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Early life behavioural differences in wild caught and domesticated sea bass (*Dicentrarchus labrax*)

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Abstract:

Behavioural studies comparing hatchery and wild-caught fish are useful to improve selection for aquaculture and restocking programmes. We examined swimming behaviour characteristics in wild captured and domesticated sea bass juveniles before and after eliciting a startle response at 8 different ages and always on naive individuals. We specifically investigated whether domestication impacts juvenile sea bass behaviour and whether the first months of captivity induce behavioural modifications in wild juveniles. An apparatus was designed to mimic a predator attack by presenting a sudden visual and mechanical stimuli simultaneously in 8 arenas where single individuals were placed and video recorded. The reactivity response was evaluated and different swimming variables including angular velocity, total distance travelled, mean velocity, immobility and distance from stimulus point were analysed from videos taken 5 min before stimulus actuation, 5 and 15 min after. Otolith readings showed that wild and domesticated juveniles were of similar age (~55 days at the start of the experiment and ~125 at the end of experiment). There were consistent behavioural differences (*e.g.* higher angular velocity and distance from stimulus point in wild fish) demonstrating that domestication reduces flight response behaviour. There were also similarities between both fish origins (similar response to stimulus actuation: decrease of total distance travelled and mean velocity, increase of angular velocity and immobility). A decrease over time in reactivity and variability in swimming responses among fish of both origins showed that captivity only does not fully explain wild fish behaviour changes and ontogenic modifications are likely interplaying.

Keywords : Domestication ; Swimming activity ; Restocking ; Selection ; Coping styles

1. Introduction

According to Price (1999) domestication is the process in which a population of animals becomes adapted to man and to the captive environment through genetic changes occurring over generation and environmentally-induced developmental events recurring in each generation. This can lead to phenotypical changes *e.g.* appearance of modified morphological and behavioural characters compared with the ancestral wild forms (Bilio, 2007). Some of these variations have been stabilised because of beneficial interests to humans. For example**,** chickens were selected to be larger, wild cattle (aurochs) to be smaller, and sheep to lose their bristly outer hairs (the kemp) and not to shed their soft inner hairs (the wool) (Diamond, 2002)**.** Most wild animals that yielded valuable domesticates were large terrestrial mammalian herbivores and omnivores and their domestication started 10 500 years ago (Diamond, 2002)**.**

When compared to terrestrial agriculture, aquaculture is still a new industry. Fish domestication is so recent that most fish in culture are still exploited captives but a few are on the threshold of becoming domesticated (Balon, 2004). However it is also the fastest growing animal food-production sector and the number of farmed fish species has increased rapidly during the last few decades, some as food fish, others for stocking in the wild (Balon, 2004). Furthermore, the domestication process includes inadvertent and artificial selections (Price, 1999). Artificial selection is the process of changing the characteristics of animals by artificial means such as directional selection, genomic selection (Hamblin *et al.*, 2011), or familial selection (Theodorou and Couvet, 2003). Artificial selection has substantially contributed to modern agriculture and animal husbandry, though aquaculture has yet to gain much from efficient breeding and selection programmes (Jobling, 2007). Furthermore, domestication may play a role in the distribution of individual characteristics such as behavioral and physiological responses which, if they are consistent over time and characteristic of a certain group of individuals, define a coping style (Koolhaas *et al.*, 1999). For example, genetic selection of pigs that are more adapted to farming conditions may indirectly result in the selection for one type of coping style and a consequent reduction in individual variation (Ruis *et al.*, 1999). Indeed, domestication and selection could rapidly impact fish behaviour, sometimes as soon as at the first generation of domestication

(Vandeputte and Prunet, 2002; Bégout Anras and Lagardère, 2004; Huntingford, 2004) and it is therefore important to check for the distribution of behavioural traits among populations, expecting bimodal distributions when coping styles are defined (Verbeek *et al.*, 1994).

Among behavioural characteristics, several studies have implied that antipredator behaviour is highly sensitive to artificial rearing (Johnsson and Abrahams, 1991; Berejikian, 1995; Dellefors and Johnsson, 1995; Johnsson *et al.*, 1996; Einum and Fleming, 1997; Fernö and Järvi, 1998; Johnsson *et al.*, 2001). The single most important effect of domestication on behavior is reduced emotional reactivity or responsiveness to fear-evoking stimuli (*i.e.* environmental change, Price, 2002). Behavioral measures of reactivity are also sensitive indicators of the complex of biochemical and physiological changes occurring in response to stress (Schreck *et al.*, 1997). In particular, swimming performances in brook trout, *Salvelinus fontinalis* and in Guppy, *Poecilia reticulate* (Beamish, 1978; Walker *et al.*, 2005) were reported to be better in wild stocks of fish *versus* domestic stocks. Changes in swimming behaviour were good indicators of the effects of the domestication process on the stress response (Millot *et al.*, 2009a; Millot *et al.*, 2009b). Standardized stimulation has been used to study the startle response in fish which is an important aspect of the swimming performances for escaping a predator (Wardle, 1993) and particularly the "C-start" response in relation to different environmental constraints: group *versus* solitary response (Domenici and Batty, 1997); pollution (Faucher *et al.*, 2006); water temperature (Johnson *et al.*, 1996) and hypoxia (Lefrançois and Domenici, 2006).

Little is known about the antipredator behaviour of hatchery-reared and wild-caught juveniles of other non-salmon fish species (Malavasi *et al.*, 2004) or on the behavioral response to fear evoking stimuli in the early life stages of fish**.** The European sea bass, *Dicentrarchus labrax* is a major species in Mediterranean aquaculture although little is known about the effects of the early phases of domestication or selection on growth apart from classical traits of commercial interest (Dupont-Nivet *et al.*, 2008; Vandeputte *et al.*, 2009). Attempts have been made to analyse behavioural responses to challenges in fish aged 12 to 24 months (Millot *et al.*, 2010; Millot *et al.*, 2011). Increased understanding of early behavioral swimming responses in sea bass should help determine early indicators that could be used for further domestication and selection programs or for restocking.

The present study aimed at comparing the swimming behavior characteristics of juvenile wild-caught sea bass with domesticated counterparts using an apparatus specifically designed to elicit a standardized and synchronized startle response in several arenas. The comparison between origins was done using always naive individuals over time to address the following questions:

(i) Does domestication have an impact on juvenile sea bass behavior, especially regarding swimming activity before and after applying a visual and mechanical stimulus mimicking some aspects of a predator attack?

(ii) Do the first months of captivity induce behavioral modifications in wild juveniles?

2. Material and methods

2.1. Experimental animals and housing conditions

Domestic sea bass larvae (five generations of domestication) were hatched at a farm in Aquanord SA (France). They were transferred on February 23rd, 2009 to the experimental station of INTECHMER (Cherbourg) when they were 3 days old (D3) and grown in a recirculated system. In total, 150 000 individuals were placed into a 1 m^3 cylindrical tank with conical bottom. All parameters were set according to the protocol used by the Aquanord hatchery. The tank was supplied with water treated by both sand and biological filters (flow rate between 150 and 500 L h^{-1} and 10% water renewal per hour). Light regime was 12:12 LD (light onset at 08:00 U.T. + 1) and intensity was between 0 and 500 Lux. Salinity was maintained at 35 g L^{-1} except during the twenty first days where it was gradually decreased to 25 g L^{-1} and increased again to 35 g L^{-1} to facilitate the swimbladder formation. The oxygenation level was 7.8 ± 0.2 mg L^{-1}, temperature was 15.2 ± 0.53°C. The temperature usually reaches 21°C in a sea bass hatchery but here it was intentionally maintained lower to avoid creating large size differences with the wild stock that was thought to be captured later according to the natural conditions. Larvae were fed Artemia nauplii from D9 to D21 (5 nauplii per ml), a mixture of Artemia nauplii and enriched meta-nauplii (SUPER SELCO®) from D22 to D27 (2.5 nauplii and 2.5 meta-nauplii per ml) and enriched meta-nauplii from D28 to D54 (5 meta-nauplii per ml). Twenty four hours before the arrival of wild fish (D53), 560 individuals were placed in a 20 L container supplied with pure oxygen and transported by car several times in the day to place them under similar conditions to those of their wild counterparts during transport.

Wild sea bass juveniles were captured off the Mediterranean coast of France (Harbour of Cap d'Agde, Southern France, 43° 58' N; 03° 30' 19'' E) by Aquarid, a society specialized in catching fry for restocking and aquariology purposes. A whole school of 560 wild fish observed from the boat was collected at low depth (280 cm). Immediately after capture they were transported in a 20 L container supplied with pure oxygen to the experimental station (INTECHMER, Cherbourg) where they arrived 24 h later, on April 15th (D54).

On D54, both fish groups (wild *vs.* domesticated) were transferred in two separate hatching trays (42.5 x 39.5 x 17.2 cm) placed in a 200 L tank (215 x 42 x 17 cm). Two more trays were placed into this tank to separate tested individuals from naive ones. This tank was connected to the recirculated system described above. Water flowed through the left side and bottom of the trays and exited through the bottom of a 500 µm diameter stitched grid replaced at D90 with a 1mm diameter grid. At this stage, all parameters were maintained stable for the total experiment duration. Temperature was 16.7 ± 0.5°C, salinity, 33.9 ± 1.0 g L^{-1}, Oxygen level, 7.24 ±0.43 mg L^{-1} and flow rate was 200 L h^{-1}. Fish were fed enriched meta-nauplii from D54 to D76 (5 meta-nauplii per ml), enriched meta-nauplii and Marine Start (150-300 µm, Le Gouessant) from D77 to D80, Marine Start (150-300 µm) from D81 to D85, Marine Start (150-300 and 300-500 µm) from D86 to D91, Marine Start (300-500 µm) from D92 to D94, Marine Start (300-500 and 500-800 µm) from D95 to D98, Marine Start (500-800 µm) from D99 to D102 and Marine Start (500-800 and 800-1200 µm) from D103 to D125. The amount of food to be automatically distributed over 12 h was calculated according to feeding tables provided by Le Gouessant.

2.2. Experimental setup

Observations were made in a dedicated room. The apparatus (Figure 1) was composed of 8 circular arenas (diameter 11 cm, height 9 cm) with opaque white walls and a transparent floor filled with 300 ml of water (from the recirculated system) which represented a water level of 5 cm. Arenas were numbered from 1 to 8 and placed on an infrared waterproof casing (1 x 1 m, Noldus, The Netherlands) that enabled recording of videos at low light intensity. The upper and internal part of each arena was composed of a piece of transparent plastic pipe (diameter 1.5 cm, length 5.5 cm) that guided a falling stimulus (a black plastic tube, diameter 0.5 cm, length 15 cm). One extremity of a fishing wire was attached to the end of the tube while the other was fixed to a plastic tablet located 50 cm above the infrared casing. The upper extremity of the wire was composed of a screw nut hanging on an electromagnet. The fall of the tube was then triggered by interrupting the electromagnet. The length of each wire was adjusted for eliciting a standardized and synchronized stimulus in each arena, with the tube coming into contact with the arena bottom. The color of the stimulus as well as the acoustic and shock waves therefore provided a mechanical and visual stimulus to the fish. A camera (Imaging Source DMK 21AUO4) with a frame rate of 60 Hz and a resolution of 640 x 480 pixels was positioned at 42.5 cm above the infrared casing. Two 60 W light bulbs were horizontally placed on walls located on the left and right sides of the infrared casing. They were located 100 cm above the infrared casing and provided an indirect lighting on the arenas. The light intensity measured at the water surface of each arena was 25 Lux.

2.3. Age determination of wild fish

At D138 *i.e.* 13 days after completing all observations, 30 wild and 30 domesticated individuals were randomly sampled from the tested fish. They were weighed and measured before dissection. The age of the domesticated fish was known but they were used as a control in the age-determination method. The fish were sacrificed using a lethal dose of 2-phenoxyethanol. The left and right sagitta otoliths were removed from the cranium, cleaned, encased in resin and mounted with CrystalBondTM glue on microscope slides. They were polished in the sagittal plane to the central primordial. All increment counts were made using the TNPC®5 image analysis software for calcified structure (Ifremer, Noesis) (Fablet and Ogor, 2005). Age was estimated as the mean of the left and right otolith values.

2.4. Experimental protocol

At each observation session, fish were collected after a two-hour feeding period. Then, four wild and four domesticated fish were gently collected from the trays and placed inside 8 opaque one liter beakers which were covered and numbered. Care was taken to visually select juveniles of similar size. They were individually transferred into arenas where order was randomly predetermined. Video capture started after a 15 min acclimatization period. Arenas were filmed for 20 min, the stimuli being actuated at the fifth minute. At the end of the video recording, individuals were anesthetized with 2-phenoxyethanol (0.3 ml L^{-1}) for measuring (total body length, BL to the nearest mm: D55 to D125) and weighing (BW, to the nearest mg, D91 to D125). They were then observed under stereo microscope to check for stomach fullness (0: no food inside stomach; 1: food inside stomach; D59 to D69). Swimbladder presence was also verified on D59 to D91. Finally, each individual was returned to its beaker and when they had recovered, they were placed into separate trays dedicated to tested individuals (one for wild and one for domesticated).

This procedure was repeated 8 times in a day *i.e.* 32 wild and 32 domesticated individuals were observed during a session. In total, 8 sessions were performed at D55, 59, 63, 69, 91, 98, 111, and 125 accounting for a total of 256 individual observations per group.

2.5. Video analyses

The video recordings were analyzed using the software EthoVision XT (Noldus, The Netherlands), which allowed a virtual point to be defined in each arena (position of the stimulus on the bottom of arena) and to track the fish swimming behavior. Six dates were analyzed from D63 to D125. A technical temporary problem with the power supply of the infrared casing did not allow the swimming activity to be analyzed (*i.e.* no tracks extraction but only qualitative observations made) on the two first dates (D55 and D59).

Each video recording was analyzed in three sequences of 5 min:

- sequence 1 (S1): 5 min before the stimulation,
- sequence 2 (S2): 5 min just after the stimulation, and
- sequence 3 (S3): 5 min recording 10 min after the stimulation.

Each video (including the two first dates) was also viewed and analyzed to evaluate whether the reactivity (React) was modified after stimulus actuation: 0: the fish does not display neither escape response nor any swimming change, 1: the fish displays a clear escape response.

The position of the fish just before the end of the fall stimulus was noted (F: fish head oriented toward the stimulus and axis of the fish making an angle between -45 and +45° to the stimulus; S: axis of the fish making an angle between 180 and 225° or between -90 and -135°; B: fish head oriented facing away from the stimulus and axis of the fish making an angle between 225 and 315° to the stimulus; Figure 2a).

2.6. Behavioural variables

For each 5 min sequence, different variables of interest were chosen to characterize fish behavior:

- Distance moved: the distance travelled by the centre point of the subject between two consecutive X-Y coordinates acquired (Dtot in mm);
- Mean Velocity: the distance moved by the centre point of the individual fish per unit time between two consecutive X-Y coordinates acquired expressed in body lengths per second (Vel in BL s^{-1});
- Time immobile: the total duration the fish displayed no movement (Im in s);
- The fish absolute angular velocity expressed in degrees per second (Vang in ° s^{-1}) was calculated by the software as followed:

  $Vang_n = RTA_n / t_n - t_{n-1}$ where RTA_n is the relative turn angle for sample n, and $t_n - t_{n-1}$ is the time difference between the current and previous sample. Here, the rate of change in direction is unsigned. The turn angle is calculated as the difference between two subsequent values for heading direction. This variable was an indicator of the amount of turning per unit time and quantified the swimming path complexity.
- The mean distance of the fish from the stimulus point (Dstim in mm),

For each challenge, the fish reaction to the stimulus (React) was reported as a binary occurrence (0: no response, 1: response),

To assess for individual variability between wild and domesticated fish and between the first and the last date of the experiment (D63 and D125), two indexes based on previous behavioral variables were calculated for each individual as follows:

(1) Reactivity index (RI):

$$RI = \frac{Xi(S1) - Xi(S2)}{Xi(S1)} - \frac{1}{32}\sum_{1}^{32} Xi(S1) - Xi(S2)$$

(2) Recovery index (RcI)

$$RcI = \frac{Xi(S3) - Xi(S2)}{Xi(S3)} - \frac{1}{32}\sum_{1}^{32} Xi(S3) - Xi(S2)$$

RI and RcI were calculated for each behavioural variable expressed as Xi. Xi(S1), Xi(S2), and Xi(S3) representing Xi values during sequences 1, 2, or 3; N = 32 referred to the number of individuals observed during a session per origin.

2.7. Statistical analysis

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

Logarithmic regressions of wild fish size *vs.* age, and body weight *vs.* age were calculated to estimate wild fish age at each observation session. The correlation between estimated domesticated fish age *vs.* weight or size was also examined. Wild and domesticated fish ages were compared using a Mann-Whitney test.

A Kruskal-wallis test was used to compare the reaction to the stimulus (React) with Origin (Wild *vs.* Domesticated fish), Date (8 ages), and Fish position just before the end of the fall stimulus (F, S and B) as independent variables.

All variables related to swimming activity were compared using ANOVA with Sequence (S1, S2 and S3) as the within-subjects factor, Origin (Wild *vs.* Domesticated fish) and Date (6 ages) as between-subjects factors. For the variable Dstim, the Sequence factor was reduced to 2 levels (S2 and S3).

RI and RcI were compared inside each Origin (wild or domesticated fish) using a Kruskal-wallis test with Date (Day 63 and Day 125) as an independent variable. They were also compared between fish origins at each date (Day 63 or Day 125) using the same test with Origin (wild or domesticated) as an independent variable.

3. Results

3.1. Wild fish age

The estimated age of wild fish at D139 (age of domesticated fish when sacrificed) was (Mean ± SD) 167.3 ± 22.9 days. Estimation of fish age based on domesticated fish otoliths was 133.2 ± 7.5 days. Both relationships between wild fish age and body length ($Y = 155.5\ \mathrm{Ln}(x) - 422.3$, $R^2 = 0.76$), age and body weight ($Y = 49.9\ \mathrm{Ln}(x) + 169.8$, $R^2 = 0.75$) were highly significant ($P < 0.001$). These data allowed estimation of the age of wild fish at each observation session (Table 1). There were no correlations between domesticated fish age and size ($R^2 = 0.01$, $P = 0.89$) or between domesticated fish age and weight ($R^2 = 0.001$, $P=0.97$). In conclusion, there was no significant difference between the ages of wild and domesticated fish used in the experiments ($U=16.5$, $P=0.81$).

3.2. Stomach fullness and swimbladder rates

Swimbladders were observed in all fish observed from D55 to D91. At D55, artemias were identified in 87.5% of domesticated fish stomachs, but were only present in 50% of wild fish stomachs at this time. At D59, 63, and 69, these percentages had increased (Mean ± SD) to 96.9 ± 5.4% in domesticated fish, and 93.8 ± 0.0% in wild fish.

3.3. Reactivity to the stimulus

During the first five assessment dates, the percentages of reactivity were between 75 and 90% in domesticated fish and between 62 and 94% in wild fish (Figure 2b). During the last three assessment dates, the percentages decreased in both fish groups: they were between 44 and 59% in domesticated fish and between 44 and 65% in wild fish. There was no significant difference between domesticated and wild fish ($H_{1,\ 480} = 1.9$, $P=0.17$), but there was significant effect of date ($H_{7,480} = 36.4$, $P<0.001$) with reactivity being significantly higher at D55 and D59 than at D111 ($P < 0.01$).

Fish positions before the end of the fall stimulus were similar in both groups regardless of whether there was a subsequent reaction. Fish were oriented in front of the stimulus 30-35% of the time, behind it 40-45% of the time, and on the side 15-20% of the time (Figure 2c and d). The fish position before the end of the fall stimulus was not linked to the subsequent escape response *i.e.*, the initial orientation of the fish did not significantly differ ($H_{2,469} = 1.3$, $P=0.53$).

3.4. Swimming activity

There was a significant interaction between Date and Origin for the angular velocity (Vang) (Table 2). On the first four assessment dates, wild fish performed higher Vang than domesticated fish; the values (Mean ± SE) during S1, S2, and S3 were respectively 1118 ± 117, 1539 ± 134, 1454 ± 135° s^{-1} for domesticated fish and 1308 ± 133, 1629 ± 139, 1751 ± 143° s^{-1} for wild fish (Figure 3). This tendency was reversed on the last two assessment dates where domesticated fish had higher Vang than wild fish (1163 ± 110, 1423 ± 105, 1278 ± 114° s^{-1} and 996±89, 1191 ± 98, 1025 ± 101° s^{-1} for S1, S2 and S3 respectively). Further Newman-Keuls tests showed that Vang was significantly higher in wild fish at D63 during S1 compared with wild and domesticated fish at D125 (Table 2, Figure 3). During S2, Vang was significantly lower in wild fish at D125 compared with all other categories at any date except for domesticated fish at D69, D98 and D125; significantly lower in domesticated fish at D125 compared with wild fish at D63, D91 and domesticated fish at D111; significantly higher in wild fish at D63 compared with domesticated fish at D69. During S3, Vang was significantly higher in wild fish at D63 compared with all other categories at any date except for wild fish at D91; significantly lower in wild and domesticated fish at D125 compared with all other categories at any date; higher in wild fish at D91 compared with domesticated fish at D98 and wild fish at D111.

The total distance travelled (Dtot) was not significantly different between wild and domesticated fish but there was a significant Date effect (Table 2). On average, values were 4732 ± 766, 3390 ± 596, 3567 ± 645 mm from S1 to S3 in domesticated fish and 4713 ± 677, 3341 ± 529, 3389 ± 539 mm in wild fish (Figure 3). Newman-Keuls tests on Date showed that Dtot was significantly higher at D125 compared with all other dates during S1. During S3, Dtot was significantly higher at D125 compared with all other dates except for D91 and significantly higher at D91 than at D63 (Table 2).

There were significant effects of Date and Date*Origin on Velocity (Vel) (Table 2). During S1, domesticated fish at D63 performed significantly higher Vel than wild fish at D98, D111 and domesticated fish at D111; domesticated fish at D111 performed significantly lower Vel than domesticated fish at D69. During S2, domesticated fish at D63 performed significantly higher Vel compared with all other categories at any date except for domesticated fish at D69 performing itself significantly higher Vel than all other categories at any date except for wild fish at D69, wild and domesticated fish at D91 (Table 2). During S3, domesticated fish at D91 performed higher Vel compared with all other categories at any date except for domesticated fish at D63, D69 and wild fish at D69.

Immobility (Im) was not significantly different between wild and domesticated fish but there was a significant Date effect (Table 2). Newman-Keuls tests on Date during S1 and S3 showed that Im was significantly lower at D125 compared with all other dates. During S2, Im was significantly higher at D111 compared with all other dates.

The mean distance of the fish from the stimulus point (DStim) was significantly higher in wild fish than in domesticated fish, with a significant effect of date. During S2, DStim was higher at D63 and 69 (Figure 3, Table 2) than at all other dates but lower at D91 and 111 compared with D125. During S3, DStim was higher at D63 and 69 than at all other dates.

3.5. Effects of fish age: comparing responses at D63 and D165

In domesticated fish, Reactivity index (RI) and Recovery index (RcI) calculated from Vang did not significantly differ between D63 and D125. RI and RcI calculated from Im both significantly increased at D125 ($H_{1,64} = 36.2$, $P<0.001$ and $H_{1,64} = 47.3$, $P<0.001$ respectively). RI calculated from Dtot and Dstim did not differ between dates but RcI from Dtot (ranging from -4.7 to 1.6 at D63 and from -1.9 to 0.4 at D125) and Dstim (ranging from -0.9 to 1.1 at D63 and from -0.5 to +0.5 at D125) were significantly higher at D63 ($H_{1,64} = 6.1$, $P<0.05$ and $H_{1,64} = 18.2$, $P<0.001$, respectively) (Figure 4a1 and b1). RI calculated from Vel did not differ between dates and RcI decreased at D125 although the difference was not significant ($H_{1,64} = 2.9$, $P=0.09$).

In wild fish, RI calculated from Vang increased at D165 but the difference was not significant ($H_{1,64} = 3.3$, $P=0.07$) and RcI did not differ between dates. RI calculated Im did not differ between dates but

RcI significantly increased at D125 ($H_{1,64}$ = 20.7, P<0.001). RI calculated from Dtot and Dstim did not differ between dates but RcI for Dtot (ranging from -2.7 to 3.4 at D63 and from -0.9 to 0.5 at D125) and Dstim (ranging from -1.1 to 0.5 at D63 and from -1.3 to 0.3 at D125) were significantly higher at D63 ($H_{1,64}$ = 26.4, P<0.001 and $H_{1,64}$ = 4.6, P<0.05 respectively) (Figure 4a2 and b2).

At D63, RI calculated from Dtot did not differ between fish origins but RcI calculated from Dtot was significantly higher in wild fish than in domesticated fish ($H_{1,64}$ = 22.3, P<0.001). RI calculated from Dstim did not differ between fish origins but RcI was significantly higher in domesticated fish than wild fish ($H_{1,64}$ = 13.1, P<0.001). At D125, no differences were recorded between wild and domesticated fish in any of the variables.

4. Discussion

This study compared the swimming behavior of wild and domesticated juvenile sea bass before and after applying a visual and mechanical stimulus. This allowed two questions to be addressed. First was the assessment of the effect of domestication. The results showed consistent behavioral differences as well as similarities between both groups of fish developed hereafter. Second was the assessment of behavioural modifications of wild fish during the first months of captivity. Some changes were indeed recorded but most were also recorded in domesticated fish showing that the captive environment was not the only factor involved in such behavioral modifications. Furthermore, individual variability was strongly reduced among fish from both origins from the first to the last day of the experiment.

4.1. Experimental conditions and wild fish age

The apparatus designed in this experiment could elicit a standardized and synchronized response whatever the initial position of the fish in the arena. It allowed collection of behavioral data on a large sample of fish (a total of 480 individuals observed over 8 sessions). The age of captured wild sea bass was verified whereas most published studies comparing wild and domesticated juveniles have only selected individuals of similar average size (Malavasi *et al.*, 2004). The age estimation method used in this study was precise and accurate *i.e.*, the estimated age of domesticated fish was close to the actual age. Otolith readings showed that wild caught sea bass were in the same age range as domesticated fish and both were also of similar size at each experimental date. All of the observed fish did not present any deformities: 100 % had a swimbladder and most of them fed during periods preceding the observation sessions. However, the very first session showed a lower percentage of stomach fullness in wild fish (only 50%). This could be explained by the effect of transport that may have been greater in wild fish than in domesticated fish or by a short-term accommodation to artemia (7 days later, stomach fullness percentage doubled).

4.2. Impact of domestication: comparing wild and domesticated fish responses

Differences were recorded between wild and domesticated fish demonstrating an impact of domestication on behavior. During the first four sessions, angular velocity was higher in wild fish and mean velocity was lower than in domesticated fish even before stimulus actuation. The most consistent variable was the distance from the stimulus point, which was always higher in wild fish. This can be linked to anti-predator response which has already been shown to be eroded in several farmed species: Atlantic salmon, *Salmo salar* (Einum and Fleming, 1997), Steelhead trout, *Oncorhynchus mykiss* (Johnsson and Abrahams, 1991), Brown trout, *Salmo trutta* (Fernö and Järvi, 1998), and Atlantic cod, *Gadus morhua* (Nordeide and Svasand, 1990). A similar result was already recorded on Japanese flounder *Paralichthys olivaceus* juveniles where predator-experienced fish showed a longer response distance to the predator, reflecting a fear response or increased caution (Arai *et al.*, 2007). The difference in mean velocity and angular velocity reflected a lower swimming complexity in domesticated fish that could be linked to a decrease in the vigilance threshold (Bégout and Lagardère, 2004). Indeed, the environment experienced by cultured fish is strikingly different from that experienced by their wild counterparts *e.g.* the physical environment is much simpler, space is restricted and migration is not possible, it is less challenging in that good quality food is readily available and fish are protected against predators (Gross, 1998; Price, 1999; Waples, 1999). Furthermore, the hatchery environment is known to favour ‘high-risk high-gain’ phenotypes (Swain and Riddell, 1990). In our study, wild fish were captured when they were approximately 43±7 days old meaning that they had survived natural predation which is very high at this stage. Indeed, this natural selection also leads to some behavioral phenotypes that increase rates of survival (Huntingford, 2004).

Some similarities were also recorded between both fish origins. They reacted similarly to the stimulus presentation: the distance travelled decreased as did the mean velocity and mobility while the angular velocity increased. These are typical indicators of fish avoidance of a dangerous area and of risk assessment (Millot et al, 2009). Ten minutes after the stimulation, the fish tended to recover a higher swimming activity though it remained at higher level than before stimulation. As already indicated by Millot et al. (2009), this means that fish remained fearful toward the stimulus. These results confirm that in nearly all cases, behavioral differences between wild and domestic populations are quantitative rather than qualitative in character and are best explained by differences in response threshold (Price, 2002).

4.3. Impact of captivity on wild fish

Some behavioural modifications occurred in wild fish over time although they also occurred in domesticated fish in most cases. The reactivity decreased from the first to the last day of the experiment (75% to 53% in domesticated fish, 72 to 66% in wild fish after 70 days).

Swimming differences were mainly recorded during the last two stimuli exposures where mean velocity tended to decrease. Distance from the stimulus was especially high during the two first exposures and it decreased during the four following ones. Angular velocity was higher in wild fish during the first four assessments but became lower than domesticated fish on the last two assessments. However, angular velocity was lower for both fish origins at the end of the study. It seems that the vigilance threshold decreased in both domesticated and wild fish over time. Therefore, it is not possible to conclude that the captive environment was the only factor involved in the behavioral modifications of wild fish. The ability to evade predators may be particularly important during the early stages of life history (Houde, 1997). Gibb et al. (2005) proposed a model explaining performance changes across life-history stages in teleost fishes that could be related to our results. In this model, performance increases during early development, peaks at the larva-juvenile transition, and declines in juveniles and adults. At the juvenile stage, the performance decreases because the axial muscle crosssectional area cannot increase rapidly enough to match the concomitant increase in body mass. We hypothesize that these performances modifications due to the biomechanical consequence of interrelated developmental changes in the size and shape of fish as they metamorphose from larvae to juvenile could also be associated with behavioral modifications *e.g.* decrease of vigilance threshold. Several studies showed that behavior and morphology were intimately related *e.g.* size and aggressiveness in salmonids (Abbot *et al.*, 1985).

Nevertheless, the captive environment was also likely to play a role in wild fish behavioural modifications. Indeed, as with other behaviors, anti-predator responses have both inherent and learned components shaped by rearing conditions and most behavior patterns should be viewed as lying somewhere on the continuum between these two extremes (Kieffer and Colgan, 1992).

At last, a decrease of intra-group variability was shown in both wild and domesticated fish between the first observation session and the last session performed 62 days later. This was mostly seen in the recovery indexes calculated for the distance travelled and the distance from the stimulus in both fish origins. At D63, the recovery index for the distance travelled was higher in wild fish whereas the recovery index for the distance from the stimulus was higher in domesticated fish. This variability decreased in both fish origins and could be related to the rearing environment which provided a constant plain environment influencing a range of behavioral traits (Salvanes and Braithwaite, 2006). Deficits have been already shown in virtually all aspects of hatchery-reared fish behavior owing to the impoverished conditions in which they are raised (Brown and Laland, 2001). Millot et al. (2009a) showed that selected 2-years old sea bass were characterized by a higher swimming activity and a lower path complexity than wild and domesticated strains before and after stimulus actuation but no significant differences were recorded between domesticated and wild ones. In this experiment, wild fish were not caught in the sea but were issued from wild caught parents. Therefore, the authors hypothesized that a 20-month period of rearing (first generation fish) could be sufficient to obtain fish presenting the same behavioural characteristics than second generation fish. In the present study, we hypothesize that an even shorter period could lead to behavioral modifications in wild-caught fish. However, after 70 days of captivity, wild fish were still characterized by a higher distance from stimulus than domesticated counterparts. At the same time it is interesting to note that domesticated fish response to aging was similar: at the beginning of the experiment *i.e.* at an early stage of development, reactivity was higher than 70 days later.

4.4. Conclusions: implications for selection and restocking programs

Our study showed consistent behavioural differences between wild-caught and domesticated sea bass juveniles which demonstrated an impact of domestication on behavior. However, it also demonstrated a wider repertoire of responses at an early stage (~ 60 days old) and selection and restocking programs could be developed on this basis. Indeed, some domesticated individuals presented similar behavioral responses to wild fish. These individuals could be selected for restocking programs that often fail because released hatchery-reared fishes show remarkable deficits in many aspects of their behavioral performance, *e.g.* antipredator response, resulting in high levels of mortality in the post-release phase (Berejikian, 1995; Brown and Day, 2002). Conversely, other domesticated individuals showing lower reactivity to stimulus and lower path complexity could represent an opportunity for primary or directed selection in aquaculture. Indeed, selected sea bass have already been shown to be characterized by behavioral traits demonstrating a better adaptation than wild and domesticated strains (Millot et al., 2009) and this could be reinforced with such primary selection. Behavioural convergence between wild and domesticated sea bass juveniles showed the strong impact of the rearing environment. Numerous behaviors are partly innate- and partly environment-dependant *e.g.* predator avoidance (Magurran, 1990; Berejikian *et al.*, 2003). In our study, the presence of a clear reactivity in the domesticated fish suggested that some innate antipredator response (*i.e.* the startle response) remained in hatchery-reared sea bass juveniles (Malavasi *et al.*, 2004). However, the fact that behavioral responses modifications occurred in both wild and domesticated fish also shows that behavior can be rapidly modified by the environment. The behavioural patterns of fish result from innate patterns of maturation (developmental changes) and learning processes (Kieffer and Colgan, 1992). In our study, a sensitive period could also be involved at an early stage that could have long-term effects on the individual's development (Bateson and Martin, 1999). Indeed deficiencies originating in early life are likely to affect later success (Salvanes and Braithwaite, 2006). However, behaviors can also arise through experience (Kelley *et al.*, 2003). Salvanes & Braithwaite (2006) showed that early experience with both variable spatial and food cues consistently produces cod that were faster in their attraction to, and their consumption of, live prey; in their speed of exploration of a new environment, and in their recovery from a stressful experience. This demonstrates behavioral plasticity in fish. Our study highlights the behavioral differences between wild-caught and domesticated fish and describes modification and repertoire variability at an early stage in sea bass life. However, further research is required to assess the developmental origin of behavioural modification and how it could be further applied to restocking and selection programs.

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Tables :

Table 1. Mean ± SD. Comparison between real domesticated fish age at each observation session and estimated wild fish age.

Domesticated fish			Wild fish			
Real age	**BL**	**BW**	**Estimated age A**	**Estimated age B**	**BL**	**BW**
55	14.88±1.83		78±4		19.97±0.86	
59	16.25±2.46		78±7		19.94±1.34	
63	18.69±1.23		79±6		20.44±1.13	
69	20.41±0.84		84±6		21.66±1.15	
91	26.28±2.29	0.14±0.05	105±15	122±5	27.38±3.01	0.14±0.07
98	31.16±2.99	0.23±0.08	123±18	128±7	32.38±3.60	0.26±0.10
111	36.66±3.69	0.41±0.14	145±16	138±9	38.44±3.21	0.46±0.13
125	42.94±4.54	0.70±0.28	165±21	154±19	44.13±4.16	0.77±0.27

Estimation is either calculated on body length vs. age logarithmic regression equation (Estimated age A^1) or on body weight vs. Age logarithmic regression equation (Estimated age B^2).

1: $Y = 155.5 \ln(x) - 422.3$.

2: $Y = 49.9 \ln(x) + 169.8$.

BL : Total body length measured on fish at each observation session.

BW: Body weight measured on fish at each observation session.

Wild fish age was determined upon otolith readings.

Table 2. Results of repeated measures ANOVA and Newman-Keuls post-hoc tests used to analyse the mean differences between Origins (Wild vs. Domesticated fish) and Dates (6 ages). Origin and Date are between-subjects factors and Sequence (S1, S2, S3) is the within-subjects factor.

	Source	df	F	P	Newman-Keuls S1	Newman-Keuls S2	Newman-Keuls S3
Vang	Origin	3 & 392	1.2	0.320			
	Date	15 & 1082	4.6	< 0.001			
	Date*Origin	15 & 1082	1.8	0.003	W*D1 > W*D6=D*D6	W*D6 < all but D*D2=D*D4=D*D6; D*D6 < W*D1=W*D3=D*D5; W*D1 > D*D2	W*D1 > all but W*D3; W*D6=D*D6 < all; W*D3 > D*D4=W*D5
Dtot	Origin	3 & 392	0.4	0.730			
	Date	15 & 1082	3.1	< 0.001	D6 > all		D6 > all but D3 D3 > D1
	Date*Origin	15 & 1082	1.4	0.140			
Vel	Origin	3 & 392	1.4	0.230			
	Date	15 & 1082	3.7	< 0.001			
	Date*Origin	15 & 1082	2.0	0.01	D*D1 > W*D4, W*D5, D*D5 ; D*D5 < D*D1, D*D2	D*D1 > all but D*D2 ; D*D2 > all but D*D1, W*D2, W*D3, D*D3	D*D3 > all but D*D1, W*D2, D*D2
Im	Origin	3 & 392	0.3	0.790			
	Date	15 & 1082	4.6	< 0.001	D6 < all	D5 > all	D6 < all
	Date*Origin	15 & 1082	1.6	0.076			
Dstim	Origin	2 & 394	2.8	0.059		W > D	W > D (0.06)
	Date	10 & 788	11.8	< 0.001		D1=D2>all ; D 3 < D4=D6 ; D5 < D6	D1=D2 > all
	Date*Origin	10 & 788	1.0	0.430			

W: wild fish; D: domesticated fish; S1 : 5 min before stimulus actuation, S2: 5 min after stimulus actuation, S3: 10 min after stimulus actuation. D1 (Day 63); D2 (Day 69); D3 (Day 91); D4 (Day 98); D5 (Day 111); D6 (Day 125). For example, D1*W means domesticated fish at day 63. Vang: absolute angular velocity (° s^{-1}); Dtot: total distance travelled in the arena (mm); Vel: mean velocity (BL s^{-1}); Im: time spent in immobility (seconds); Dstim: mean distance of the fish from the stimulus point (mm).

Significant threshold was $P<0.05$.

Figures captions

Figure 1. Representative scheme of the experimental setup.

1: Digital camera (60 Hz); 2: Electromagnet; 3: Fishing wire attached to the end of the stimulus; 4: Black plastic tube used as a stimulus; 5: Circular arena; 6: Infrared casing; 7: Electrical switch button allowing to interrupt the electromagnet and then to drive the fall of the stimulus.

Figure 2. Escape response after stimulus actuation in wild-caught *vs.* domesticated fish. Black bars are wild-caught fish, white bars are domesticated fish.

(a): different positions of the fish just before the end of the fall stimulus : F: fish head oriented toward the stimulus and axis of the fish making an angle between -45 and +45° to the stimulus; S: axis of the fish making an angle between 180 and 225° or -90 and -135°; B: fish head oriented facing away from the stimulus and axis of the fish making an angle between 225 and 315° to the stimulus.

(b): Percentage of escape responses at different dates corresponding to observation sessions.

(c): Position of the fish just before the end of the fall stimulus when no escape response was observed.

(d): Position of the fish just before the stimulus fall end when escape response was observed.

Figure 3. Mean ± SE. Behavioural variables in domesticated *vs.* wild fish at different ages (D) during three sequences (S) of 5 minutes (Black bars are wild-caught fish, white bars are domesticated fish):

S1 : 5 mn before stimulus actuation. S2: 5 mn after stimulus actuation; S3: 10 mn after stimulus actuation.

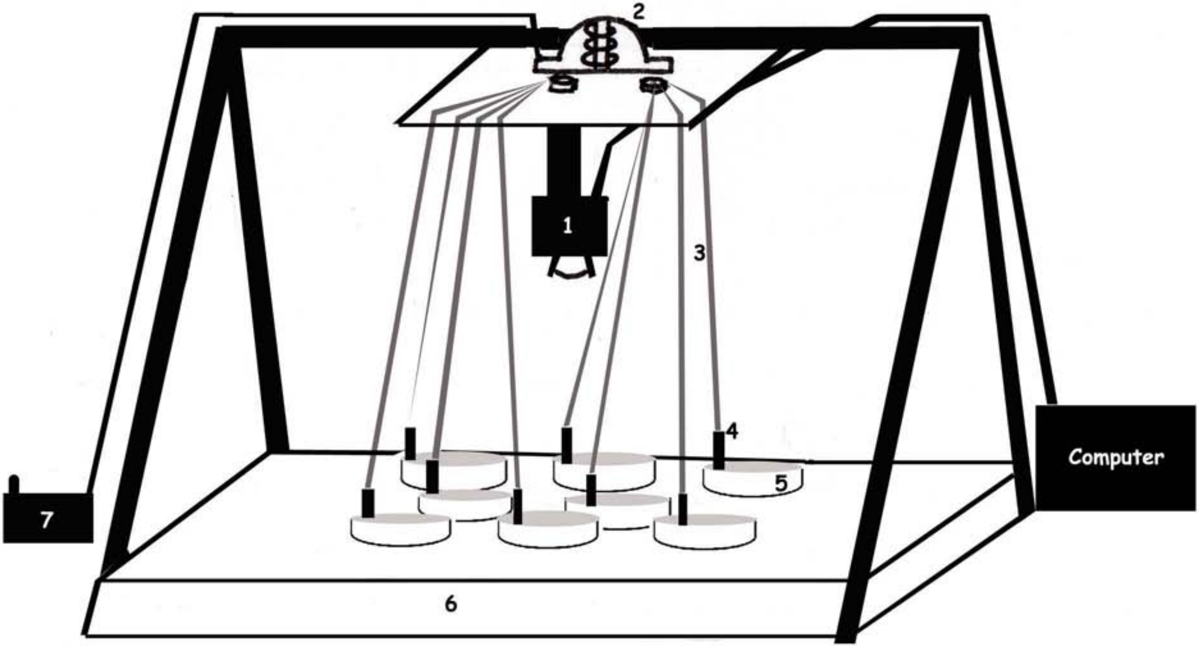
Day 63 (D63); Day 69 (D69); Day 91 (D91); Day 98 (D98); Day 111 (D111); Day 125 (D125).

Figure 4. Distribution of recovery indexes (RcI[1]) based on different behavioural variables (Xi) and ranged in ascending order (N=32 individuals). Black rhombus are data at day 63, white squares are data at day 125.

(a1): Distance from the stimulus point in domesticated fish; (a2): Distance from the stimulus point in wild fish; (b1): Cumulative distance travelled in domesticated fish; (b2): Cumulative distance travelled in wild fish.

$$1 : \ RcI = \frac{Xi(S3) - Xi(S2)}{Xi(S3)} - \frac{1}{32}\sum_{1}^{32} Xi(S3) - Xi(S2)$$

With S3: sequence of 5 min, 10 min after stimulation, S2: sequence of 5 min just after stimulation. Xi: value of the individual for the variable X.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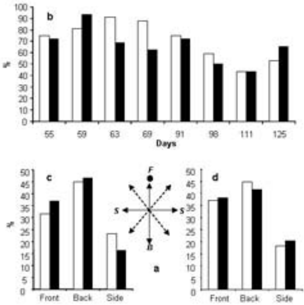
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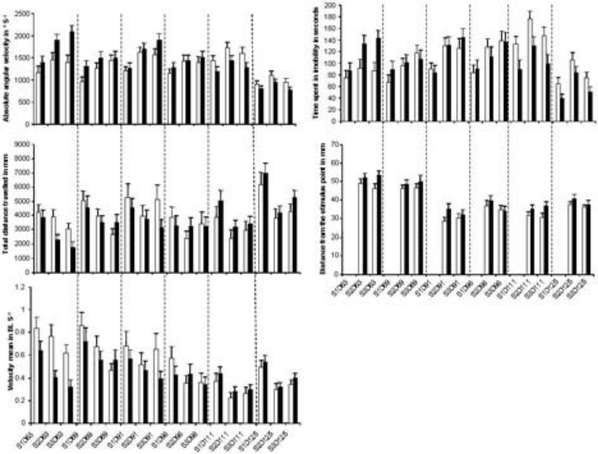
Absolute angular velocity in ° S⁻¹
0
500
1000
1500
2000
2500
Time spent in immobility in seconds
0
20
40
60
80
100
120
140
160
180
200
Total distance travelled in mm
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
Distance from the stimulus point in mm
0
10
20
30
40
50
60
70
Velocity mean in BL S⁻¹
0
0.2
0.4
0.6
0.8
1
1.2
S1D63
S2D63
S3D63
S1D69
S2D69
S3D69
S1D91
S2D91
S3D91
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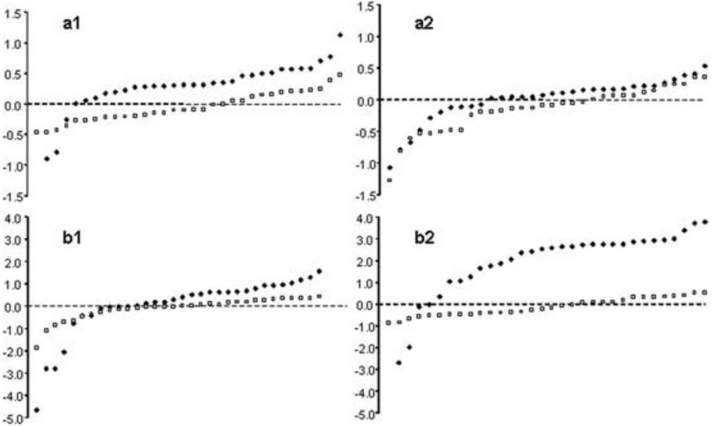
S1D125
S2D125
S3D125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a2
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3.0
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