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## First insight into personality traits in Northern pike (*Esox lucius*) larvae: a basis for behavioural studies of early life stages

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### Abstract :

Personality traits have been studied for some decades in fish species. Yet, most often, studies focused on juveniles or adults. Thus, very few studies tried to demonstrate that traits could also be found in fish larvae. In this study, we aimed at identifying personality traits in Northern pike (*Esox lucius*) larvae. Twenty first-feeding larvae aged 21 days post hatch (16.1 +/- 0.4 mm in total length, mean +/- SD) were used to establish personality traits with two tests: a maze and a novel object. These tests are generally used for evaluating the activity and exploration of specimens as well as their activity and boldness, respectively. The same Northern pike twenty larvae were challenged in the two tests. Their performances were measured by their activity, their exploratory behaviour and the time spent in the different arms of the maze or near the novel object. Then, we used principal component analysis (PCA) and a hierarchical ascendant classification (HAC) for analysis of each data set separately. Finally, we used PCA reduction for the maze test data to analyse the relationship between a synthetic behavioural index (PCA1) and morphometric variables. Within each test, larvae could be divided in two sub groups, which exhibited different behavioural traits, qualified as bold (n = 7 for the maze test and n = 13 for the novel object test) or shy (n = 9 for the maze test and n = 11 for the novel object test). Nevertheless, in both tests, there was a continuum of boldness/shyness. Besides, some larvae were classified differently between the two tests but 40 % of the larvae showed cross context consistency and could be qualified as bold and/or proactive individuals. This study showed that it is possible to identify personality traits of very young fish larvae of a freshwater fish species.

**Keywords :** Behavioural tests, Activity, Exploration, Boldness/shyness continuum, Freshwater species

## 42 **Introduction**

43 For more than twenty years, many studies focusing on behavioural variations have been conducted,  
44 and a new concept has emerged: animal personality, which is unique to each individual (Réale et al.  
45 2007; Toms et al. 2010; Conrad et al. 2011; Nakayama et al. 2012). Personality can be defined as a  
46 collection of behavioural traits, which are consistent both over time and in different environmental  
47 conditions (Gosling 2001; Dall et al. 2004; Castanheira et al. 2013). Nevertheless, this does not  
48 imply that individual behavioural traits do not evolve over time, but rather that the combination of  
49 them is rather constant whatever the period of life. Likewise, even though personality of fish is  
50 consistent (e.g., a “shy fish” will remain “shy”), it can be shaped differently according to  
51 environmental factors, such as water temperature (Biro et al. 2010), predation pressure (Brown et al.  
52 2005; Bell and Sih 2007), food predictability (Chapman et al. 2010) or life history traits (Biro and  
53 Stamps 2008; Adriaenssens and Johnsson 2011). From these studies, personality can be considered  
54 as an individual variable; it is a resultant behavioural characteristic, which could be correlated with  
55 other individual variables (morphology, genetic origin) and compared between different  
56 environmental situations.

57       Classically, five main behavioural traits are considered to establish behavioural personality  
58 in fish: activity, boldness, exploration, aggressiveness and sociability (Réale, et al. 2007). For fish,  
59 activity is defined by several variables linked to fish movements: type and characteristics of  
60 swimming (speed, angular speed) and distances covered (Norton et al. 2011). Second, boldness (or  
61 its opposite shyness) (e.g. Dyer et al. 2009) is defined as the propensity to take risks by exploring  
62 new environments and consequently taking more risks of being predated. Boldness can be tested  
63 with a lot of devices in different situations. For example, fishes can be placed in front of new types  
64 of food (Höjesjö et al. 2011) or new objects (Norton et al. 2011). The performances, such as the  
65 latency time for taking new food or for exploring the new object, are then measured. Boldness can

66 also be evaluated by the reactions of fish facing a novel environment (Norton et al. 2011); in this  
67 case special devices are used such as mazes. Besides, boldness can also be measured under stressful  
68 conditions, such as in face of a predator or a lure of predator (Moretz et al. 2007). Third, exploration  
69 is the ability of a fish to discover a new environment. It is tested by using different devices (mazes,  
70 open field). Fourth, aggressiveness is defined as the involvement in fights or aggressive interactions  
71 with congeners (e.g., Norton et al. 2011). Introducing two fish in the same space (or separated by a  
72 glass) can be used for testing aggressiveness; interactions between fishes may be characterized by  
73 aggressive behaviours, such as chases, attacks or bites (Biro et al. 2010); it can also be done using a  
74 mirror (Gerlai et al. 2000). Fifth, sociability corresponds to the interactions of an individual within a  
75 social group and its relationships with its conspecifics (e.g. Cote et al. 2010). Inter-individual  
76 distances and interactions are often used as proxies for sociability (Cote et al. 2010).

77 Most studies on fish personality have been performed either on juveniles (Westerberg et al.  
78 2004) or adults (Dahlbom et al. 2011), whereas very few studies focused on early life stages.  
79 Sundström et al. (2004) realized one of the few complete studies on larvae, yet in this case, the trout  
80 *Salmo trutta* fry have a relative large size at hatching (more than 15 mm). In general, the tiny size  
81 and the fragility of larvae are two reasons explaining why there are so few studies focusing on the  
82 personality traits of these stages. However, such knowledge at these early life stages may be  
83 important both for understanding the dynamic of recruitment of fish population in wild conditions  
84 and the management of farmed fish by identifying particular individuals that could be strongly  
85 aggressive or potentially cannibals under rearing conditions.

86 The Northern pike *Esox lucius*, is a solitary species at adult stage, which inhabits lakes,  
87 ponds and also rivers with weak current, in Eurasia and North America (Kottelat and Freyhof  
88 2007). The larvae are about 9 mm at hatching (Teletchea et al. 2009; Parlier and Corolla 2013;  
89 Trabelsi et al. 2013). The diet of the larvae is based mainly on zooplankton, and both juveniles and

90 adults are chiefly piscivorous (Kottelat and Freyhof 2007). Northern pike is a diurnal predator using  
91 vision as the main sense to localize its prey; cannibalism in larvae can appear within few days after  
92 first feeding and not only as juveniles (one month after hatching) (Giles et al. 1986, Kucharczyk et  
93 al. 1997). There is only one study on the existence of behavioural syndromes defined as a suite of  
94 correlated personality traits (Sih et al. 2004) in pike: they found that personality existed in young-  
95 of-the year pikes (47-74mm fork length) across three contexts (activity in presence of a competitor,  
96 exploration of a novel environment and boldness under predation risk) but there were no temporally  
97 personality traits (Nyqvist et al. 2013). Until now, most of the explanations of cannibalism in fish  
98 larvae are based on size heterogeneity whatever the origin of this heterogeneity (growth  
99 heterogeneity, environmental factors (quantity of food, density of larvae, luminosity, water  
100 turbidity) (for review see Baras 2012)). If these studies furnished a lot of information on the  
101 physiological origins of cannibalism, they did not answer to the question: why does a larva become  
102 cannibal? The study of the larva personality could be an answer to this question.

103         The goals of the present study were to develop a method for sorting pike larvae according to  
104 their personality traits. This classification could be used for ranking fish larvae according to their  
105 behavioural performances. Such a classification requires (1) identifying different behavioural  
106 responses, (2) testing the variability of these behaviours, and (3) dividing the studied population  
107 into groups separated by their behavioural traits.

108

## 109 **Material and methods**

### 110 Rearing conditions

111 Eggs were obtained from wild adults caught in ponds located in the Lindre Centre (Moselle, France,  
112 (L: 48.799, l: 6.747). Sperm of four males was pooled for fertilizing oocytes of one female, so  
113 tested fish could be siblings or half-siblings. Eggs were incubated at 11°C. After hatching, larvae

114 were maintained in small nets (15x15x10cm) (n=15; 80-100 individuals per net) in a large tank at a  
115 temperature between 11°C and 13°C. larvae were collected at the beginning of the hatching period  
116 and there was no age difference between them. During the period of larval rearing, a photoperiod of  
117 12 hours (day light from 8:00 to 20:00 h) was applied; the luminosity in the room was set at  
118 100 Lux. The air temperature of the rearing room was set at 13°C ( $\pm$  2°C). Water pH was stabilized  
119 at 7.4. Food (*Artemia nauplii*) was distributed *ad libitum* from 9:00 to 16:30 hours starting at 10  
120 days post hatching (dph), that was the beginning of the exogenous feeding period, and was  
121 completed during five days with food granulates (100  $\mu$ m, Biomar, Brande (Danemark), chemical  
122 composition: NFE 14%, protein 58%, lipids 12%, Vitamin A (IU Kg<sup>-1</sup>) 17500 , DHA (g kg<sup>-1</sup>) 11,  
123 EPA (g kg<sup>-1</sup>) 9). After five days of co-feeding, a larva received only granulates.

124

#### 125 Tests

126 Three personality traits were studied: activity, exploration and boldness. The behavioural  
127 performances of fish were evaluated using two devices: the maze and the novel object. For the two  
128 tests, we used a luminous table with a low light (less than 100 Lux); the different devices (maze and  
129 small aquariums for the novel object test) had a translucent bottom and all behaviours were  
130 recorded via a camera (Sony Handycam DCR-SR72E) set just above the device. For each test, the  
131 device was filled with water from the hatching room to keep the same water quality. In order to  
132 avoid differences in temperature between the water of the device and those of the hatchery, we  
133 maintained the temperature of the test room at 13°C. During the tests, the water temperature was  
134 monitored in all devices. For both tests, the same twenty larvae were used. Larvae were between 18  
135 and 28 dph. We tested two larvae per day; for each larva, the order of the test was randomly  
136 distributed. Each larva was thus evaluated in the two tests during the same day; so we had to take  
137 the larva from one device after the first test and place it into an individual holding rack in the  
138 hatching room before the second test was conducted.

139

#### 140 Maze test

141

142 We used a plus maze (Fig.1A); the size of each arm (16x7x3 cm) was defined in order to fit  
143 with the size and the swimming abilities of larvae. At the extremity of one arm, we put a small box  
144 (9x7x3 cm) as a start box, for acclimating the larva to its new environment. This start box was  
145 separated from the first arm by a removable wall, which we removed after five minutes, which was  
146 typically (Ninkovic and Bally-Cuif 2006) used in such experiments. The larvae were allowed to  
147 swim freely in the maze during 30 min. For video analyses, we divided the maze into five zones  
148 (the four arms and the square part in the middle).

149

#### 150 Novel object test

151

152 To evaluate this test, we made two small aquaria (19.5x5x4.5 cm) stuck together by an  
153 opaque common wall: each aquarium was divided into two unequal compartments by an opaque  
154 divider (fig. 1B). This device allowed us testing two fish at the same time. Before the beginning of  
155 the test, we introduced a steel nut in the separated novel object compartment. At the beginning of  
156 the test, each larva was put into each aquarium and the removable divider was maintained in place.  
157 After 15 min, the divider was removed and the larva was free to explore the novel object in their  
158 environment during 30 min. For video analyses, the aquaria were divided into two equal zones (Fig.  
159 1B).

160

#### 161 Video data collection

162

163 We used the software “The Observer XT” (Noldus, version 10.0) to analyse the data. From  
164 preliminary observations, we had defined the relevant behaviours (see below) exhibited by the

165 larvae. We encoded all behaviours (variables of interest, Table 1), and measured the time laps  
166 between them. For each test, we chose to analyse six periods of two minutes separated by a period  
167 of three minutes for video of the twenty larvae. By comparing such sampled analysis with the  
168 analysis of an entire video recording, we found that this method allowed us reducing time without  
169 losing behavioural information.

170

#### 171 Behaviours

172 In the two tests, we considered fish activity by measuring the time spent swimming or immobile  
173 (Table 1). Swimming was subdivided into rapid swimming (RS) or slow swimming (SS). RS was  
174 defined as instantaneous speed more than one larva body length  $s^{-1}$  ( $BL s^{-1}$ ) and SS less than one  $BL$   
175  $s^{-1}$ . Immobility (IM) was defined by the fact that the fish did not move from its position (the head  
176 did not change its position in space but the body may present some undulations) and measured by  
177 its duration (s). The second set of variables dealt with exploration of the space and time spent in the  
178 different zones of the devices in the two tests. In the maze, we measured the latency for the larva to  
179 go out from the start box (LA), the number of arms visited (NA), the total number of zones visited  
180 (NZ1) and the time spent in the first arm of the maze added to the time spent in the start box (TT).  
181 In the novel object test, we counted the number of zones visited (NZ2) by the larva and the time  
182 spent at a distance of less than one  $BL^{-1}$  from the object (TO).

183

#### 184 Morphological data

185 Just after running the tests, each fresh larva was weighted (Sartorius Scale, accuracy 0.1 mg) and  
186 stored individually in formalin (4%). Thereafter, they were placed under a binocular microscope  
187 (OPTIKA microscope, SZP-10), connected to a camera (MICROVISION Instruments, Lw1235C-  
188 GT1). This system allowed us obtaining photographs. From the photographs, we measured two

189 morphological variables (software: Archimed, MICROVISION Instruments 6.0.14): the total length  
190 of the body from the extremity of the head to the end of the caudal fin and the eye diameter. We  
191 used the total length of the larva, because it is a characteristic directly linked to growth and to  
192 muscular abilities. The eye diameter could be a relevant parameter of the perception of the  
193 environment and demonstrated as a good correlate of personality in fish (e.g.: bigger eye fish  
194 showed bolder than smaller eye one, Rey et al. 2013) even though we have no information on the  
195 maturation of the visual system of the northern pike larvae.

196

197 Data analyses

198

199 Data on the behavioural variables of interest were summed for the six periods of two minutes  
200 leading to twelve minutes of observation per fish in each test. The normality for all the data was  
201 tested with the Shapiro-Wilk method. Mean, standard deviation (SD), and coefficient of variation  
202 (CV in percentage) were calculated for each variable.

203         The analysis was conducted in three steps. First, we characterized the personality traits of  
204 fish in each of the two tests, second we compared the personality traits of fish between the two tests  
205 (cross context consistency analysis) and third we evaluated the possible links between these traits  
206 and morphological traits for the maze test. We analysed both kinds of data (behavioural and  
207 morphological) by using a principal component analysis (PCA). In the PCA, only principal  
208 components with Eigen values greater than 1 were considered for further analyses. . For each PCA,  
209 we gave the percentage of variance of each variable on the first axis (e.g.; the NZ1 represented 15%  
210 of the variance expressed by the first axis, PC1). For each test separately, a hierarchical ascending  
211 classification (HCA) was performed on the scores of the larvae along the axis 1 of the PCA (PC<sub>1</sub>  
212 scores of PCA reduction). This analysis was done to identify groups of individuals displaying



213 similar personality traits. The level of group discrimination was considered as the level 1 when the  
214 dissimilarity was higher (method used is the smaller distance between two individuals). After each  
215 HCA, the data on variables of interest were compared between the two groups with non-parametric  
216 tests (Mann-Whitney U test) due to the low number of individuals in each group. We compared the  
217 different positions of the individuals after the HCA between the two tests and determined the  
218 proportion of individuals, which were or not in the same group. We used these two statistical  
219 methods (PCA and HCA) because they are complementary and present different powers with non-  
220 transformed data. For the maze test, the correlations between the scores of the larvae along the axis  
221 1 of the PCA and the morphological data were analysed using Pearson regression. All the statistical  
222 analyses were performed with Statistica (version10).

223

## 224 **Results**

225

### 226 Morphological and behavioural data

227

228 The morphological data and the mass weakly differed between the 20 larvae studied (Table 2). The  
229 behavioural variables showed a large variability between individuals and the CV varied from 53%  
230 to 206% (Table 2). The morphological variables showed less variation; their CV varied from 10%  
231 to 43% (Table 2).

232

### 233 Activity and Exploration of pike larvae (maze test)

234

235 The first two axes of the PCA represented around 60% of the total variability (fig. 2A, F1=40.25%,  
236 F2=19.02%), thus they were the only ones considered further. On the first axis (Fig. 2A),  
237 morphological variables were associated positively with behavioural variables, which corresponded

238 to exploratory behaviours (NA, SS); they were opposite to non-exploratory behaviours (LA, and  
239 TT). For example, the number of arms visited represented 16% of the total variance and the latency  
240 to go out of the start zone 11%. There was a great variability in the individual contribution to the  
241 total variability (from 0% to 17%). This implies that larvae exhibited a great variation in their  
242 behavioural responses in the device. The second axis represented more the morphological variables  
243 (body length (12%), eye diameter (11%) of the total variance) opposed to two behavioural variables  
244 (NA (11%) and NZ1 (15%) of the total variance).

245 Two main groups were found in the hierarchical ascending clustering (Fig. 2B) (Variance  
246 within class=49.8% and between classes=50.2%). The limit of the dissimilarity between the two  
247 groups was clearly established. The first group gathered thirteen individuals (G1) and the second  
248 group seven (G2). The larvae of G2 were more active (Mann-Whitney U test for all comparisons,  
249  $N_{G1}=13$ ,  $N_{G2}=7$ ): they visited more arms ( $U=8.5$ ,  $p=0.002$ ), spent more time slow swimming ( $U$   
250  $=21.0$ ,  $p=0.057$ ), stayed less time in the first zone ( $U=8.5$ ,  $p=0.002$ ), and had a shorter latency time  
251 ( $U=8.3$ ,  $p=0.003$ ). These individuals had a higher body length ( $U=14.0$ ,  $p=0.014$ ) and a greater eye  
252 diameter ( $U=17.0$ ,  $p=0.025$ ).

253

254 Activity and boldness of pike larvae (novel object test)

255

256 The first two axes of the PCA represented around 66 % of the total variability ( $F1=38.64\%$ ,  
257  $F2=27.85\%$ , fig. 3A). Exploratory behaviours (SS and NZ2) were associated with the first axis and  
258 as well as morphological variables with IM and opposed to RS (Fig.3A). Activity variable (TO) was  
259 associated with the second axis (Fig. 3A). Therefore, it appeared that the variables were strongly  
260 divided by their nature on the first axis; exploration variables (SS represented 32% and NZ2 32% of

261 the variance), and morphological data (body length represented 23% of the total variance, eye  
262 diameter 20%),

263

264 The results of the hierarchical ascending clustering showed a clear division of the larvae into  
265 two groups (Fig. 3B) (Variance within class=53.8% and between classes=46.2%). The limit of the  
266 dissimilarity between the two groups was clearly established: a group of nine (G1) and a group of  
267 eleven larvae (G2). The larvae of G2 were more active (Mann-Whitney U test for all comparisons,  
268  $N_{G1}=11$ ,  $N_{G2}=9$ ): they spent more time slow swimming ( $U=18.0$ ,  $p=0.003$ ), tended to spent more  
269 time rapid swimming ( $U=26.0$ ,  $p=0.074$ ), they spent more time near the novel object ( $U=3.0$ ,  
270  $p<0.001$ ), and visited a higher number of zones ( $U=6.0$ ,  $p=0.001$ ) with less immobility ( $U=42$   
271  $p=0.57$ ). There was no difference in morphological variables between the two groups (Total length  
272 of the body  $U=46$   $p=0.79$ , eye diameter  $U=48$ ,  $p=0.91$ ).

273

274 Cross context analysis and relationships between behavioural, morphological data and age

275

276 Among the twenty larvae tested in the two situations, eight fish gathered in the same group of more  
277 active, more exploratory and bolder individuals. Twelve fish differed in their responses depending  
278 on the test. There was no morphological difference between fish that were or not in the same group.  
279 For this correlation analysis, we used only the data of the maze test, because the PCA showed more  
280 divergent results on the first axis (F1) for the measured traits. We found no significant correlation  
281 between the  $PC_1$  scores of each larva and body length ( $r^2=0.05$ ,  $t=0.98$ ,  $p=0.34$ ), eye diameter  
282 ( $r^2=0.03$ ,  $t=0.69$ ,  $p=0.50$ ), and body mass ( $r^2=0.02$ ,  $t=0.57$ ,  $p=0.57$ ). There was also, no significant  
283 correlation with the age of the larvae at the moment of the test ( $r^2=0.05$ ,  $t=0.15$ ,  $p=0.88$ ).

284

285 **Discussion**

286 One of the most important results of the present study is that different behavioural traits were  
287 observed in very young fish larvae using a maze and a novel object tests. These tests allowed us to  
288 discriminate different groups of fish. This implies that personality traits appeared very early in the  
289 life of the fish, and personality could be an inheritable character (Wright et al. 2003). For instance, a  
290 simple gene mutation linked to growth factors may modify fish personality in zebra fish (*Danio*  
291 *rerio*) (Norton et al. 2011). Other works suggested that a genetic basis of behavioural traits related  
292 to personality could exist. For instance, the study of behavioural syndromes of 12 populations of  
293 three-spined stickleback *Gasterosteus aculeatus*, showed that some syndromes existed in fish reared  
294 in ponds where their piscivorous predators were present (Dingemanse et al. 2007), or boldness  
295 varied between four wild-caught populations of zebra fish (Wright et al. 2007). For the syndrome  
296 aggression-boldness there were differences across but not within populations of Zebra fish (Martins  
297 and Bhat 2014), further isogenic clones clones developed different behavioural syndromes (Millot  
298 et al. 2014). Domestication may also act as a selection process for personality traits (activity,  
299 boldness) (Moretz et al. 2007). As we had no information on the genetic relationships between the  
300 pike larvae we tested, we cannot exclude that some observed differences in personality could be the  
301 consequences of genetic differences.

302

303 The behavioural tests

304

305 In our study, the device for each test had been chosen and designed to evaluate a particular  
306 behavioural trait. It is why there could be some differences between individual responses to the test.  
307 For example, Bell (2005) found a correlation between three behavioural traits in one population of  
308 sticklebacks but not in another, tested using different devices. The maze gives us information on the  
309 abilities of fish to explore a new environment, and the novel object is used for testing the stress and

310 the fear facing a new element in the fish surroundings (Frost et al. 2007). According to the  
311 definition of the five axes of personality and their clustering in two groups proactive vs. reactive  
312 (Koolhaas et al. 1999), animals that are more aggressive will also be bolder and more explorative in  
313 a novel environment (Huntingford 1976). Boldness measured as the amount of time spent close to a  
314 novel object, gave more reproducible results (Wright et al. 2003) and could be more adapted to the  
315 study of larva. Exploration was quantified as the amount of time needed to explore the arms of a  
316 maze. In this latter device, exploratory behaviour was measured as the time taken to explore and  
317 swim in each arm. Thus, this device seemed more dependent on the activity and consequently on  
318 the physical strength of the larvae. Consequently, the two devices (maze and novel object) did not  
319 measure the same behavioural trait linked to the characteristics of the larva development.

320

321 Personality and Northern pike biology

322

323       Even if there exists a genetic background and there are some probabilities that the fish we  
324 tested were not genetically equivalent, it is known that fish personality also varies according to  
325 environmental factors. For instance, an increase in water temperature modifies activity, boldness  
326 and aggressiveness of two damselfishes *Pomacentrus moluccensis* and *P. bankanensis* (Biro et al.  
327 2010). Other environmental factors such as predation pressure (Brown et al. 2005), food  
328 predictability (Chapman et al. 2010) or period of activity (Millot et al. 2009) may also influence the  
329 expression of personality traits and especially boldness. Personality varies also with the social  
330 context (Budaev and Brown 2011). In Eurasian perch *Perca fluviatilis*, if shy fish are grouped,  
331 they foraged during a longer period of time in open area (without vegetation) than when they were  
332 with bold individuals (Magnhagen and Staffan 2005). Social similarity has been demonstrated in a  
333 social-learning context in which sticklebacks copied the choices of demonstrator fish

334 proportionately more as the number of demonstrator increased (Pike and Laland 2010). The  
335 experiments that we realized with northern pike larvae were done under standardized conditions for  
336 temperature and light, so there was no change in the environmental conditions between the two  
337 tests. Moreover all fish larvae were reared under the same social conditions, by groups of  
338 individuals of the same age. The only difference introduced between them is the size and the mass  
339 of the larva, which might be linked to personality (Brown and Braithwaite 2004). Our results  
340 showed that in the maze test, the more active and exploratory larvae were also longer with a bigger  
341 eye. For the latter, it was also observed to vary in relation to personality in sticklebacks particularly  
342 with risk-taking behaviour but not with shoaling behaviour (Kim and Velando 2015).

343         Our results showed a clear separation between the two main groups of larvae, the bold and  
344 shy ones; in the two tests that did not evaluate the same components of the larva personality, there  
345 were some similarities (40% of fish were in the same groups) between the two rankings. Larvae  
346 may be ranked also, along a bold–shy continuum (Réale et al. 2007). The degree of boldness (or  
347 shyness) is determined by a trade-off between foraging gains (and/or mating opportunities) and the  
348 associated risks (Wilson et al. 1993; 1994). Hence, variation in boldness is driven by the balance of  
349 costs and benefits, and could be affected by metabolic rate (Krause et al. 1998), food deprivation  
350 (Godin and Smith 1988) and the perception of predation risk (Coleman and Wilson 1998). Our  
351 larvae were reared in the same conditions, but we cannot be sure that the individuals have the same  
352 abilities to react to environmental constraints such as the introduction of exogenous food. We noted  
353 that there were differences between the tests and that the majority of the fish changed of groups and  
354 seemed to show different behavioural traits between the two tests. First, we found that 40% of the  
355 individuals did not change groups and it was a good result compared to previous studies showing  
356 less than 20 % of behavioural cross context consistency (Ferrari et al. 2014). Second, the different  
357 tests did not target the same behavioural traits and larvae may differ in their behavioural responses

358 to different environmental constraints. This does not mean that they present different personality but  
359 that, for the same behavioural trait, they showed graduate responses. This variability may be  
360 correlated to age (the larvae tested at the beginning of the experiments were ten days younger than  
361 the latest ones) or to small differences in development variables for individuals of the same age.

362 In conclusion, our study put forward the possibility to discriminate personality traits in  
363 young fish larvae, such as the Northern pike. Such results open new opportunities for testing  
364 behavioural abilities of very young fish. The existence of behavioural syndromes or personality in  
365 fish larvae could be used in different situations: during the domestication process, with the selection  
366 of fish with particular morphological and physiological traits, for resolving bottlenecks in  
367 aquaculture (i.e. cannibalism between young larvae may limit the farming success of species of  
368 economical interest; cannibalism could be linked to particular fish personality and this trait could be  
369 selected as morphological or physiological traits), or for predicting the invasive abilities of new  
370 species (Zhao and Feng 2015). The personality traits vary between individuals in a bold-shy  
371 continuum, with a clear separation between bold and shy larvae, and in our study, most of the fish  
372 changed their status between the two tests. Nevertheless, the differences are sufficient to rank the  
373 larvae along a gradient of boldness-shyness. This information could be used to sort out and to select  
374 fish and to study their behaviours during their development. In this case, each individual should be  
375 marked individually, i.e. with a micro-tag (Ferrari et al. 2014) and followed during its development.

376

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381

382 **Compliance with Ethical Standards**

383 Conflict of interest: the authors declare that they have no conflict of interest.

384 Ethical approval: All fish treatments and procedures used in this study were in accordance with the  
385 general guidelines of the Council of European Communities (1986, No. 86/609/CEE) and the  
386 French Animal Care Guidelines (Animal approval No. C54-547-18).

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519 **Figures legends**

520

521 **Figure 1:** Scheme of the two devices used for the behavioural tests. Figure 1A represents the maze  
522 with four arms (A1, A2, A3 and A4), the central zone (CZ) and the start zone (SZ). Figure  
523 1b represents two devices (D1 and D2) used for the novel object test with the two different  
524 zones: near (ZN) or far (ZF) the novel object.

525

526 **Figure 2:** PCA (2A) and HAC (2B) realized with seven behavioural variables and integrating two  
527 morphological variables for the maze test. In the PCA, ED: Eye Diameter and BL: Body  
528 length. For the other indexes see the mean in Table 1. For the HCA, the X-axis gives the ID  
529 number of each larva and each has the same ID number in the two tests (see also figure 3B).

530

531 **Figure 3:** PCA (3A) and AHC (3B) realized with seven behavioural variables and integrating two  
532 morphological variables for the novel object test. (For indexes see Figure 2 legend and Table  
533 1)

534

534 **Table 1:** Variables of interest used to describe activity, exploration and boldness in the maze and  
535 novel object test by Northern pike larvae.

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538 *Rapid swimming (RS)* This variable is defined as the movement of the fish larva in less than one  
539 second on a distance equal to the length of the body. It stops when the fish is immobile.

540 *Slow Swimming (SS)* The contrary as above. We measured the time during which the larva moved  
541 more than a body length during one second.

542 *Immobility (IM)* As the time of observation was limited (30 minutes), immobility was considered as  
543 a correlative variable of swimming; when fish did not swim. We considered immobility as the time  
544 spent by a larva to swim (undulations of the body) but without any displacement of the body.

545 *Duration of exploratory behaviour (TT)* It is the time during which, in the maze test, the larva went  
546 out of the start zone and explored only the first arm; this duration comprised also returns in the start  
547 zone.

548 *Latency to go out the start zone (LA)* In the maze test, it was the time that an individual used to go  
549 out the habituation zone and venture in the first arm. *Number of arms (NA)*: in the maze, it was the  
550 total number of arms that the larva visited during the period of observation.

551 *Number of zones (NZ)* In novel object test, it was the total number of zones that the larva visited  
552 during the period of observation. It could concern the labyrinth (NZ1) or novel object (NZ2).

553 *Time spent near the novel object (TO)* It was the total time during which the larva was in contact or  
554 at less than one centimetre from the novel object. On the opposite, for from the novel object (FO).

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559 **Table 2:** Morphological and behavioural data used in the two tests (Mean, Standard deviation and  
 560 coefficient of variation (CV) (n= 20 individuals).

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563	Variables		Mean	SD	CV (%)
564	Total body length (mm)		16.13	0.37	10
565	Eye diameter (mm)		1.23	0.03	10
566	Body Mass (mg)		19.4	1.84	43
567					
568	Behaviour in	SS (s)	107.4	12.8	53
569	Maze test	RS (s)	3.9	1.6	187
570		IM (s)	25.0	3.3	60
571		TT (s)	343.9	55.3	72
572		LA (s)	164.3	43.6	118
573		NZ1	6	1	56
574		NA	2	0	65
575					
576	Behaviour in	SS (s)	179.6	24.7	61
577	Novel object test	RS (s)	8.2	3.8	206
578		IM (s)	20.5	3.7	80
579		TO (s)	218.6	30.4	62
580		NZ 2	8	2	99

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584 **Pasquet et al. Figure 1A**

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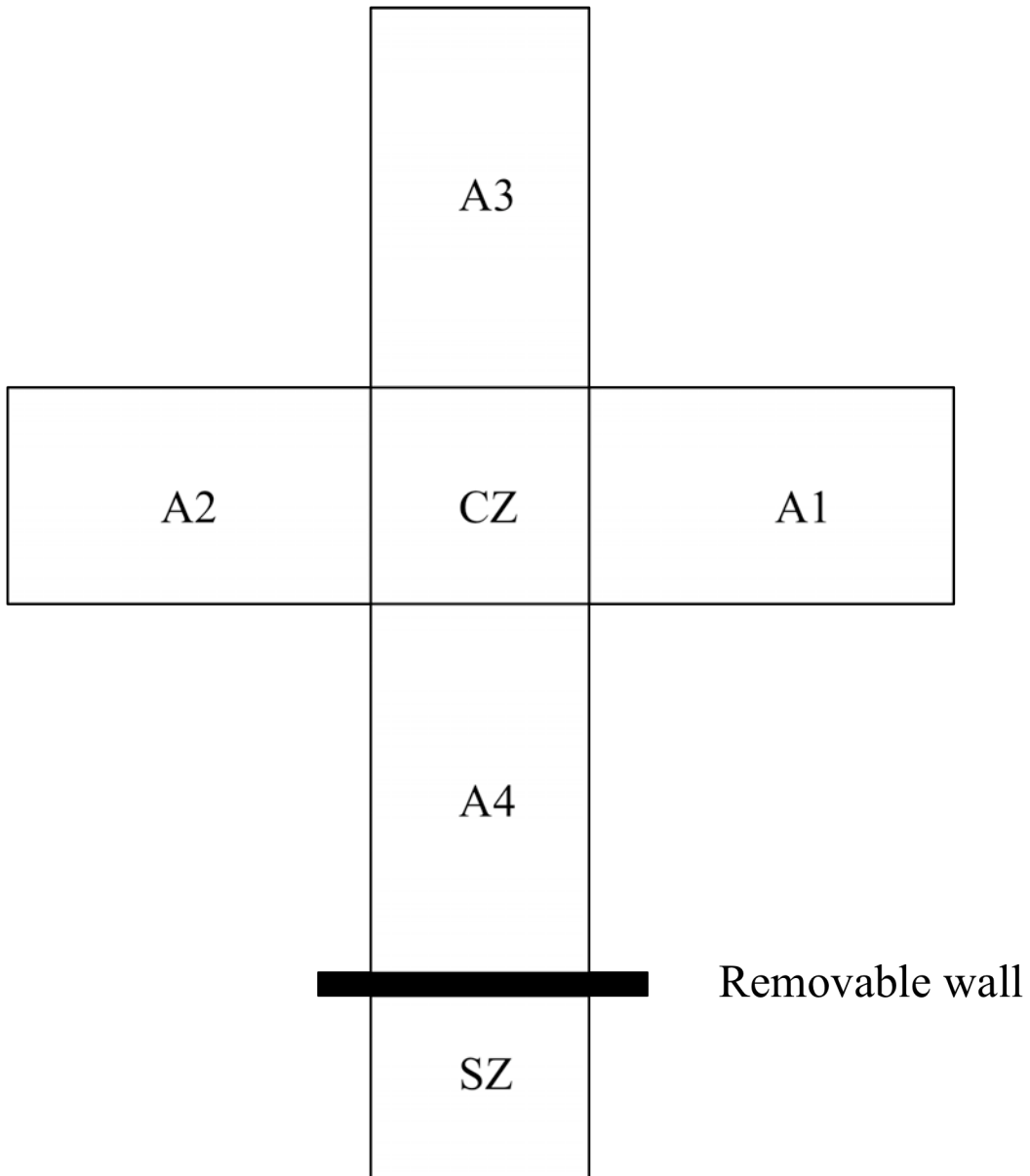
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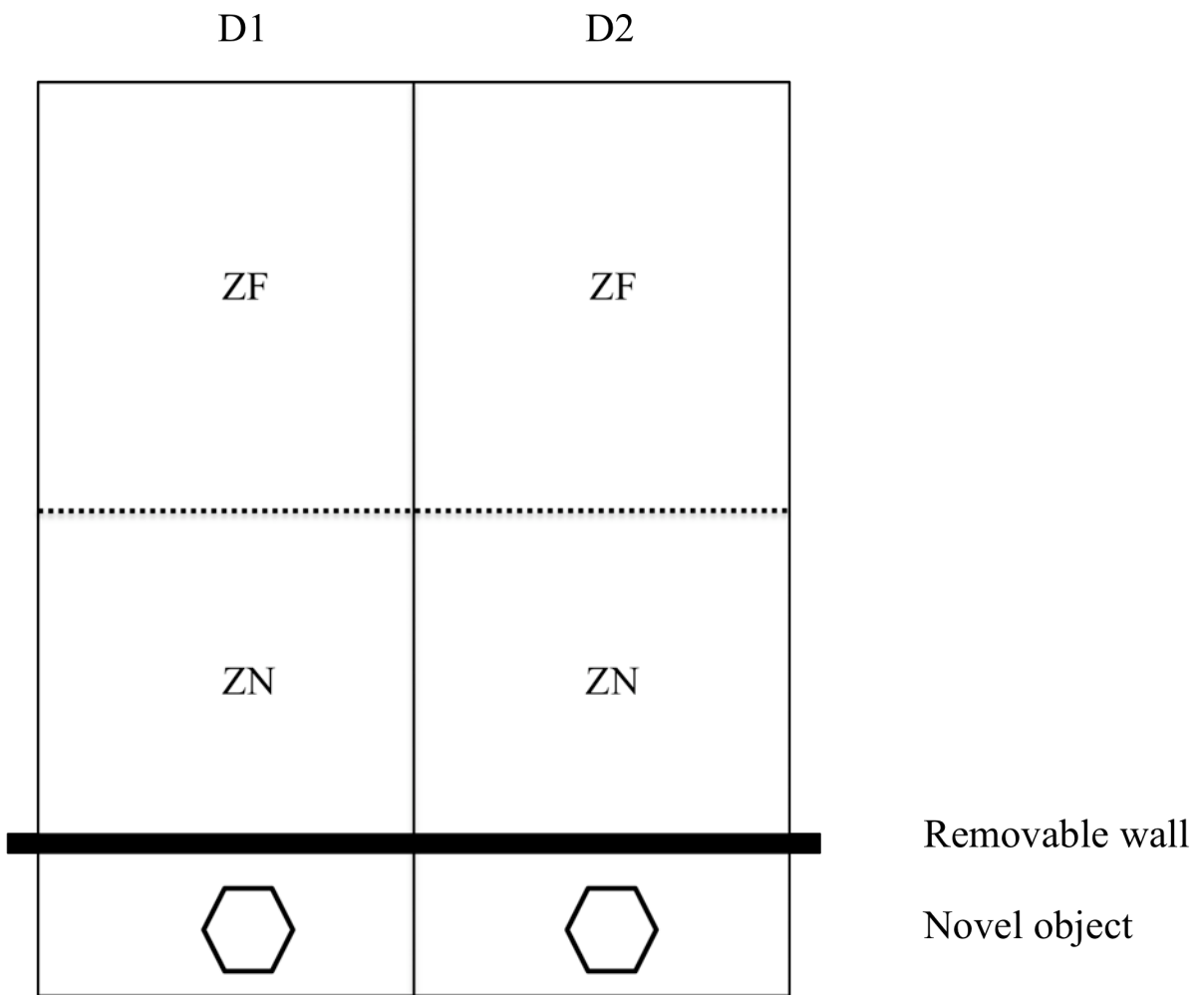


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Pasquet et al. Figure 1B

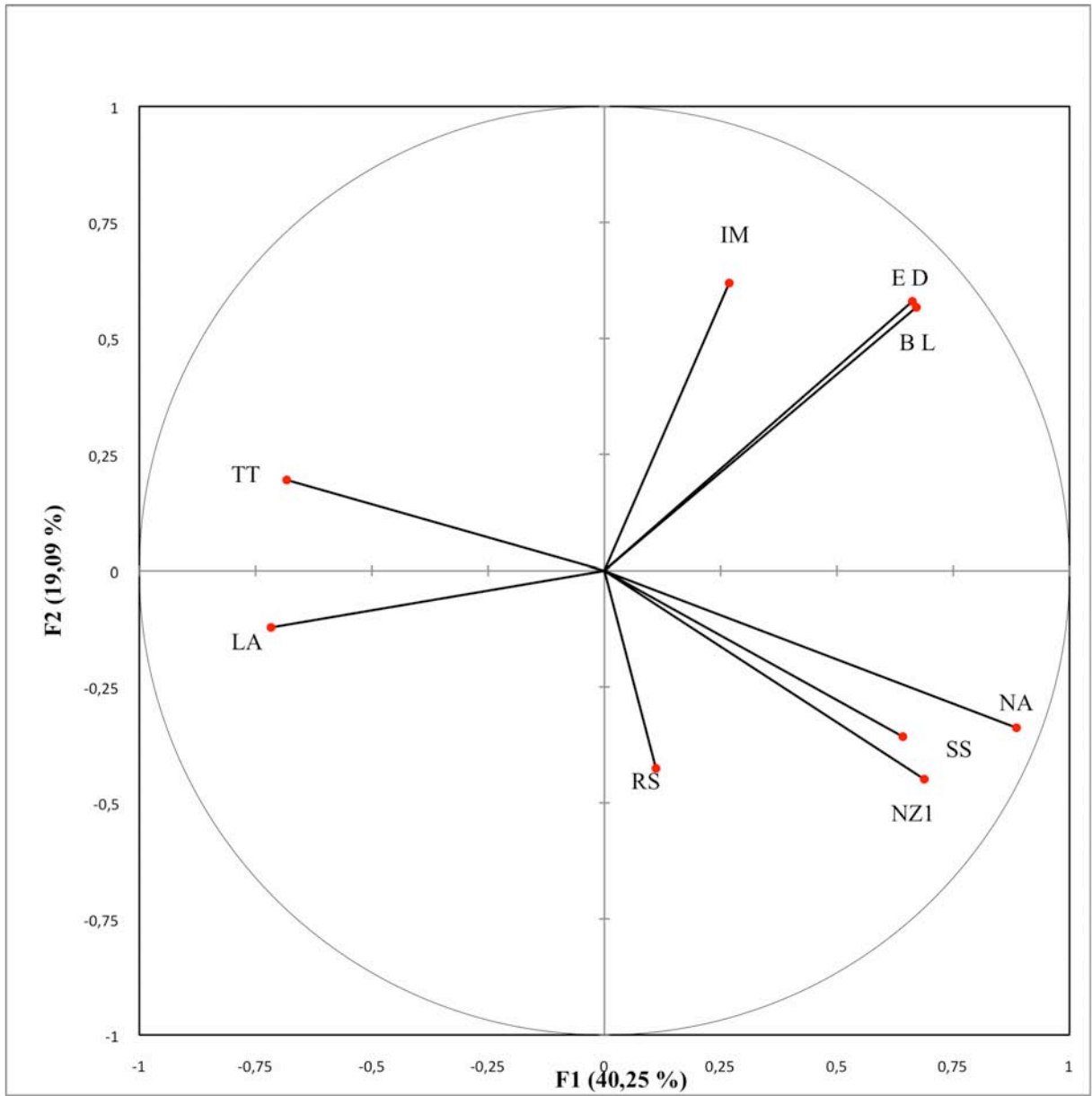


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**Figure 2**

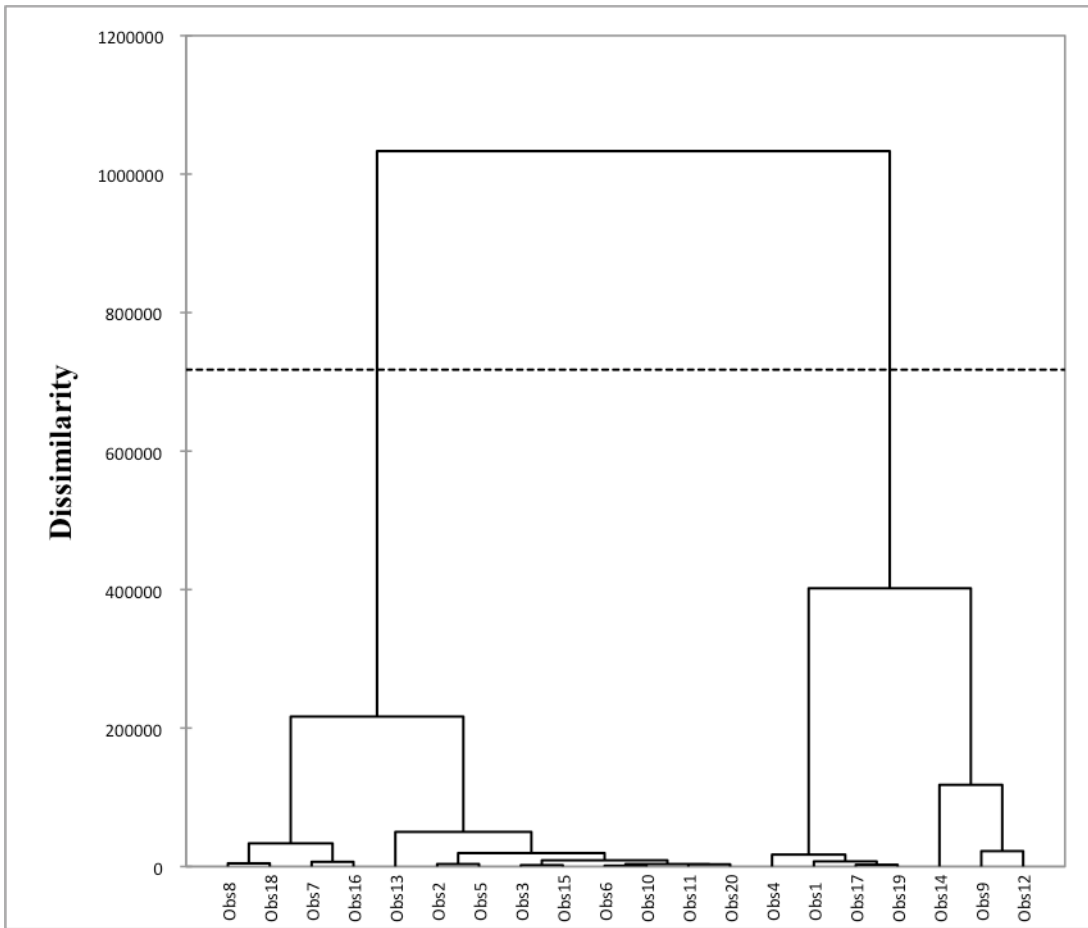
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**B)**



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639 **Figure 3**

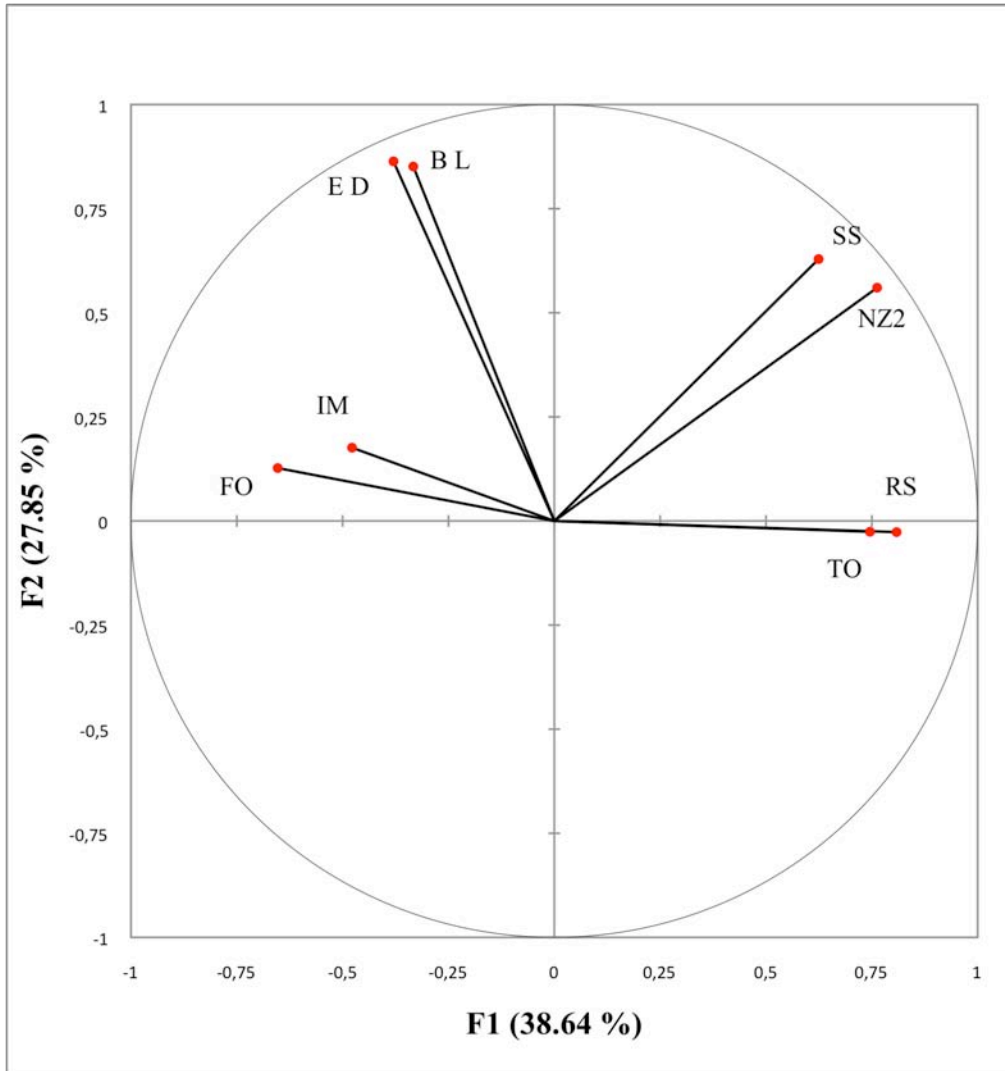
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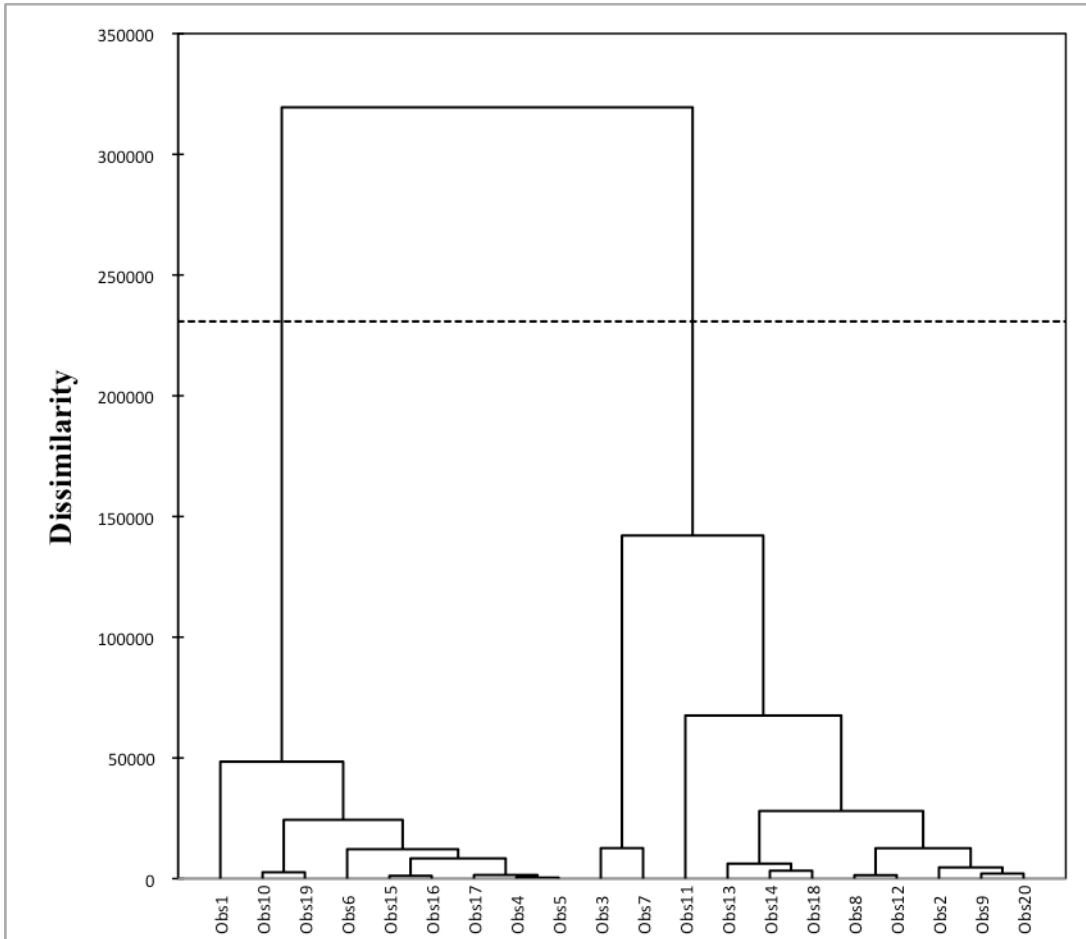
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**B)**



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