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

Pasquet Alain [1, *], Sebastian Anthony [1], Begout Marie-Laure [2], Ledore Yannick [1], Teletchea Fabrice [1], Fontaine Pascal [1]

[1] CNRS, UR AFPA, USC INRA 340, University of Lorraine, 54506, Vandoeuvre les Nancy, France
[2] Ifremer, place Gaby Coll, BP 7, 17137, La Rochelle, L'Houmeau, France

* Corresponding author : Alain Pasquet, email address : alain.pasquet@univ-lorraine.fr

**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 (*Exos 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

**Introduction**

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

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

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

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

The Northern pike *Esox lucius*, is a solitary species at adult stage, which inhabits lakes, ponds and also rivers with weak current, in Eurasia and North America (Kottelat and Freyhof 2007). The larvae are about 9 mm at hatching (Teletchea et al. 2009; Parlier and Corolla 2013; 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 (lU $Kg^{-1}$) 17500 , DHA (g $kg^{-1}$) 11,

123　EPA (g $kg^{-1}$) 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 (MICROVISON Instruments, Lw1235C-

188  GT1). This system allowed us obtaining photographs. From the photographs, we measured two

189    morphological variables (software: Archimed, MICROVISON 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 ($PC_1$

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

**Discussion**

285

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 fluaviatilis*, 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

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

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

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).

387

388

389 **References**

390

391 Adriaenssens B, Johnsson JI (2011) Shy trout grow faster: exploring links between personality and

392     fitness-related traits in the wild. Behav Ecol 22, 135–143

393 Baras E (2012) Cannibalism in fish larvae: What have we learned? In: Larval Fish Aquaculture

394     (Qin JG, ed.). Nova Science Publishers, New York, pp. 1-37

395 Bell AM (2005) Behavioural differences between individuals and two populations of

396     stickleback(*Gasterosteus aculeatus*). J Evol Biol 18**,** 464–473

397 Bell AM, Sih A (2007) Exposure to predation generates personality in three-spined

398     sticklebacks (*Gasterosteus aculeatus*). Ecol Lett 10, 828–834

399 Biro PA, Stamps JA (2008) Are animal personality traits linked to life-history productivity?

400     Tends Ecol Evol 23, 361–368

401 Biro PA, Beckmann C, Stamps JA (2010) Small within-day increases in temperature affects

402     boldness and alters personality in coral reef fish. Proc R Soc B Biol Sci 277, 71–77

403 Brown C, Braithwaite V (2004) Size matters: a test of boldness in eight populations of the

404     poeciliid *Brachyraphis episcopi*. Anim Behav 68, 1325–1329

405 Brown C, Jones F, Braithwaite V (2005) In situ examination of boldness-shyness traits in the

406     tropical poeciliid, *Brachyraphis episcopi*. Anim Behav 70, 1003–1009.

407    Bry C, Basset E, Rognon X, Bonamy F (1992) Analysis of sibling cannibalism among pike, *Exox*

408        *lucius* juveniles reared under semi-natural conditions. Env Biol Fish  35, 75-84.

409    Budaev S, Brown C (2011) Personality Traits and Behaviour. In: Fish Cognition and Behaviour.

410        (Brown C, Laland K, Krause J, eds). Wiley-Blackwell, Oxford, pp 135–165

411    Castanheira MF, Herrera M, Costas B, Conceicao LEC, Martins CIM (2013) Can we predict

412        personality in fish? Searching for consistency over time and across contexts. PLOS ONE*,*

413        e.0062037

414    Chapman BB, Morrell LJ, Krause J (2010) Unpredictability in food supply during early life

415        influences boldness in fish. Behav Ecol 21, 501–506

416    Coleman K, Wilson DS (1998) Shyness and boldness in pumpkinseed sunfish: individual

417        differences are context-specific. Anim Behav 56, 927–936

418    Conrad JL, Weinersmith KL, Brodin T, Saltz JB, Sih A (2011) Behavioural syndromes in

419        fishes: a review with implications for ecology and fisheries management. J Fish Biol 78,

420        395–435

421    Cote J, Fogarty S, Weinersmith K, Brodin T, Sih A (2010) Personality traits and dispersal

422        tendency in the invasive mosquitofish (*Gambusia affinis*). Proc Roy Soc B Biol Sci 277,

423        1571–1579

424    Dahlbom SJ, Lagman D, Lundstedt-Enkel K, Sundström LF, Winberg S (2011) Boldness

425        predicts social status in zebrafish (*Danio rerio*). PLOS ONE 6: e23565

426    Dall SRX, Houston AI, Mc Namara JM (2004) The behavioural ecology of personality: consistent

427        individual differences from an adaptive perspective. Ecol Lett 7: 734-739

428    Dingemanse NJ, Wright J, Kazem AJN, Hickling r, Dawnay N (2007) Behavioural syndromes

429        differ predictability between 12 populations of three-spined stickleback. J Anim Ecol 76,

430        1128-1138

431  Dyer JRG, Croft DP, Morrell LJ, Krause J (2009) Shoal composition determines foraging
432      success in the guppy. Behav Ecol 20, 165–171

433  Ferrari S, Benhaïm D, Colchen T, Chatain B, Begout ML(2014) First link between self-feeding
434      behaviour and personality traits in European seabass, *Dicentrarchus labrax*. Appl Anim
435      Behav Sci (In press) doi: 101016/j.applanim.2014.09.019

436  Frost AJ, Winrow-Giffen A, Ashley PJ, Sneddon LU (2007) Plasticity in animal personality
437      traits: does prior experience alter the degree of boldness? Proc Roy Soc B Biol Sci 274**,**
438      333–339

439  Gerlai R, Lahav M, Guo S, Rosenthal A (2000) Drinks like a fish: zebra fish (*Danio rerio*) as a
440      behavior genetic model to study alcohol effects. Pharma Biochem Behav 67, 773–782

441  Goding JGJ, Smith SA (1988) A fitness cost of foraging in the guppy. Nature 333, 69-71

442  Gosling SD (2001) From mice to men: what can we learn about personality from animal
443      research? Psycho Bull 127**,** 45–86

444  Höjesjö J, Adriaenssens B, Bohlin T, Jönsson C, Hellström I, Johnsson JI (2011)
445      Behavioural syndromes in juvenile brown trout (*Salmo trutta*); life history, family
446      variation and performance in the wild. Behavioral Ecology Sociobiology 65, 1801–1810

447  Huntingford FA (1976) The relationship between anti-predator behaviour and aggression
448      among conspecifics in the three-spined stickleback, *Gasterosteus aculeatus*. Anim
449      Behav 24, 245–260

450  Kim SY, Velando A (2015) Phenotypic integration between anti-predator behaviour and
451      camouflage pattern in juvenile sticklebacks. Evolution doi: 10.1111/evo.12600

452  Koolhaas JM, Korte SM, De Boer SF, Van Der Vegt BJ, Van Reenen CG, Hopster H, De
453      Jong IC, Ruis MAW, Blokhuis HJ (1999) Coping styles in animals: current status in,
454      behavior and stressphysiology. Neurosci Biobehav Rev 23, 925-935

455     Kottelat Mc, Freyhof J (2007) Handbook of European freshwater fishes. Publications Kottelat,

456         Cornol, Switzerland. 646 pp

457     Krause J, Loader SP, McDermott J, Ruxton GD (1998) Refuge use by fish as a function of

458         body-length-related metabolic expenditure and predation risk. Proc Roy Soc  B Biol Sci

459         265, 2373–2379

460     Magnhagen C, Staffan F (2005) Is boldness affected by group composition in young-of-the-year

461         perch (*Perca fluviatilis*)? Behavioral  Ecology Sociobiology 57, 295–303

462     Martins ET, Bhat A (2014) Population level personalities in Zebra fish: aggression-boldness across

463         but not within populations. Behavioural Ecology 25, 368-373

464     Millot S, Pean S, Labbe L, Kemeis T, Quillet E, Dupont-Nivet M, Begout ML (2014) Assessment

465         of genetic variability of fish personality traits using rainbow trout isogenis lines. Behavior

466         genetics 44, 383-393

467     Millot S, Bégout ML, Chatain B (2009) Risk-taking behaviour variation over time in sea bass

468         *Dicentrarchus labrax*: effects of day-night alternation, fish phenotypic characteristics and

469         selection for growth. J Fish Biol 75, 1733–1749

470     Moretz JA, Martins EP, Robison BD (2007) The effects of early and adult social

471         environment on zebrafish (*Danio rerio*) behavior. Env Biol Fish 80, 91–101

472     Moretz JA, Martins EP, Robison BD (2007) Behavioral syndromes and the evolution of correlated

473         behavior in zebrafish? Behav Ecol 556-562

474     Nakayama S, Harcourt JL, Johnstone RA, Manica A (2012) Initiative, personality and leadership in

475         pairs of foraging fish. PLOS ONE 7: e36606

476     Ninkovic J, Bally-Cuif L (2006) The zebrafish as a model system for assessing the reinforcing

477         properties of drugs of abuse. Methods, 3é, 262–274

478    Norton WHJ, Stumpenhorst K, Faus-Kessler T, Folchert A, Rohner, N, Harris MP, Callebert J,

479        Bally-Cuif L (2011) Modulation of Fgfr1a signaling in zebrafish reveals a genetic basis for

480        the aggression-boldness syndrome. J Neurosci 31, 13796–13807

481    Nyqvist M, Gozlan RE, Cucherousset J, Britton JR (2013) Absence of a context-general

482        behavioural syndrome in a solitary predator. Ethology 119, 156-166

483    Parlier E, Corolla JP (2013) In: Doris, *Esox lucius* (Linnaeus, 1758),

484        http://doris.ffessm.fr/fiche2.aspj?fiche_numero=336

485    Pike TW, Laland KN (2010) Conformist learning in nine-spined sticklebacks' foraging decisions.

486        Biol Lett 6, 466-468

487    Réale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal

488        temperament within ecology and evolution. Biol Rev Camb Philos Soc 82, 291–318

489    Rey S, Beltana S, Vargas R, Roher N, Mackenzie S (2013) Combining animal personalities with t

490        ranscriptomics resolves individual variation within a wild-type zebrah fish population and

491        identifies underpinning molecular differences in brain function. Molecular Ecology 22, 6100-

492        6115

493    Sih A, Bell A, Johnson JC (2004) Behavioural syndromes: an ecological and evolurionary

494        overview. TRENDS Ecol and Evol 19, 372-378

495    Sundström LF, Petersson E, Höjesjö J, Johnsson JI, Järvi T (2004) Hatchery selection promotes

496        boldness in newly hatched brown trout (*Salmo trutta*): implications for dominance. Behav

497        Ecol 15, 192–198

498    Teletchea F, Fostier A, Kamler E, Gardeur JN, Le Bail PY, Jalabert B, Fontaine P (2009)

499        Comparative analysis of reproductive traits in 65 freshwater fish species: application to the

500        domestication of new fish species. Fish Biol Fisheries 19, 403-430

501    Toms CN, Echevarria DJ, Jouandot DJ (2010) A methodological review of personality-related
502         studies in fish: focus on the shy-bold axis of behavior. Int J Comp Psychol  23, 1–25

503    Trabelsi A, Gardeur JN, Telechea F, Brun-Bellut J, Fontaine P (2013) Hatching time effect on the
504         intra-spawning larval morphology and growth in the Northern pike (*Esox Lucius* L.).
505         Aquaculture Research 44, 657-666

506    Westerberg M, Staffan F, Magnhagen C (2004) Influence of predation risk on individual
507         competitive ability and growth in Eurasian perch, *Perca fluviatilis*. Anim Behav 67,
508         273–279

509    Wilson DS, Coleman K, Clark AB, Biederman L (1993) Shy-bold continuum in pumpkinseed
510         sunfish (*Lepomis gibbosus*): an ecological study of a psychological trait. J Comp Psycho
511         107**,** 250–260

512    Wilson DS, Clark AB, Coleman K, Dearstyne T (1994) Shyness and boldness in human and
513         other animals. Trends Ecol Evol 9**,** 442–445

514    Wright D, Rimmer LB, Pritchard VL, Butlin RK, Krause J (2003) Inter and intra-population
515         variation in shoaling and boldness in the zebrafish (*Danio rerio*). Naturwissenschaften 90,
516         374-377

517    Zhao DP, Feng PSH (2015) Temperature increases impacts personality traits in aquatic non-native
518         species: implications for biological invasions under climate change. Cur Zool (on line)

519

**Figures legends**

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

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

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

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

536

537

| |
|---|
| 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). |

555

556

557

558
559

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

561

562

| Variables | | Mean | SD | CV (%) |
|---|---|---|---|---|
| Total body length (mm) | | 16.13 | 0.37 | 10 |
| Eye diameter (mm) | | 1.23 | 0.03 | 10 |
| Body Mass (mg) | | 19.4 | 1.84 | 43 |
| | | | | |
| Behaviour in | SS (s) | 107.4 | 12.8 | 53 |
| Maze test | RS (s) | 3.9 | 1.6 | 187 |
| | IM (s) | 25.0 | 3.3 | 60 |
| | TT (s) | 343.9 | 55.3 | 72 |
| | LA (s) | 164.3 | 43.6 | 118 |
| | NZ1 | 6 | 1 | 56 |
| | NA | 2 | 0 | 65 |
| | | | | |
| Behaviour in | SS (s) | 179.6 | 24.7 | 61 |
| Novel object test | RS (s) | 8.2 | 3.8 | 206 |
| | IM (s) | 20.5 | 3.7 | 80 |
| | TO (s) | 218.6 | 30.4 | 62 |
| | NZ 2 | 8 | 2 | 99 |

581

582

583

584

25

**Pasquet et al. Figure 1A**

585
586
587
588
589
590



Removable wall
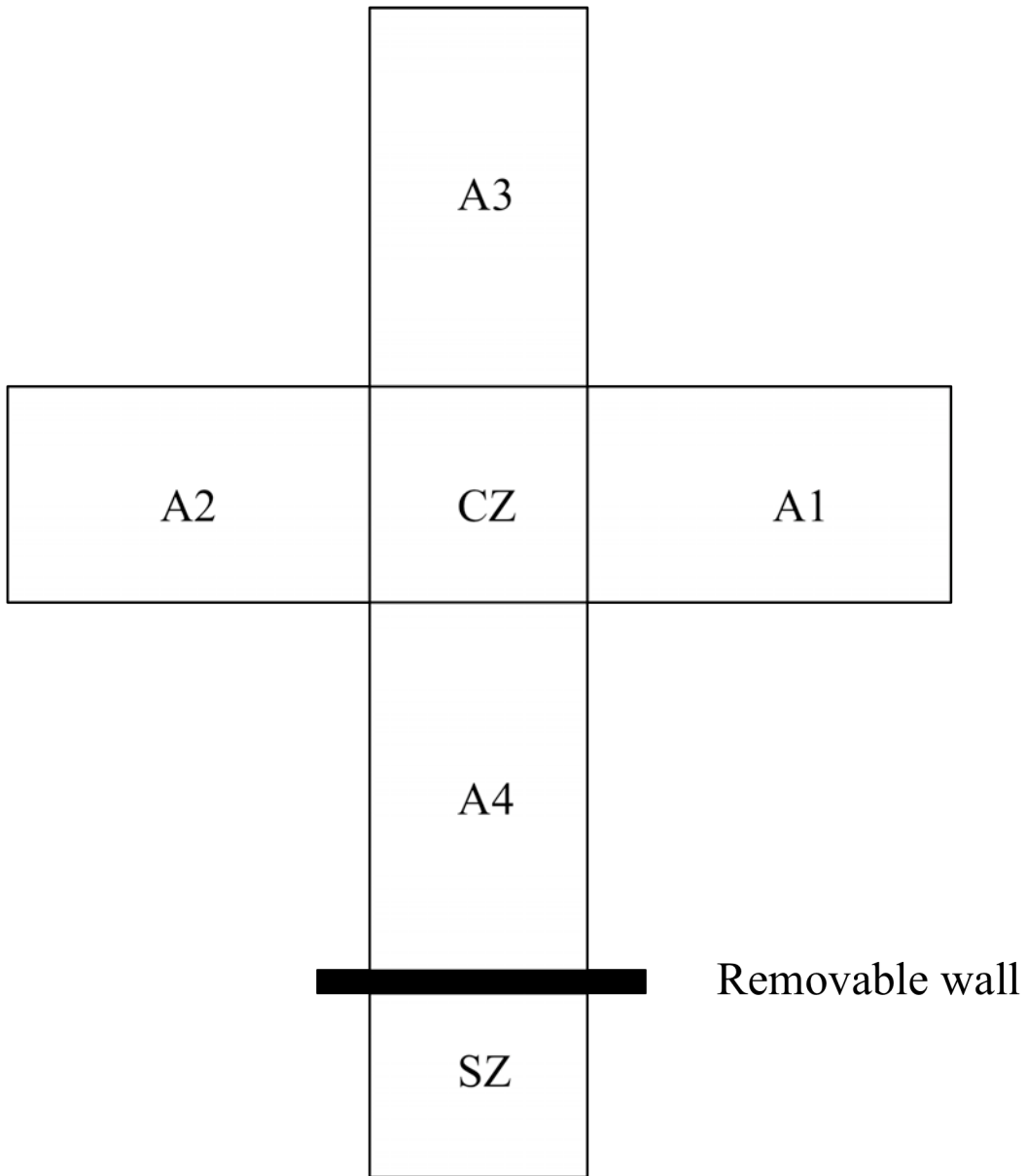
**Pasquet et al. Figure 1B**

Removable wall

Novel object

599
600 **Figure 2**
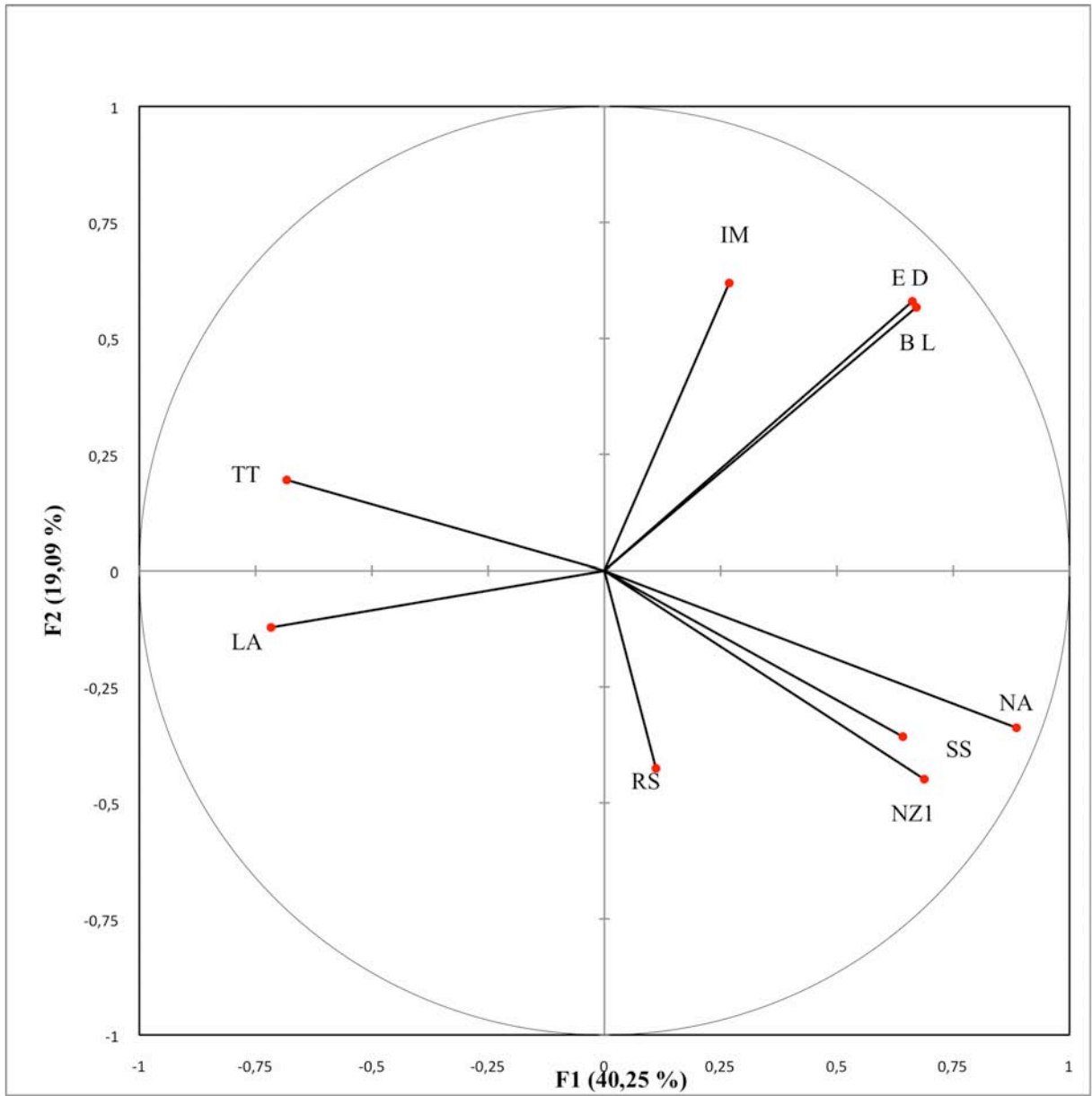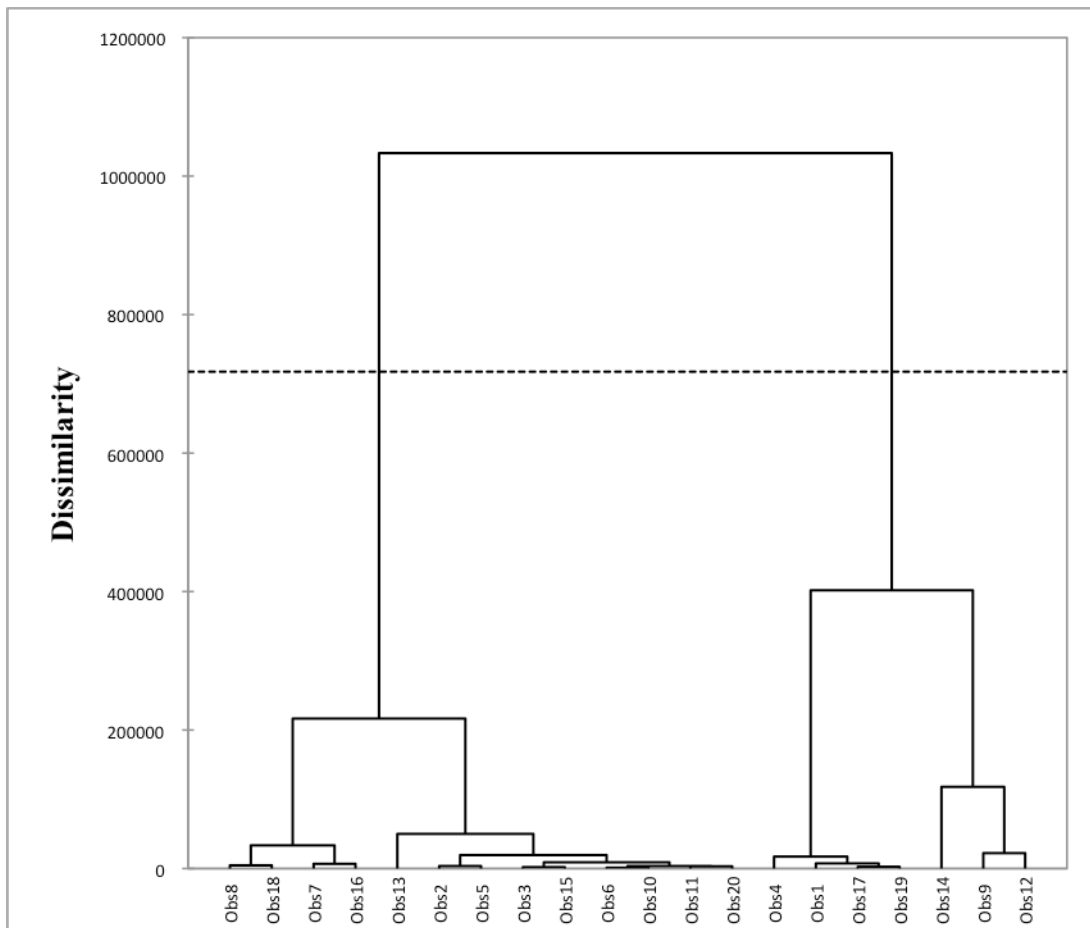601
602 **A)**
603
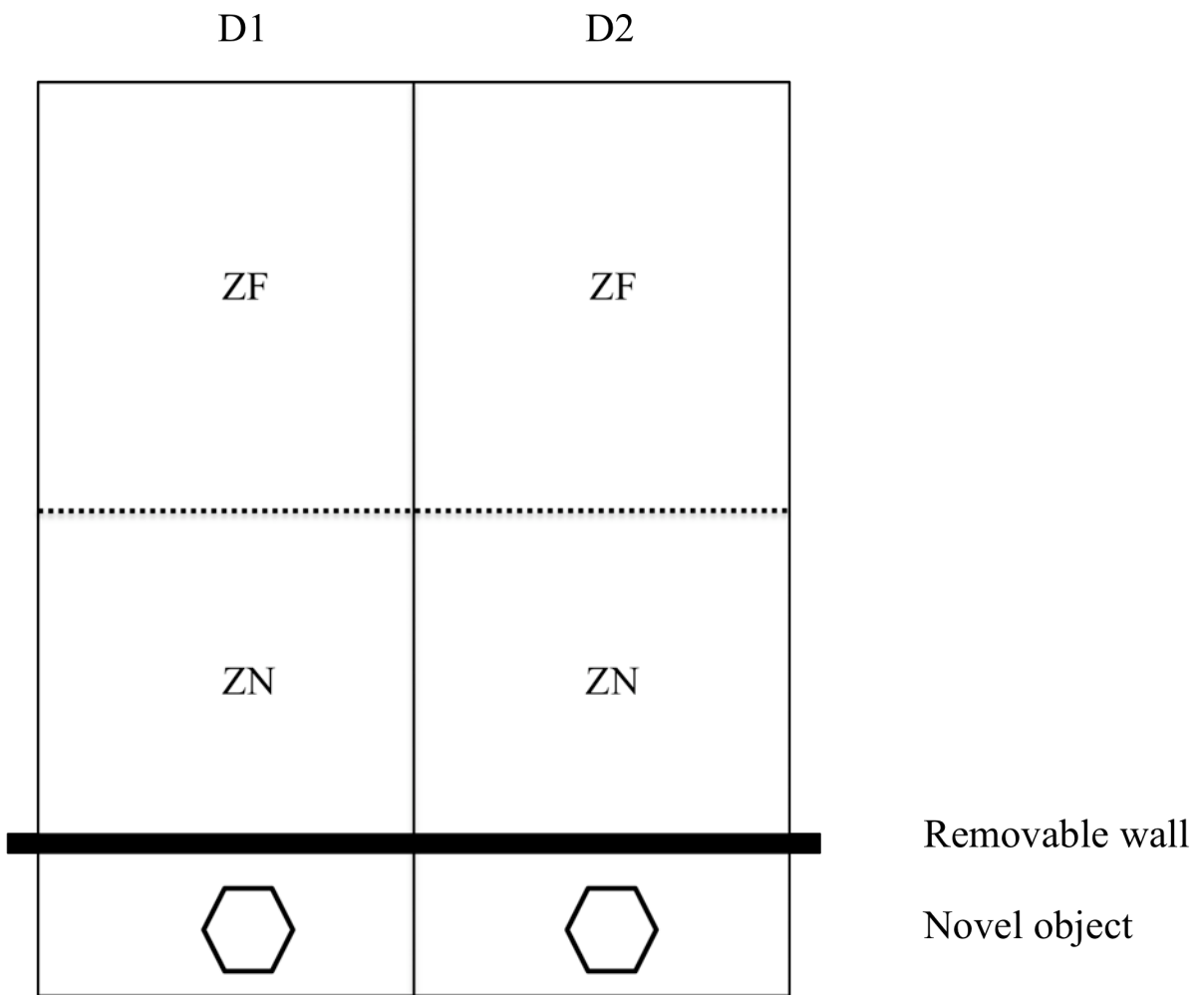


604
605
606
607
608
609
610
611
612
613
614

615
616
617
618
619
620
621
622
623
624
625
626
627
628    **B)**
629
630
631
632
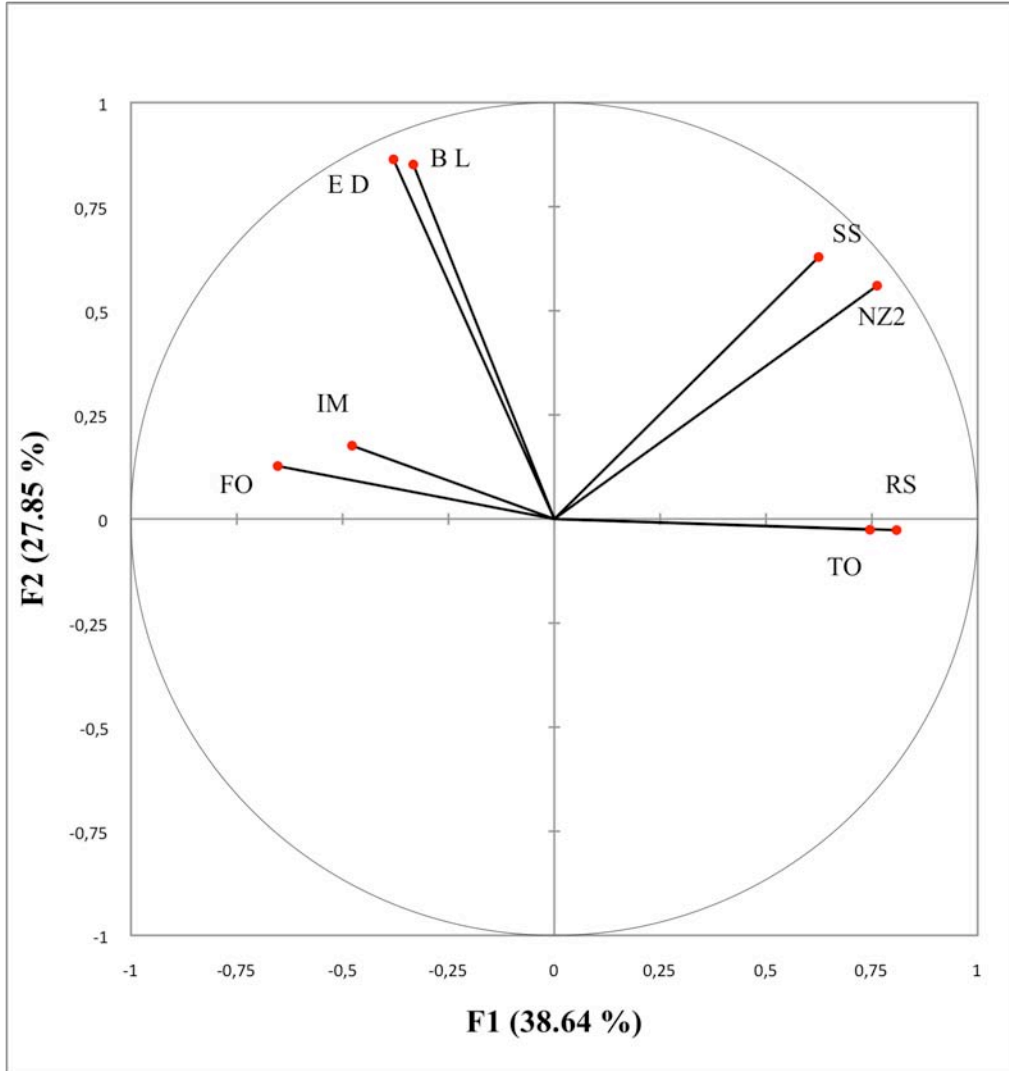


633
634
635
636
637
638
639

639  **Figure 3**
640
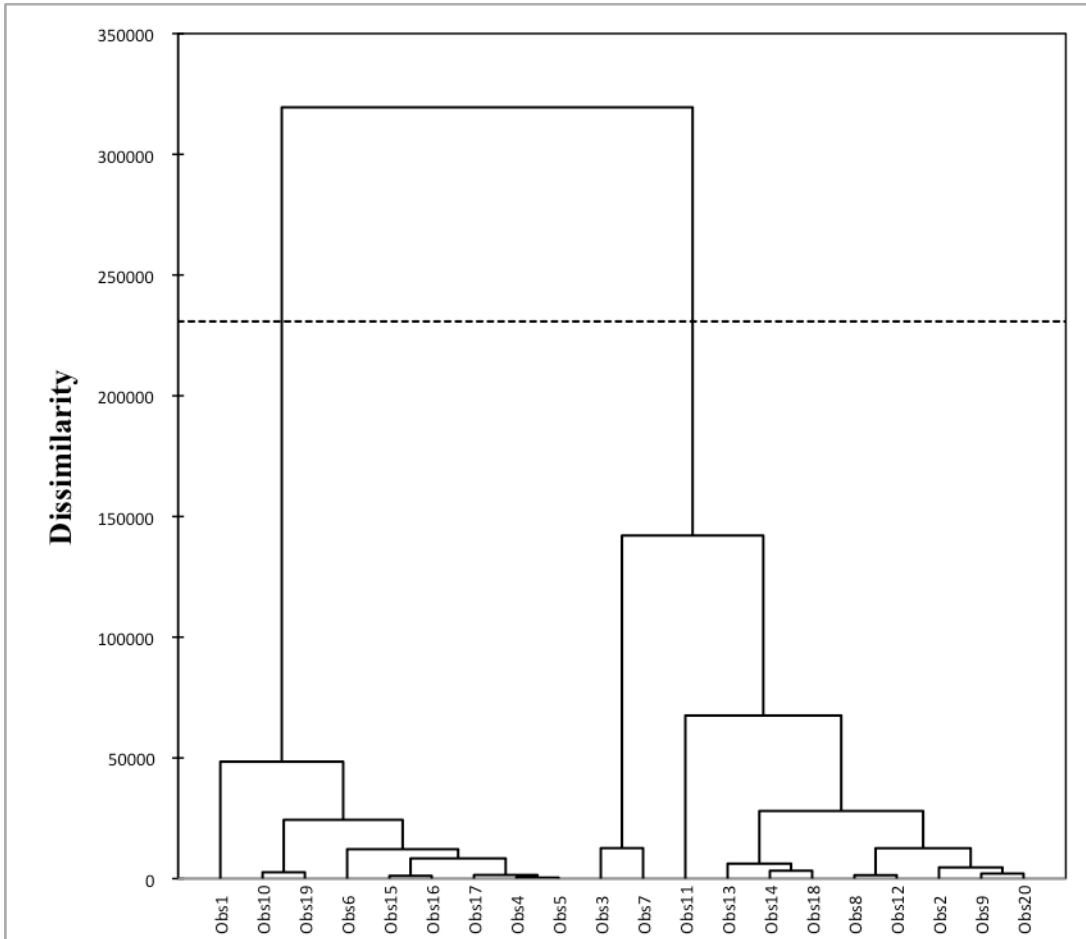641  **A)**
642
643
644



645
646
647
648
649
650
651
652
653
654
655
656
657
658

659
660
661
662
663
664 **B)**
665
666
667
668



669
670