth evaluations and very low mortality rate showed no evidence that any injuries occurred over the course of the experiment. However, low aggression level

does not mean absence of social interactions between fish. Indeed, in exploitation competition, there are no direct interactions between competitors; instead, one individual exploits and depletes a food patch before a second individual arrives (Ward et al. 2006). Recent work based on growth heterogeneity modelling in fish rearing systems rejected the hypothesis of competition decrease within time (Campéas 2009). According to this author, growth heterogeneity stability is not correlated with the stabilisation of social interactions (i.e., size had little or no effect on fight determinism), and CV_w stability is the result of the dominance pressure of large fish on small fish. Indeed, according to Drews (1993), dominance can be defined as the priority to food access, and small subordinates may have a reduced feeding rate in the presence of dominant fish (Abbot et al. 1985) despite surplus feed availability (Jobling and Wandsvik 1983; Øverli et al. 1998; Sloman and Armstrong 2002). In our study, this idea is supported by the fact that correlations between initial and final weights tended to be lower in T_{low} groups, but at the end of experiment, when CV_w increased, correlations reached the same values as those of the T_{high} groups. Indeed, Campéas (2009) showed that this tendency was related to a modification of fish rank (weight rank between the beginning and the end of the studied period) in low CV_w groups.

Growth heterogeneity was frequently shown to be accompanied by food wastage (McCarthy et al. 1992; Jobling and Baardvick 1994; McDonald et al. 1996), but in our trial neither feed-demand nor feed wastage were different between the high and low CV_w groups. In both cases, all the food delivered was ingested and, since all fish grew positively, it is very likely that they all had access to food.

Feeding rhythms were identical in both treatments, with two main peaks at 06:00 and 11:00 h confirming that sea bass given free access to the self-feeder have a predominantly diurnal feeding pattern. Nevertheless, the greatest activity was not concentrated at the end of the day, as has been described in previous work (Azzaydi et al. 1999). Such morning activity has already been observed for this species under similar conditions (Millot 2008; Millot and Bégout 2009), but varying patterns of feeding rhythms have also been reported by several authors (Anthouard et al. 1993; Sánchez-Vázquez et al. 1994, 1995, 1998; Bégout-Anras 1995; Boujard et al. 1996).

Over $P2$ and $P3$, the social structure was similar in low and high CV_w groups, and in line with that described by several authors for the species (Covès et al. 2006; Di-Poï et al. 2007; Millot et al. 2008). In both treatments, only two or three fish were responsible for about 35% of the triggering activity and in most cases, there was only one high-triggering fish. However, at the end of $P3$, there were HT fish in all of the T_{low} groups, but in only one tank of the T_{high} groups. The remainder of the population was divided into two groups: about 20% of LT fish and 78% of ZT fish. Size structure is well known to play an important role in social interactions (Abbot and Dill 1989; Dou et al. 2004), and relative size is the most determinant factor in fighting ability during aggressive behaviour, as well as for attacking opponents and obtaining preferential access to food (Dou et al. 2004). However, our study shows that the juvenile sea bass group structure (according to the triggering activity) was not influenced by the size structure under self-feeding conditions. This group structure seems to be very stable and self-organised even after the removal of the regular feed-demand leader (Di-Poï et al. 2007; Millot and Bégout 2009) or chronic and acute stress experiments (Millot et al. 2010, 2011). In all cases, these changes were followed by the reconstruction of the group with the same structure and function as the initial group (Di-Poï et al. 2008).

In low and high CV_w groups, HT fish showed similar culture performances as the other fish, which confirmed results obtained by previous authors (Di-Poï et al. 2007, 2008; Millot et al. 2008). Covès et al. (2006) also showed that HT sea bass did not monopolize the trigger or the distributed pellets, therefore indicating that they were not displaying hierarchy. This could explain why the group structure was not influenced by the size structure. It should be remembered that the self-feeder was regulated to provide tanks with a reward level optimizing feed allocation to the group, and that direct competition for food only occurs when food is limited and defensible (Karplus et al. 2000). There is a need for further behavioural research in order to better understand the link between size-based social structure, and group structure according to triggering activity. For instance, it would be interesting to induce competition by restricting food delivery under self-feeding conditions, and to look at the influence of these two overlapping constraints on feed demand and social interactions.

Finally, all measured physiological variables were very similar in both low and high CV_w groups, and were within the usual values for sea bass (Coeurdacier et al. 1997; Dosdat et al. 2003; Millot et al. 2008) indicating that no important physiological changes were promoted by the population heterogeneity.

5 Conclusion

In the light of these results, it appears that grading practices, which are common in fish farming, induce only a transient modification of feed demand behaviour under self-feeding conditions. Juvenile sea bass seemed to adapt very quickly to new situations occurring in self-feeding conditions, including modifications in the size distribution of the population. The stability of the social structure built up around the self-feeder devices already known to be better in terms of feed conversion (Azzaydi et al. 1998), constitutes a new argument for their use (instead of automatic-feeders) Furthermore, grading practice does not improve the growth of graded fish in self-feeding conditions and the remarkable stability of CV_w over time indicates that size-grading frequency could be largely reduced, not only to avoid social structure disturbances but also to limit handling which is a labour-intensive and costly procedure.

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