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Evaluation of behavioral changes induced by a first step of domestication or selection for growth in the European sea bass (*Dicentrarchus labrax*): A self-feeding approach under repeated acute stress

S. Millot[a, *], S. Péan[a], D. Leguay[a], A. Vergnet[b], B. Chatain[b] and M.-L. Bégout[a]

[a] Ifremer, Place Gaby Coll, BP 5, 17137 L'Houmeau, France
[b] Station expérimentale d'aquaculture, Ifremer, chemin de Maguelone, 34250 Palavas-les-Flots, France

*: Corresponding author : S. Millot, Tel.: +33 5 46 509 440; fax: +33 5 46 500 600, email address : sandiemillot@yahoo.fr

Abstract:

Among other strategies to improve fish welfare in rearing environment, domestication and/or selective breeding was proposed to minimize fish responsiveness to husbandry practices. To verify this hypothesis on a recently domesticated specie, the sea bass, *Dicentrarchus labrax*, L., an experiment was realized, using four populations differing according to their level of domestication or selection: one population produced from wild parents (*Wild*), one population produced from parents domesticated for one generation (*Domesticated*) and two produced from parents selected for growth for one generation (*Selected A* and *Selected B*). The experiment was carried out over 91 days with 600 fish (50 fish per tank, 150 fish per population). After a control period, the fish were submitted from day 35 and during 56 days to a stress treatment including frequent and random application of 4 acute stressors (pursuing fish with a net during 1 min, switching off the light for 2 s during the day or, conversely, switching on the light for 2 s during the night, and overflying a bird predator silhouette above the tank during 30 s). The two variables that were measured, *i.e.*: fish self-feeding behavior and growth performance [at days (D) 14, 35, 63, and 91] were both altered, albeit differentially according to populations, by the stress treatment. During the first stress period (from D35 to D63), all groups modified their feeding rhythm and highly increased their feed intake while their growth rate decreased (*Domesticated* and both *Selected* fish groups) or remained stable (*Wild*). During the second stress period (from D64 to D91) fish continued to modify their feeding rhythm (being more and more diurnal) and increased again their feed intake; conversely to what happened during the first stress period, here, these modifications were associated with an improvement of the growth rate of all populations. During the whole experiment, both *Selected* groups and *Domesticated* fish were always characterized by a higher body mass, specific growth rate and body condition factor than *Wild* fish. In conclusion, and according to the results of this study, a first generation of domestication or selection improved fish growth performance but, at this early stage do not modify behavioral responses to repeated acute stress exposure.

Keywords: Feed intake; Feeding rhythm; Specific growth rate; Adaptation capacities; 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 used to improve traits strongly associated to production cost (*e.g.* growth rate, disease resistance, age at maturity, flesh quality), but very little is known on selected fish capacities to tolerate stress *per se*. It was nevertheless shown that fish responsiveness to stress has a genetic component that could be, therefore, modified by selective breeding (Pottinger and Pickering, 1997). Indeed, (Pottinger and Pickering, 1997) and (Pottinger and Carrick, 1999) have shown that it was possible to select rainbow trout (*Oncoryhnchus mykiss*, Walbaum) strains presenting a high or low cortisol response to confinement stress. These strains have also shown other clear behavioral and physiological differences such as a quicker resumption of feeding, when placed in a novel environment, for the low cortisol responding strain ([Overli et al., 2004] and [Overli et al., 2002]), and a lower brain serotonin concentration (Overli et al., 2005). According to these results, it seems feasible to generate strains displaying a high stress tolerance, and thus, improved performances in aquaculture,

across a number of traits (***e.g.*** improvement of feed conversion efficiency, growth, fecundity, egg quality, post-slaughter flesh quality and also reduction in the incidence of disease), and in addition an improvement of their welfare (Pottinger & Pickering, 1997).

The sea bass (***Dicentrarchus labrax***, L.) is an important species in Mediterranean and Atlantic aquaculture that was recently domesticated. Therefore, very little is known on effects of the very early step of domestication or selection for growth apart from classical traits of commercial interest (Dupont-Nivet et al, 2008; Vandeputte et al., 2009) and specially nothing is know, on behavioral responses to stress exposure and welfare potential. Though, stress is an unavoidable component of finfish aquaculture environment (Pottinger & Pickering 1997), and is also largely associated to fish welfare, which is an important issue for the industry, not just for public perception, marketing and production acceptance, but also often in terms of production efficiency, quality and quantity (Broom, 1998; Southgate & Wall, 2001; Huntingford et al., 2006). Therefore, even if stress responses do not highlight all welfare disturbances, it is generally admitted that they strongly indicate a poor welfare (Broom, 1988; Huntingford et al., 2006). Such evidences led to an active research on potential methods to reduce stress responses in aquaculture species (Ashley, 2007). Among them, domestication and selective breeding to minimize fish responsiveness to stressors, was a major axis of research of the last few years (Pottinger, 2003).

The present study thus proposes to evaluate the early effect (one generation) 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 wasted) by repeated acute stress exposure (stress tolerance used as a

100 screening procedure). Growth performance (body mass, body condition factor, specific
101 growth rate) was recorded as complementary traits.
102
103 **2. Material and methods**
104
105 ***2.1. Experimental set up***
106
107 The four populations from where the fish tested in this experiment were sampled, were
108 produce to evaluate the response to selection for growth in the frame of a genetic EU project
109 (Competus COOP-CT-2005-017633) and the details of rearing conditions and sizes of these
110 populations can be found in Vandeputte et al., 2009. In summary, the four tested populations
111 have been hatched and reared at the experimental research station of Ifremer in Palavas-les-
112 Flots (France). Until the start of the experiment, fish were reared according to sea bass rearing
113 standards (Chatain, 1994). They were produced from a full factorial crossing (each female
114 was crossed with each male) of 13 wild Mediterranean females with (i) 20 Atlantic wild
115 males (***Wild*** group; which will represent here the "control" strain of the experimental design)
116 (ii) 20 Atlantic domesticated males (***Domesticated*** group), (iii) 19 and (iv) 17 Atlantic males
117 selected for growth according to different procedures (***Selected A*** and ***B*** groups). The ***Wild***
118 parental males were chosen among an Atlantic wild population kept in captivity for one to
119 three years. The domesticated and the ***Selected A*** males have been obtained by choosing fish
120 in a population reared for two years (one generation) according to sea bass rearing standards
121 (Chatain, 1994): the domesticated ones were chosen at random while the selected ones were
122 the 5% longest fish at the same age (20 months, 400 g). The ***Selected B*** males were also the
123 5% longest fish of this population but in a group that had undergone the PROSPER selective
124 procedure (Chevassus et al., 2004): fish graded at the age of 200, 444 and 685 days to be

reared in homogeneous body mass class. Thus, all fish tested in this experiment never experienced the natural environment, had the same life history, and only differed by their male parent presenting different levels of domestication or selection:

- wild sires captive for at least one year (***Wild*** group)
- sires, descendant of the previous wild parents that has completed an entire cycle of rearing (***i.e.*** first generation of domestication), and were chosen at random (***Domesticated*** group) or among the 5% longest (***i.e.*** first generation of domestication and selection; ***Selected A*** and ***B*** groups).

The present experiment was carried out with a triplicate per strain from 14/03/07 to 12/06/07. The 12 tanks (1m^3 each) were supplied with semi-recirculated seawater; all tanks were in the same room. For each tank, the flow rate was 4 m^3 h^{-1} and the water renewal 30 % per day. Water temperature was maintained at 20.3 ± 1.1°C, oxygenation above 90 % of saturation in the water-outlet, and salinity was 36.3 ± 1.5. Water ammonia and nitrite compounds were measured every day and were never above recommended levels for sea bass. Tanks were lit by a neon lamps hanged 1.5 m above the water surface. Light regime was 16:8 LD (light onset at 06:00) with twilight transition periods of 30 min. Fish were fed a commercial diet for sea bass (Neo Grower Extra Marin 5.0, France) containing 45 % of crude protein and 20 % of lipid according to the manufacturer. The experiment was realized over 91 days with 600 fish (50 fish per tank, 150 fish per strain). One tank of ***Selected B*** fish has never learned to use the self-feeder and was therefore removed from our analysis.

At the beginning of the study, fish were 24 months-old and four groups were randomly sampled from the larger populations. ***Wild*** group weighted an average of 468 ± 7 g (coefficient of variation (CV) = 17%, n = 150 fish), ***Domesticated*** group an average of

443 ± 6 g (CV = 18%, n = 150 fish), ***Selected A*** group an average of 530 ± 8 g (CV = 19%, n = 150 fish) and ***Selected B*** one an average of 523 ± 10 g (CV = 20%, n = 100 fish)**.** Fish were again weighted (to the nearest mg and measured for length to the nearest mm) 14 (D14), 35 (D35), 63 (D63) and 91 (D91) days after the beginning of the experiment. Experimental periods were defined as the period between two measuring day: P1 from D1 to D14; P2 from D15 to D35; P3 from D36 to D63 and P4 from D64 to D91. All measuring days were done under anesthesia using clove oil (0.08 ‰).

The feeder device comprised a screened type sensor (a metal rod protected by a PVC cylinder; Covès et al., 2006; Millot et al., 2008) and a control box. After each actuation, fish were rewarded with 25 pellets, feed dispensers thus achieving a mean distribution of 0.1 to 0.08 g kg^{-1} fish at the beginning and at the end of the experiment respectively. Such a set up allowed monitoring the number, the date and the hour of feed demand in each tank.

Each fish was implanted with a PIT-tag to follow individual body mass and length over time. Fish were placed under self-feeding conditions at D1 and food access was possible during the whole day along (24 h) even during waste counts from 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 done continuously for 77 days except 24 hrs before and during fish handling (8 days off in total).

2.2. Stress treatment

After a first phase of rearing (P1 + P2), which represented the control phase of the experiment, stress events screening procedures were applied; P3 + P4 therefore represented the phase of stress treatment. P1 + P2 was used to compare before ***versus*** after stress treatment for all strains. Such an experimental design was chosen because all tanks were in

the same room and same water circuit, and disturbances to one tank were unavoidably transmitted to adjacent tanks. The stress treatment screening consisted in: pursuing fish with a net during 1 min, switching off the light for 2s during the day or, in contrary, switching on the light for 2 s during the night, and overflying a bird predator silhouette above the tank during 30 s. To prevent any fish habituation, each stressor was applied randomly over time, fish being not disturbed at all during some days, or, on the contrary, submitted to one, two or three stress per day (with the same or with different stressors; Table 1).

2.3. Statistics

To account for fish growth in between periods, all feeding related variable were relative to fish biomass.

The variables chosen to measure the different performances were the following:

- The amounts and the coefficient of variation of feed demanded (FD), intaken (FI) and wasted (FW) (g per kg of biomass present in the tank and per day). These variables were used to evaluate feeding behavior changes.
- 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) allowed to appreciate growth pattern modifications and to hypothesize changes in fish metabolic rate using feed intake as a proxy.
- The amounts of feed demands per hour (g per kg of fish biomass) was chosen to follow the group feed demand rhythm and changes over time.

- The specific growth rate was calculated as: SGR (% body mass per day) =100 (Ln M_f – Ln M_i) x t^{-1}, where M_f and M_i are the final and the initial body mass (g) respectively, and t the total number of days.
- The body condition factor was calculated as: K (g cm^{-3}) = 100 x M x L^{-3} where M is mass (g) and L the standard body length (cm).
- The coefficient of variation was calculated as: CV (%) = 100 x SD x X^{-1} where SD is standard deviation and X is average.
- The feed efficiency (FE) was calculated from biomass and feed consumption: FE = (final biomass (kg) –initial biomass (kg)) x (feed intake (kg))$^{-1}$.

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

Data were checked for normality with Shapiro-Wilk test and for homogeneity of variances with the Bartlett's test; they all complied for parametric tests to be used. For fish body mass, body condition factor and specific growth rate variables, a repeated ANOVA was used to analyze the average differences between populations (fixed factor), periods (fixed factor), and tanks (random factor nested to population). The different periods considered here were: during the control phases; P1 and P2, and during the stress phases; P3 and P4. For the variables related to feeding behavior, P1 was not included on the statistical analysis because for each population, feed demand activity only began 14 days after the study started. Therefore, for the amount of feed demanded, wasted or intaken, the same type of ANOVA described above, was used but the periods considered here were only P2, P3 and P4. For the feed demand rhythm, a repeated ANOVA was used to compare the differences between populations (fixed factor), periods (fixed factor), hour (fixed factor) and tanks (random factor nested to population). The number of data for this variable corresponded to the number of recorded feeding day (68) x 24 hours x number of tank (11). Homogeneous groups were

225 determined with ***a posteriori*** Newman and Keuls test (Dagnélie, 1975). For all tests,
226 significant threshold was $p< 0.05$, and analyses were performed using the Statistica software
227 (Statsoft, USA).
228
229 **3. Results**
230
231 During the experiment, some fish died for different reasons ***i.e.*** some jumped out of the tank
232 or for unidentified causes, however, no mortality could be allocated to stress or anesthesia: it
233 concerned 1 ***Wild*** fish during P1, 1 ***Wild*** and 1 ***Domesticated*** fish during P3; 2 ***Domesticated***
234 and 2 ***Selected A*** fish during P4. These changes in the number of individuals were taken into
235 account in all measured variables.
236
237 ***3.1. Amount of feed demanded, intaken and wasted over time***
238
239 ***Wild*** fish systematically demanded ($F_{3,703} = 9.9$, $p< 0.001$) and ate ($F_{3,703} = 9.7$,
240 $p< 0.001$) less than ***Selected A*** and ***B*** or ***Domesticated*** ones (Fig.1). During P2, ***Wild***
241 demanded, and entirely ate, an average of 2.66 ± 0.39 g kg^{-1} day^{-1} while the three other groups
242 demanded in average 4.17 ± 0.24 g kg^{-1} day^{-1}, ate 4.15 ± 0.24 g kg^{-1} day^{-1} and wasted $0.02 \pm$
243 0.01 g kg^{-1} day^{-1}. During P3, demanded ($F_{3,703} = 27.8$, $p< 0.001$) and intaken ($F_{3,703} = 28.1$,
244 $p< 0.001$) food increased significantly for all groups. FD and FI being 3.97 ± 0.41 g kg^{-1} day^{-1}
245 (no waste) for ***Wild*** and FD = 5.30 ± 0.24 g kg^{-1} day^{-1} and FI = 5.25 ± 0.24 g kg^{-1} day^{-1} for the
246 other groups. During P3, FW did not change for ***Selected B*** and ***Domesticated*** groups ($0.02 \pm$
247 0.01 g kg^{-1} day^{-1}) while it increased by 3 fold for ***Selected A*** (0.09 ± 0.04 g kg^{-1} day^{-1}, which
248 represented about 2% of the demanded amount; $F_{3,703} = 2.2$, $p< 0.05$). During P4, these
249 amounts of FD and FI increased again being 5.06 ± 0.30 g kg^{-1} day^{-1} (no waste) for ***Wild***

250 group, and FD = 5.97 ± 0.14 g kg^{-1} day^{-1} and FI = 5.95 ± 0.14 g kg^{-1} day^{-1} for the others;
251 their FW being 0.01 ± 0.01 g kg^{-1} day^{-1} .
252 Observing the immediate day-to-day stressor effect on feeding behavior was difficult but the
253 CV of feed intake (CV_{FI}) highlighted fish appetite variation over each experimental period.
254 Thus, during P2, CV_{FI} were equal to 89%, 33%, 55% and 53% for ***Wild***, ***Domesticated***,
255 ***Selected A*** and ***B*** respectively. During P3, ***Domesticated*** fish showed a slight CV_{FI} increase
256 (CV= 39%) while the three other populations showed a CV_{FI} decrease (CV= 45%, 35%, 47%
257 for ***Wild***, ***Selected A*** and ***B*** respectively). During P4, a high CV_{FI} decrease was observed for
258 all fish strains: 26% for ***Wild***, 19% for ***Domesticated*** and 23% for ***Selected A*** and ***B***.
259
260 ***3.2. Variations over time of fish growth and feed efficiency***
261
262 At the beginning of the study, selected (***A*** and ***B***) and non selected (***Domesticated*** and
263 ***Wild***) fish presented a difference of 14% in body mass. ***Selected*** fish, nevertheless issued from
264 a single generation of selection for growth were characterized by a growth improvement of
265 20%, which is generally obtained in two generations of selection in most breeding programs
266 dealing with fish (Vandeputte et al., 2009). This difference between selected and non selected
267 fish was maintained more or less during the whole experiment; except at D91 where the
268 difference of body mass was 13% with ***Domesticated*** and 19% with ***Wild*** ($F_{12,2718}$ = 3.3, p<
269 0.001; Fig.2 A). In general, fish lost body mass during P1 (-3% for ***Domesticated*** and -7% for
270 the other groups). Then, during P2 and P3 fish body mass slightly increased (around +3% for
271 ***Wild*** strain and +6% for the other strains). During P4, fish body mass increased rapidly
272 reaching a rate of +9% for ***Wild*** group and +12% for the others.
273 Whatever fish strain, gonads weighted an average of 0.23 ± 0.02 g for males (0.04 %of BW) and 3.56
274 ± 0.17 g for females (0.59 % of BW). These results highlighted that tested fish were not sexually
275 mature.

276 Fish specific growth rate during P1 was negative for all groups, ***Selected*** (***A*** and ***B***) and ***Wild***
277 populations being more affected than the ***Domesticated*** population (-0.19 ± 0.01 and
278 -0.08 ± 0.01 % day^{-1} respectively; $F_{9,2172}$ = 11.9, p< 0.001; Fig.2 B). During P2, all
279 populations showed a high SGR increase, the ***Wild*** group being the less performing. This
280 difference was maintained more or less during the whole experiment. During P3 the SGR of
281 ***Selected*** and ***Domesticated*** groups decreased significantly (around -25%) while ***Wild*** SGR did
282 not really change (-5%). Finally, during P4, the SGR of all strains highly increased, especially
283 in the ***Wild*** group (3 fold higher than during P3).
284 At D1, the body condition factor (K) of ***Selected A*** group was higher than in other populations
285 ($F_{12,2718}$ = 4.9, p< 0.001; Fig.2 C). During P1, the K factor highly decreased in all populations
286 and at D14 ***Domesticated*** and ***Selected A*** were characterized by a higher body condition factor
287 than those of ***Selected B***. During P2, only the ***Selected B*** group showed a significant body
288 condition factor increase (+3%). During P3, the K factor was stable in all populations. Finally,
289 during P4, the K factor increased significantly for all groups except for ***Wild*** fish.
290 All populations had similar feed efficiency (FE) during the whole experiment ($F_{6,21}$ = 0.5,
291 p> 0.05). However, even if the FE changes over time were not significant, the values varied
292 from 0.63± 0.11 during P2 to 0.35 ± 0.14 during P3 and returned to 0.60 ± 0.05 during P4.
293
294 ***3.3. The daily rhythm of feeding activity***
295
296 As a general feature, all groups realized more feed demands during the night than
297 during the day period (Fig.3). However, some differences appeared between groups over time
298 ($F_{138,17664}$= 3.5, p< 0.001). According to the stress treatment timetable (Table 1), the fish
299 feeding rhythm change did not correspond to the time where stressors were performed.
300 Indeed, no real difference appeared at 01:00, 04:00, 10:00 and 14:00. The changes seemed

more correlated to dawn (06:00) and dusk (22:00) and more visible when the data were analyzed by period. Thus, during P2, fish realized 53% (***Wild***), 56% (***Selected B***), 77% (***Selected A***) to 94% (***Domesticated***) of their feed demands during the night period with a peak at 22:00. During P3, the percentage of feed demands during the night period decreased but the majority was still nocturnal for all groups (51% for ***Wild***, 54% for ***Selected B***, 69% for ***Selected A*** to 79% for ***Domesticated***) with again a peak at 22:00. However, all populations increased their feed demands activity at 06:00 (3 fold more for ***Selected B*** and ***Wild***; 4 fold more for ***Selected*** and 20 fold more for ***Domesticated***). During P4, the feed demands during the night period decreased again and especially for ***Selected*** fish which were characterized at this moment by a diurnal feeding (69% for ***Selected B*** and 59% for ***Selected A***). ***Domesticated*** fish increased also their diurnal feed demands (+46% at 06:00) but continued to realize 75% of their feed demands during the night period. ***Wild*** fish, on the contrary, showed an increase of their nocturnal feed demands (+17%) and a decrease of their feed demands at 06:00 (-11%).

4. Discussion

At the beginning of the experiment fish were naive facing the self-feeder and whatever the group they really began to correctly activate it after 14 days. This period was thus synonym of food deprivation and as a consequence, characterized by a loss of fish body mass, a negative growth rate and a decrease of K factor for all populations. The loss of body mass during this period was comparable between ***Selected A, B*** and ***Wild*** groups indicating an analogous metabolic utilization that was higher than that of the ***Domestic*** group. During the second part of the control period, all groups showed an increase of their growth performance especially noticeable in ***Selected*** and ***Domesticated*** fish. As for brown trout (***Salmo trutta***, L.; Mambrini

et al., 2004), sea bass were able to display compensatory growth after a long period of food deprivation. In the different salmonid species studied so far, this growth compensation is realized by an increase of feed intake (Bull and Metcalfe, 1997; Bull et al., 1996; Metcalfe and Thorpe, 1992), feed efficiency (Boujard et al., 2000; Dobson and Holmes, 1984; Kindschi, 1988; Quinton and Blake, 1990) or both (Miglavs and Jobling, 1989). In our study, the growth increase was mainly attributable to an increase in feed intake (during this period, ***Selected*** and ***Domesticated*** fish ate 57% more food than ***Wild*** fish), with no effect on feed efficiency. It can therefore be put forward that, as observed by Mambrini et al. (2004) on brown trout, feed efficiency in sea bass is not affected by a first generation of domestication or selection for growth processes.

The rhythm of feeding activity confirms that sea bass do not feed continuously during the day (Sánchez-Vázquez et al., 1995). They displayed a nocturnal feeding behavior with an important peak of feed demands at dusk (22:00) especially for ***Selected*** and ***Domesticated*** fish. This result was in accordance with the observation of Mambrini et al. (2004) on brown trout, showing that feeding rhythm was affected significantly by the line, the peak of feeding being more pronounced for ***Selected*** fish than for control ones. Repeated intermittent acute stressors are generally admitted to alter behavior (Pickering & Pottinger, 1989; Pankhurst & Van der Kraak, 1997), the most common change in fish being a reduction of the feeding activity during the stress period (Pickering et al., 1991; Farbridge & Leatherland, 1992, Pankhurst & Van der Kraak, 1997) associated with a growth rate reduction (Pickering & Stewart, 1984; McCormick et al., 1998; Liebert & Schreck, 2006). However, in our study, none of the sea bass groups exposed to a repeated stress treatment screening presented a reduction in feeding activity but, on the contrary, a significant increase of feed demand and intake during the first stress treatment period (+49% for ***Wild*** and +30% for ***Selected*** and

351 ***Domesticated***) leading for ***Selected A*** fish to a wastage that was already suggested as an
352 indicator of stress level by Millot et al. (2008). This period was also characterized by a high
353 feed intake CV, which seemed to indicate an important perturbation of fish feeding behavior.
354
355 During the second period of stress, all fish groups showed again an increased of feed intake
356 (+28% for ***Wild*** and +12% for ***Selected A*** and ***B*** or ***Domesticated***), of SGR, of body mass and
357 of body condition factor (except for ***Wild*** fish) and a high decrease of feed intake CV. During
358 this period, food wastage for the ***Selected A*** fish returned to the level observed before any
359 stressor application. Moreover, at the same time, the feed efficiency of all populations reached
360 again the level observed before the stress period (0.60). All these observations could be
361 explained by fish adaptation to stress treatment challenge according to two processes: 1)
362 habituation, which is characterized by a progressive decrease of the animal response to an
363 unreinforced stimulus (stressor) presented repeatedly or continuously (Humphrey, 1933;
364 Thorpe, 1963; Hinde, 1970; Peeke & Petrinovich, 1984), and/or 2) a compensation for a
365 higher metabolic rate caused by stress through an increase of feed intake. This adaptation was
366 also accompanied by a feeding rhythm change, where fish presented a more and more diurnal
367 pattern. This observation was particularly true for ***Selected*** and ***Domesticated*** fish which were
368 also characterized by a higher body mass, SGR and K factor than ***Wild*** fish at the end of the
369 experiment. These results, thereby plead in favor of a modification of the feeding rhythm to
370 adjust meal timing to the metabolic rate variations imposed by stressors in order to improve
371 food utilization and assimilation, as previously showed by Spieler (1977) and Parker (1984)
372 on mammals.
373
374 **5. Conclusion**
375

The results of this study, pointed out that the improvement of growth performance induced by a first generation of domestication or selection for growth in sea bass was mainly due to a higher appetite rather than a better feed efficiency but that, at this early stage, behavioral responses to repeated acute stress were not modified. Finally, to better evaluate the effects of domestication or selection processes, it will be useful to investigate, in future experiments, the effect of additional generations for which the rearing condition pressure would be enhanced. Furthermore, if one goal in the future is to select fish for stress tolerance, it will be necessary to develop dedicated indicators (traits) on which selection pressure could be made.

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Brief Vitae of Authors

S. Millot, PhD in Oceanography from Ifremer and University of La Rochelle, France. Master degree in Biology of Behaviour from the University of Paris XIII, France.

S. Péan, Second-year Ph.D. Student in Oceanography from Ifremer and University of La Rochelle, France. Master degree in Coastal Ecology from the University of La Rochelle, France.

D. Leguay, engineer at Ifremer, with specialities in design, realization and development of scientific tools in the fields of ecotoxicology, physiology and fish welfare.

A. Vergnet, engineer at Ifremer, with specialities in design, realization and development of scientific tools in the fields of in aquaculture of marine fishes.

Béatrice Chatain, PhD, is a senior scientist in aquaculture of marine fishes, with specialities in larval zootechny and genetics. She has coordinated 4 EU research projects in the field of sea bass genetics.

Marie-Laure Bégout, PhD, is a senior scientist studying biological basis of behavioral adaptation and analysing underlying physiological mechanisms. She has participated in several Welfare EU projects.

Table 1: Stress treatment timetable. Netting: pursuing fish with a net during 1 min; light on: switching on the light for 2 s during the night; light off: switching off the light for 2s during the day; bird: overflying a bird predator silhouette above the tank during 30 s.

Experimental day	Hour of the day			
	At 01:00	At 04:00	At 10:00	At 14:00
35	Measuring day			
36				
37	light on			
38	light on			
39	light on	light on		
40				
41	light on			
42		light on	netting	
43		light on		netting
44	light on			light off
45		light on	netting	
46	light on	light on		
47		light on		
48	light on			
49		light on		
50		light on		
51	light on		netting	light off
52		light on		netting
53	light on	light on		
54		light on		
55	light on		bird	netting
56		light on		
57		light on		bird
58	light on		netting	light off
59		light on		
60	light on	light on		
61		light on		
62	light on			bird
63	Measuring day			
64		light on		
65	light on			light off
66		light on		
67	light on	light on		
68		light on		
69	light on		bird	
70		light on		netting
71		light on	netting	
72	light on		netting	light off
73		light on		
74	light on	light on		
75		light on		
76	light on			bird
77		light on	bird	
78		light on		netting
79	light on		netting	light off
80		light on		bird
81	light on	light on		
82		light on		
83	light on		bird	netting
84		light on	netting	
85		light on		netting
86	light on			light off
87		light on		bird
88	light on	light on		
89		light on		
90				
91	Final measuring day			

Figure captions:

Figure 1. Amount of food intake over time

Mean (+ SE) intaken (demanded – wasted) food amounts for 4 strains of sea bass: ***Wild, Domesticated, Selected A*** and ***Selected B***. In white: during a control period (period 2; 21 days), in light grey: during the first period of stress treatment (period 3; 28 days), in dark grey during the second period of stress treatment (period 4; 28 days).

Figure 2. 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, Domesticated, Selected A*** and ***Selected B*** sea bass strains. In white: during a control period (period 2; 21 days), in light grey: during the first period of stress treatment (period 3; 28 days), in dark grey during the second period of stress treatment (period 4; 28 days). Letters indicate significant differences between date for each strain (ANOVA and Newman & Keuls test, $p<0.05$).

Figure 3. Feeding rhythm over time

Pattern of daily mean (± SE) feed demands per hour during a control period (period 2; 21 days; A), during the first period of the stress treatment (period 3; 28 days; B) and during the second period of the stress treatment (period 4; 28 days; C) for ***Wild, Domesticated, Selected A*** and ***Selected B*** sea bass strains. The grey boxes indicate the night period.

Selected B

Amount of intaken food (g kg -1 day -1)

0 2 4 6 8 10 12 14

14 18 22 26 30 34 38 42 46 50 54 58 62 66 70 74 78 82 86 90

Selected A

Amount of intaken food (g kg -1 day -1)

0 2 4 6 8 10 12 14

14 18 22 26 30 34 38 42 46 50 54 58 62 66 70 74 78 82 86 90

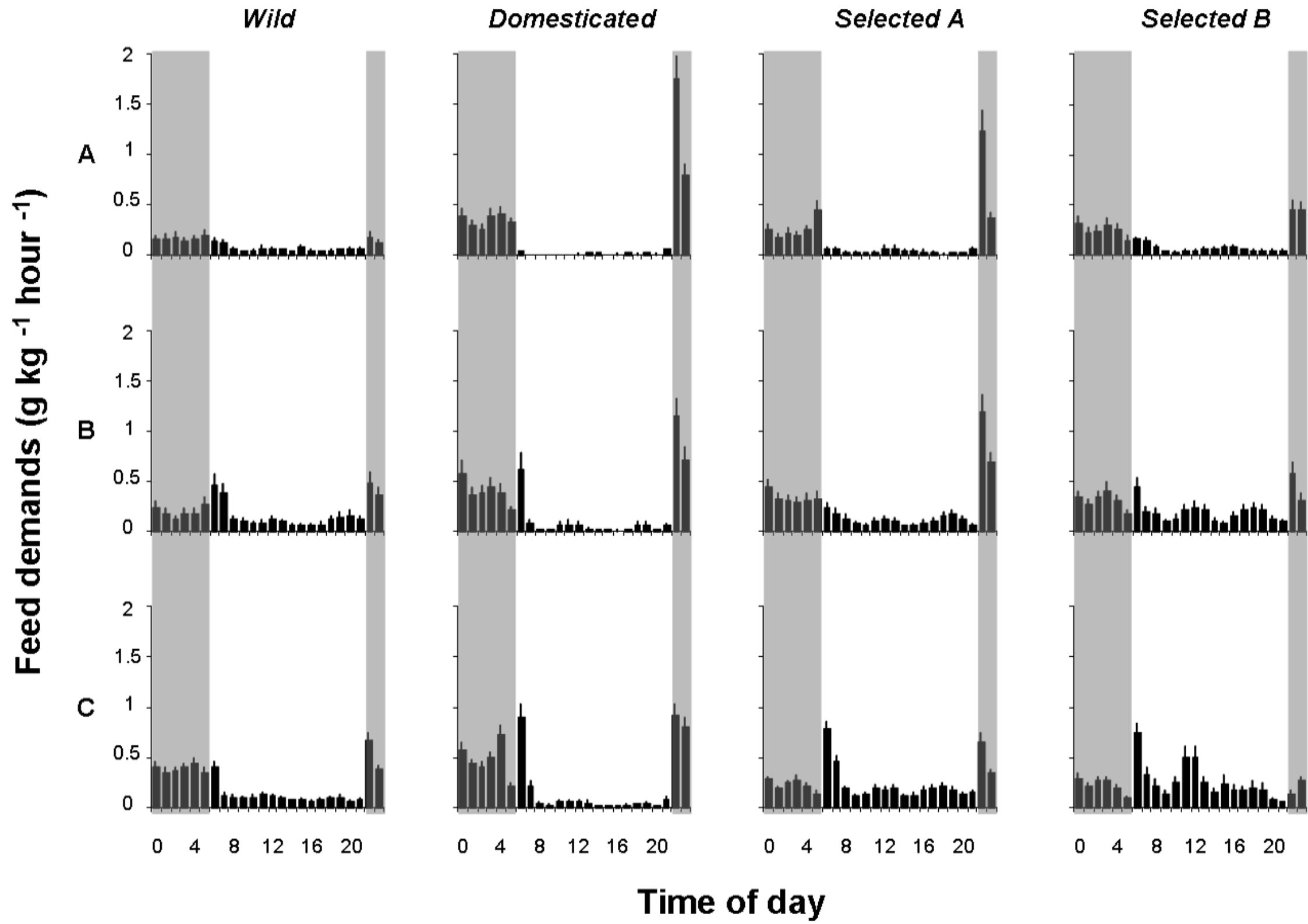
Domesticated

Amount of intaken food (g kg -1 day -1)

0 2 4 6 8 10 12 14

14 18 22 26 30 34 38 42 46 50 54 58 62 66 70 74 78 82 86 90

Body mass (g)
SGR (% day -1)
K (g cm-3)
Selected β
A
B
C


Wild
Domesticated
Selected A
Selected B
A
B
C
Feed demands (g kg -1 hour -1)
0
0.5
1
1.5
2
0 4 8 12 16 20
Time of day