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Self-feeding behavior changes induced by a first and a second generation of domestication or selection for growth in the European sea bass, *Dicentrarchus labrax*

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Abstract – Among the strategies that can be used to improve fish welfare in a rearing environment, domestication and/or selective breeding was proposed to minimize fish responsiveness to husbandry practices. To verify this hypothesis on a recently domesticated species, the sea bass *Dicentrarchus labrax*, two experiments were realized, each using two populations differing according to their level of domestication or selection. For the first experiment, we used one population produced from wild parents (*Wild;* initial body mass: 106 ± 3 g), and one population from parents selected for growth for one generation (*Selected 1;* initial body mass: 129 ± 4 g). For the second experiment, we used one population produced from parents domesticated for two generations (*Domesticated;* initial body mass: 72 ± 3 g), and one produced from parents selected for growth for two generations (*Selected 2;* initial body mass: 89 ± 4 g). The first experiment was carried out over 112 days with 240 fish (60 fish per tank, 120 fish per population), and the second one over 84 days with 200 fish (50 fish per tank, 100 fish per population). Two variables, self-feeding behavior and growth performance, were measured over the time of the experiments. After a control period, the fish were submitted twice, at three-week intervals, to an acute stress treatment consisting of draining the tank and leaving the fish out of water for one minute. Both self-feeding behavior and growth performance were altered by the acute stress treatment. During the first post-stress period, the *Domesticated* and *Selected* (*1* and *2*) groups showed more pronounced post-stress exposure responses than the *Wild* fish: they modified their feeding rhythm, their feed intake, and their growth rate. During the second post-stress period, feeding rhythm was still affected (being more diurnal with a well defined peak), but the feed intake and growth rate results showed that the *Domesticated* and *Wild* groups seemed less affected than the *Selected* (*1* and *2*) populations, which continued to express a high post-stress response.
According to these results, it can be concluded that: (1) an application of two acute stress treatments, at three-week intervals, modified fish feeding behavior and growth performance; (2) the domestication process seemed to improve fish adaptation abilities to this kind of stress; and (3) the process of selection for growth led to a final, better growth, but did not seem to improve fish acute stress tolerance.

Key words: Feed intake / Feeding rhythm / Specific growth rate / Adaptation capacities / Fish welfare

1 Introduction

Fish domestication can be defined as "the process by which a population of animals becomes adapted to humans and to the captive environment, by some combination of genetic changes occurring over generations and environmentally induced developmental events re-occurring during each generation" (Price 1984). Selection is usually applied to improve traits strongly associated with production cost (e.g., growth rate, disease resistance, age at maturity and flesh quality), and very little is known on selected fish capacities to tolerate stress. Nevertheless, fish responsiveness to stress was shown to have a distinct genetic component, and may therefore be modified by selective breeding (Pottinger and Pickering 1997). Thus, it may be feasible to generate strains displaying high stress tolerance and therefore improve performance within aquaculture across a number of traits (e.g., improvement of feed conversion efficiency, growth, fecundity, egg quality, post-slaughter flesh quality, and reduction in the incidence of disease), and in addition an improvement of their welfare (Pottinger and Pickering 1997).

Alongside these improvement and perhaps partly due to the rapid expansion of the aquaculture industry, the welfare of farmed fish has received increasing attention. However, the concept of welfare is complex and difficult to define, although it is commonly admitted that it responds to one of the three

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following conditions: (1) the animal can adapt to its environment and is in good health, with all its biological systems working appropriately; (2) the animal is able to meet what are often called its "behavioral needs"; and (3) the animal is free of negative experiences such as pain, fear, and hunger (Huntingford and Kadri 2008). When cultured, fish are commonly exposed to repeated acute stresses that differ from those they face in the wild, such as: handling, grading, transport, and prophylactic treatment (Pottinger and Pickering 1997). Fish reaction to stress is generally divided into primary and secondary responses (Mazeaud et al. 1977), and even tertiary level responses according to Wedemeyer et al. (1990). In aquaculture, this tertiary level (Barton 2002; Conte 2004; Huntingford et al. 2006) includes both direct and indirect maladaptive effects, such as growth reduction (Barton et al. 1987; Pickering et al. 1991; Pankhurst and Van der Kraak 1997), suppressed reproductive function (Contreras-Sanchez et al. 1998; McCormick 1998, 1999; Schreck et al. 2001), and reduction in immune capacities (Einarsdottir et al. 2000), and disease resistance (Pickering 1992; Balm 1997). Therefore, even if stress responses do not reveal all welfare disturbances, it is generally accepted that they strongly indicate poor welfare (Broom 1988; Huntingford et al. 2006). Such evidence led to active research on potential methods to reduce stress responses in aquaculture species (Ashley 2007). Among these methods, domestication and selective breeding to minimize fish responsiveness to stressors has been a major research direction over the last few years (Pottinger 2003).

The European sea bass, *Dicentrarchus labrax* (Linnaeus 1758) is an important species in Mediterranean and Atlantic aquaculture. Sea bass was only recently domesticated, so very little is known about the effects of the early steps of domestication or selection for growth, apart from effects on the classically-measured traits of commercial interest (Dupont-Nivet et al. 2008; Vandeputte et al. 2009), and specifically, little is known about behavioral responses to stress exposure and welfare potential. The authors have already evaluated the behavioral changes induced by a first step of domestication or selection for growth in the European sea bass (Millot et al. 2010). They found that a first generation of domestication and selection improved fish growth performance, but at this early stage did not modify behavioral responses to repeated acute stress exposure (one to four acute stressor applications per day). The present study, thus proposes to evaluate and compare the effects of first and second generations of fish domestication and selection for growth on behavior changes. The chosen approach was an evaluation of the modifications induced in self-feeding (feed demand rhythm, quantities of food intake and food waste) by two acute stress exposures at an interval of three weeks (stress tolerance used as a screening procedure). Growth performance (body mass, body condition factor and specific growth rate) was recorded as a complementary trait.

2 Materials and methods

2.1 Experimental set-up

The four populations of fish tested in this experiment were produced as part of a genetic EU project to evaluate the response to selection for growth (Competus COOP-CT-2005-017633). The details of rearing conditions and sizes of these populations can be found in Vandeputte et al. (2009). In summary, the four tested populations were hatched and reared at the experimental research station of Ifremer in Palavas-les-Flots (France). Until the start of the experiment, fish were reared according to usual sea bass rearing standards (Chatain 1994). All fish had been bred using full factorial designs where each female was crossed with each male. All fish had the same rearing history, had never experienced the natural environment, and only differed in levels of domestication or selection of their male parent:

- The *Wild* group is a progeny issued from the crossing of 13 Mediterranean wild (F0) dams with 20 Atlantic F0 sires: thus parents had not experienced any domestication or selection pressure (Millot et al. 2010).
- The *Selected 1* group is a progeny issued from the crossing of the same 13 Mediterranean wild (F0) dams with 19 Atlantic F1 sires produced from a single generation with selection for growth (Millot et al. 2010).
- The *Domesticated* group is a progeny issued from the crossing of 6 dams with 6 sires, both Atlantic F1 domesticated, which had only been exposed to domestication pressure.
- The *Selected 2* group is a progeny issued from the crossing of the same Atlantic F1 domesticated dams with 14 Atlantic F1 sires that had been subjected to two generation of selection for growth.

Important features of this method include:

- The wild parents had been in captivity for one to three years before they were used to breed the progenies.
- The F1 sires were the descendants of these same wild parents, and had completed an entire cycle of rearing (i.e., first generation of domestication) before they were chosen for reproduction.
- The choice of sires was made at the age of 20 months (400 g), and was carried out at random for the domesticated group, but among the 5% longest for each selected group (i.e., first or second generation for the groups *Selected 1* or 2, respectively).

Thus, the comparison of:

- *Wild* versus *Selected 1* (Experiment 1) would show the effects of a first generation of domestication and selection for growth.
- *Domesticated* versus *Selected 2* (Experiment 2) would show the effects of one pressure of selection for growth.
- Fish from Experiment 1 versus fish from Experiment 2 would show the effects of one more generation of domestication pressure.

The experiments were carried out with duplicates tank for each strain. The 4 tanks (400 L each) were supplied with semi-recirculated seawater, and all tanks were installed in the same room. For each tank, the flow rate was 4 m^3 h^{-1} and the water renewal was 10% per day. Water temperature was maintained at 20.2 ± 1.5 °C, oxygenation was above 90% of saturation in the water-outlet, and salinity was 22.3 ± 3.3.

Table 1. Control and stress phases duration in both experiments.

	Code	Duration (number of days)	
		Exp. 1	Exp. 2
Control phase			
control phase	P1	27	21
Second control phase	P2	26	21
Phase following the:			
stress treatment	P3	24	21
Second stress treatment	P4	35	21

Water ammonia and nitrite compounds were measured every day, and were never above recommended levels for sea bass. Tanks were sheltered with black curtains, individually lit by a 120 W lamp, 90 cm above the water surface. The light regime was 16:8 LD (light onset at 06:00 h) with twilight transition periods of 30 min. Fish were fed a commercial diet for sea bass (Neo Grower Extra Marin 4.0; `www.aqua.legouessant.com`) containing 45% crude protein and 20% lipid according to the manufacturer.

The first experiment (Exp. 1; *Wild* and *Selected 1* groups) was realized over 112 days (5 May 2006–24 August 2006) with 240 fish (60 fish per tank, 120 fish per strain), and the second experiment (Exp. 2; *Domesticated* and *Selected 2* groups) over 84 days (5 February 2008–28 April 2008) with 200 fish (50 fish per tank, 100 fish per strain). At the beginning of the study, fish were 14 and 12 months-old for Exp. 1 and 2 respectively. The *Wild* group weighed an average of 106 ± 3 g (coefficient of variation (CV) = 32%, n = 120 fish), the *Selected 1* group an average of 129 ± 4 g (CV = 34%, n = 120 fish), the *Domesticated* group an average of 72 ± 3 g (CV = 30%, n = 100), and the *Selected 2* group an average of 89 ± 4 g (CV = 29%, n = 100). Fish were again weighed to the nearest mg and measured for length to the nearest mm at 27 (D27), 53 (D53), 77 (D77), and 112 (D112) days after the beginning of Exp. 1; and 21 (D21), 42 (D42), 63 (D63), and 84 (D84) days after the beginning of Exp. 2. Experimental periods were defined as the period between two measuring days: P1 from D1 to D27 and from D1 to D21; P2 from D28 to D53 and from D22 to D42; P3 from D54 to D77, and D43 to D64; and P4 from D78 to D112, and D65 to D84 for Exp. 1 and Exp. 2 respectively (Table 1). All measures were done under anaesthesia using clove oil (0.08‰).

The feeder device included a screened type sensor (a metal rod protected by a PVC cylinder, Covès et al. 2006; Millot et al. 2008, 2009) and a control box. After each activation, fish were rewarded with 50 pellets; the feed dispensers, thus performed a mean distribution of 0.5 to 0.3 g kg^{-1} fish (Exp. 1), and 0.8 to 0.6 g kg^{-1} fish (Exp. 2) at the beginning and at the end of each test, respectively. Such a set up allowed the number, the date, and the hour of feed demand to be monitored in each tank.

Each fish was implanted with a PIT-tag (Passive Integrated Transponder) so that individual body mass and length could be monitored over time. Fish were placed under self-feeding conditions at D1, and food access was possible throughout the day (24 h), even during waste counts (10:00 to 11:00). Apparent feed consumption within each tank (feed amount dispensed minus wasted pellets collected in the sediment trap) was monitored daily. Triggering activity recordings were taken continuously for 112 and 84 days for experiments 1 and 2, respectively, except for the 24 h before and during fish handling (8 days out of the total for each experiment).

2.2 Stress treatment

After a first phase of rearing (P1 + P2), which represented the control phase of the experiment, acute stress events were applied twice between 10:00 and 12:00 on D53 and D77, and D42 and D64 for Exp. 1 and 2, respectively; P3 and P4 therefore represented the phases of post-stress treatment (Table 1). P1 + P2 results were compared with post-stress results for all strains to look for stress-induced effects. Such an experimental design was chosen because all tanks were in the same room with the same water circuit, and disturbances to one tank were, therefore, unavoidably transmitted to adjacent tanks. The stressors consisted of draining the tank, and leaving the fish out of water for 1 min before being caught and anesthetised for weight and length measurement.

2.3 Statistics

To account for fish growth between the different defined periods, all feeding-related variables were considered relative to fish biomass. The variables chosen to measure the different performances were the following:

- the amounts of feed demanded (FD), food intake (FI) and food waste (FW) (g per kg of biomass present in the tank and per day). These variables were used to evaluate feeding behavior changes;
- the amount of feed demands per hour (g per kg of fish biomass) was chosen to monitor the group feed demand rhythm and changes over time;
- the evolution over time of fish body mass (g), body condition factor (K in g cm^{-3}), specific growth rate (SGR in % day^{-1}), and feed efficiency (FE) was examined to find any growth pattern modifications, and to formulate hypotheses on changes in fish metabolic rate based on feed intake.

The calculated variables were obtained as follows:

- SGR (% body mass per day) = 100 (Ln M_f – Ln M_i)t^{-1}, M_f and M_i being the final and the initial body mass (g) respectively, and t the total number of days;
- K (g cm^{-3}) = 100 $M L^{-3}$, M and L being the fish mass (g) and standard length (cm), respectively;
- FE = (final fish biomass – initial fish biomass) × (feed intake)$^{-1}$, Fish biomass is expressed in kg.

All mean values were expressed with their standard error (±SE).

For each experiment, data was checked for normality using the Shapiro-Wilk test, and for homogeneity of variances with the Bartlett's test; the experiments all complied with the assumptions of the parametric tests used thereafter. For the variables related to feeding behavior, fish body mass, body condition factors, and specific growth rate variables, a repeated

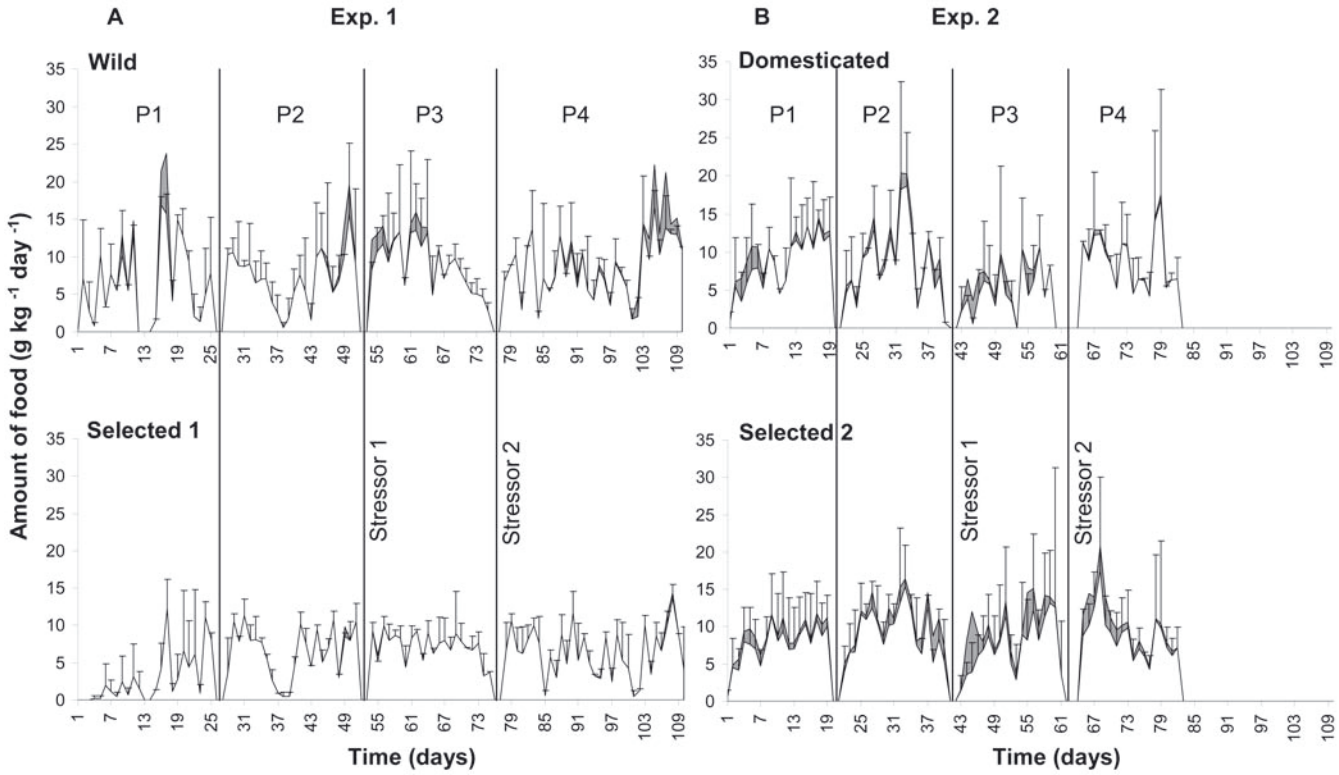

Fig. 1. Daily food intake and wastage. Daily mean (±SE) intaken (white) and wasted (grey) food for 2 strains of sea bass in two experiments (Experiment 1 = *Wild* and *Selected 1* (A), Experiment 2 = *Domesticated* and *Selected 2* (B)) during P1, P2, P3 and P4 periods. Stressors 1 and 2 represented the day when fish where submitted to acute stress event.

ANOVA was used to analyse the average differences between populations (fixed factor), periods (fixed factor), and tanks (random factor nested to population). The different periods considered here were: the control phases, P1 and P2; and the post-stress phases, P3 and P4. For the feed demand rhythm, a repeated ANOVA was used to assess the differences between populations (fixed factor), periods (fixed factor), hour (fixed factor), and tanks (random factor nested within population). The number of data for this variable corresponded to the number of recorded feeding days (104 or 76) × 24 h × number of tank (4). Homogeneous groups were determined using the a posteriori Newman and Keuls test (Dagnélie 1975). For all tests, the significant threshold was $p < 0.05$, and the analyses were performed using the Statistica software package (`www.statsoft.com`).

3 Results

During the experiment, some fish died for different reasons. Some of these individuals jumped out of the tanks, others died due to unidentified causes, but no mortality could be attributed to stress or anesthesia. These losses concerned: 1 *Wild* and 2 *Selected 1* fish during P1; 1 *Selected 1* fish during P3; and 2 *Selected 1* fish during P4. These changes in the number of individuals were taken into account in all measured variables.

3.1 Feed demand, intake and wastage over time

The amount of feed demand and intake fluctuated highly from one day to another, and it was difficult to observe the immediate day-to-day stressor effect. However, in analysing the feeding behavior by period it was possible to observe variations over time.

The results of Exp. 1 (Fig. 1A) showed that the *Wild* groups learned how to use the device in only 2 days, contrary to the *Selected 1* fish, which only began to correctly activate it after 14 days. This experiment also showed that, whatever the period, the *Wild* fish systematically demanded and ate more food than the *Selected 1* fish ($F_{3,412} = 15.2$, $p < 0.001$). During P1, *Selected 1* demanded and ate entirely an average of 2.85 ± 0.87 g kg^{-1} day^{-1} while the *Wild* fish demanded on average 6.89 ± 0.98 g kg^{-1} day^{-1}, ate 6.23 ± 0.79 g kg^{-1} day^{-1} and wasted 0.67 ± 0.28 g kg^{-1} day^{-1}. During P2, demand and intake of food increased significantly for the *Selected* 1(+124%) while it stayed stable for the *Wild*. During P3, demand and intake of food remained stable for *Selected* 1 while it increased significantly for the *Wild* (+ 25%). During P4, the amounts of FD and FI stayed stable for both groups.

The results of Exp. 2 (Fig. 1B) did not show any difference in learning ability between the *Domesticated* and *Selected 2* fish, they both learned after about 2 days. This experiment highlighted that the *Domesticated* and *Selected 2* demanded and ate the same amount of food during the whole experiment, except during P3 where the *Selected 2* fish had a higher feeding activity than the *Domesticated* fish ($F_{3,280} = 7.98$, $p < 0.001$).

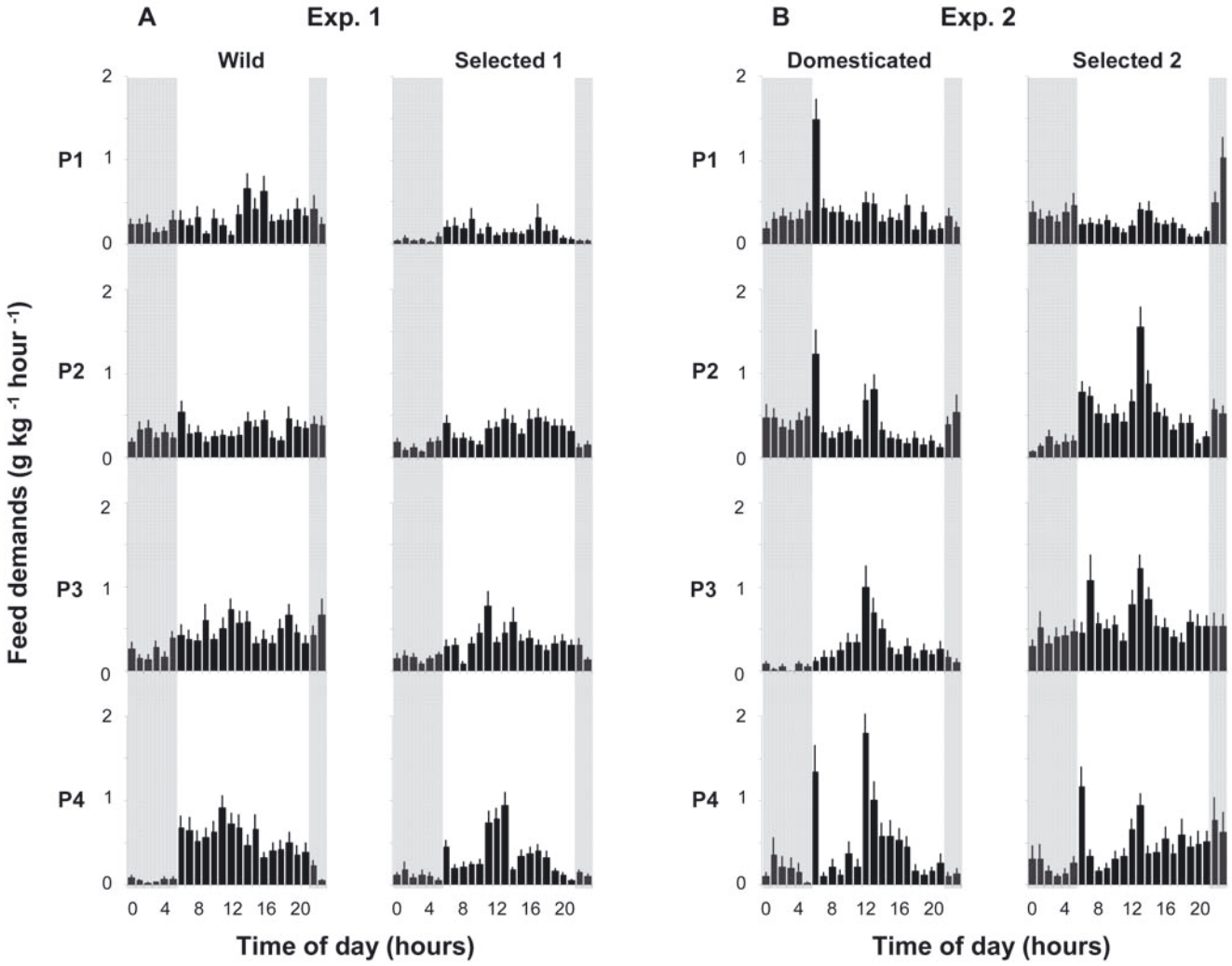

Fig. 2. Daily feeding rhythm**.** Pattern of daily mean (+SE) feed demands per hour for 2 strains of sea bass in two experiments (Experiment 1 = *Wild* and *Selected 1* (A), Experiment 2 = *Domesticated* and *Selected 2* (B)) during P1, P2, P3 and P4 periods. The grey boxes indicate the night period.

During P1, the *Domesticated* and *Selected 2* groups demanded on average 8.57 ± 0.67 g kg^{-1} day^{-1}, ate 7.58 ± 0.58 g kg^{-1} $day^{-1}$and wasted 0.99 ± 0.20 g kg^{-1} day^{-1}. During P2, demand and intake of food increased significantly for *Selected* 2 (+53%) while it stayed stable for the *Domesticated* group. During P2, *Selected 2* fish also showed a slight increase of food wastage (1.84 ± 0.38 g kg^{-1} day^{-1}). During P3, demand and intake of food remained stable for *Selected* 2 while it decreased significantly for the *Domesticated* group (–40%). During this period, *Selected 2* fish showed again, an increase of food wastage (3.06 ± 0.77 g kg^{-1} day^{-1}). During P4, *Selected 2* fish showed a slight decrease of FW, but the amounts of FD and FI stayed stable. In contrast, the *Domesticated* fish showed a stable FW, but an increase of 61% of food demanded and eaten.

3.2 Daily rhythm of feeding activity

The results of Exp. 1 (Fig. 2A) showed that the *Wild* and *Selected 1* fish performed more feed demands during the day than during the night period. However, some differences appeared between groups over time ($F_{69,9090}$ = 1.77, $p < 0.001$). During P1, the *Wild* and *Selected 1* fish performed respectively 73% and 88% of their feed demands during the day period. During P2, the *Wild* and *Selected 1* fish still showed a diurnal feeding (98% and 84% respectively) spread over the whole day. During P3, the highest percentages of feed demands were still diurnal for both groups (75% for *Wild* and 81% for *Selected 1*). During this period the *Selected 1* fish presented a feeding peak at 11:00. During P4, the feeding rhythm for all groups was sharper. The *Wild* fish were more and more diurnal (94%) and presented a feeding peak at 11:00 (10% of feed demand; FD). The *Selected 1* group performed 87% of their feed demands during the day period, especially from 11:00 to 13:00 (38% FD).

The results of the Exp. 2 (Fig. 2B) showed that the *Domesticated* and *Selected 2* fish performed more feed demands during the day than during the night period. However, some differences appeared between groups over time ($F_{69,6912}$ = 2.74, $p < 0.001$). During P1, the *Domesticated* fish performed part

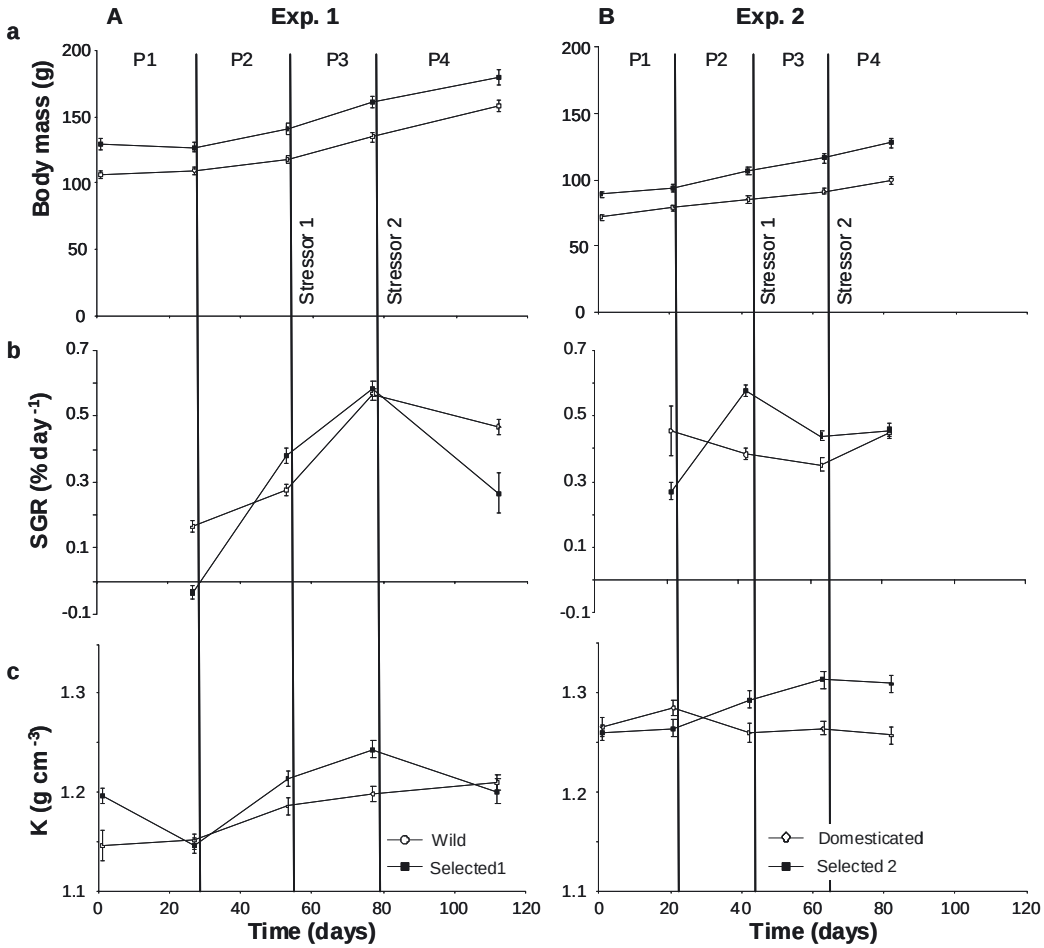

Fig. 3. Growth performance over time**.** Variations over time of mean (±SE) body mass (a), specific growth rate, SGR (b) and body condition factor, *K* (c) for *Wild*, *Selected 1* (Experiment 1, A), *Domesticated* and *Selected 2* (Experiment 2, B) sea bass strains during P1, P2:, P3 and P4 periods. Stressors 1 and 2 represented the day when fish where submitted to acute stress event.

of their feed demand during the day period (73%) with a peak at 06:00, while the *Selected 2* fish seemed more nocturnal (51%) with a feeding peak at 23:00. During P2, the *Domesticated* and *Selected 2* fish performed 62% (with a peak at 06:00) and 82% (with a peak at 13:00), respectively, of their feed demands during the day period. During P3, the highest percentages of feed demands were still diurnal for both groups (91% for *Domesticated* and 74% for *Selected 2*). During this period the *Domesticated* fish presented a feeding peak at 12:00 and the *Selected 2* fish at 07:00 and 13:00. During P4, the feeding rhythm for all groups was sharper. The *Domesticated* fish presented two main feeding peaks during the day period: at 06:00 (15% FD) and at 12:00 (20% FD). The *Selected 2* fish continued to perform 75% of their feed demands during the day period with a peak at 06:00 (11% FD) and one at 13:00 (9% FD).

3.3 Temporal variation in fish growth and feed efficiency

The results of Exp. 1 (Fig. 3Aa) showed that during the whole experiment the *Selected 1* fish presented a body mass around 18% higher than the *Wild* fish ($F_{1,1163}$ = 80.8, p < 0.001). Both groups were characterized by a stable body mass at D1 and D27, then a slight increase at D53 (+8%), and finally a rapid increase at D77 and D112 (+15%; $F_{4,1163}$= 63.8, p < 0.001). Fish-specific growth rate was slower in the *Selected 1* group than in the *Wild* group during P1 (–0.03 ± 0.02 and 0.16 ± 0.07% day^{-1} respectively; Fig. 3Ab). During P2, the *Selected* 1 showed a high SGR increase, while the *Wild* group was performed less well. After the first stress treatment (e.g., during P3), SGR increased for both groups. During P4, the *Wild* fish showed a stable SGR while it decreased by 55% for the *Selected 1* group ($F_{3,933}$ = 8.9, p < 0.001).

At D1, the body condition factor (*K*) of the *Selected 1* group was higher than in the *Wild* population (Fig. 3Ac). At D27, the *K* factor decreased greatly in the *Selected 1* group and remained stable for the *Wild* fish. At D53, both groups showed a significant body condition factor increase. At D77, only the *Selected* 1 group showed a body condition factor increase (+2%). Finally, at D112, the *K* factor stayed stable for the *Wild* group and decreased for the *Selected 1* fish (–3%; $F_{4,1163}$ = 4.2, p < 0.01). The *Wild* and *Selected 1* populations had similar feed efficiency (FE) during the whole experiment

($F_{3,8}$ = 1.15, p = 0.38). However, the FE values varied over time; it was of −0.33 ± 0.46 during P1, then increased during P2 (0.52 ± 0.07) and P3 (0.82 ± 0.18), and finally returned to 0.51 ± 0.04 during P4.

The results of Exp. 2 (Fig. 3Ba) showed that the *Selected 2* fish presented a body mass of around 25% more than the *Domesticated* fish during the whole experiment ($F_{1,980}$ = 151.5, p < 0.001). Both groups were characterized by a constant increase of body mass between each measuring day (+9%; $F_{4,980}$ = 47.5, p < 0.001). Fish-specific growth rate was slower in the *Selected 2* group than in the *Domesticated* group during P1 (0.27 ± 0.03 and 0.45 ± 0.07% day^{-1} respectively; Fig. 3Bb). During P2, *Selected* 2 showed a high SGR increase while the *Domesticated* group was characterized by a SGR decrease of 17%. After the first stress treatment (e.g., during P3), *Selected 2* fish showed a SGR decrease of 24% while it stayed stable for the *Domesticated* ones. During P4, the *Selected 2* fish showed a stable SGR while SGR increased by 29% for the *Domesticated* group ($F_{3,784}$ = 42.2, p < 0.001).

At D1, there was no difference in body condition factor (K) between the *Domesticated* and *Selected 2* groups (Fig. 3Bc). At D21, the K factor was still stable for both groups. At D42, *Selected 2* fish showed a significant body condition factor increase, but this remained stable for the *Domesticated* ones. Finally, at D63 and D84, the K factor was still stable for both groups ($F_{4,980}$ = 7.9, p < 0.001).

The *Domesticated* and *Selected 2* populations had similar feed efficiency (FE) during the whole experiment ($F_{3,8}$ = 1.47, p = 0.29) without variation over time. Their average FE value was 0.59 ± 0.06.

4 Discussion

4.1 Control phase

According to the results of the first study experiment, it seemed that a first generation of domestication and selection did not improve the capacity of the fish to learn how to use the self-feeder. Indeed, at the beginning of this experiment, fish were naive about using the self-feeder; the *Wild* groups then learned how to use the device in only 2 days, in contrast to the *Selected 1* fish, which only began to activate it correctly after 14 days. This early period was thus one of food deprivation and was characterised by a slight loss of fish body mass, a negative growth rate, and a decrease in K factor for this population. The second experiment did not show any difference in learning ability between the *Domesticated* and *Selected 2* fish. Indeed, despite the fact that fish were also naïve when faced with the self-feeder, both groups of fish started to use the device after only 2 days. Thus, it seemed that the selection process did not influence fish learning ability. Nevertheless, the comparison between the two experiments showed that a second generation of domestication might improve fish adaptation toward self-feeder triggering for the selected fish.

During the second part of the control period, the *Selected* 1 group, which presented the lowest SGR at the beginning of Exp. 1, showed a high increase of its growth performance. The fish growth increase was mainly attributable to an increase in feed intake (+124%) and to an increase in feed efficiency, which corresponded to the growth compensatory phenomenon as defined by Jobling (1994). However, the feed efficiency was the same for the *Wild* and *Selected 1* strains. The same result was observed for the *Selected 2* fish in Exp. 2 (+53% of feed intake) with the same feed efficiency between domesticated and selected strains. Thus, the better growth performance of *Selected* 1 and 2 fish could be explained by their cumulative feed intake, and as observed by Mambrini et al. (2004) on *Salmo trutta* L., feed efficiency was not affected by the selection processes. The level of fish domestication also did not seem to influence the compensatory growth capacities of sea bass.

The rhythm of feeding activity indicated that sea bass did not feed continuously during the day. They displayed a diurnal feeding behavior, with an important peak of feed demands at dawn (06:00) and at 13:00, for fish issued from the second generation of domestication and selection. This result was in accordance with the observation of Mambrini et al. (2004) on *S. trutta*, which showed that feeding rhythm was affected significantly by the strain, the peak of feeding being more pronounced with fish domestication and selection level.

4.2 Post-stress phase

Fish handling, associated with capture and confinement, is generally considered to alter behavior (Pickering et al. 1982; Mesa 1994; Olla et al. 1995). The most common change in fish is a reduction of the feeding activity during the stress period (Pickering et al. 1991; Farbridge and Leatherland 1992; Pankhurst and Van der Kraak 1997) associated with a reduction in growth rate (Pickering and Stewart 1984; McCormick et al. 1998; Liebert and Schreck 2006), and a probable increase in energy demand, and thus metabolic rate, as shown by Barton and Schreck (1987), Wendelaar Bonga (1997) and Pankhurst and Van Der Kraak (1997). In our studies, none of the sea bass strains presented such behavioral patterns after acute stress treatments, but instead showed different reactions.

In Exp. 1, the *Wild* fish appeared less negatively affected by the stressor (showing an increase in feed demand and SGR) than the *Selected 1* fish, which were characterized by an increase in SGR, but a stable feed intake. According to McCarthy and Siegel (1983), such a phenomenon could result from an increasing amount of energy allocated to growth versus maintenance cost, and thus could be interpreted as rapid stress adaptation. The second stress treatment seemed to have no effect on the *Wild* fish. Indeed, these fish ate the same amount of food and grew at the same rate as during the preceding period. In contrast, the *Selected 1* fish showed a decrease in SGR, and a stable feed intake during this period. According to these results, it seems that a first generation of domestication and selection for growth do not improve the fish stress adaptability.

In Exp. 2, the *Domesticated* fish also seemed affected by the first stressor, but capable of rapid adaptation. Indeed, they showed a decrease in feed intake and a stable SGR. As for the *Selected 1* fish, such phenomena could result from an increasing amount of energy allocated to growth versus maintenance cost. The *Selected 2* fish seemed more affected by the first stress treatment. This population showed a high SGR decrease

(–24%), and the same feed intake, but a significant increase of wasted food, which was already identified as an indicator of stress level by Millot et al. (2008). After the second stress treatment, the *Domesticated* group showed the same feed intake and SGR as observed before the stress treatments. These results could be interpreted as a total stress recovery. The *Selected 2* fish presented some recovery indicators such as a decrease of food wastage, but still kept a constant SGR. Also, for this experiment, selection for growth process did not seem to improve fish stress adaptability. The comparison of the results of these two studies showed that the domestication process did not seem to influence fish stress response.

The post-stress treatment period was also characterized by feeding rhythm changes. Indeed, in the first experiment, the *Wild* fish group presented an increasingly diurnal feeding pattern over time, reaching 94% of their feed demand during the day period at the end of the experiment, and the *Selected 1* fish showed a feeding activity progressively restricted to the 11:00–13:00 period. This preference for an increasingly diurnal feeding over time was also observed for these two populations when they were exposed to frequent, repeated, acute stress (Millot et al. 2010). In the second experiment, the *Selected 2* fish concentrated their feeding activity on two peaks: at 06:00 and at 13:00. The *Domesticated* fish presented a particularly interesting feeding rhythm change. Before the stress treatment, this population was characterised by a feeding peak at 06:00, but after the first acute stress treatment this feeding peak completely disappeared and resumed after the second stress treatment for the *Domesticated* fish, indicating a full recovery.

In conclusion, and according to the results of these studies, application of two acute stress treatments at three-week intervals, modified fish feeding behavior and growth performance. These strain comparisons also suggest that the domestication process promotes fish environmental adaptation (i.e. self-feeder use) and that fish issued from selection for growth processes seemed to have different adaptation abilities, which despite post-stress behavioral modifications ultimately led to better growth. Considering the economic importance of fast fish growth in the aquaculture industry, it seems that the domestication and selection processes could be even more profitable if meal timing and quantity could be flexible, and thus respect fish needs in relation to environmental constraints; it would, hence, potentially enhance fish welfare under culture conditions. An additional option would be to use stress tolerance criteria as selection objectives. Nevertheless, to improve our understanding the effects of domestication and selection processes, we recommend that further experiments should be carried out on fish issued from subsequent generations of these lines.

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