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Is personality of young fish consistent through different behavioural tests?

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Keywords

Behavioural syndromes, individual personality, behavioural tests, pikeperch juveniles, Sander lucioperca.

Abstract

Most studies carried out on personality recognized that personality is defined by behavioural traits consistent through time and/or contexts. In fish, most studies on personality were performed either on juveniles (aged between 6 months and 1 year) or adults, but very few focused on the early life stages. The main goal of this study is to characterize behavioural syndromes and to highlight the existence of a personality in young juvenile pikeperch, a species with a strong economic value. To study the consistency of behavioural responses of juvenile (50 and 64 days post-hatch) pikeperch Sander lucioperca ($n = 41$, total length = 5.8 ± 1.0 cm and mass = 1.6 ± 0.7 g), we performed three tests per fish in one day: exploration (cross-maze), dyadic and restraint test. In the cross-maze test, exploratory fish were more active and bolder. In the dyadic test, fish with the highest number of contacts, showed also more approaches, orientations and avoidance behaviours. In the restraint test, bolder fish were more active and tried to escape more often. Consequently, the investigation of the different behavioural responses of each fish highlighted behavioural syndromes in this species. Furthermore, for the first time, we showed, with across-context analysis, that young juvenile pikeperch, responded in the same way to exploration and dyadic test but their responses were opposite in the restraint test. Our results opened new opportunities for testing individual personality in very young fish that may help solving some aquaculture problems, such as intra-cohort cannibalism.

1. Introduction

Animal behaviour studies have allowed establishing that individuals exhibit consistent behavioural differences (Koolhaas et al., 1999; Bell et al., 2009; Killen et al., 2016), which are described by different terms, among which personality or behavioural syndromes. Yet, these two terms are not equivalent. Personality is defined as behavioural individual differences stable over time and/or in varying environmental contexts (Réale et al., 2007). Behavioural syndrome is a suite of correlated behaviours reflecting between- individual consistency in behaviour across multiple (two or more) situations (Sih et al., 2004). A population or species can exhibit a behavioural syndrome (Sih et al., 2004).

Different personality traits are recognized in animals in relation to diverse behavioural responses of individuals within the same population and the performances of these traits followed a continuum along the personality axis. Five traits have been identified: boldness-shyness, exploration-avoidance, activity, aggressiveness, and social tolerance (Sih et al., 2004; Réale

et al., 2007; Cote et al., 2008). The boldness-shyness trait is a measure of the individual's reaction to a situation perceived as dangerous (Réale et al., 2007). Several tests exist to test boldness-shyness among which the open-field test, maze-test or novel object test (Adriaenssens and Johnsson, 2013; Castanheira et al., 2013; Ferrari et al., 2014). In fish, several kinds of measures have been used to assess boldness-shyness (Toms et al., 2010). Behavioural variables include the latency to approach a novel object (Pasquet et al., 2015), or to explore a novel environment (Fraser et al., 2001), the time to emerge from a shelter (Ferrari et al., 2014), or to respond to a frightening stimulus (Millot et al., 2009). The exploration-avoidance trait includes behaviours that involve the individual willingness to investigate novel environments, food items or objects in the absence of predators or other frightening stimulus (Conrad et al., 2011). In fish, these behaviours could be analysed by the number of explored zone in a maze (Ferrari, 2014), as well as by the latency to explore a novel space in an open-field test and by the travelled distances in an

open area (Adrianssens et al., 2012; Ferrari et al., 2014). The activity trait is measured by the number or the frequency of movement in a familiar environment or by the time spent swimming in a given area (Pasquet et al., 2015; Colchen et al., 2016). The aggression is often measured by either the defence of a territory or by food resource competition (Conrad et al., 2011). For this trait, another approach is the assessment of 'competitive abilities' (Huntingford et al., 2010) rather than a direct measure of aggression, such as the number of attacks or bites. To study aggressiveness, the mirror test is classically used (Höjesjö et al., 2011; Adrianssens et al., 2012) or direct confrontations are realized in a dyadic test (Sundström, 2004). The sociability or social tolerance trait refers to an individual's reaction towards conspecifics, which does not correspond to an aggressive interaction. In fish, sociability is classically measured by avoidance or attraction to conspecifics (Conrad et al., 2011).

In fish, most studies on personality were performed either on juveniles (between 6 months and 1 year age) or adults (Dahlbom et al., 2011). In contrast, very few studies focused on the early life stages (larval and the first juvenile's stages) (Sundström, 2004; Pasquet et al., 2015; Tudorache et al., 2015). The small size as well as the difficulty to manipulate these first developmental stages could explain why there are so few studies focusing on personality. Nevertheless,

understanding personality of the early life stages is an essential question to improve our knowledge on these stages and may contribute to solve one of the main bottlenecks in the rearing of new species, which is the intra-cohort larval cannibalism (Conrad et al., 2011; Baras, 2012; Teletchea and Fontaine, 2014), as observed in pikeperch *Sander lucioperca* (Kestemont et al., 2007).

Pikeperch has a strong economic potential for inland aquaculture diversification in Europe (Wang et al., 2009). To our knowledge, personality in larvae and juveniles of pikeperch has never been performed. To establish a personality profile, it is necessary that individuals respond consistently to different behavioural tests. To be sure that a behavioural test allows us to show personality, it is necessary to realize cross-context validations of behavioural measures (Réale et al., 2007). In our study, we aim at exploring the relationship between the fish behavioural responses, and for that we used three among the most common behavioural tests (see Conrad et al., 2011 for review). For a determined fish, the three tests (novel environment, social and net restraint) were all performed within the same day. The main goals of this study are: (i) to characterize behavioural syndromes between behaviours in each test, (ii) to highlight the existence of a personality and (iii) to realize across-context analysis of behavioural syndromes between tests in young pikeperch juveniles.

2. Material and Methods

2.1. Fish and experimental conditions

Eggs were obtained from stripping of two domesticated females reared in a fish farm (Asialor, Pierrevillers 57, France) on January 26th, 2015. After hatching (January 27th, 2015), larvae were raised at URAFPA (<http://www.urfpa.fr/>, France) in a RAS (Recirculating Aquatic System). Fish were hand fed commercial food (first with nauplii of *Artemia* (550–600 µm, Catvis, Hertogenbosch, The Netherlands) from 4 dph to 18 dph, followed by Larviva PROWEAN 100, 300, 500, 700 µm (BIOMAR", France) until 35 dph and INICIOplus 0.8 mm (BIOMAR", France) according to the protocol used by Schram and Philipsen (2003). Fish were fed seven times per day (between 8:30 to 17:30). At 44 dph (stage 2, Ott et al., 2012), 41 fish were randomly sampled (total length = 5.8 ± 1.0 cm and mass = 1.6 ± 0.7 g). After anaesthesia (Tricaine methanesulfonate (MS 222): 80 mg L⁻¹), fish were tagged with Visible Implant Elastomers (VIE tag, Northwest Marine Technologies, USA; Bell and Sih, 2007). We used two different colours (blue and pink) and tags were injected in the operculum and/or in flanks of each fish on the right or the left side. Then, fish were distributed in three 700 L tanks (13–14 fish per tank) without enrichment. Throughout the entire experiment, artificial lighting followed a 12 h light/12 h darkness cycle with light on from 08:00 to 20:00 and a flow rate of 100L h⁻¹. Water temperature was maintained at 20 ± 0.3 °C. The physico-chemical properties of the water were monitored twice per week (dissolved oxygen: 7.66 ± 0.35 mg L⁻¹; ammonia 0.16 ± 0.09 mg L⁻¹; nitrite: 0.02 ± 0.02 mg L⁻¹). Tanks were cleaned every day in the morning.

2.2. Characterization of behaviours

All behavioural tests were carried out on juveniles (N = 41) aged between 50 and 64 dph (stage 2, Ott et al., 2012). For

each 700 L tank, fish were caught, identified and isolated in a bucket, one hour before the first test. Then each fish was submitted to the three tests during the same day: cross-maze test, dyadic test and net restraint test. Exploration and restraint test were performed randomly and dyadic test was always performed the last one. All tests were executed in a dedicated room separate from the rearing tanks. The water in the test devices was changed between each fish. Tests were video recorded (Sony HDR-CX550VE). All variables in the three tests were measured with continuous focal observations considering all the recording period (30 min) except for the swimming activity that was recorded during three time periods of two minutes. Behavioural data were obtained with the Observer XT software (Noldus, The Netherlands, version 10.0). After the tests, all fish were euthanized by over-anesthetizing them in MS 222 bath (240 mg L⁻¹).

2.2.1. Behaviours in the cross-maze test

Swimming activity, exploration and boldness were evaluated using a cross-maze test. The cross-maze (16 cm × 5 cm with 2.5 cm of water) was divided into five zones (Fig. 1), and placed on a translucent table with a light below. Fish were tested one by one. Each fish was placed in the acclimatization zone (7 cm × 5 cm) separated from the maze by a vertical divider. After a five-minute acclimatization period, the divider was opened and fish behaviour was video recorded for 30 min. If a fish did not go out of the acclimatization zone (AZ) during the 30 min' period, a latency of 1800s was attributed. For exploration and boldness, three variables were analysed: the individual latency to emerge from the acclimatization zone LE (in s), the time spent outside the acclimatization zone TOAZ (in s) and the total number of visited zones TNVZ (for definitions see Table 1). They were analysed over the whole 30 min period. Swimming activity SA (for definition see Table 1) was calculated over three periods of time: from the 5th to

the 8th minute, from the 15th to the 18th minute and from the 25th to the 28th minute (adapted from Pasquet et al., 2015).

2.2.2. Behaviours in the dyadic test

Dominance relationships between fish were evaluated with a dyadic test. Tests were carried out in an aquarium (20 cm × 12.7 cm × 20.4 cm with 13 cm of water) divided into two zones separated by a divider. One fish was placed in each zone. After 15 min, the divider was removed and fish were left together for 30 min. Four behaviours were noted throughout the entire 30 min period of observation: the number of orientations O, approaches App, avoidances Av and physical contacts C (for definitions see Table 1).

2.2.3. Behaviours in the restraint test

A restraint test was used to evaluate the behavioural responses of fish to a stressful situation. Just before running the test, fish were placed in an emerged net (15 cm × 13 cm × 9 cm) fixed on an aquarium (filled with five cm of water in case of the fish jumped out of the net) for a period of two minutes. Individual behaviour was analysed and individual variables of interest were calculated for the whole duration of the two-minute test. The three behaviours analysed throughout the entire duration of the test were: the latency (in s) before the first escape attempt, the number of escape attempts and the total escape duration (in s). An escape attempt was defined as an elevation of the body in the net (Ferrari et al., 2014a) (Table 1).

3. Results

3.1. Cross-maze test

All significant correlations between behavioural variables are presented in Fig. 2. Fish that took a longer latency to emerge from the acclimatization zone showed significantly a lower number of visited zones (Fig. 2A). Furthermore, latency was neither correlated to swimming activity ($r_s = -0.08$; p -value = 0.6) nor with time spent outside the acclimatization zone ($r_s = 0.01$; p -value = 0.9). However, the time spent outside the acclimatization zone was significantly positively correlated with swimming activity (Fig. 2B) and with the total number of visited zones (Fig. 2C). The total number of visited zones was also significantly positively correlated with swimming activity (Fig. 2D).

3.2. Dyadic test

For dyadic test fish, those that approached more congeners, did more orientations ($r_s = 0.54$; p -value = 0.0003), and also had more physical contacts with them ($r_s = 0.43$; p -value = 0.005). Consequently, approach behaviour vs physical contacts and approach behaviour vs orientation behaviour were positively correlated. Furthermore fish, which oriented themselves more towards congeners, had more physical contacts with them ($r_s = 0.48$; p -value = 0.0013).

3.3. Restraint test

2.2.4. Statistical analyses

Normality and homogeneity of variance for all data were tested on residuals, with the Shapiro-Wilk method for normality and Levene method for homogeneity of variance. Non-parametric tests were performed due to the non-normality of data. Mean, standard deviation (SD), median, first and third quartiles were calculated for each variable (Table 2).

The statistical analysis was conducted in three steps. First, we characterized the personality traits of fish in each of the three tests looking for behavioural syndromes between traits with correlations

between each variable within each test. For each behavioural test, a Spearman correlation was performed between all variables analysed during videos. Second, we highlighted personality of fish between the three tests. To do that, a Principal Component Analysis (PCA) was realized with all variables of the three tests. In the PCA, only principal components of the two first axes (Eigen values superior to 1) were considered for further analyses. For each PCA, we gave the percentage of variance of each variable on the first and second axis. Third, we realized a cross context consistency analysis, with Spearman correlations between variables of the three tests to determine whether behavioural syndromes exist between tests. All the statistical analyses were performed with R (version 3.2.3) and results were presented as mean ± SE and significant at $P < 0.05$.

All significant correlations are presented in Fig. 3. The total escape duration was significantly positively correlated with the number of escape attempts (Fig. 3A), and was longer for individuals that tried to escape more. This was due to the number of an increase of escape attempts rather than the mean duration of escape attempts. Furthermore, Latency before the first escape attempt was significantly negatively correlated to the total escape duration (Fig. 3B) and the total number of escape attempts (Fig. 3C). Thus, fish waiting more before their first escape attempt, had lower escape attempts and consequently a shorter escape duration.

3.4. Characterization of personality and cross context analysis

The first two axes of the PCA represented around 50% of the total variance (First axis = 33.6%, Second axis = 16.4%, Fig. 4). On the first axis (Eigen Value = 3.69), all behavioural variables of the cross-maze test (TNVZ, SA, TOAZ) and the dyadic test (O, App, C) were significantly positively correlated to the first axis, excepted for the latency to emerge from the acclimatization zone (LE), which was significantly negatively correlated to the first axis (Table 3). Furthermore, for the restraint test, the latency before the first escape attempt (L1E) was significantly negatively correlated to the first axis (Table 3) and escape duration was positively correlated (Table 3). The second axis (Eigen Value = 1.8) represented chiefly the restraint test variables (NE (28%), L1S (25%), and ED (14%) of the total variance respectively) (Fig. 4). Latency before the

first escape attempt was positively correlated with the second axis ($r = 0.67$; p -value < 0.0001 ; Table 3), but the number of escape attempts, and the total escape duration were negatively correlated to the second axis ($r = -0.7$; p -value < 0.0001 and $r = -0.5$; p -value < 0.001 , respectively; Table 3). In the PCA, there was an important variability in the individual contribution to the total variance (from 0% to 26%). This implied that some fish exhibited a great variation in their behavioural responses in the restraint test.

Spearman correlations between the three behavioural tests (cross-context consistency analysis) gave few significantly

4. Discussion

The aim of this study was to characterize behavioural syndromes in very young pikeperch juveniles and to investigate the existence of personality. The comparison of the different behavioural responses from each fish in three common behavioural tests allowed us to assess behavioural syndromes. In cross-maze test, the exploratory fish were more active and bolder. In dyadic test, fish with the highest number of contacts, had also more approaches, orientations and avoidance behaviours. In restraint test, the bolder fish were more active in the net. However, when we realized across-context analysis, we found that there were two types of response in function of the tests. One of the most important results of the present study was that young pikeperch juveniles in exploration test and in dyadic test respond in the same way but their behavioural responses were opposite in the restraint test.

These results imply that first, behavioural syndromes appeared very early in the fish life. Few studies showed the existence of behavioural syndrome in first life stages and highlight personality (Pasquet et al., 2015; Tudorache et al., 2015). Considering the results of each test, we found behavioural syndromes in accordance with the literature. For exploration test, all variables were correlated and only the latency to emerge from the acclimatization zone was negatively correlated to the others. Fish with a high latency to emerge explored less (shorter period of time outside the acclimatization zone, less visited zones, less active). The exploration test is classically performed in several fish families, among which Clariidae, Cottidae, Cyprinidae or Centrarchidae (see Conrad et al., 2011 for review). However, conclusions on exploration test are often various and may be species and/or context-specific (Conrad et al., 2011). It would be interesting to test several fish families in the same exploratory test whether it is species-dependant or context-specific. In restraint test, individuals showing lower latency to escape, showed higher number of escape attempts and spent more time escaping (see Castanheira et al., 2013). Escaping behaviour during restraint test or confinement has been used to discriminate personality in other animals, such as pigs (Ruis et al., 2000; Bolhuis et al., 2005) as well as in fish (Silva et al., 2010; Castanheira et al., 2013; Ferrari et al., 2014, 2015; Magnhagen et al., 2015). In fish studies as in our work, individuals, which attempt to escape more often, showed a bolder profile [in senegalensis sole *Solea senegalensis* (Silva et al., 2010); in seabream *Sparus aurata* (Castanheira et al., 2013); in seabass *Dicentrarchus labrax* (Ferrari et al., 2014); in arctic charr *Salvelinus alpinus* (Magnhagen et al., 2015)]. In their study, Castanheira et al. (2013) studied five behavioural

results. Between the exploration test and the dyadic test, approach behaviour was significantly positively correlated to time spent outside the acclimatization zone ($r_s = 0.34$; p -value = 0.03) and to the total number of visited zones ($r_s = 0.32$; p -value = 0.04). The total number of visited zones was positively correlated with the number of physical contacts ($r_s = 0.39$; p -value = 0.01) and to the orientation behaviour ($r_s = 0.31$; p -value = 0.04). Between the exploration test and the restraint test, the time spent outside the acclimatization zone was significantly positively correlated to the escape duration ($r_s = 0.34$; p -value = 0.03). Moreover, the latency to emerge from the acclimatization zone and the latency before the first escape attempt were not correlated (Fig. 5).

tests in seabream and classified the restraint test as the most repeatable in time, and also, the most repeatable test through fish species. In fact, species cited above and pikeperch respond always in the same way to restraint test.

Furthermore, the correlation between behavioural responses in restraint or confinement tests with those in other behavioural tests is largely documented: with hypoxia test (Castanheira et al., 2013), with open-field test (Ferrari et al., 2014), with feeding test (Øverli et al., 2007). Consequently, we attempt to find an obvious relationship between behavioural variables of restraint test and the two other tests (exploration and dyadic tests). First, between exploration test and restraint test, we found only a positive correlation between the time spent outside the acclimatization zone and the escape attempt duration. This correlation allowed us to classify as bolder the individuals that had a higher score in those two tests. Individuals with a passive response during the restraint test tended to be shy during the exploration test as in the seabass (Ferrari et al., 2014). A positive correlation between latency to emerge of shelter and latency before the first escape attempt has already been found (Ferrari et al., 2014). In our study, we found no correlation between these two variables. We could explain this result by the design of our acclimatization zone in the exploration test. In Ferrari et al. (2014), this zone was a real shelter totally closed and opaque (protected from light), contrary to our test where the acclimatization zone was opened and not protected from light and so could not be considered as a refuge for the fish. In our study, we showed that there was no clear link between exploration and restraint test. Pikeperch is a species that shows a very high intra-cohort cannibalism rate (Molnár et al., 2004; Kestemont et al., 2007). Consequently, pikeperch exhibited some behaviours during cannibalism (attacks, bites), which are the same as during aggressive attacks. It has been shown that fish, which were more aggressive when being placed in a social dominant contest, had lower cortisol responses to a confinement test (Øverli et al., 2004). As more active fish in restraint test have also lower cortisol rate (Magnhagen et al., 2015), we could expect that it exists a behavioural syndrome between dyadic test and restraint test in pikeperch. In juvenile pikeperch, we found no significant correlation between these two tests. This lack of correlation could be first explained by the design of our dyadic test. Conrad et al. (2011) found that fish aggression was often measured in territory defence or in feeding contests, which was not the case in our study. Second, the number of fishes that become dominant depends mainly on the difference in boldness rank between the two fish in the pair (Sundström, 2004). In our study, fish used in the dyadic test came from the

same tank. Consequently, we could hypothesize that a dominance relationship was already established between the two fish that could explain why there was no aggressive behaviour (attack, bite) between them but just few orientations, approaches, physical contacts, and avoidance behaviour. Under our dyadic context, fish did not need to show aggressive behaviour to establish the status between the two individuals, which could explain the lack of correlation. Nevertheless, between exploration test and dyadic test, PCA results showed a high positive correlation between the different variables. We can conclude that exploratory fish were more aggressive in dyadic test that confirm the correlation between exploration/boldness and dominance social rank (Sundström, 2004).

Each test allows us to show behavioural syndromes but there was no cross-context validation between the three tests. Exploration and dyadic tests resulted in the same conclusions, but responses in the restraint test were totally opposite. Responses observed in restraint test are similar to other studies (Castanheira et al., 2013; Magnhagen et al., 2015) and authors conclude that bold individuals showed more escape attempts during net restraint compared to shy individuals (Magnhagen et al., 2015). Consequently, it remains unclear why no cross-context consistency was observed in pikeperch for restraint test compared to the others. Other studies, also, did not show clear cross-context consistency between responses to behavioural tests (see: Coleman and Wilson, 1998; Ferrari et al., 2015). Coleman and Wilson (1998) showed that individuals were consistent for two traits (exploratory and risk-taking), but that the individual's exploration activity is not predictive of risk taking. In our study, the most active individuals in exploration test or in dyadic test were also individuals more active to escape from the net but behavioural responses in exploration test or in dyadic test cannot predict results in restraint test. This result could be due to the high intra-individual variability observed and may support a lack of consistency in cross-context analysis (Réale

et al., 2010; Ferrari et al., 2015). Behavioural individual plasticity was highly context-specific. Exploration was quantified with the time spend to explore, the swimming activity or the number of visited zones. Consequently, this test could be more dependent of the physical strength of the larvae (Pasquet et al., 2015) than the resistance to a stress situation as in the restraint test (Magnhagen et al., 2015). Confinement or restraint test is commonly used for quantifying stress response, especially in fish (Cockrem, 2013).

The fact that the behavioural traits in exploration test and in restraint test did not covariate implies that plasticity between these traits is not limited. It means that individual variations of these traits are adaptive and allow animals to behave in an optimal fashion in all situations (Conrad et al., 2011).

In conclusion, our study highlighted the possibility to discriminate behavioural syndromes in young juveniles of pikeperch. This result opens new opportunities for testing individual personality in the early life stages where there are major bottlenecks during rearing period, such as growth heterogeneity, high deformity rate and a very high mortality rate due to cannibalism (up to 29% in that species, Kestemont et al., 2007). Several studies worked on the impact of environmental (light, temperature, turbidity of water), nutritional (composition of food, quantity of food) and populational factors (density, dominance hierarchy) on the intra-cohort cannibalism rate (Smith and Reay, 1991; Hecht and Pienaar, 1993; Baras, 2012). Even though the rate of cannibalism decreased, none of these studies succeeded to stop cannibalism. Consequently, in captive populations, cannibalism seems to be more explain by individual characteristics. As the personality is also an individual behavioural characteristic, trying to establish a relationship between personality and cannibalism could be a promising way to answer the question why some larvae and juveniles of pikeperch become highly cannibals under rearing conditions.

Table 1. Description of behaviour variables analysed in the three behaviour tests. All these variables were used in PCA. Abbreviations used in the PCA are also described.

Cross-maze test	<p>Latency to emerge from the acclimatization zone (LE): time before going out of the acclimatization zone</p> <p>Time spent outside the acclimatization zone (TOAZ): sum of time spent in all zones except the acclimatization zone.</p> <p>Total number of visited zones (TNVZ): sum of number of visited zones during all time periods analysed.</p> <p>Swimming activity (SA): time when fish was active</p>
Dyadic test	<p>Approach (App): the movement of the focal fish towards the other fish with slow swimming</p> <p>Physical contact (C): a collision between two individuals, which could be a frontal or a lateral collision (Colchen et al., 2016)</p> <p>Orientation (O): the focal fish turned its body head-first towards the conspecific and visually tracked it (Bell and Sih, 2007)</p> <p>Avoidance (Av): when a congener approach, the focal fish go away</p>
Restraint test	<p>Escape duration (ED): sum of durations of each escape attempt.</p> <p>Number of escape attempts (NE): total number on two minutes' period of escape attempt.</p> <p>Latency before the first escape attempt (L1E): time before the first escape attempt</p>

Table 2. Mean \pm SD, Median, First and Third quartile of each behaviour variables tested in the three tests: cross-maze test, dyadic test and restraint test. For abbreviation see Table 1.

Behaviour variables	Mean	Standard deviation	Median	First quartile	Third quartile
App	1.08	1.71	0	0	1
C	0.25	0.62	0	0	0
Av	1.72	2.64	1	0	2
O	1.08	1.70	0	0	1
ED	18.15	8.51	18	11	23
NE	16.13	6.04	17	12	20
L1E	8.95	8.38	6	3	13
LE	522.85	536.05	470	4	801
TOAZ	613.05	516.72	769	0	1108
TNVZ	211.88	210.08	185	5	372
SA	232.85	194.98	315	29	377

Table 3. Correlation table of PCA analysis. For abbreviation see Table 1.

Behaviour variables	Axis 1		Axis 2	
	rho	p-value	rho	p-value
Empty Cell				
TNVZ	0.88	<0.0001	–	–
SA	0.85	<0.0001	–	–
TOAZ	0.76	<0.0001	–	–
LE	–0.42	0.006	–	–
O	0.61	<0.001	–	–
App	0.60	<0.001	–	–
C	0.58	<0.001	–	–
Av	–	–	0.63	<0.001
NE	–	–	–0.71	<0.001
L1E	–0.33	0.03	0.68	<0.001
ED	0.45	0.003	–0.51	<0.001

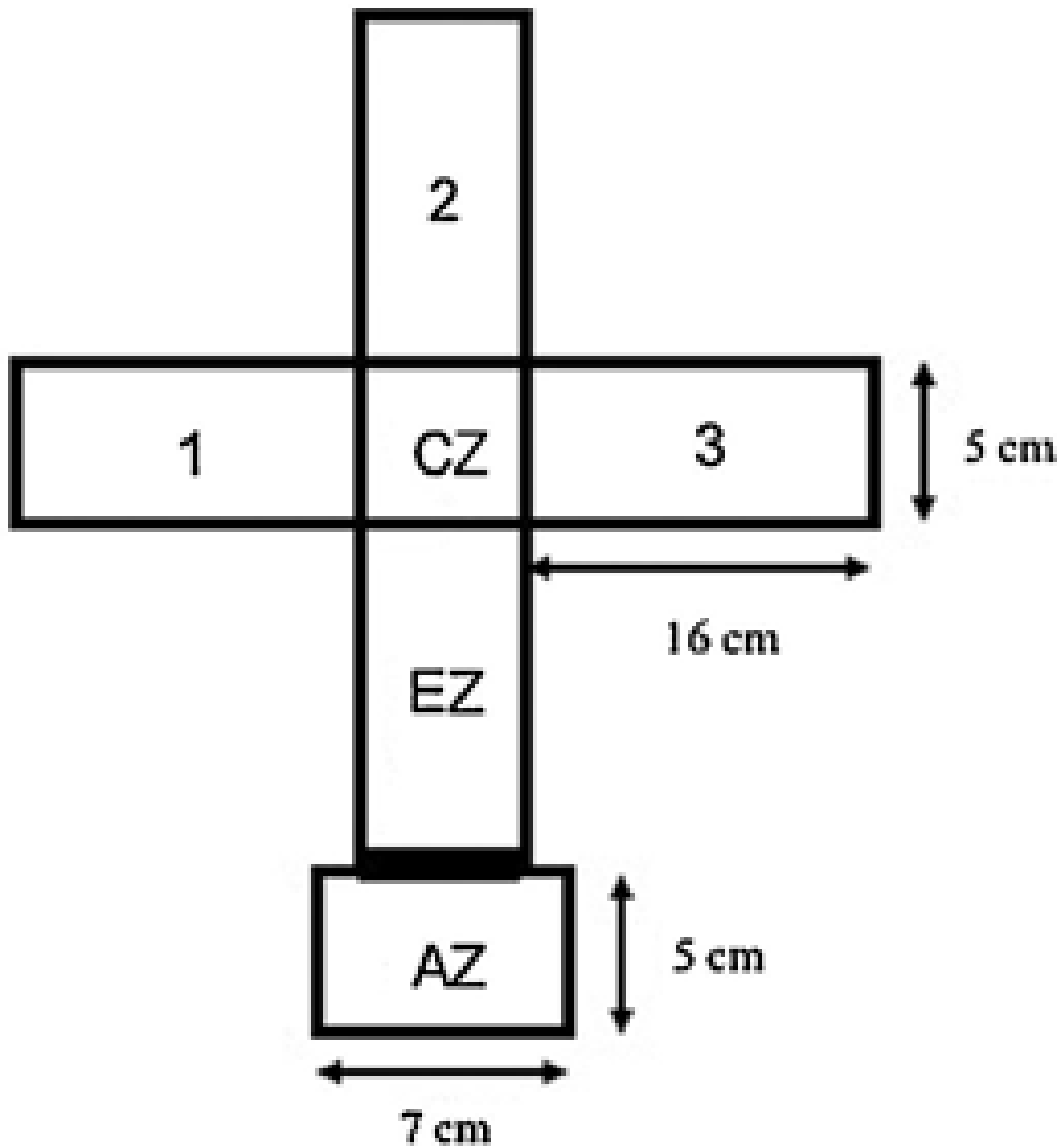


Figure 1. Scheme of the device used for cross-maze tests. Maze was divided in six zones: acclimatization zone (AZ), entry zone (EZ), central zone (CZ) and three others zones (1,2,3). Between AZ and EZ, there was a removable divider.

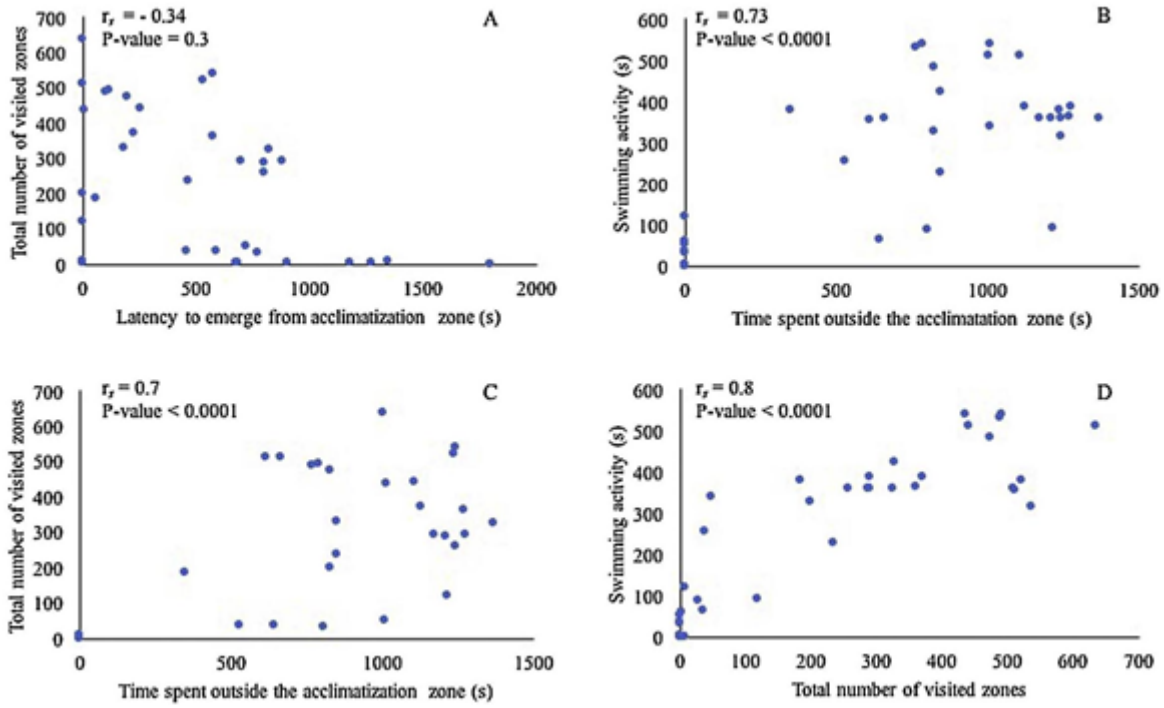


Figure 2. Significant Spearman correlations between each variables of cross-maze test. The Rho-value (r_s) and p-value are indicated. Each point represents a tested fish.

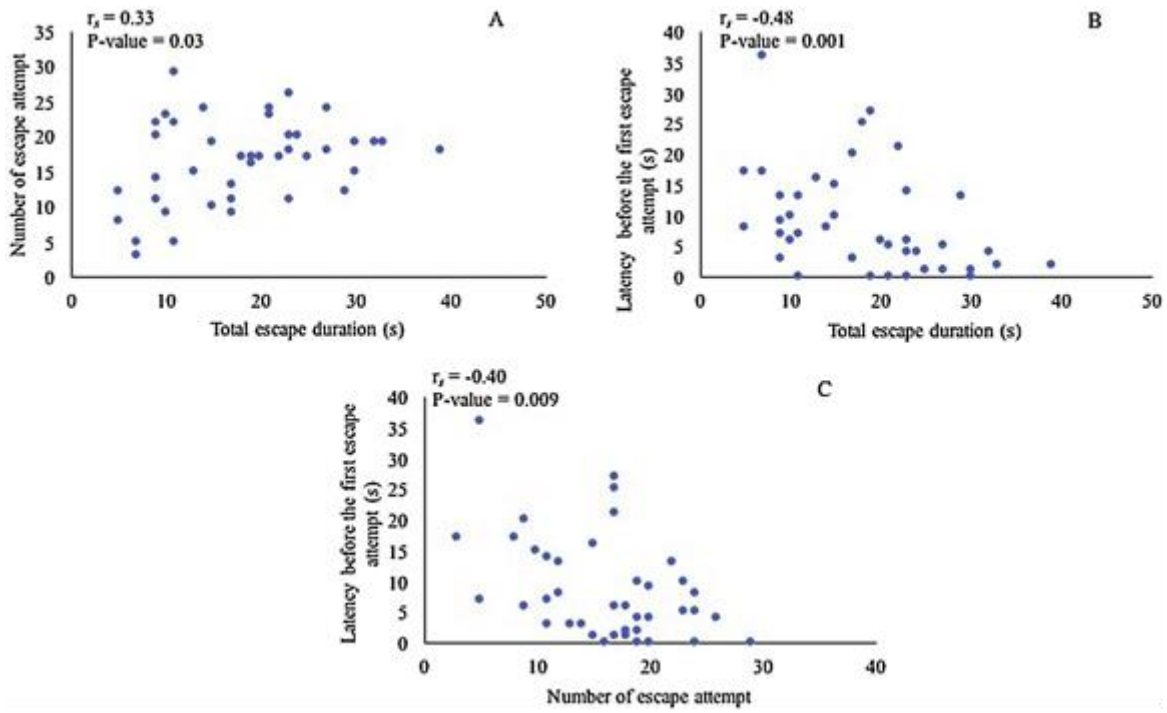


Figure 3. Significant Spearman correlations between each variables of restraint test. The Rho-value (r_s) and p-value are indicated. Each point represents a tested fish.

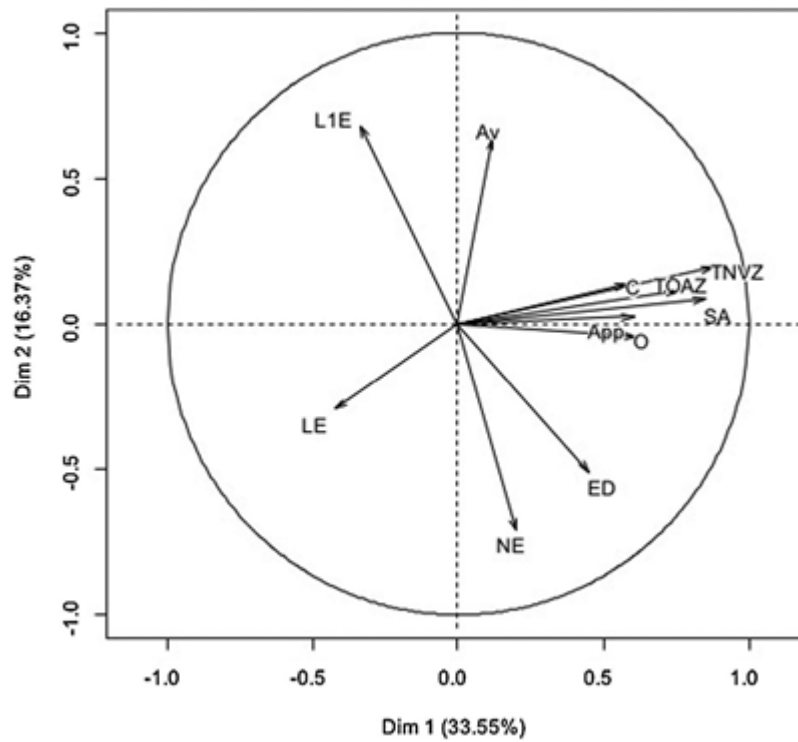


Figure 4. PCA realized with 11 behavioural variables of the three tests (cross-maze test, dyadic test, restraint test). For cross-maze test: LE: Latency to Emerge from the acclimatization zone, NVZ: Number of Visited Zones, TOAZ: Time spent Outside of the Acclimatization Zone, A: Activity; for dyadic test: App: Approach, O: Orientation, Av: Avoidance, C: Contact; and for restraint test: NE: Number of Escape attempt, ED: total Escape Duration, L1E: Latency before the first Escape attempt.

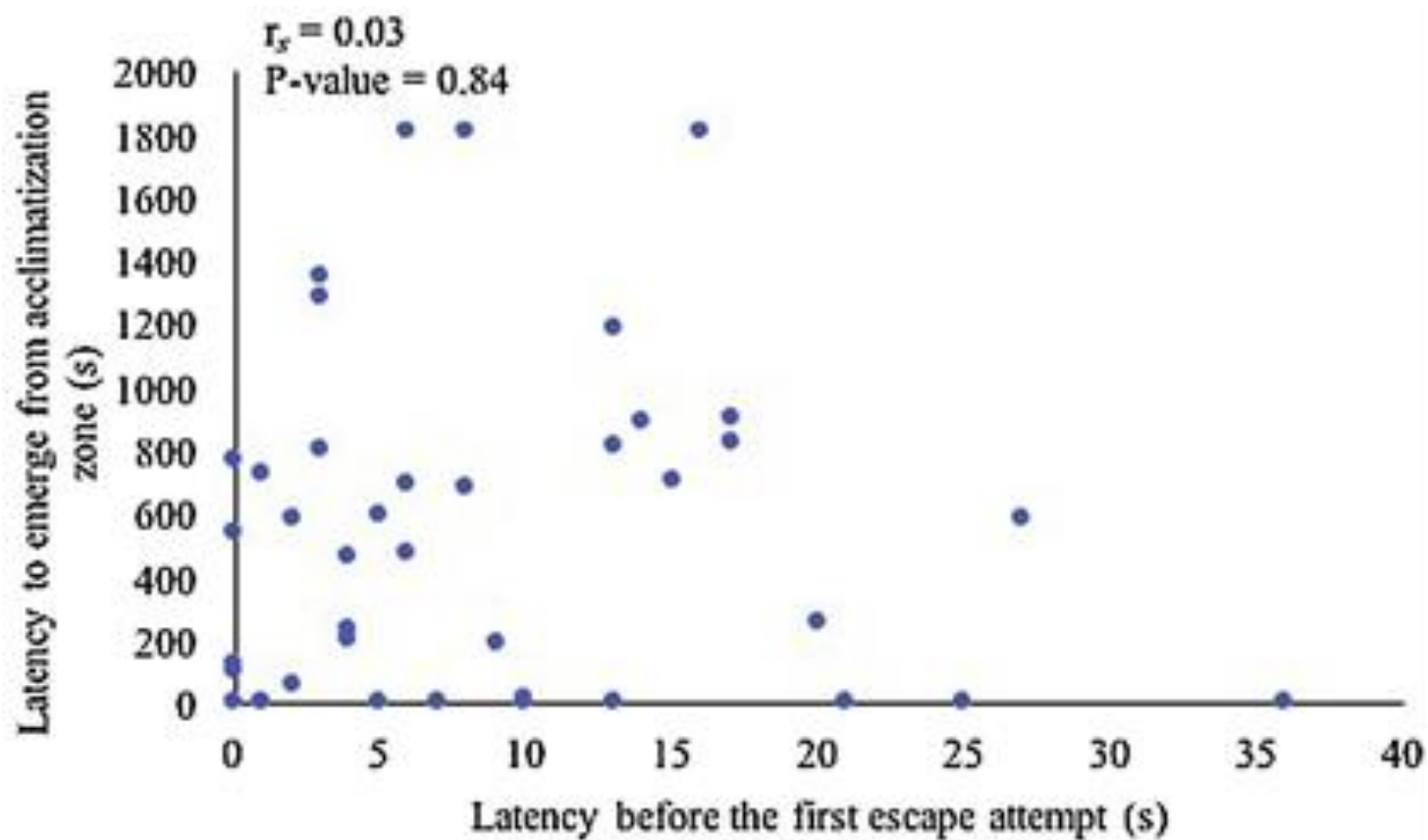


Figure 5. Spearman correlation between latency to emerge from the acclimatization zone from cross-maze test and latency before the first escape attempt from restraint test. Rho-value (r_s) and p-value are indicated. Each point represents a tested fish.

5. References

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