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The shy prefer familiar congeners

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Abstract :

The shy–bold continuum is both a fundamental aspect of human behavior and a relatively stable behavioral trait for many other species. Here we assessed whether shy individuals prefer familiar congeners, taking the European sea bass, a recently domesticated fish showing similar behavioral responses to wild fish, as a model to better understand the inter-individual variability in social behavior previously observed in this species. In the wild, the link between familiarity i.e. the preference of fish for familiar congeners and boldness could be part of the mechanism underlying shoaling formation in fish. Thirty fish were individually tested in a device designed to assess the preference for a familiar vs. an unfamiliar congener on the basis of visual cues only. An open field test (OFT) with shelter was performed on the same fish 32 days later to assess the boldness of each individual. Variables of interest included the proportion of time spent in the shelter, border and center zone of the arena and variables of activity. Variables measured in OFT were collapsed into first principal component scores using Principal Components Analysis (PCA) which allowed characterizing a shy-bold continuum. Time spent near the familiar congener was negatively correlated with boldness i.e. shy individuals spent most of the time near the familiar congener. We discuss the relevance of these findings to the understanding of the behavior of European sea bass and suggest that the link between familiarity and shyness is a general aspect of both animal and human behavior.

Highlights

► We assessed sea bass preference for familiar congener. ► We assessed sea bass boldness. ► Shy individuals prefer to spend time near familiar congeners. ► We suggest the link between familiarity and shyness is a general aspect of both animal and human behaviour.

Keywords : familiarity, personality, open field, social behavior, swimming activity, shyness, shoaling behavior, visual cue

INTRODUCTION

The shy–bold continuum is both a fundamental aspect of human behavior and a relatively stable behavioral component for many other species (Wilson, 1994). Coleman and Wilson (1998) suggested that shyness and boldness may be context specific, but they also form one of the five axes of animal personality defined as a correlated set of individual behavioral and physiological characteristics that are consistent over time and across situations (Koolhaas et al., 1999). These five axes are: boldness (response to potentially risky situations), activity, exploration (response to novel situations), aggressiveness and sociability. Correlations between different personality traits may exist, leading to the formation of a behavioral syndrome (Sih et al., 2004). The shy-bold continuum has been studied in numerous vertebrates (Benus, 1991; Boon, 2007; Careau, 2010; Verbeek et al., 1994) , including teleosts e.g. the rainbow trout *Oncorhynchus mykiss* (Øverli et al., 2006; Sneddon, 2003) and the European sea bass (Ferrari et al., 2014; Ferrari et al., 2015) as well as in invertebrates (Briffa and Greenaway, 2011).

In fish species, boldness is usually associated with a proactive strategy whereas shyness is associated with a reactive strategy. Proactive animals tend to engage in active avoidance or cope with stressful stimuli (Koolhaas, 2008; Koolhaas et al., 1999) through a “fight or flight” response contrary to reactive ones which display a passive behavior through a “freeze and hide” response. Bold fish take more risks and explore their environment faster (less cautiously) when exposed to novelty (MacKenzie, 2009; Øverli et al., 2006), and they are more aggressive, dominant (Castanheira et al., 2013; Øverli et al., 2004), and less flexible than shy fish i.e. they show a lower variability in their behavioral responses with environmental changes (Ruiz-Gomez, 2011). By contrast, shy individuals tend to be risk averse and are generally neophobic, show higher behavioral flexibility and are more responsive to their environment (Sneddon, 2003), and more sociable than bold individuals (Ward et al., 2004). Intermediate fish are in the middle of these two extremes.

In the wild, gregarious species form shoals that represent non-random assemblages according to species, size, parasite load and familiarity i.e. individuals prefer to join groups with which they have had a previous experience (Griffiths, 2003; Griffiths and Ward, 2007; Hoare et al., 2000; Krause et al., 1996; Krause and Ruxton, 2002; Pitcher and Parrish, 1993). Shoaling behavior in fish is also an important antipredator strategy (Magurran, 1990; Pitcher and Parrish, 1993). Familiarity maximizes the benefits of grouping and mediates association decisions in shoaling species (Griffiths and Ward, 2007). However, the importance of social dynamic and fidelity of fish shoals in the wild remains unclear because these variables are difficult to monitor (Helfman, 1984; Hilborn, 1991; Hoare et al., 2000; Svensson et al., 2000). Furthermore, the decision to shoal strongly depends on the context encountered by the fish e.g. European minnows *Phoxinus phoxinus* shoal to obtain shelter, but only when there is insufficient physical structure available (Orpwood et al., 2008). Thus, one could expect that the preference for familiar fish increases under the threat of predation, but several studies have found that the preference of fish for familiar individuals is not affected by the appearance of a model predator (Brown, 2002; Griffiths, 1997). This suggests that fish have adapted to maintain a consistent preference for familiar congeners in habitats where they are often exposed to predators.

It is well known that boldness can have potential fitness consequences by influencing many traits including mate choice (Godin and Dugatkin, 1996), parental care (Budaev, 1999), reproductive success (Armitage and Van Vuren, 2003) and anti-predator behavior (Réale and Festa-Bianchet, 2003), but the link between familiarity, i.e. the preference for familiar congeners, and boldness as a mechanism underlying shoaling formation in fish, has not yet been addressed.

The aim of the current study was to assess whether shy individuals prefer familiar congeners, taking the European sea bass as a model. This species was recently domesticated and shows high interindividual behavioral variability, with some domesticated individuals presenting similar responses to wild fish (Benhaïm et al., 2012). In a previous study (Benhaïm et al., 2013a) comparing wild and domesticated juvenile sea bass, we found that fish were similarly attracted to unfamiliar congeners, on the basis of visual cues alone. However, some individuals (both wild and domesticated) spent most of their time on the opposite side (empty compartment) of the unfamiliar congener. Here, we used a preference test for a familiar *vs.* unfamiliar congener and a boldness test to examine whether this inter-individual variability could be explained by a link between the shy-bold continuum and familiarity.

METHODS

Experimental animals and housing conditions

Two separate batches of sea bass were hatched at the Aquanord SA farm (France). On November 11th 2011, when the fish were 3 days old, they were transferred to the experimental station of INTECHMER (Cherbourg) and grown in a recirculated system. All parameters were set according to the protocol used by Aquanord hatchery.

Fish (about 300 individuals per batch) were later grown in an open water system (i.e. physico-chemical parameters resembling natural conditions) in two separate 2 m^3 tanks until the beginning of this experiment which started on March 22nd 2014. At this time, the fish were 865 days old.

Fish from both tanks were anesthetized with benzocaine (200 ppm) and 40 individuals from each tank were selected based on previous biometric data. Total length (mean ± SD) was 13.9 ± 0.9 cm in the first tank and 13.8 ± 0.8 cm in the second; weight was 28.4 ± 3.2 g in the first tank and 28.1 ± 2.8 g in the second. All the selected individuals were PIT-tagged (Iso, 9 x 1.4

mm) on the same day by inserting a tag horizontally just behind the head to prevent any change of position after implantation.

To optimize the familiarization process between individuals, the two groups of 40 selected individuals were placed in two 200 L tanks for 75 days. The tanks were provided with water from a recirculated system. During this period, the light regime was a 16:8 LD cycle (light onset at 06:00 U.T. +1). In both tanks, temperature, salinity and oxygen level were (Mean ± SD), 18.5 ± 0.3°C, 35.0 ± 0.0 g L^{-1}, 6.5 ± 0.2 mg L^{-1}, respectively. Fish were fed manually until satiation three times daily with a commercial diet (Neo Grower Extra Marin, 4 mm, Le Gouessant, France).

Observation room

Observations were made in a dedicated room. All experiments were video recorded at 25 frames per second (Ethovision XT recording, Noldus, the Netherlands; camera Ikegami CD48E; 2.8 - 12 mm Computar® lens equipped with an IR filter positioned at 180 cm above the water surface). An infrared casing (1x1 m, Noldus, The Netherlands) was placed under the apparatus used in this experiment to enable the recording of videos at low light intensity and to improve video analysis. Two 60 W light bulbs were horizontally placed on walls located on the left and right sides of the infrared casing. They were located 180 cm above the infrared casing and provided an indirect and homogenous lighting on the apparatus. The light intensity measured at the water surface was 100 Lux.

Familiarity test

Individuals were tested one by one in a device (90 * 40 cm with a water height of 15 cm) constructed from opaque white plastic and transparent Plexiglas® (Fig. 1). The start box (40 x 20 cm) was separated from the rest of the device by a removable transparent wall. The end of the device (located in front of the start box) was occupied by two separate compartments (precluding olfactory cues) with one transparent wall. These compartments, continuously supplied with air (air pump), were used to place a congener that was either familiar or unfamiliar to the test individual. The familiarity test was therefore based on visual cues that have been shown to underpin individual recognition in a variety of species (Balshine-Earn et al., 1998; Fricke, 1973; Hert, 1985). Shortly before observations, the device was filled with water, the level of which was maintained at 15 cm. Temperature, salinity and oxygen level were verified before and after the end of observations performed on each fish and were respectively 18.4 ± 0.5°C, 35.0 ± 0.0 g L^{-1}, 6.6 ± 1.3 mg L^{-1} before and 18.4 ± 0.3°C, 35.0 ± 0.0 g L^{-1}, 6.6 ± 1.2 mg L^{-1} after.

Before the beginning of observations, the position of the unfamiliar or familiar congener on the left or right side inside the independent boxes located at the end of the device was randomly determined for each individual. This device shape was used instead of a T-maze to minimize potential bias related to side-turning preference. The first tested fish was gently collected from the tank using a net and immediately placed inside a bucket closed by a cover and then placed in the start-box. After a 10 min acclimatization period, the transparent wall was removed and the video capture started. The device was filmed for 30 min. The experiment was carried out when the fish were 939 days old and three days were required to test all individuals (N = 30). The water was entirely renewed every two hours. The unfamiliar and familiar fish used as the attractors were changed every two hours to minimize the stress of confinement and handling (10 individuals from each category were used for the experiment).

Before being returned to their initial tank, fish were anesthetized and their length and weight were measured.

The video recordings were analyzed using EthoVision XT software (Noldus, The Netherlands), which allowed six virtual zones to be defined in the device (Fig. 1) and to track the swimming behavior of the fish.

Different variables of interest (position preference or activity) were chosen to analyze the behavior of the fish:

-The proportion of time spent in each of the six defined zones (%): Start-Box in front of the familiar (*Start-Fam*) or the unfamiliar congener (*Start-UnFam*), Pre-Familiar and Pre-Unfamiliar located between *Start-Fam*/*Start-Unfam* and *Fam*/*UnFam* (*Pre-Fam* and *Pre-Unfam*), zone near the familiar congener (*Fam*), zone near the unfamiliar congener (*UnFam*).

-The distance traveled by each fish in the device (*Dtot* in mm).

-The velocity mean expressed in body length per second (*Vel* in BL s^{-1}).

The last two variables quantified the fish swimming activity level in the device.

-The absolute angular velocity of the fish expressed in degree per second (*Vang* in ° s^{-1}), which was calculated by the software as follows:

$Vang_n = RTA_n / t_n - t_{n-1}$ where RTA_n is the relative turn angle for the sample n, and $t_n - t_{n-1}$ is the time difference between the current and previous sample. The rate of change in direction was unsigned. The turn angle was calculated as the difference between two subsequent values for the direction of the head. This variable was an indicator of the amount of turning per unit time and quantified the swimming path complexity, high Vang values being linked to higher level of vigilance (Benhaïm et al., 2012).

Boldness test in the open field apparatus

An open field test (OFT) with shelter was performed when the fish were 973 days old i.e. 32 days after the familiarity test to ensure the fish had recovered from the first test. Among numerous behavioral tests used to assess the shyness-boldness axis, open field tests (Budaev 1999, 1999; Yoshida 2005) are widely used and was chosen in this case because it has been successfully applied in previous studies on sea bass (Ferrari et al., 2014). The open field (90 * 40 cm with a water height of 15 cm, Fig. 2) contained a shelter (opaque PVC box 20 * 20 * 20 cm closed by a vertically sliding opaque trapdoor) placed in one corner and was divided into four virtual zones using the software EthoVision XT: Entry, Pre-shelter, Border and Center. The center zone was considered a risky area because thigmotaxis (staying close to the walls of an arena) is a common measure indicative of a high degree of shyness in such an apparatus (Dahlbom, 2011; Maximino, 2010). Selected fish (N=30) were individually placed in the shelter. After a 10 min acclimatization period, the door was gently opened. If the individual did not leave the shelter within 20 minutes following the acclimatization time, the experiment was stopped and a latency of 1200 seconds was attributed. . Before being returned to their initial tank, fish were anesthetized and their length and weight were measured.

Variables of interest were extracted with Ethovision XT and were as follows:

- The latency of each individual to emerge from the shelter (*Lat* in s), the proportion of time (%) respectively spent in the shelter (*Shelter*), the center zone (*Center*), the border zone (*Border*), the pre-shelter zone (*Pre-Shelter*), and at the entrance of the shelter (*Entry*).
- The mean distance from the shelter (*DtoShelter* in cm) and the number of returns to the shelter (*FreqShelter*).
- The distance travelled by each fish in the device (*Dtot* in mm), the absolute angular velocity of the fish expressed in degrees per second (*Vang* in ° s^{-1}), and its mean velocity expressed in body length per second (*Vel* in BL s^{-1}).

Statistical analysis

In both experiments, the spatial distribution of fish was analyzed using parametric analysis of variances (ANOVA) with zone as an independent factor after verification of distribution normality and homoscedasticity (Dagnélie 1975). If the data did not fulfill these requirements, non-parametric Kruskall-Wallis tests were used. Significant ANOVA were followed by a posthoc multiple comparison test (Newman–Keuls) and significant Kruskall-Wallis tests by rank-based multiple comparisons.

All the variables measured in OFT were collapsed into first principal component scores using Principal Components Analysis (PCA). A correlation matrix was used to verify multicollinearity, i.e., to identify variables that did not correlate with any other variable, or correlate very highly ($r = 0.9$) with one or more variables. Those latter variables were removed from downstream analyses. The remaining selected variables used for the PCA were: *Shelter*, *Center*, *Border*, *Pre-Shelter*, *Entry*, *DtoShelter* and *FreqShelter*. Each individual fish was then affected a PC1 score later used in cross-context analysis. The too small number of variables issued by the familiarity test prevented running PCA analysis.

Associations within tests were assessed by non-parametric Spearman’s rank order correlation. The correlations within tests were corrected using the Bonferroni method with $n = 9$ for the familiarity test and $n = 11$ tests for the OFT, thresholds for significance being 0.0055 and 0.0045, respectively. For cross-context analysis (between tests), we analyzed the correlation between individual values for either the proportion of time spent in the *Fam* zone or *UnFam* zone and PC1 individual scores from OFT using Pearson's correlation coefficient.

RESULTS

During the experiment, no mortality was recorded and all fish grew similarly: Body weight and Total length were (Mean ± SD) 32.6 ± 3.2 g and 14.3 ± 0.7 cm, respectively, after the familiarity test, and 38.0 ± 4.2 g and 15.1 ± 0.7 cm, respectively after the open field test.

Familiarity test

All the fish tested left the start zone after the wall was removed, i.e. none of the fish spent 100% of the time in the *Start-Fam* or *Start-UnFam* zone.

Fish spent most of the time in the *Fam* zone (mean ± SE, 28.9 ± 6.7%, Fig. 3) but also some time in the *UnFam*, *Start-Fam* and *Start-UnFam* zones (19.3 ± 5.2%, 18.3 ± 5.7% and 19.3 ± 6.4%, respectively). The time spent in the device was not randomly distributed between zones (ANOVA: $F5, 180 = 2.7$, $p = 0.02$), with fish spending significantly more time in the *Fam*, *UnFam* and *Start-Fam* zones than in the *Pre-Fam* and *Pre-UnFam* zones (Fig. 3). There was high inter-individual variability with 11 individuals spending most of the time in the *Fam* zone, six in the *UnFam* zone, four in the *Start-UnFam* zone, five in the *Start-Fam* zone, two in the *Pre-Fam* zone, and two in the *Pre-UnFam* zone. The proportion of time spent in the *Fam* zone was significantly correlated with *Vang* (Spearman rank correlation: $rs = -0.58$, N = 30, $p = 0.002$) and with *Vel* ($rs = 0.67$, N = 30, $p = 0.0002$).

Open field test

Six individuals (out of 30) spent all the time inside the shelter. Including those fish with a latency of 1200 s, the mean latency to emerge from the *Shelter* was 460.3 ± 88.1 s. Fish spent most of the time in the *Shelter* and *Border* zones (56.1 ± 7.9% and 24.4 ± 6.0%, respectively, Fig. 4). The proportion of time spent in the apparatus significantly differed between zones (Kruskal-Wallis test: H4,150 = 30.05, $p < 0.0001$), with fish spending more time in the *Shelter* zone than in the *Center*, *Pre-shelter* and *Entry* zones (Fig. 4). There was a significant correlation between *Dtot* and the proportion of time spent in *Border* or *Shelter* zones (r = 0.46, N = 30, p = 0.04, and rs = -0.49, N = 30, p = 0.03, respectively). *Dtot* and *DtoShelter* were correlated (r = 0.57, N = 30, p = 0.01), and *Vang* and *FreqShelter* were inversely correlated (r = -0.52, N = 30, p = 0.02). Finally, the proportion of time spent in *Shelter* was strongly inversely correlated with that spent in *Border* zone (r = -0.98, N = 30, p < 0.0001).

The first component (PC1) explained 44% of the variation in the data (loadings: *Shelter* = 0.97. *Border* = 0.85. *Center* = -0.55. *PreShelter* = 0.29. *Entry* = 0.36. *FreqShelter* = 0.16. *DtoShelter* = 0.93)). PC1 was therefore related to two extremes: the negative pole to high values of *Shelter* (contribution to the first component was 0.31 and the correlation was -0.974), the positive one to high values of *Border and DtoShelter* (contributions to the first component were 0.23 and 0.28 and the correlations were 0.85 and 0.93 respectively). PC1 represented therefore a gradient from shy (high values of *shelter*) to bold individuals (high values of *Border* and *DtoShelter*).

Links between the Familiarity and open field test results

The proportion of time spent in the *Fam* zone was negatively correlated with PC1 individual scores (r = - 0.41, N = 30, p = 0.04, Fig. 5). Proportion of time spent in the *UnFam* zone was not significantly correlated with PC1 individual scores (r = - 0.17, N = 30, p = 0.41).

DISCUSSION

The aim of this study was to assess whether shy individuals prefer congeners with whom they are familiar, taking the European sea bass, a recently domesticated fish species, as a model. We determined whether fish were attracted to a familiar or an unfamiliar congener and then assessed boldness using an open field test.

Fish tended to spend more time in the zone near the familiar congener but this was not significantly different from that spent near the unfamiliar congener. This is likely due to high variability between individuals that reduced the statistical power of the analysis. This variability has already been observed under similar situations where we showed that some individuals voluntarily avoided the zone located near an unfamiliar congener, preferring instead to spend time in the opposite zone near an empty compartment, whereas others spent most of the time in the start box (Benhaïm et al., 2013a). Overall, fish spent almost 60% of the time near a familiar or unfamiliar congener, possibly because social or gregarious species may greatly benefit from social interactions regulating the stress response, especially when placed in a novel and therefore stressful environment (Allen et al., 2009). Indeed, group behavior promotes growth as a result of social facilitation (Peuhkuri et al., 1995; Stirling, 1977) and limits the risk of predation (Roberts, 1996). Vigilance is much lower when the distance between neighbors is small, because information about whether other group members have detected a predator is easier to obtain from close individuals than from distant ones (Pöysä, 1994). Such gregarious behavior is widespread among fishes, and swarms, flocks, herds-group formation are also a widespread phenomenon in many different animal species populations (Krause and Ruxton, 2002). Further, the time spent near the familiar congener was positively correlated with velocity and negatively correlated with angular velocity. Velocity and angular velocity are linked to a high level of visual interaction between the tested fish and the familiar congener through the transparent wall and a lower level of vigilance as already shown in previous studies (Benhaïm et al., 2013a).

Several theories can explain the observed variability in behavior. First, the decision to shoal strongly depends on the context encountered by the fish, in accordance with Hamilton's theory of the selfish herd (Hamilton, 1971). Fish choosing not to shoal with familiar or unfamiliar congeners may be considered subordinate. Indeed, staying alone is the best strategy for subordinates (Pitcher and Parrish, 1993), because it protects them from suffering injuries in an escalated contest (Hilborn, 1991). Second, congener avoidance or preference for the familiar or the unfamiliar congener may also be linked to particular behavioral traits such as boldness, as discussed below.

The proportion of time spent in the shelter, which provides a relevant indication of boldness, varied substantially between individuals, consistent with previous studies (Biro and Stamps 2008; Brown, 2007; Budaev 1999; Eriksson, 2010; Fraser, 2001). When the fish left the shelter, they spent most of the time in the Border zone and only a very short time in the Center zone. The proportion of time spent in the border zone was negatively correlated with the proportion of time spent in the shelter, consistent with a previous study on the same species under similar conditions (Ferrari et al., 2014). Experiments in such devices involve handling and isolating the fish, which is clearly stressful for all individuals regardless of their boldness level, as already shown by previous studies on Zebrafish, *Danio rerio* (Blaser and Vira, 2014) and sea bass (Benhaïm et al., 2013a; 2013b; 2013c). This may explain why all individuals preferred to swim in the border zone, because this area is perceived by the fish to be safer than the central zone. Unsurprisingly, the total distance traveled in the device was positively correlated with the time spent in the border zone and the distance to shelter which showed that the fish were not motionless when they left the shelter as already observed in a previous study (Ferrari et al., 2014). The PCA approach allowed us to identify a clear Shelter-Border/Distance to shelter axis which can be used as a proxy for evaluating the shy-bold continuum in sea bass (Ferrari et al., 2015).

Linking the results of the familiarity and open field tests, we found a strong negative correlation between the proportion of time spent near the familiar congener and boldness. Fish which spent more time inside than outside the Shelter (i.e. shy fish) preferred to stay near the familiar congener. On the contrary, bold fish did not show any preference for the familiar or unfamiliar congener *i.e.* they were equally attracted to both congeners. Shy fish tend to be risk averse (Wilson, 1994); which is supported by our work, as they preferred to shoal with familiar congeners with whom they have established a stable relationship over a long period of time without encountering major aggression or other incidents that they were unable to cope with previously. However, our study did not show any obvious avoidance of unfamiliar congeners in shy individuals. Bold fish have been shown to take more risks and explore their environment less cautiously when exposed to novelty (Øverli et al., 2006; MacKenzie et al., 2009), which may explain why they spent the same amount of time near familiar and unfamiliar congeners. We hypothesize that these individual differences in behavior could be related to personality traits even though the consistency over time was not tested in the present study. The repeatability of personality tests is still a major concern in sea bass as well as in other animal species. For example, Bell et al. (2009) reported that repeatability was greater for experiments separated by short intervals than for those separated by longer intervals. Further, a previous work on sea bass showed the lack of consistency over time in the results obtained for individual-based tests (OFT was however not used in this experiment) that is likely due to the stress induced by repeated trials (Ferrari et al., 2015). The results of these repeated tests could also be biased by the high memory and learning abilities observed in this species, defined as a change of behavior with experience (Dill, 1983).

The familiar congener preference could also be related to sociability and the link between shyness and the familiar congener preference could be indicative of a behavioral syndrome in sea bass. As already shown by previous studies on birds, shy individuals tend to be more sociable than bold individuals (Verbeek et al., 1994). The potential existence of behavioral syndromes in sea bass has already been suggested in previous work e.g. individuals with a passive response during a restraint test tended to be shyer during an open field test (Ferrari et al., 2014). The present study and a previous one on the same species (Benhaïm et al., 2013a) showing that individuals were attracted to unfamiliar congeners when they had no other choice apart from an empty compartment in a T-maze, enable a better understanding of the decision process in sea bass. If they have the choice, bold individuals will spend the same amount of time near familiar and unfamiliar congeners whereas shy individuals will spend more time near the familiar congener. If they have no choice, both bold and shy individuals will spend more time near the congener whether familiar or not. Finally, because our study showed a preference in shy individuals to stay near the familiar congener, it also confirms that sea bass are able to discriminate familiarity on the basis of visual cues alone as already shown on the same or different species (Brown, 2002; Brown and Colgan, 1986; Di-Poï, 2008; Griffiths, 1997; Waas and Colgan, 1994)

When assessing the significance of these results in the wild, it is important to consider that sea bass pelagic eggs are largely dispersed by estuarine currents and individuals are gregarious especially at the juvenile stage (Barnabé, 1978); however, little is known about dispersal, shoal site fidelity and shoal fidelity in wild sea bass. Some authors however mentioned post-larvae and fry congregating in upper estuaries, creeks and harbors i.e., a few dozen to many thousands remaining in distinct groups for several years at a time (Aprahamian and Barr, 1985; Claridge and Potter, 1984; Dando and Demir, 1985; Kelley, 1988). Furthermore, shoaling behavior is known to be generated by visually stimulating attractions between individuals but it could also involve the recognition of familiar congeners on the basis of visual cues.

Further research is needed to confirm that the significant individual differences and the significant correlation between familiarity and shyness in our sea bass population point to personality traits differences and behavioral syndromes.

Subject to the confirmations of these conditions, our results may apply to many other species, including humans. Indeed, personality has been identified in a broad array of species and some aspects of personality show considerable cross-species generality (Capitanio, 1999; Gosling, 2001; Gosling and John, 1999; Seyfarth et al., 2012). Neuman (2014) argues for a cognitive-biological theory of personality stating that the common denominator of various personality theories are neural systems of threat/trust management and their emotional, cognitive, and behavioral dimensions. The world is a challenging place and both human and non-human organisms have to cope with many threats (Neuman, 2014). Variability of personality traits may result from the dynamics of evolutionary game theory that reach a particular optimum of trade-offs (Costa, 1980). To cope with a stressful environment, we found that shy fish chose to join the familiar congener on the basis of visual cues only, much like human newborn babies who prefer their parents' and other familiar voices over those of strangers (Zajonc, 1968), or shy adults who prefer to avoid meeting strangers (Crozier, 2001). Individual recognition and experience of past encounters strongly influences social behavior (Hinde, 1985) i.e. we act differently in the company of somebody we know than in that of a person we meet for the first time.

In conclusion, our study reveals an interesting link between familiarity and shyness that may be a general aspect of both animal and human behavior. Further research is needed to confirm this hypothesis. Within sea bass species, it would be useful to understand better the link between boldness and shoal preference in different environmental and social contexts i.e. trade-offs that individuals with different behavioral characteristics face when considering competition for foraging and predation risk. Behavioral studies on different social species including humans are required to verify the generality of the link between familiarity and shyness.

Ethical Statement

This study was conducted under the approval of the Animal Care Committee of France under the official licence of M.L. Bégout (17-010).

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Figure legends

Fig.1. Schematic representation and picture of the social interaction device and the virtual zones defined for the video recording analysis.

(a) Schematic configuration of the device with the familiar congener placed on the left side. Dotted lines are transparent Plexiglas® walls, continuous lines are white opaque plastic. The bottom of the device is made of transparent Plexiglas®. Virtual zones defined for the video recordings analysis are:

Start-Fam and StartUnfam closed by a removable transparent wall where the fish is placed at the beginning of the experiment; Pre-Fam and Pre-Unfam: areas located after *Start-Fam* and Start-Unfam; Fam: the area located near the compartment where the familiar congener was placed; Unfam: the area located near the compartment where the unfamiliar congener was placed; 1 and 2: left and right separate compartments closed by a transparent Plexiglas® wall where congeners (familiar or unfamiliar) were placed.

(b) Picture of the device showing the focal individual located in Fam.

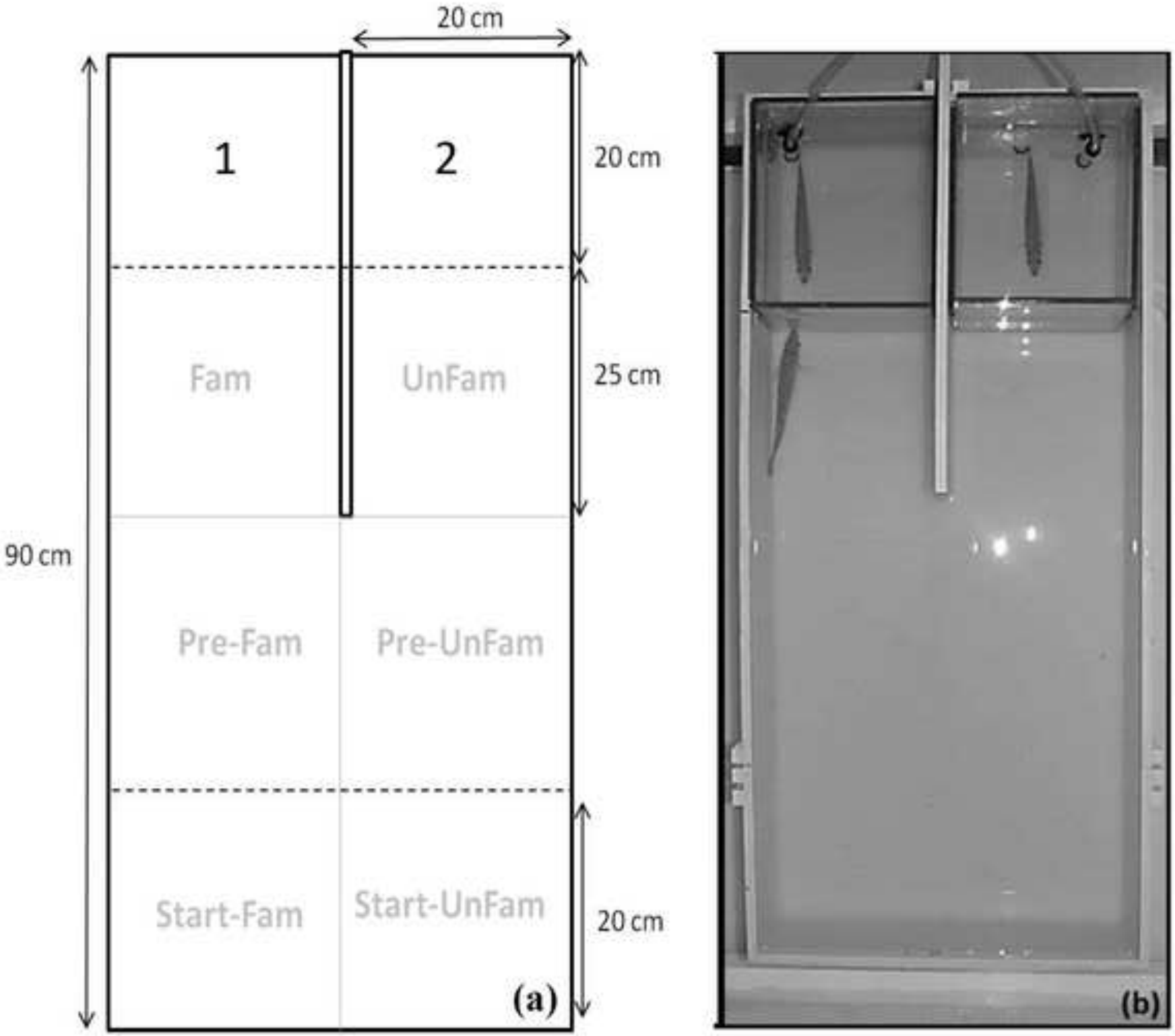
Fig. 2. Scheme and dimensions of the open field apparatus

Fig. 3. Proportion of time spent (mean ± SEM in %) by fish in each zone of the familiarity test device. *Start-Fam* and Start-UnFam: Start-Box in front of the familiar or the unfamiliar congener, Pre-Fam and Pre-Unfam: Pre-Familiar and Pre-Unfamiliar zones, Fam: zone near the familiar congener, UnFam: zone near the unfamiliar congener. Significant differences ($P < 0.05$) between zones are shown by different letters above the bar (rank-based multiple comparisons).

Fig. 4. Proportion of time spent (mean ± SEM in %) by fish in each zone of the open field. Significant differences ($P < 0.05$) between zones are shown by different letters above the bar (rank-based multiple comparisons).

Fig. 5. Pearson's correlation between the proportion of time spent in the zone near the familiar congener (Fam) in the familiarity test and the first principal component scores using Principal Components Analysis (PC1) in the open field test (OFT), $r = -0.41$, N = 30, $p = 0.04$. Equation for the best linear fit is: $y = -7.5195x + 25.8$..

Figure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(a)
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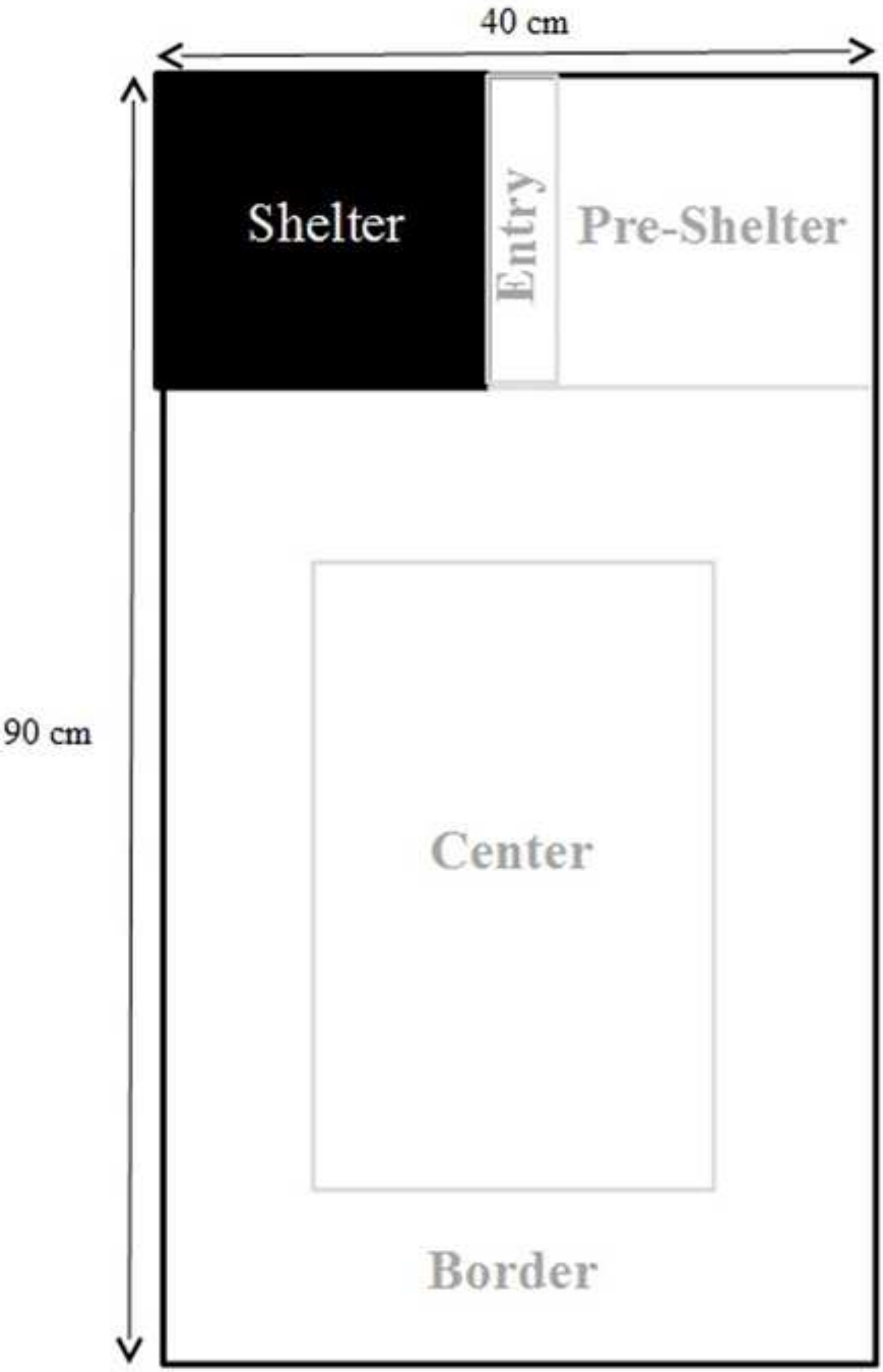
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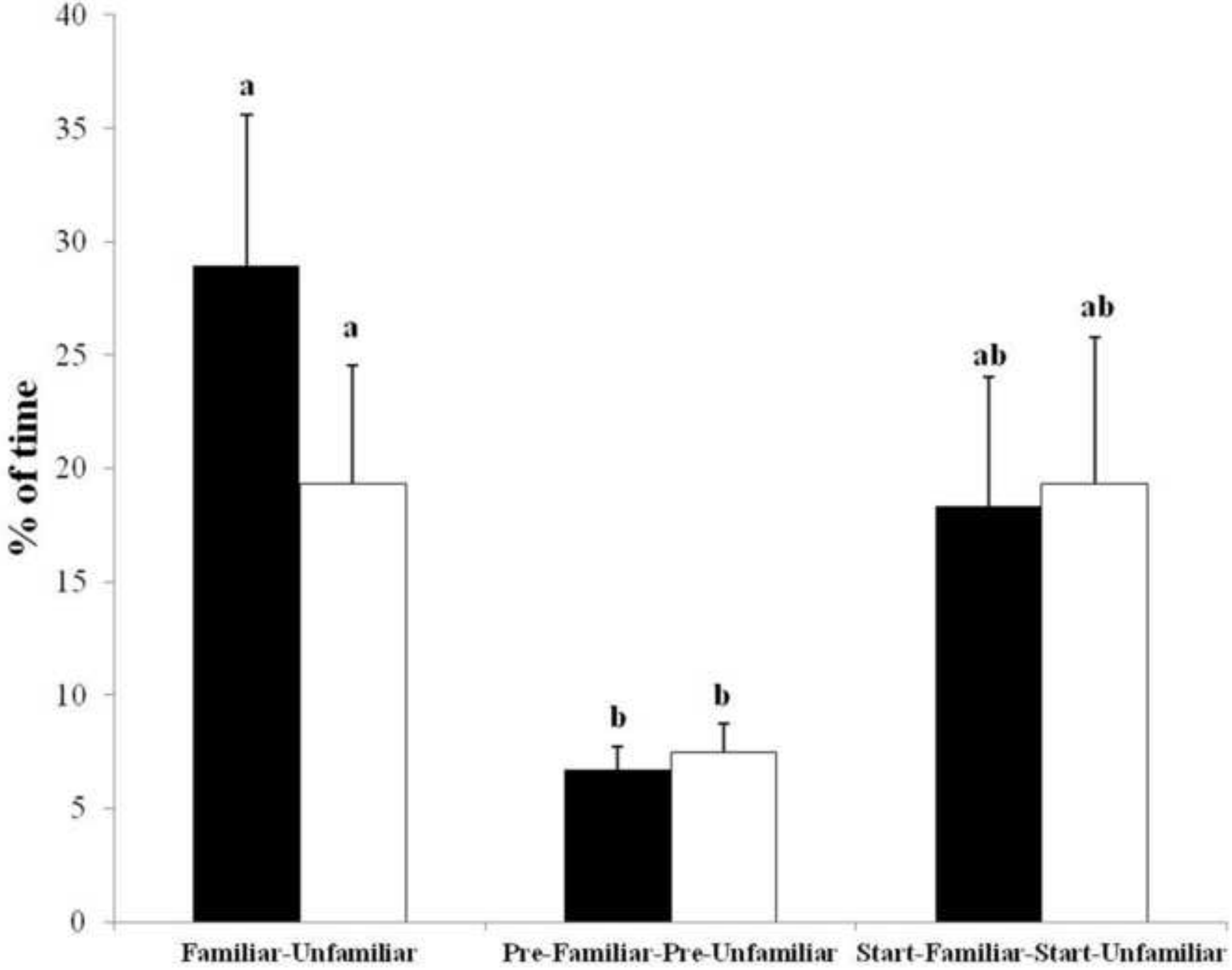
Figure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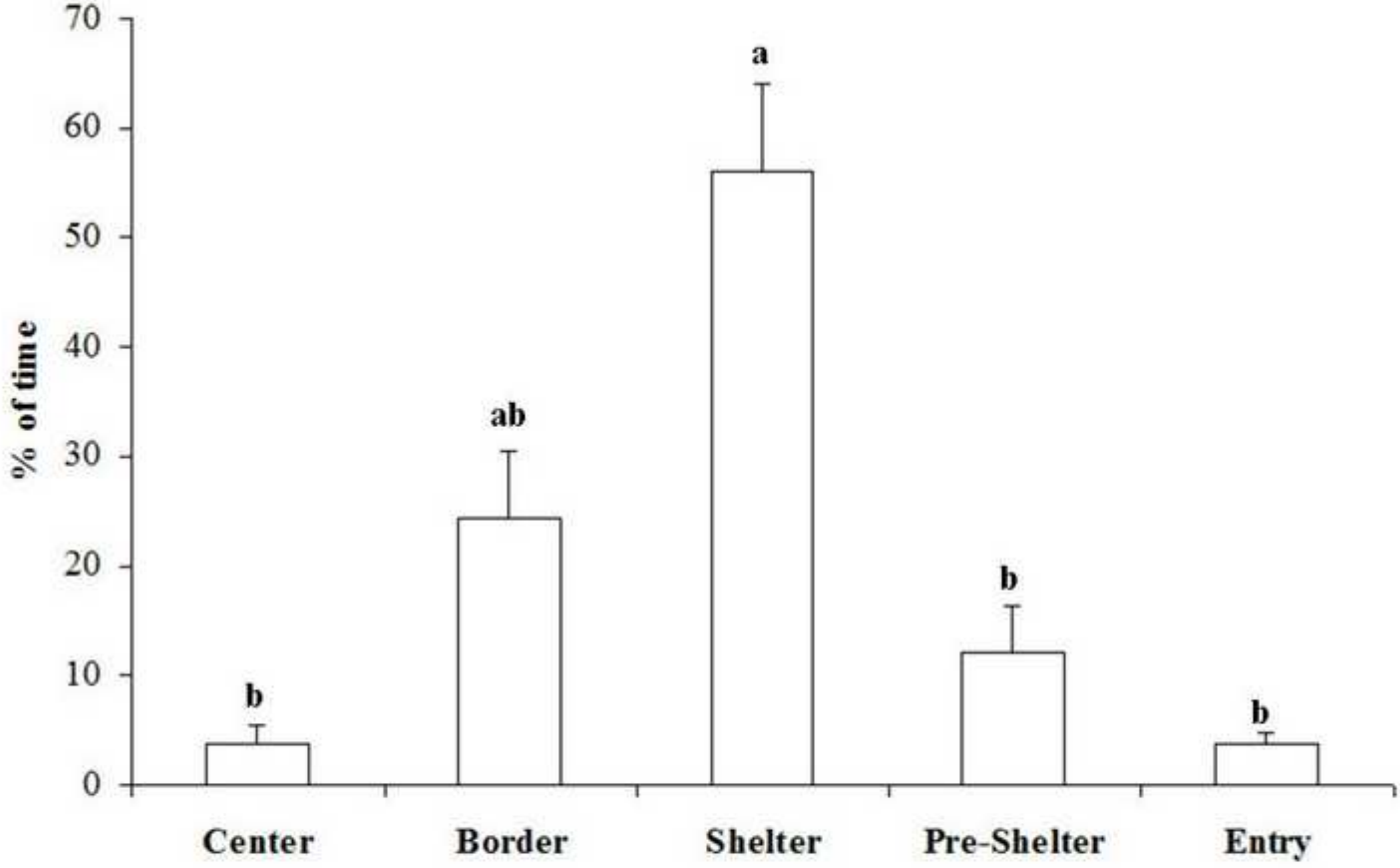
Start-Familiar-Start-Unfamiliar

Figure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Figure