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Feed demand behavior in sea bass juveniles: Effects on individual specific growth rate variation and health (inter-individual and inter-group variation)

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Abstract:

Feeding motivation is one major indicator of fish welfare and an investigation on the link between feed demand, growth and physiological variables in sea bass juveniles was developed. A computerized on-demand feeding system coupled with a PIT tag monitoring device was used to continuously record for 219 days the triggering activity of 150 individuals (initial average body weight 131.6 ± 1.80 g and coefficient of variation 16.8%). Each group was held in 400 l tanks at 22.2 ± 1.5 °C and light regime was 16:8 LD. In all the tanks, 89% of the fish actuated the trigger, but only two or three fish accounted for 45% of the total triggering activity. These few high-triggering individuals had a transient higher growth i.e. at the time an individual was the high-triggering fish in the tank, its Specific Growth Rate (SGR) increased and was higher than that of the other fish. However, high-triggering fish did not exhibit a higher initial and final body weight nor a higher average SGR than low- and zero-triggering fish. Fish of different triggering categories did not show differences in physiological variables (muscle composition, blood and tissues biochemistry). This study also revealed that when an imbalance between apparent daily feed tank consumption and feed demand was observed (i.e. wastage), it was mostly due to an increasing demand rather than a decreasing consumption; such wastage could often be linked to particular stressors (measuring day, population sampling or social interactions) and therefore, feeding motivation disturbances could be a relevant operational fish welfare indicator.

Keywords: Feeding activity; Growth; Social interaction; Welfare

Introduction

Aquaculture is growing more rapidly than all other animal food production sectors (FAO, 2002). In parallel, an increasing concern for fish welfare in general and under aquaculture conditions in particular can be noticed in recent years (Ellis et al., 2002). However, unlike other major forms of livestock production, there is a lack of scientific information on the welfare of fish raised under intensive aquacultural conditions (F.S.B.I., 2002; Chandroo et al., 2004; Huntingford et al., 2006). As for now, fish welfare definition raises two main challenges related to the lack of basic knowledge about emotional expressions in fish, the difficulty to observe individual fish in its farm environment (Wolffrom, 2004; Huntingford and Adams, 2005; Dámsgard et al., 2006; Huntingford et al., 2006) and the physiological basis themselves of welfare concept (Korte et al., in press). The current attempts are mainly to control welfare in farmed fish through regulation of management practices in order to minimize stress and its consequences: reduced appetite and growth, decreased general immune competence, reproductive changes, diseases, and mortality (Turnbull et al., 2005).

Hence, in aquaculture there is a need to identify welfare indicators, species specific and operational. Feeding motivation is one candidate since farmers can access such indicator and it is well known that fish appetite can fluctuate between days or at a bigger scale in weeks or months (Madrid, 1994), and with environmental conditions (Alanärä, 1992; Anthouard et al., 1993; Rubio et al., 2003b). Therefore, understanding the major causes of feeding motivation and associated growth variation and how the group and individual Specific Growth Rate (SGR) evolve is crucial for the success of fish farming (Martins et al., 2005). The minimization of individual variation in food consumption and growth contributes to maximizing production efficiency, reducing food waste and improving water quality (Jobling and Baardvik, 1994).

To investigate feeding activity, there is a wide choice of methods from direct or video observations, dyestuffs and markers, the quantitative determination of gastrointestinal content and the recording of feeding activity using on-demand feeders with feed waste monitor to X-ray methods (Jobling et al., 2001). Among all existing techniques to investigate feeding behavior, the study with a self-feeder coupled with a PIT tag detection antenna, gives the possibility to reveal individual and group feed demand behavior. Such combination of techniques was successfully used in sea bass (Covès et al., 1998; Rubio et al., 2004; Covès et al., 2006). The potential for synergy between that method and a long study period provide opportunities to increase our knowledge on the influences of various facets of fish behavior and physiology. Indeed, that approach, allows to study fish demand feeding behavior and motivation (levels, rhythms, alteration) and to correlate it with physiological variables, individual growth and SGR in a marine cultured species like sea bass.

The aim of this study was to determine how individual demand feeding activity influence individual growth rate, group feed consumption and physiological variables. The influence of common stresses in aquaculture such as handling for measurement and sampling (fish removal) were taken into account in order to understand better sea bass feed demand behaviour variation in relation to management practices.

1. Material and methods

1.1. Apparatus

The device to operate the feeders comprised 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 and feed dispensers were regulated to distribute between 0.5 g kg^{-1} of fish at the beginning and 0.4 g kg^{-1} at the end of the experiment. The reward level (about 50 pellets each time, one per individual) 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 behavior and the group (*i.e.* tank) apparent feed consumption (uneaten pellets were counted). Each individual fish was horizontally PIT-tagged by inserting a tag just behind the head to prevent any change of position subsequent to implantation.

1.2. Experimental set up

The experiment was carried out with sea bass using one condition in triplicate. Three 400 l seawater tanks were supplied with sand filtered water in a recirculated system (flow rate of $4 \text{ m}^3 \text{ h}^{-1}$ in each tank and 10 % water renewal per day). Water temperature was maintained at $22.2 \pm 1.5^\circ\text{C}$, the oxygenation above 70 % saturation in the outlet and salinity was 28.6 ± 3.3 . Tanks were surrounded by black curtains and for each a 120 W lamp was located about 90 cm above the water surface. Light regime was 16:8 LD (light onset at 06:00 U.T.+1) with twilight transition periods of 30 min. Fish were fed by a commercial sea bass diet (Neo Grower Extra Marin 4.0, France: 45 % crude protein, 20 % lipid according to the manufacturer, 4 mm). The experiment was realized over 219 days, with 150 fish in 3 tanks (50 sea bass in each tank). Sea bass were hatched in Aquanord (France) and grown at FMB-Aquapole (France). At the beginning of the study, fish were 13 months old with an average initial body weight (BW_i) of $131.6 \pm 1.80 \text{ g}$ (coefficient of variation (CV) = 16.8%, N = 150 fish). Fish were again weighed (to the nearest mg and measured for length to the nearest mm) after 25 (D25), 80 (D80), 148 (D148), 197 (D197) days and at the end of the experiment 219 days (D219, Measuring day, Fig. 1). Fish were placed under self-feeding conditions at D0 (after one week of acclimation) and food access was possible all day (24 h) even during rearing unit cleaning and waste counts from 10:00 to 11:00 (U.T.+1). Apparent feed tank consumption (food quantity dispensed minus waste counted on the bottom of the tank and in the sediment trap) was monitored daily. Triggering activity recordings were done continuously for 219 days and were only stopped before (48 h in advance, with no recordings and fasting of fish) and during fish manipulation (20 days off in total).

1.3. Analytical methods

In order to evaluate physiological status (i.e. muscle composition, plasma and tissues biochemistry), fish were sampled on 3 occasions after 48 h of fasting (Population sampling, Fig. 1). At day 25, day 106 and day 219, respectively 27 fish, 32 fish and 31 fish were rapidly taken and immediately euthanized with a lethal dose of ethylene-glycol-monophenyl-ether (2 ‰). The D25 fish sampling was carried out randomly, but only the high-triggering fish were reanimated by intensive oxygenation in order to prevent a disruption in the recently established social structure. This sampling was composed of 8 zero-triggering fish and 19 low-triggering fish. The two other samplings were made randomly and were composed of 5 zero-, 26 low- and 1 high-triggering fish for D106 and 2 zero-, 25 low- and 4 high-triggering fish for D219. In each fish a blood sample (c. 1 ml) taken within 1 min from caudal vessels was separated in two aliquots. One was centrifuged immediately (3500 rpm, 5 min) and plasma samples were stored at -16°C until analysis. The second aliquot was kept at room temperature to isolate serum and was thereafter stored at -16°C until analysis. In the same fish, different tissues were taken as follows: gill arch immediately washed in 0.25 M sucrose and then frozen and stored in liquid nitrogen; whole liver and about 8 cm^2 of dorsal muscle (taken under the first dorsal fin and skin was removed) weighed and immediately frozen in liquid nitrogen and stored at -80°C until analysis. Muscle samples were ground and moisture content was determined on homogenated samples (24 h at 105°C) and subsequently freeze-dried and ground before further analysis. Chemical analyses were performed in triplicate for each sample according to AOAC (Association of Official Analytical Chemists, 1984) methods: ash (7 h at 550°C), crude fat (Folch et al., 1957), crude protein (Dumas method with an Elementary NA 2000[®], $N \times 6.25$). Fish brain were dissected and immediately frozen at -80°C until dosing of 5-HIAA/5-HT ratio to evaluate serotonergic activity. Indeed, brain serotonergic activity is estimated using brain 5-HIAA/5-HT ratio, where 5-HIAA (5- hydroxyindoleacetic acid) is the major metabolite of 5-HT (5-hydroxytryptamine, serotonin) (Di-Poï et al., 2007).

Blood plasma analyses were performed as follow: osmolarity using an Advanced Instrument Osmometer[®] and chloride, sodium and potassium using an Electrolyte Beckman Analyser[®]. Total IgM were measured in serum using an ELISA test (Coourdacier et al., 1997). Gill ($\text{Na}^+ \text{-K}^+$)-ATPase activity was determined according to the method described in Boeuf et al. (1989). Frozen whole liver was ground and glycogen content was determined on homogenated samples according to Carr and Neff (1984) method using a Diagnostic Enzymatic Kit, (Biomérieux ref 61269). Dosing of 5-HIAA/5-HT ratio in all brain tissue was achieved using ELISA kits (IBL Hamburg, Germany) following the method described in Di-Poï et al. (2007).

1.4. Data analysis

Primarily, the individual and group feeding behavior were studied, by determining fish individual triggering level and the resulting population composition in different categories of triggering activity. Individual feed demand behavior (i.e. their proportional contribution to the total number of trigger actuations within a group) was calculated and used to distinguish between high-triggering (> 25% actuations), low-triggering (< 25%) and zero-triggering (0%) individuals (Covès et al., 2006). Average and standard error (\pm SE) were calculated.

Then, the daily apparent feed tank consumption (DFtC) and feed conversion ratio (ratio between the DFtC and the biomass difference in time) were evaluated for each tank.

Finally, fish growth, group and individual Specific Growth Rate (SGR) were calculated using the following formula: SGR (% body weight per day) = $100 (\ln BW_f - \ln BW_i) / t$, where BW_f and BW_i are the final and the initial body weight (g) respectively and t the total number of days. Reduction in sample size was taken into account for each analysis (Table 2).

Weight coefficient of variation (CV_w) was calculated as: $100 * SD * BW^{-1}$, where SD is standard deviation of the individual weight and BW is average weight. Fulton index (K) was calculated as: $100 * BW * (L^3)^{-1}$, where L is standard length and its coefficient of variation (CV_K) as: $100 * SD * K^{-1}$, where SD is standard deviation of the individual Fulton index and K is average Fulton index.

Parametric statistical test (Tukey HSD) was used to compare average weights between tank and non-parametric statistical tests (Kruskal-Wallis, KW) were used to analyze differences in BW_i , BW_f and SGR for the 3 classes (low, high and zero triggering-fish). For all the tests, the significant threshold was $p < 0.05$.

All physiological results were expressed as average \pm SE. Statistical analysis were conducted using Statistica® for Windows, data in percent were arc-sin square-root transformed. For D25, D106 and D109, the effects of the factor "tank" on physiological variables were checked. For D 106 and D219 the effects of the factor "triggering categories" were checked. Since no effects of these factors were distinguished, changes in muscle composition and physiological variables were tested versus time only by an ANOVA. Significant ANOVA were followed by a post-hoc multiple comparison test (Newman-Keuls test). Differences were considered significant at $p < 0.05$.

2. Results

2.1. Categories of triggering activity

The first actuation occurred on average 10 days after the experiment started, for all high and low triggering fish. The composition of each population of 50 sea bass was compared and no tank effects were seen ($KW = 2.24$; $p = 0.33$; $df = 2$; $N = 150$) which allowed data pooling. Indeed, the populations were composed of 11.3 ± 0.86 % of zero-triggering fish, 83.3 ± 0.26 % of low-triggering fish and 5.3 ± 0.29 % of high-triggering fish (Table 1). Each triggering category took part differently in the group feed demand : 45.6 ± 7.5 % of total fish actuation activity was realised by the high-triggering fish (8 in total). The remaining 54.4 ± 3.8 % of total actuation was attributed to the low-triggering fish, since zero-triggering fish did not make any feed demand. During the experiment, there were 2 to 3 high-triggering fish per tank, generally only one high-triggering fish at one time and that structure was stable in time.

2.2. Apparent feed tank consumption

The apparent daily feed tank consumption (DFtC) was unsettled from one day to another (Fig. 1). The stressful events (i.e. measuring day and population sampling), did not have pronounced effects on the DFtC but sometimes showed an effect on food wastage (Fig. 1). Immediately or some days after a stressful event (D106, population sampling), the quantity of uneaten pellets could represent up to 70 % of the quantity distributed in tank 1 and 2. Wastage appeared in some cases during a change from one high-triggering individual to another in the tank. Such change in individual function was observed seven times: Two of these changes could be related to a measuring day, two others to a population sampling but 3 changes appeared spontaneously. When there were two high-triggering fish

in the same tank, that transient period was often accompanied by a significant pellet's wastage (e.g. Day 55, Tank 3, Fig. 1). In general such period was concluded by a change of high-triggering individual which ended the food wastage.

2.3. Fish growth

Initial average weight were not homogeneous between the 3 tanks (Tukey HSD, $p < 0.05$, $N=150$) but presented an average CV_w of $16.54 \pm 0.78\%$. That difference in initial average weight between groups was due to fish distribution in the tank aiming at a low CV_w of each group and not at an initial homogeneous average. However, that initial difference was levelled off beyond D148 and average weight in the 3 tanks became homogeneous (Tukey HSD, $p > 0.05$, $N=63$; Fig. 2A).

CV_w increased during the first part of the experiment: for tank 2, CV_w increased from 17.78% to 24.01%. After this time, the coefficient decreased, and returned to a level equivalent to that of other tanks (Fig. 2B).

Fulton index (K) was not significantly different for all tanks (Tukey HSD, $p > 0.05$, $N=150$) and it increased in time (D0: 1.17 ± 0.01 , D25: 1.21 ± 0.01 , D80: 1.36 ± 0.01 , D148: 1.36 ± 0.01 , D197: 1.39 ± 0.01 and D219: 1.40 ± 0.01). CV for Fulton index (CV_K) was initially of 8.75 ± 0.43 . During the first 100 days and for tank 2, CV_K increased from 8.57% to 13.65%. Thereafter, CV_K decreased, and returned to a level equivalent to that of other tanks (Fig. 2C).

Average feed conversion ratio for tanks 1, 2 and 3 were respectively 1.97 ± 0.35 , 2.32 ± 0.31 and 2.36 ± 0.31 . These values were not significantly different (KW= 1.14, $p= 0.56$, $df= 2$, $N= 15$) giving an overall average feed conversion ratio of 2.22 ± 0.17 . Concerning mortality, there were 30 sampled fish and 11 deaths of different origins (e.g. jumps, death following anesthesia).

Group specific growth rate

Tanks 1, 2 and 3 showed the same group SGR evolution in time: SGR increased significantly from D25 to D80 in each tank (Tukey HSD, $p < 0.05$, $N=150$; Fig 3), but group SGR was not significantly different between tanks at the same period (Tukey HSD, $p > 0.05$; Fig. 3).

Individual specific growth rate

Generally, at the time when a fish became a high-triggering individual, its SGR increased or was higher than the average SGR for the tank at the same period. This phenomenon was observed in all tanks. Among the fish which were soon to become high-triggering fish, the forthcoming one was characterized by a markedly negative SGR (Fig. 3). In tank 1, when a fish toggled to become high-triggering, its SGR increased whereas the SGR of the current high-triggering fish decreased (Fig. 3). The same was observed for fish number 43 in tank 3, but its SGR decrease appeared only 55 days after it had lost its high-triggering status.

For individual SGR calculated over the total duration of the experiment, the differences between triggering fish categories disappeared within each tank (T1, T2 and T3) ($KW_{T1}= 2.68$, $p= 0.26$, $df= 2$, $N= 42$; $KW_{T2}= 4.48$, $p= 0.11$, $df= 2$, $N= 40$; $KW_{T3}= 4.01$, $p= 0.14$, $df= 2$, $N= 41$). Similarly for CV_{SGR} , differences could not be seen between categories ($KW_{T1}= 8.16$, $p= 0.02$, $df= 2$, $N= 42$; $KW_{T2}= 5.08$, $p= 0.08$, $df= 2$, $N= 40$; $KW_{T3}= 3.98$, $p= 0.10$, $df= 2$, $N= 41$).

These observations were confirmed by the fact that the high-triggering fish were not characterized by a final weight significantly higher than that of other fish ($KW_{T1}= 0.52$, $p= 0.77$, $df= 2$, $N= 22$; $KW_{T2}= 0.25$, $p= 0.88$, $df= 2$, $N= 21$; $KW_{T3}= 1.06$, $p= 0.59$, $df= 2$, $N= 19$).

There was also no relation between the initial weight of fish and their capacity to become a high-triggering fish ($KW_{T1}= 2.73$, $p= 0.25$, $df= 2$, $N= 42$; $KW_{T2}= 2.94$, $p= 0.23$, $df= 2$, $N= 40$; $KW_{T3}= 3.80$, $p= 0.15$, $df= 2$, $N= 41$). Nevertheless, the initially largest fish have a slightly higher SGR than that of other fish (however not always significant, $KW_{T1}= 12.26$, $p= 0.02$, $df= 4$, $N= 42$; $KW_{T2}= 2.86$, $p= 0.41$, $df= 3$, $N= 40$; $KW_{T3}= 8.71$, $p= 0.07$, $df= 4$, $N= 41$).

2.4. Muscle composition and fish physiology

Some changes in muscle composition occurred during the course of the experiment: statistically significant differences were observed in water, protein and lipid content between D25, D106 and D219 while ash content remained stable (Table 2).

For plasma and tissues biochemistry, values were measured within the usual range of variation (Pichavant et al., 2001; Person Le Ruyet et al., 2002, 2003; Dosdat et al., 2003; Table 3). CV was low for all blood plasma variables except potassium, IgM and for 5-HIAA/5-HT ratio. Significant differences in the osmolarity and hydromineral balance (mainly in sodium and calcium content) were observed between D25, D106 and D219; they were concomitant to a decrease in liver glycogen content and to a transient increase in gill (Na⁺-K⁺)-ATPase activity (Table 3). For IgM values no significant changes occurred in time. For 5-HIAA/5-HT ratio, a significant change could be seen over the duration of the experiment with a 2.5 fold decrease between the first and the two subsequent sampling dates (Table 3).

3. Discussion

In the present study, sea bass feeding behavior complexity was revealed by the simultaneous analysis of a set of variables (feed-demand, apparent daily feed tank consumption and wastage, fish growth) in relation to aquaculture practices prone to be source of stressors. Analysis of physiological variables was added to document fish health.

3.1. Feed demand activity

Sea bass used in the experiment were naive to the self-feeding apparatus, and triggering activity started 9-10 days after the beginning of the experiment which is in accordance with previous results (Rubio et al., 2003a, 2004; Covès et al., 2006).

Our results confirmed that within a group of 50 sea bass, only two or three fish ("high-triggering") were responsible for around 45% of the triggering activity under a reward regime equivalent to 1 pellet per individual given after each actuation. The rest of the population could be divided in two groups: individuals that seldom actuated the trigger and fish that never actuated the device (respectively "low-triggering" and "zero-triggering" fish, Covès et al., 2006). We demonstrated that this population composition in 3 categories was homogeneous and stable in time over 200 days which was three times longer than in earlier surveys (Covès et al., 2006). Further, we observed changes in fish individual role in the population at the time of stressful events (measuring day, population sampling) or in a spontaneous way, keeping the overall same composition only assumed by a different fish. That observation follows earlier works of Alanära and Brännäs (1993) and Brännäs and Alanära (1993) in salmonids.

In general, feed intake patterns vary mostly in relation to photoperiod and temperature (Madrid et al., 2001). In our study under controlled conditions, we could observe that tank feed consumption was relatively constant in time with some day to day variations and steadily accounted for approximately 2% of the biomass present in the tank. Generally, all distributed feed was ingested by the group, and it is interesting to note that feed distribution and thus, high-triggering fish activity, seemed to be adjusted to the group needs (evolving biomass) rather than to the high- or low-triggering individual needs.

At the time of stressful events, apparent feed tank consumption did not vary, as opposed to feed demand and food wastage could represent up to 70% of the delivered quantity and could be related to an increase in triggering activity more than a decrease in feed consumption. The latter was also seen during a spontaneous change of high-triggering fish that could involve a transition over a period of variable length (5 to 20 d). In general, a high-triggering fish kept its status during approximately 20 d, but it happens that this duration was longer (e.g. fish # 3 in tank 2, 186 days). Once the high-triggering fish lost its status it became again a low-triggering fish, but it could sometimes recover its high-triggering status (alike individual # 1 in tank 1 at day 142). Thus, the frequent change of high-triggering fish in the tank could be regarded as the consequence of an imbalance group social structure. The excessive triggering activity during a stress period could also be explained by that imbalance or by the fact that, when two fish were active at the same time, a competition could appear. Alike in salmonids, where social rank is directly correlated with self-feeder device triggering, the "dominant" fish had the higher actuation level (Alanära and Brännäs, 1993; Brännäs and Alanära, 1993).

Thus, the monitoring of self-feeding activity, its variation from expected levels, and monitoring of uneaten food quantity in the tank could enable the detection of either a change of the group social structure or the appearance of an environmental stress within the rearing units.

3.2. Triggering activity, fish growth and physiology

Average apparent feed conversion ratio was 2.22 ± 0.17 , which corresponded to the value already observed by Azzaydi et al. (2000) when sea bass have a free access to a demand-feeder. Thus, fish feeding activity was likely in phase with their natural rhythm and prone to optimize their growth performance (Boujard and Leartherland, 1992; Jobling et al., 1995; Thorpe and Cho, 1995).

Average fish weight increased regularly in time with a significant difference appearing between tanks during the first part of the experiment only. During that period, Fulton condition index increased for all the tanks, which indicated a general improvement in fish condition. However, at the same time, tank 2 presented a particular increase in CV_W and CV_K meaning that some individuals grew quicker and realized a better condition than others, despite the fact that the biggest fish in the tank were not always those which had the best condition. For tank 2, CV_W and CV_K increase was accompanied by a catch of weight rate significantly less important than that of the two other tanks. As such, an increased CV in time and consequently, differences in fish growth, is likely to indicate individual competition within fish group as shown for salmonids (Jobling, 1995). After 100 days, average weight, CV_W and CV_K became homogeneous in all the tanks. According to Jobling (1995) when the fish achieve rapid and homogenous growth rates, and uniform body weight, it would be reasonable to conclude that the social environment is favorable and that the food is evenly distributed among the fish in the group.

SGR values (min: 0.30 ± 0.02 , max: 0.73 ± 0.03) observed during the experiment corresponded to the values already published for sea bass (same size and under similar conditions, Muller-Feuga, 1990). At the beginning of the experiment $16 \pm 2\%$ of the population presented a negative SGR, then the percentage decreased in time to reach 0% of the population (except 1 fish out of 19 for tank 3). That differential SGR in time could be related to the process of self-feeder learning. According to different authors (McCarthy et al., 1992; Jobling, 1995; Alanärä et al., 1998) individual SGR and CV_{SGR} can be considered as a significant indicator of social ranking within a group: "dominant" or in our case high-triggering fish display high and constant SGR (i.e. low CV_{SGR}), and "dominated" or other triggering categories fish display low and variable SGR (i.e. high CV_{SGR}). In salmonids, fish with the highest bite counts has the highest growth rate, indicating that the competitive ability to release food is beneficial for food intake (Abbott and Dill, 1989; Alanärä and Brännäs, 1993; Brännäs and Alanärä, 1993). A preferential access to the feed zone, as well as a better preparation for the meal (anticipation of pellets delivery and/or better assimilation through early secretion of digestive enzymes) would explain that when a fish becomes a high-triggering individual, its SGR increases or is higher than the average group SGR. Such differential SGR was not visible on the long term but was hereby linked to a triggering time window. Indeed, the high-triggering sea bass were not characterized by an overall higher SGR (already observed by Landless, 1976; Alanärä and Brännäs, 1993; Chen et al, 2002 and Covès et al, 2006) and by a lower CV_{SGR} than the other individuals in the group. When the high-triggering fish lost its status, its SGR became again equivalent to the average SGR, which in turn tended to homogenize all the SGRs in time. It is interesting to note that at the beginning of the experiment, when all the individuals were naïve, the future high-triggering individuals had a negative SGR. We could hypothesize that the individual variations in learning could be linked to the initial fish growth. Thus, fish with a negative growth might be searching more for pellets and/or might spend more time in the feeding zone which in turn may enhance the self-feeder learning process. That hypothesis remains to be tested in the future by a thorough study of individual feed intake as well as fish space partitioning in the various zones of the tank. The fact that there were both i) no relation between initial body weight and fish capacity to become a high-triggering fish and ii) no significant difference in growth between the three triggering categories, leads to hypothesize that fish can employ different strategies to achieve the same end (Magurran, 1993). Indeed, despite the fact that short-term benefits may be unequal (i.e. an SGR increase) different behavioral patterns could appear in the rest of the group in order to compensate.

All measured physiological variables including IgM levels (Coourdacier et al, 1997) were within the usual values for marine fish (sea bass and turbot) measured over 3-6 months using the same sampling procedures (Pichavant et al., 2001; Person Le Ruyet et al., 2002, 2003; Dosdat et al., 2003). No major disturbances in fish physiological variables occurred during the course of the experiment only time related changes were observed in some blood plasma and tissues biochemistry and they

could be related to fish growth and aging. Indeed, the observed differences between initial and final sampling could be related to i) fish status at the onset of the experiment related to initial conditioning and ii) to changes that may have occurred during the course of the 7 months experiment with regards to environmental conditions and/or to fish status (e.g. hormonal changes related to maturation process). The moderate changes in osmolarity and to some extent in ions concentrations were concomitant with the decrease in liver glycogen content, which was at its highest at the onset of the experiment. Muscle composition changes with time and consequently with fish size may be related to fish feeding behavior. It is well known that feeding behavior and feed intake determine fish growth rate but also flesh composition in particular the lipid content which may be quantitatively and qualitatively modified; in comparison flesh protein content is less influenced by external feeding conditions since it is mainly dependent on intrinsic factors such as the fish species variety and size (Borresen 1992, Shearer 1994). Information concerning the biochemical composition of sea bass flesh prior to commercial size is scarce and incomplete in comparison with whole body composition (Person Le Ruyet et al., 2004 a, b; Montero et al., 2005, Skalli et al. 2006). The wide heterogeneity of lipid distribution in fish flesh with wide gradients from the anteroposterior to ventral regions made comparison difficult (Toussaint et al., 2005). The relative low fat muscle content observed in this study may result from a moderate feed intake over all the experiment, only at the end was total lipid content within the usual range for sea bass juveniles fed a similar diet (Skalli et al., 2006). Concerning brain serotonergic activity, 5-HIAA / 5-HT values observed in the present survey were within the same range as those measured by Di-Poï et al. (0.072 – 0.142 of wet weight; 2007). However, our long term survey protocol did not allow us to underline differences between triggering categories but initial *i.e.* learning phase values (with an average of 0.115 ± 0.068 of wet weight and a large CV) were similar to values measured for low- or zero-triggering fish (Di-Poï et al, 2007). Therefore, we could hypothesized that the establishment of a stable population composition after 100 days also induced a general reduction of stress levels as confirmed by the homogeneous growth rate achieved. Further work should be pursued in the direction of linking such neurophysiological indicator with behavioral flexibility in sea bass in order to select for fish better adapted to aquaculture.

According to the present results, feed-demand behavior more clearly highlights the inter-individual differences than physiological variables. A long-term study is also important to underline fish group demand-feeding behavior and makes it possible to reveal individual characteristics, like SGR evolving with individual triggering-activity period. Further, it illustrates that more than the high-triggering fish identity itself, it is the role, indeed the function, that a fish assumes which is important and conditions the group feeding behavior. Finally, the measurement of the difference between tank feed consumption and feed demand in each group seems to be a relevant indicator in order to access the occurrence of a stressing event in the rearing environment. The monitoring of that variable in aquaculture should make it possible to rapidly pinpoint a fish welfare alteration and to remedy for it.

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Figures

Figure legend

Figure 1. Apparent daily feed tank consumption (DFtC, g per day) for each tank. The difference between tank consumption and food quantity delivered in tank is represented by the black area which represents food wastage. The vertical black line represents the stress events and the dotted vertical straight line represents a change of high-triggering fish in the tank. The horizontal black bar on the top represents the period when there were two high-triggering fish at the same time in the tank.



Represents high pellets wastage events (more than 70% of the quantity distributed in the tank).

Figure 2. (A) Average weight in relation to time for each tank (\pm SE, g). Letters indicate significant differences between tanks (Tukey HSD test, $p < 0.05$). **(B)** Coefficient of variation of weight (CV_W) in relation to time for each tank. **(C)** Coefficient of variation of the Fulton condition index (CV_K) in relation to time for each tank.

Figure 3. Evolution of the group average \pm SE and the high-triggering fish SGR ($\% \text{ day}^{-1}$) per period and for each tank. Averages with a different letter were significantly different (Tukey HSD test, $p < 0.05$, average \pm SE). The full straight line represents the zero-triggering period, the dotted line represents the low-triggering period, and the discontinuous straight line represents the high-triggering period for each of the high-triggering fish identified. The initial triggering activity of fish is defined with HT for high-triggering and ZT for zero-triggering. † represents fish sampled.

Figure 1

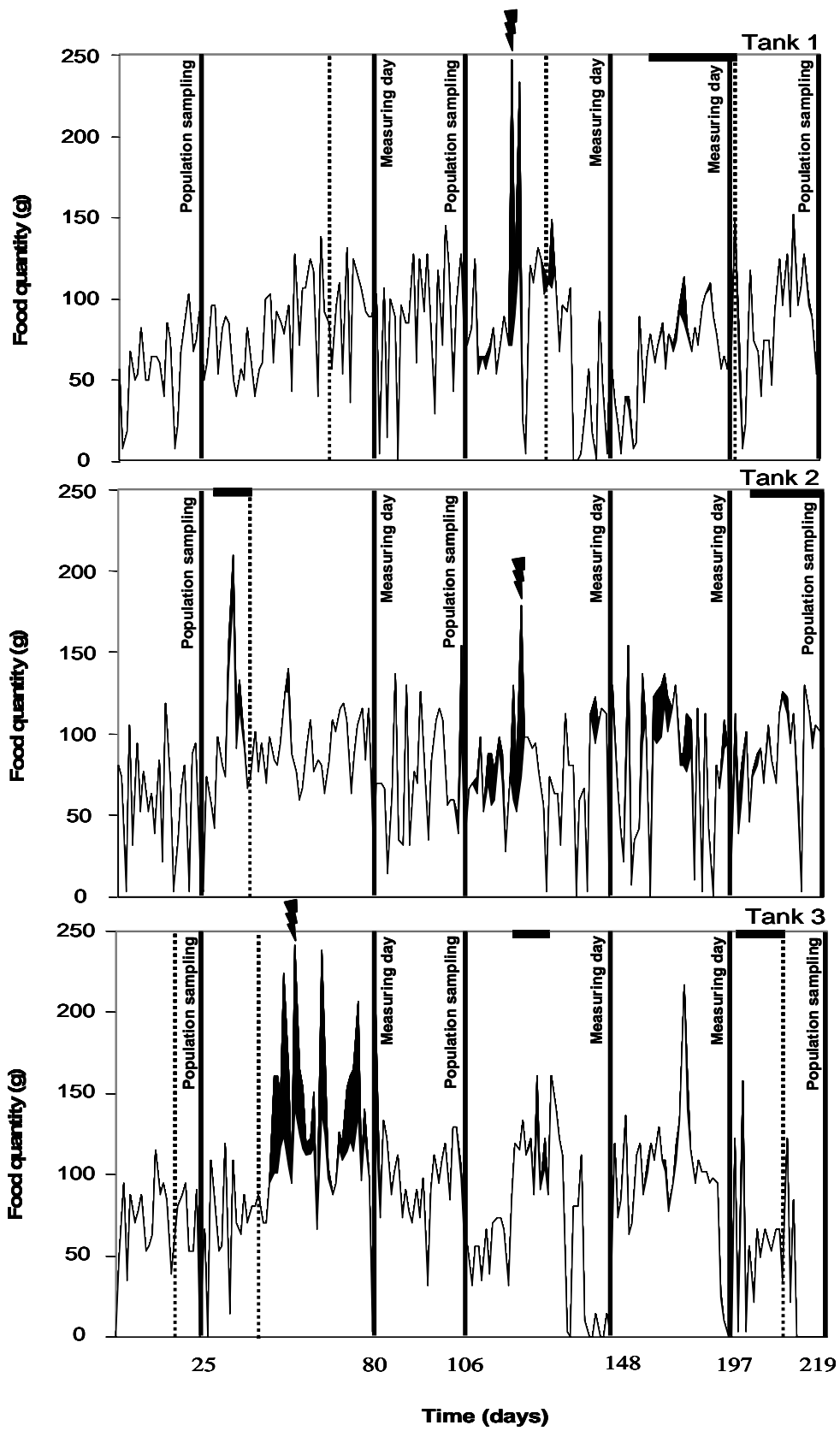


Figure 2

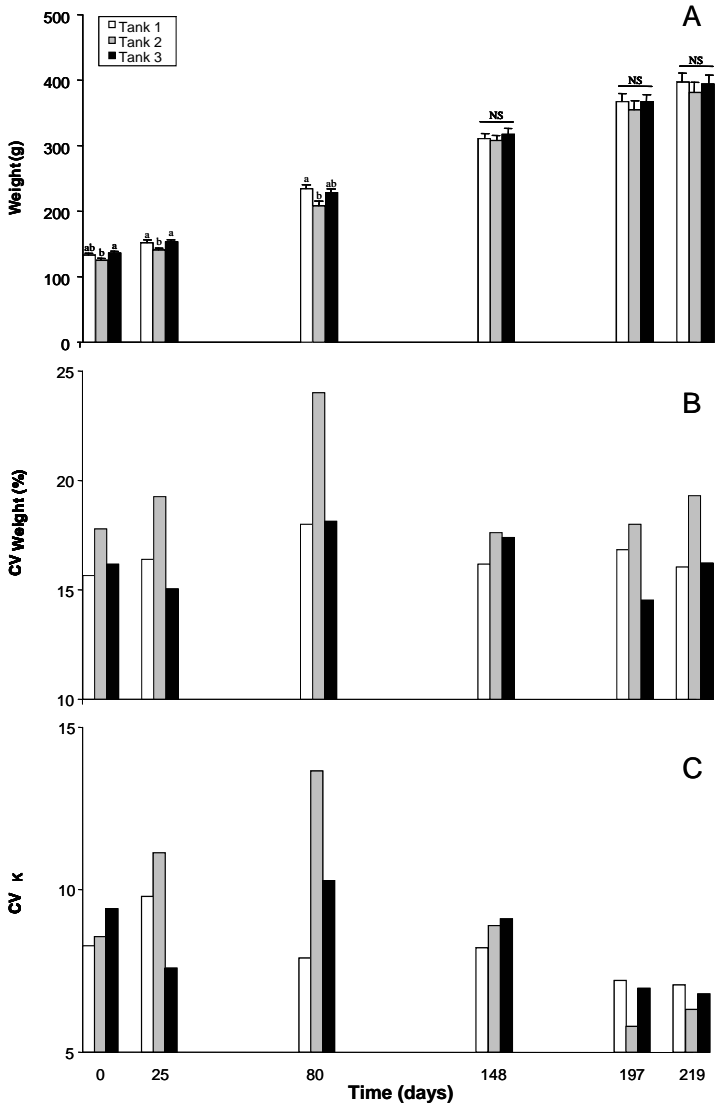


Figure 3

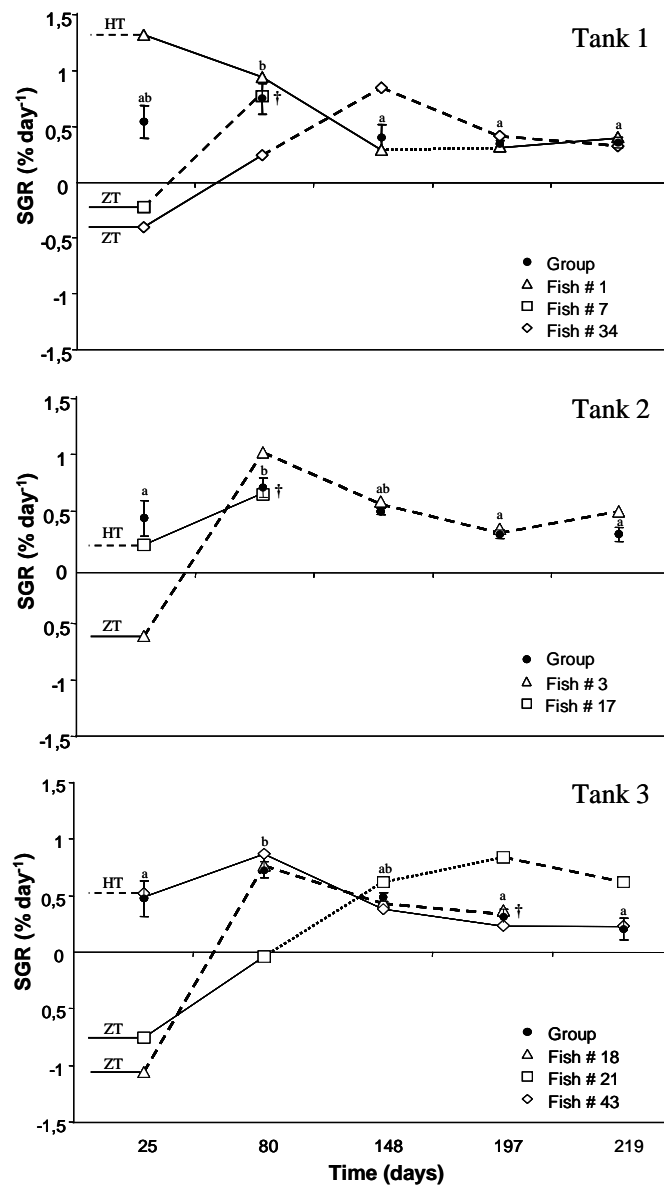


Table 1. Occurrence of the different feed-demand behaviour categories per tank and for the total experimental duration.

<i>Tank</i>	<i>High-triggering</i> (%)	<i>Low-triggering</i> (%)	<i>Zero-triggering</i> (%)
1	6	82	12
2	4	80	16
3	6	88	6

Table 2. Muscle composition of sea bass in relation to time.

	Day 25	Day 106	Day 219	
Water content (% ww)	76.0 ± 0.2 ^c (1.3)	74.6 ± 0.2 ^b (1.8)	73.1 ± 0.2 ^a (1.5)	***
Protein content (% ww)	19.9 ± 0.1 ^a (3.2)	21.5 ± 0.2 ^b (4.5)	21.5 ± 0.1 ^b (3.2)	***
Lipid content (% ww)	1.6 ± 0.2 ^a (53.1)	1.8 ± 0.2 ^a (24.1)	2.9 ± 0.2 ^b (47.8)	***
Ash content (% ww)	1.4 ± 0.1 (8.7)	1.4 ± 0.1 (4.5)	1.4 ± 0.1 (2.2)	NS
N	27	32	31	

Data are averages ± SE and coefficient of variation (%) inside brackets. Letters following averages indicate statistical differences between dates, averages not sharing a common letter are significantly different and no letter means no significant differences. * P<0.05, ** P<0.01 and *** P<0.001.

Table 3. Blood plasma and tissues biochemistry in sea bass juveniles.

	Day 25	Day 106	Day 219	
Osmolarity (mmol l ⁻¹)	352 ± 1 ^a (1.4)	362 ± 1 ^b (1.7)	361 ± 1 ^b (2.0)	***
Chloride (mmol l ⁻¹)	146.8 ± 0.6 ^b (1.9)	142.5 ± 0.7 ^a (2.9)	147.6 ± 1.1 ^b (3.9)	***
Sodium (mmol l ⁻¹)	180.1 ± 1.3 ^b (3.6)	173.8 ± 1.0 ^a (3.2)	175.3 ± 0.8 ^a (2.2)	***
Potassium (mmol l ⁻¹)	5.1 ± 0.3 (32)	5.2 ± 0.4 (37)	5.5 ± 0.3 (26)	NS
Calcium (mmol l ⁻¹)	3.1 ± 0.1 ^a (7)	3.5 ± 0.1 ^b (11)	3.4 ± 0.1 ^b (7)	***
Liver glycogen (µmol g ⁻¹)	79.1 ± 3.7 ^b (23)	55.4 ± 3.0 ^a (30)	61.6 ± 2.8 ^a (25)	***
Gill (Na ⁺ -K ⁺)-ATPase (µmol iP mgP ⁻¹ h ⁻¹)	12.3 ± 1.4 ^a (59)	16.5 ± 1.0 ^b (32)	14.4 ± 0.8 ^{ab} (32)	*
IgM (mg ml ⁻¹)		13.2 ± 6.0 (45.5)	15.9 ± 6.8 (42.5)	NS
5-HIAA / 5-HT	0.115 ± 0.068 ^a (59)	0.044 ± 0.008 ^b (19)	0.045 ± 0.010 ^b (23)	***
Average weight of sampled fish (g)	^a 149.25 ± 4.45 (16)	^b 244.68 ± 9.80 (23)	^c 396.10 ± 11.88 (17)	***
N	27	32	31	

Data are averages ± SE and coefficient of variation (%) inside brackets. Letters following averages indicate statistical differences between dates, averages not sharing a common letter are significantly different and no letter means no significant differences. * P<0.05, ** P<0.01 and *** P<0.001.