

## How does a domestication process modulate oogenesis and reproduction performance in Eurasian perch?

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1 **How does a domestication process modulate oogenesis and reproduction**  
2 **performance in Eurasian perch?**

3

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28 **Abstract:**

29 The domestication process is accompanied with adaptation of the animals to captive  
30 conditions. It induces changes at different levels thereby affecting a variety of biological  
31 functions. While there is abundant literature on the domestication effects on growth and stress  
32 response in teleosts, the effects on reproduction have received limited attention. In this work,  
33 we investigated the domestication effect on reproductive ability of Eurasian perch (*Perca*  
34 *fluviatilis* L.), a promising candidate for the development of European aquaculture and whose  
35 reproductive physiological processes through the domestication history has not been  
36 questioned yet.

37 To address this question, two populations of F1 and “domesticated” females were submitted  
38 to a photothermal program allowing the control of gonadogenesis advancement. Gonado-  
39 somatic index, 17  $\beta$ -estradiol levels and oocyte diameter were significantly higher in  
40 domesticated population than in F1 one. In contrast, testosterone, 11-ketotestosterone, and  
41 vitellogenin levels were found to be higher in F1 females than in their domesticated  
42 counterparts. Lower reproductive performance were observed in the domesticated population  
43 compared to the F1 one in terms of survival rate, hatching rate, percentage of eyed stage  
44 larvae and percentage of malformations. In conclusion, this study shows that, in this example  
45 of domestication process, despite a positive effect on the precocious gonadogenesis  
46 advancement and vitellogenesis progress this domestication route negatively affected the  
47 reproductive performance.

48 **Keywords:** *Perca fluviatilis*, Recirculating Aquatic System, GnRH, Gonadotropins, Sexual  
49 steroids.

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## 55 **1. Introduction**

56 Price (1984) defined the domestication as “the process whereby a population of animals  
57 becomes adapted to humans and captivity conditions through one or several combinations of  
58 genetic changes throughout the generations”. Digard (2003) proposed to define domestication  
59 as “the action that men exercise over animals they own, even by raising them”. Thus the list  
60 of domesticated fish species goes from the range of 25-30 in the first definition to more than  
61 200 according to the definition of Digard (2003). This shows that the concept of  
62 domestication still divides opinions within the scientific community. However, everyone  
63 agrees that fish populations may undergo, through the domestication process, changes in their  
64 genome due to adaptation to environmental stressors, genetic selections (deliberate or not) or  
65 inbreeding phenomena (Balon 2004 ; Price 1984). It induces changes at anatomical,  
66 physiological and molecular levels thereby affecting a variety of biological functions (e.g.  
67 Brummet et al. 2004). There is abundant literature on the domestication effects on growth  
68 (Withler and Beacham 1994; Hassin et al. 1997; Tymchuck et al. 2006) and stress response in  
69 teleosts (Pickering and Pottinger 1989, 1997; Vandeputte and Prunet 2002; Douxfils et al.  
70 2011). On the one side, it has been shown that globally domestication reduces fish stress  
71 response, by rendering them less fearful as showed in salmonids (Pickering and Pottinger  
72 1989,1997; Vandeputte and Prunet 2002), or less sensitive to acute handling stressors in  
73 Eurasian perch (Douxfils et al. 2011). But in European sea bass (*Dicentrarchus labrax*) it has  
74 been shown that heritability of cortisol response to stress was low (Volckaert et al. 2012). On  
75 the other side, the domestication seems to boost the growth performance (Withler and  
76 Beacham 1994; Hassin et al. 1997; Tymchuck et al. 2006).

77 Contrary to growth and stress response, the effects of domestication process on reproduction  
78 have received limited attention. Moreover, it appears that this field of research has conducted  
79 to conflicting results. In the ide (*Leuciscus idus*), Krejszeff et al. 2009 showed that  
80 domestication positively influenced the ovulatory responses following the Ovopel™  
81 hormonal treatment. In contrast, they showed that domestication exhibited a negative effect  
82 on embryo survival. More recently, a study on the same species showed different results by  
83 pointing out a lower ovulatory response to Ovopel™ in the pond-cultured group relatively to  
84 wild fish (Ciesla et al. 2013) and no domestication effect was observed on the embryo  
85 survival. Moreover, the effect of domestication can be different from one species to another.  
86 In Atlantic salmon (*Salmo salar*) domestication led to an 83% decrease of the hatching rates  
87 (Jonsson and Jonsson 2006). In cod (*Gadus morhua*), domestication negatively affected  
88 fertilization rates, hatching and larvae survival (Salze et al. 2006). In brown trout (*Salmo*  
89 *trutta*) domestication negatively affected sperm quality (Dannewitz et al. 2004) but had a  
90 positive effect on spawning and egg weight (Randak et al. 2006). Consequently, notable  
91 differences also exist between the fish families, the fish species and the fish populations.  
92 These differences led us to investigate the domestication effects on reproductive ability of  
93 Eurasian perch (*Perca fluviatilis*), a species whose domestication history is still recent, and  
94 belonging to a fish family poorly investigated.

95 Eurasian perch is a freshwater fish species highly valuable in Europe (Fontaine, 2009). The  
96 perch market is local and the current production of market-size perch in Europe is thus unable  
97 to satisfy the demand, which is mainly centered in the Alpine region (Setälä et al. 2008;  
98 Watson, 2008). Consequently to a relative decline of wild stocks due to intensive fisheries,  
99 and to an increasing demand for small sized perch especially in Switzerland, intensive perch  
100 culture has been developed in the past two decades, and Eurasian perch has become a relevant  
101 species in European aquaculture (Fontaine 2004; Watson 2008). Up to now, only one study

102 has examined the effect of domestication on Eurasian perch reproduction (Kristan et al. 2012).  
103 Results showed a positive effect of domestication on fertilization rates, but a negative effect  
104 on ovulation and hatching. Actually, there is no detailed study aiming at understanding the  
105 influence of domestication on both the mating performance and the reproductive mechanisms  
106 underlying these effects. Our hypothesis is that disruption in the level of the endocrine actors  
107 that control the reproductive cycle explains the changes of the reproductive performance  
108 along the domestication process.

109 The aim of this study was to compare two perch populations (F1 and “domesticated”),  
110 exposed to a special photothermal program allowing the control of gonadogenesis  
111 advancement, in terms of morphological parameters (gonado-somatic index, hepato-somatic  
112 index), levels of GnRH and gonadotropins gene expression, sexual steroids and vitellogenin,  
113 as well as the oocyte developmental stages and reproductive performance.

114

## 115 **2. Materials and methods**

116

### 117 *2.1 Fish*

118 Two populations of F1 and “domesticated” perch were studied in this experiment, both  
119 originally came from the Geneva Lake (French-Swiss border). F1 breeders came from wild  
120 broodstock (F0) collected as eggs directly taken from Geneva Lake. According to the local  
121 producer (Lucas Perches, Hampont, France), “domesticated” breeders are generated from  
122 seven generations fish raised in captivity, even if the introduction of wild individuals during  
123 reproduction seasons cannot be totally excluded. The domestication history of these fish is  
124 thus a "black box". The F1 and domesticated breeders were issued from an artificial  
125 reproduction completed on November 1st, 2011 and were then raised on the producer  
126 facilities, before coming to the URAFPA-MAN facilities located in Nancy (France) on

127 February 6<sup>th</sup>, 2012, where they were placed in two 6000 L. tanks for growth. At their arrival  
128 to our facilities, fish of both populations were 3-months-old. From their hatching, the two  
129 studied fish populations (F1 and “domesticated”) have thus been raised in equal and  
130 standardized husbandry conditions, at the Lucas perches facilities, fish were raised at a  
131 temperature range of 18°C-20°C and a photoperiod of 13L/11D with a blue light or a red  
132 light. At the URAFPA-MAN facilities fish were kept at 20°C and a photoperiod of 16L/8D  
133 during the growth period. Fish were daily fed to apparent satiation during the growth period  
134 and during the experiment (Le Gouessant pellets n°5, proteins 47%, lipids 13%, ash 6.90%  
135 and fiber 2.2%). Fish were handled in accordance with national and international guidelines  
136 for protection of animal welfare.

137

## 138 *2.2 Experimentation and water quality management*

139 Experiment started on May 17<sup>th</sup>, 2013, fish were 18 months old and never spawned before.

140 Breeders of both populations were transferred to 500 L. independent experimental tanks (3  
141 tanks per population with 48 fish per each tank), each tank had his own filtration system and  
142 25% of the water was changed every month. At the beginning of the experiment, fish weight  
143 ranged between 67-137.8g for the “domesticated” fish and in the range of 70.4-129.8g for the  
144 F1 ones. Both populations were exposed to a photothermal program enable to induce and  
145 drive the gonadogenesis (Fontaine et al. 2015) (Fig. 1).

146 In each tank, water quality was measured three times per week. pH was maintained between  
147 7.0 and 7.5 by NaCO<sub>3</sub> additions. The dissolved oxygen was maintained over of 6 mg/L. The  
148 total ammonia (0,36 ± 0,53 mg/L) and nitrite (0,08 ± 0,12 mg/L) nitrogen were measured  
149 using a CARY I spectrophotometer and remained always below 1 mg/L.

150 Fish were sampled at days T0, T35 (decrease of temperature), T157 (vernalization period) and  
151 295 (increasing of temperature ) after the start of the photothermal program (Fig. 1). Six

152 females per tank were caught at each sampling period. The first sampling occurred just before  
153 the initial photoperiod decrease. At each sampling date, fish were anesthetized into a Tricaine  
154 methanesulfonate (MS-222) bath (120 mg/L, Sigma). Blood was sampled from the caudal  
155 vein using a syringe, then stored on ice in heparinized microtubes until centrifuged at 3000g  
156 for 10 min (Centrifuge Jouan C-412). Aliquots of plasma were stored at -80°C until ELISA  
157 analysis was performed. Then, each fish was euthanatized by overanesthetizing in Tricaine  
158 methanesulfonate (MS-222) bath (240 mg/L), weighted and dissected for gonad and liver  
159 weighting and calculation of the gonadosomatic index ( $GSI=100\times \text{gonad weight}/\text{total fish}$   
160  $\text{weight}$ ) and hepatosomatic index ( $HSI= 100\times \text{liver weight}/\text{total fish weight}$ ). For gene  
161 expression analysis, the whole brain and pituitary were snap frozen (separately) in liquid  
162 nitrogen and stored at -80°C.

163

### 164 *2.3 Gonadal histology*

165 Samples of ovaries were stored in a Bouin-Holland solution for 1 week, washed once with  
166 water, twice with 70 % ethanol and stored in absolute ethanol (Abdulfatah et al. 2010). Then,  
167 fragments of gonads were cut into 6-mm-thick slices, dehydrated with OTTIX solution  
168 (DIAPATH SpA, Italy/MM France) and embedded in a paraffin substitute dubbed Diawax  
169 (DIAPATH SpA, Italy/MM France) (adapted from Langeron 1942). Sections of 6  $\mu\text{m}$  were  
170 cut from the Diawax block and stained with a Masson's trichrome-haematoxylin Gill III  
171 (Merck, Darmstadt, Germany), 0.5 % phloxine B (VWR, California, USA) and 0.5 % light  
172 green (Sigma, Saint Quentin Fallavier, France) according to Rinchar and Kestemont (1996).  
173 The stage of oocyte development was determined according to Wallace and Selman (1981).  
174 Observations have been completed with a light upright optical microscope (Nikon Eclipse Ni-  
175 U), Nikon BR software (Nikon France, Champigny sur Marne, France).

176



177 *2.4 Total RNA extraction and reverse transcription*

178 Total RNA was extracted from the brain and pituitary using TRIzol reagent (VWR,  
179 CTRIZ02) according to the manufacturer's protocol. Concentration of total RNA was  
180 measured by UV absorption spectrometry using a Jasco V-630 Spectrophotometer. DNase  
181 treatment (DNase I Thermo Fischer Scientific, EN0521) was performed according to  
182 manufacturer's recommendations. Reverse transcription was performed with M-MLV reverse  
183 transcriptase (Sigma, M1302) according to the manufacturer's recommendations. For each  
184 sample 1µg of total RNA was used. Reaction mixes have been done following manufacturer's  
185 recommendations. Each reverse transcription mix was incubated at 37°C for 50 min and then  
186 heated at 90°C for 10 min, c-DNA samples were finally kept at -80°C.

187

188 *2.5 Gene expression analysis*

189 Primers were designed based on a multiple alignment of sequences available in the NCBI data  
190 base, targeting conserved regions of the FSHb and LHb-subunits, the GnRH 3 as well as the  
191 β-Actin as housekeeping gene.

192 In order to isolate and to sequence *P. fluviatilis* FSHb, LHb-subunits and the GnRH 3 cDNAs  
193 from the brain, a PCR reaction was performed. PCR conditions were as follows: denaturing  
194 cycle of 95°C for 2 min, followed by 35 cycles of 95°C for 1 min, 58°C for 1 min, 72°C for 1  
195 min, followed by a final elongation step of 72°C for 5 min. PCR products were checked on  
196 1% agarose gel electrophoresis. The single obtained band corresponding to the expected  
197 product size was excised from the gel and the amplicon was purified with E.Z.N.A. Pure Kit  
198 (VWR, D6493-01). Purified products were cloned employing the InsTAclone PCR Cloning  
199 KIT (Thermo Fischer Scientific, K1214), and plasmids of 2 days cultures of bacterial colonies  
200 (*Escherichia coli*). Plasmids were analyzed by PCR using M7 universal primers and agarose  
201 gel to confirm the size of the inserted cDNA. Automated sequencing was performed

202 (ABI3730XL DNA sequencer, Macrogen, Korea). Upon gene identification, primers for  
203 quantification were designed on two different exons with the FastPCR software V6.5. (Table  
204 1).

205 Real-time PCR was carried out with a StepOne Plus q-PCR Cyclor (Applied Technologies)  
206 using QUANTA SYBR green reagent (VWR, 733-1382) in a 20  $\mu$ L reaction volume (5  $\mu$ L  
207 diluted cDNA, 2.5  $\mu$ L (600 nM) of each primer, 10  $\mu$ L of 2-fold diluted SYBR-Green  
208 solution) under the following conditions: 95°C initial denaturation for 10 min, followed by 40  
209 cycles of 95°C denaturation for 30s, 60°C primer annealing and extension for 1 min. All  
210 PCRs were run in duplicate. Expression was determined by the comparative CT method  
211 (DDCT) considering a calibrator sample (pooled brain or pituitary cDNA).

212 A melting curve analysis was carried out to ensure assay specificity. The absence of amplified  
213 genomic DNA was validated by comparing the CT obtained in samples with and without  
214 DNase treatments and by designing the primers on two different exons. Samples were  
215 normalized to  $\beta$ -Actin mRNA.

216

## 217 *2.6 Hormone assays*

218 17 $\beta$ -Estradiol (E2, ng/mL) was assayed on 50  $\mu$ L of plasma using the DIAsource E2-  
219 ELISA kit (DIAsource, KAP0621). When necessary, dilution from 1/20 to 1/70 of the plasma  
220 samples was performed. Sensitivity was 5 pg/ml. CV intra-assay varied between 3.1 and 2.6%  
221 and CV inter-assays varied between 4.7 and 2.4% for low and high levels, respectively.

222 Testosterone (T, ng/mL) was assayed on 25  $\mu$ L of plasma using the DIAsource Testosterone  
223 ELISA Kit (DIAsource, KAPD1559). When necessary, a dilution 1/2 of the plasma samples  
224 was performed. Sensitivity was 83 pg/mL, CV intra-assay varied between 1.5 and 9.5 % and  
225 CV inter-assays varied between 7.6 and 8.7% for low and high levels, respectively. The  
226 androgen 11-ketotestosterone (11KT, ng/mL) was assayed on 50  $\mu$ L of plasma diluted 1/50 in

227 ELISA buffer using the Cayman 11-keto Testosterone EIA Kit (Cayman, 582751) and  
228 sensitivity was 1.3 pg/mL. CV intra-assay varied between 2.6 and 8.4 % and CV inter-assays  
229 varied between 9.4 and 12.1% for low and high levels, respectively. Plasma  
230 17 $\alpha$ ,20 $\beta$ -dihydroxy-4-pregnen-3-one (DHP, ng/mL) was assayed using  
231 radioimmunoassay according to Fostier and Jalabert (1986) adapted by Mandiki et al. (2005),  
232 following two extractions with cyclohexane/ethyl acetate (v/v). The DHP antibody was  
233 provided by Dr A. Fostier (INRA, Rennes, France).  
234 Cortisol was assayed on 20  $\mu$ L of plasma using the DIAsource Cortisol ELISA Kit  
235 (DIAsource, KAPDB 270). The intra-assay coefficient of variation was 5.6%, the inter-assay  
236 coefficient of variation was 6.9%. The detection limit was 2.5 ng/mL.

237

### 238 *2.7 Vitellogenin assay*

239 Vitellogenin was indirectly estimated with Alkali-labile phosphate levels (ALP,  $\mu$ g/mL). ALP  
240 levels were assayed on 80  $\mu$ L of plasma. Plasma samples were first centrifuged for  
241 precipitation of high molecular weight proteins with trichloroacetic acid (20%). The pellet  
242 was then washed with absolute ethanol, chloroform solution - di-ethyl ether - absolute ethanol  
243 (1: 2: 2), acetone and finally the di-ethyl ether. The dried pellet was then dissolved in a NaOH  
244 solution (2N) brought to 100°C for 15 min, and then the phosphate release was neutralized by  
245 adding cold HCl (2N). Phosphates are then assayed by the method of Stanton (1968) using a  
246 phosphorus standard solution for calibration, addition of molybdcic acid and reducer solutions  
247 and finally by reading the absorbance at 660 nm with a spectrophotometer.

248

### 249 *2.8 Aromatase assay*

250 Aromatase activity was measured as the specific release of tritiated water produced by  
251 conversion of [1 $\beta$ -H<sup>3</sup>]-androstenedione to estrone, as previously described by Noakson et al.

252 (2001) with some modifications (Mandiki et al. 2005). Fifteen milligrams of frozen tissue  
253 (gonads) were homogenized in a buffer solution (50 mM potassium phosphate, 250 mM  
254 sucrose and 10 mM dithiothreitol pH 7,4; 1:10). A tenth of the homogenate were added of a  
255 NADPH-substrate system consisting of 1 mM NADPH, 25 nM Androst-4-ene-3, 17-dione,  
256 [ $1\beta$ - $^3\text{H}(\text{N})$ ] 275 nM Androst-4-ene-3, 17-dione in the buffer solution and incubated with  
257 gentle shaking for 60 min at 30°C. The reaction was stopped by adding trichloroacetic acid.  
258 Samples were then centrifuged and the supernatant extracted with chloroform, the bottom  
259 contained the protein. The aqueous phase was washed with dextran (5%)-activated charcoal  
260 (0.5%), centrifuged, and an aliquot of the supernatant was added to a liquid scintillating to  
261 determine the radioactivity due to the presence of [ $^3\text{H}$ ]  $\text{H}_2\text{O}$ . For this purpose a liquid  
262 scintillation counter was used. The Lowry method was used to determine protein  
263 concentration after denaturing the protein with NaOH. The aromatase activity was expressed  
264 in  $\text{fmol min}^{-1} \text{mg}^{-1}$  protein.

265

### 266 *2.9 Spawning season and reproductive performance*

267 Spawning season started on March 25<sup>th</sup>, 2014 and finished on April 3<sup>th</sup>, 2014. 77% of the  
268 females spawned during the spawning season in the F1 population and 78% of the females  
269 spawned in the “domesticated” one. Spawning was induced by temperature increase at the end  
270 of the photothermal programme and no hormonal treatment was used during the experiment.  
271 Males and females were kept together in the same tanks. Females were stripped every day  
272 during the spawning season at 5:00 AM. When a female was ovulating, ribbons were  
273 collected in a dry basin and fertilized with 1mL of sperm originated from three different  
274 males, in order to optimize the fertilization success. Ribbons were then transferred into  
275 incubators and maintained at constant temperature ( $13 \pm 0,5^\circ\text{C}$ ). After 24h, 48h, 72h and 120h  
276 post fertilization, samples of approximately 200 eggs from each ribbon were observed to

277 calculate survival rates. Hatching started at 8 days post fertilization. Larvae were measured  
278 and analyzed for malformations (sclerosis, lordosis, yolk bag malformations) with a binocular  
279 microscope, on fixed embryos (n=6 spawns).

280

### 281 *2.10 Statistical analysis*

282 Data are expressed as mean  $\pm$  standard deviation with individual fish as the experimental unit.  
283 Normality of data and homogeneity of variances were tested using Shapiro–Wilk and Levene  
284 test, and data were either log-transformed or arcsin square-root transformed (for data  
285 expressed in percentage) when needed. When data respected the assumptions of normality, a  
286 two-way analysis of variance (ANOVA) with two independent factors (population and time)  
287 was performed followed by LSD post hoc tests to identify significant differences. The level of  
288 significance used in all tests was  $p < 0.05$ . When data were not normally distributed, a  
289 Kruskal–Wallis test was performed followed by the nonparametric Mann–Whitney test. All  
290 statistical analyses were performed using the STATISTICA software (StatSoft, Tulsa, USA).

291

## 292 **3. Results**

293

### 294 *3.1. GSI and gonadal development stages*

295 During the first month of the experiment (T0 to T35), females of both populations had a low  
296 GSI (below 1%) (Fig. 2). Significant increase of the GSI was observed after five months  
297 (T157) ( $P < 0.05$ ), no significant difference was observed between the two populations until  
298 T157. At T295, the “domesticated” females showed a significantly higher GSI ( $p < 0.05$ ) in  
299 comparison with the F1 ones (Fig. 2,  $P < 0.05$ ). Histological analysis revealed that during the  
300 first five weeks of the photothermal program, both female populations showed immature  
301 gonads with more than 90% of protoplasmic oocytes. Since T157 both populations showed

302 more advanced gonad maturation and no significant difference between the two populations  
303 was pointed out (Fig. 3). However, the protoplasmic oocyte diameter was significantly more  
304 elevated for the “domesticated” females ( $109\pm 19\mu\text{m}$ ) than for the F1 ones ( $104\pm 11\mu\text{m}$ ) at T35  
305 ( $p<0.05$ ). The same observation was noticed at T295 with ( $152\pm 13\mu\text{m}$ ) for “domesticated”  
306 females and ( $138\pm 12\mu\text{m}$ ) for the F1 ones ( $p<0.05$ ). At T295, “domesticated” females showed  
307 a more advanced gonadal development than the F1 ones with respectively 54% and 39% of  
308 late vitellogenesis oocytes (Fig. 3), even if there was no significant difference in terms of the  
309 oocyte diameter for vitellogenic oocytes.

310

### 311 *3.2. Sexual steroids, vitellogenin and aromatase*

312 Values for plasma  $17\beta$ -estradiol (E2) were the lowest levels at T0 and T35 for both female  
313 populations (less than 0.5 ng/mL) (Fig. 4A), with a significantly higher level ( $p<0.05$ ) for  
314 domesticated females at T35 in comparison with F1 ones. At T157, plasma E2 levels  
315 significantly increased for both populations in comparison with T35 levels. At T295, a drop of  
316 E2 levels was noticed in the F1 population, so E2 values were significantly higher ( $p<0.05$ ) in  
317 domesticated females than in F1 ones.

318 Plasma Testosterone (T) levels started to increase since T157 until the end of the experiment  
319 for both populations (Fig. 4B). Significant difference was observed at T157 when plasma T  
320 levels were significantly higher for F1 females in comparison with the “domesticated” fish  
321 ( $p<0.05$ ). Plasma vitellogenin levels followed a similar pattern of change as those observed  
322 for T (Fig 4C), with a significant difference ( $p<0.05$ ) at T157 in favor of F1 females. 11-  
323 Ketotestosterone (11-KT) levels remained low for both female populations until T295, when  
324 11-KT levels significantly increased but no significant difference between the two groups was  
325 observed (Fig. 4D).

326 Plasma DHP levels showed an increasing pattern along the experimental time-course for both  
327 populations (Fig. 5A) and no significant difference was observed between the two groups. In  
328 the same way, the aromatase showed an increasing activity, but no significant difference was  
329 registered between the two groups (Fig. 5B).

330

### 331 *3.3. GnRH and GtHs gene expressions*

332 GnRH mRNA levels did not show any significant difference between the two populations  
333 (Fig. 6A, B), but showed a two-fold increase at T35 and remained at this level until T157.

334  $\beta$ FSH mRNA levels showed a quick increase between T0 and T35 for both populations (Fig.  
335 6C, D), and were significantly higher in the F1 perch brains in comparison with their  
336 “domesticated” counterparts at T157 and decreased at the last sampling time. In both brain  
337 and pituitary tissues (Fig. 6E, F),  $\beta$ LH mRNA levels increased coincidentally with advancing  
338 ovarian development. Significant differences in favor of the F1 population were observed at  
339 T35 and T295 in the brain ( $p < 0.05$ ) (Fig. 6E) and pituitary ( $p < 0.005$ ) (Fig. 6F).

340

### 341 *3.4. Plasma cortisol*

342 Plasma cortisol levels showed a significant difference between the two groups with higher  
343 values found in the F1 population ( $p < 0.05$ ) at T35 than the “domesticated” fish (Fig. 7). But,  
344 the difference between the two groups was not significant at the other sampling times.

345

### 346 *3.5. Reproductive performance*

347 The development rates at 24h, 48h, 72h and 120h post-fertilization were evaluated for the two  
348 populations. The F1 population showed a better survival rate than the “domesticated” one at  
349 the four observation times (Table 4) ( $p < 0.05$ ). The F1 population showed a higher eyed stage  
350 larvae percentage and a higher hatching rate than the “domesticated” population. F1

351 population larvae were of better quality than their “domesticated” counterparts in regard of  
352 percentage of deformities ( $p < 0.05$ ).

353

#### 354 **4. Discussion**

355

356 In the present study, changes in gonadosomatic index in the population of “domesticated” fish  
357 corresponds to the profile described by Migaud et al. (2002), and the maximum level of GSI  
358 reached at the last sampling time ( $18.5 \pm 3.2\%$ ), is consistent with the level obtained in  
359 Eurasian perch females by Kristan et al. (2012). These GSI values were significantly higher  
360 than those registered in F1 fish ( $15.8 \pm 3.2\%$ ), being themselves lower than those recorded by  
361 Migaud et al. (2003) and Kristan et al. (2012) in wild Eurasian perch populations (F0). In  
362 agreement with the above GSI results, “domesticated” females showed a slightly more  
363 advanced gonadal development than the F1 ones with respectively 54% and 39% of late  
364 vitellogenesis oocytes at the end of the photothermal program, and a more elevated  
365 protoplasmic oocyte diameter than the F1 ones. This further supports the more advanced  
366 gonadogenesis in the “domesticated” fish by comparison to their F1 counterparts. These  
367 results do not corroborate previous observations made by Kristan et al. (2012) in Eurasian  
368 perch females. Differences between farmed and wild perch have been suggested to be caused  
369 mainly by unsuitable feed for the farmed fish (Bell et al. 1997; Kestemont et al. 1999;  
370 Izquierdo et al. 2001; Kestemont et al. 2008; Henrotte et al. 2010). These authors stated that,  
371 to address lower reproduction in farmed perch, it is necessary to improve the diet by adding  
372 prey fish and a larger proportion of natural ingredients to the feed. In the experiment of  
373 Kristan et al. (2012) wild fish were fed with prey fish and farmed ones with both industrial  
374 food and prey fish, while in our study, both populations were fed only by industrial food. This  
375 could be one element explaining the discrepancy between the two studies.



376

377 Moreover, we cannot rule out the implication of other external factors (e.g. type of  
378 photothermal program) and internal factors (e.g. fish age) in the monitoring of divergent data.  
379 Our wild population is a F1 generation issued from F0 broodstock caught as eggs from the  
380 wild (Geneva Lake) and reared in artificial conditions during their whole life. The wild  
381 population studied by Kristan (2012) is a wild population reared in ponds and transferred in  
382 artificial conditions for the experiment, which implies acclimation phenomena due to the  
383 transfer to artificial conditions, which may in turns alter gonad maturation and reproduction.  
384 In addition, we cannot exclude the influence of internal factors like fish age that has been  
385 shown to influence reproductive capacity in teleosts (Abi-Ayad, 1998). Our fish were  
386 eighteen months old for both populations, whereas fish studied by Kristan (2012) were three  
387 years old. The geographical origins, and by consequence, potentially the genetic patrimony, is  
388 another internal factor that can impact fish reproduction and justify divergent results.

389

390 While the gonadogenesis was more advanced in the “domesticated” population, the final  
391 reproductive outcomes were markedly better in the F1 fish. The fertilization rate monitored at  
392 24h was almost 3-fold higher in the F1 fish ( $78\pm 35\%$ ) in comparison with the “domesticated”  
393 group ( $28\pm 29\%$ ). The fecundity rates observed by Kristan (2012) were more elevated (90%)  
394 in both populations. Kouril and Hamackova (1999) reported 60-95% fertilization of eggs in  
395 wild perch after artificial stripping which is in agreement with our values for the F1  
396 population. This difference of fertilization rates is an additional indication that the egg quality  
397 in Eurasian perch is highly variable between the studies and also within the same population.  
398 The highest contrast between farmed and F1 perch in the parameters of egg quality was  
399 recorded after the evaluation of hatching rates. A really poor percentage of hatching rate was  
400 obtained for the farmed fish ( $2\pm 2\%$ ), slightly lower than the data provided by Kristan et al.

401 (2012) in other farmed population. This poor ova quality could be explained by the fact that  
402 “domesticated” fish exhibited a faster gametogenesis than the F1 ones and they did not  
403 accumulate enough nutrients for embryo development. This better egg quality in F1 fish was  
404 also confirmed by comparing the percentages of malformations between both populations.  
405 Anyway these results and those of Kristan et al. (2012) suggest that “domesticated”  
406 broodstock in Eurasian perch exhibit lower reproductive performance than fish at an earlier  
407 stage of domestication.

408

409 In the present study, the better reproductive performance in F1 are *a priori* surprising  
410 regarding the gonadogenesis and oocyte development results. To understand the physiological  
411 mechanisms that are behind these differences, we investigated some physiological actors  
412 driving the control of oogenesis control. The estrogen 17- $\beta$  estradiol (E2) levels were higher  
413 in the “domesticated” fish than in the F1 fish, which is in agreement with the GSI results.  
414 Contrarily, testosterone levels were higher in F1 females than in “domesticated” ones. We  
415 tried to understand these opposite results by measuring aromatase activity (enzyme which  
416 converts testosterone to 17-beta estradiol). No significant difference in aromatase activity was  
417 found between the two populations while we expected a decline in aromatase activity for the  
418 F1 population. Our observation cannot explain this difference in sexual steroids levels  
419 between the two populations. However, E2 being the principal mediator of vitellogenin  
420 production and incorporation in the oocytes (Lubzens et al. 2010), these more elevated E2  
421 levels in domesticated fish should have been accompanied with higher levels of vitellogenin  
422 in the plasma. However opposite results were found. These conflicting observations might be  
423 explained by three simultaneous phenomena: firstly Eurasian perch displays strongly elevated  
424 testosterone levels by comparison to other fish species and these testosterone levels are 20-50-  
425 fold higher than the E2 level (Milla et al. 2009a). Secondly, like E2, testosterone is a

426 stimulator of vitellogenin production in fish (Ramezani-Fard et al. 2013). Thirdly, more  
427 elevated testosterone levels were measured in the F1 perch than in the domesticated ones.  
428 All together, we speculate that the higher vitellogenin level measured in F1 fish would be  
429 more explained by the differential testosterone level between both groups rather than the  
430 classically described E2 involvement. Concerning 11 keto-testosterone, given the low level  
431 observed in comparison with the testosterone level and the absence of contrasted  
432 concentrations between both groups, we consider that this sexual steroid was not a major actor  
433 explaining this divergence of oogenesis progress between F1 and domesticated fish.  
434 Moreover, to explain the divergence in oogenesis and reproductive performance between the  
435 two populations, we investigated the plasma  $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one (DHP),  
436 which is the maturation inducing steroid (MIS) in percids (Nagahama et al. 1987). Our  
437 hypothesis was that, a difference in kinetics of the final oocyte maturation between the two  
438 populations would be at the origin of the reproductive performance divergence. But plasma  
439 DHP levels which shows a classical kinetics of change did not display different levels  
440 between the two female groups. However it has been reported that another MIS is associated  
441 with the final oocyte maturation control in some perciform species: it is the  $17\alpha,20\beta,21$ -  
442 trihydroxy-4-pregnen-3-one ( $20\beta$ -S) (Thomas 1988; Asahina et al. 1991 ; Modesto and  
443 Canario 1993; King et al. 1994), we can imagine that in perch, final oocyte maturation could  
444 be controlled by the combined action of DHP and  $20\beta$ -S, but we cannot exclude the  
445 hypothesis that the DHP could be the principal MIS in perch but it has to be sampled more  
446 frequently during the final oocyte maturation phase. All combined, our hypothesis is that this  
447 domestication process displayed distinct effects according to the time-course of oogenesis.  
448 Indeed, farmed fish exhibited an earlier gonadogenesis and vitellogenesis than F1 ones, then  
449 turning to depreciative effects on final oogenesis, oocyte quality, finally traduced by poor  
450 reproductive performance.

451

452 Several studies have reported the effect of domestication on reproduction efficiency in many  
453 species (Dannewitz et al. 2004; Salze et al. 2005; Randak et al. 2006; Jonsson and Jonsson  
454 2006; Krejszeff et al. 2009; Kristan et al. 2012; Ciesla et al. 2013). These data suggest that  
455 physiological changes occur during the domestication process due to farming conditions.  
456 However, these studies do not provide information about the endocrine mechanisms in  
457 broodstock explaining these changes in reproductive capacity. As the level of sex steroids is  
458 modified in our study, we hypothesized that a disruption of the gonadotropic axis occurred  
459 during the domestication process. Significant differences between the two populations were  
460 monitored in the level of gonadotropin gene expressions in the brain and pituitary, while  
461 GnRH gene expression was not affected. However, we only investigated the GnRH3 form in  
462 our experiment, but there is another form involved during the reproduction in fish which is the  
463 GnRH1 (Yaron et al. 2003) and which could be affected as well during the domestication  
464 process. These results support the hypothesis of a gonadotropic axis disruption, starting at the  
465 gonadotropic level affecting in turns the secretion of sexual steroids in the gonad. As the level  
466 of FSH and LH transcripts were higher in F1 fish, in particular at the two last sampling times,  
467 we think that the more markedly rise in testosterone level in this population is due to LH rise,  
468 then leading to a better reproductive performance in F1 fish. Indeed, blood plasma  
469 testosterone is regulated after exposure to the LH agonist hCG in perch females at the end of  
470 the reproductive cycle (Milla et al. 2009a). However, we cannot rule out the fact that the  
471 measures of FSH and LH transcript levels might not reflect the circulating levels of GTHs.  
472 Moreover, the hormonal differences observed between the two populations might reflect a  
473 differential threshold of action of these hormones between the two populations.  
474 Kristan (2012) proposed that the stress state of fish would explain the differences in the  
475 reproductive performance between the two populations. Even if there was not any problem in

476 terms of water quality, infection or mortality, the level of cortisol were not low during this  
477 experiment and the level was higher in F1 fish before the alveoli cortical stage of the cycle.  
478 Consequently, in accordance with the proposal of Kristan, we cannot rule out the fact that the  
479 differential cortisol levels or sensitivity to stressful conditions led to differential advancement  
480 of early gonadogenesis between the two groups of fish, stress and cortisol being major  
481 modulators of fish reproduction (Barton 2002; Milla et al. 2009b; Schreck 2010). But, this  
482 precocious difference alone would not be sufficient to explain the major differences in the  
483 reproductive performance, as fish were probably in the same level of stress state since the  
484 vitellogenesis stage.

485 In conclusion, this study showed that this domestication route negatively affected the  
486 reproductive performance. However, some conflicting results were observed about the  
487 reproductive physiological parameters: on the one hand, this domestication process advanced  
488 the start of gonadogenesis until the start of the vitellogenesis period, probably caused by more  
489 precocious E2 rise. Then, the lower level of gonadotropins transcripts and testosterone  
490 potentially led to a drop in vitellogenin in comparison to F1 fish, further affecting the  
491 reproductive success. This study showed that this domestication process negatively affected  
492 reproduction, so more attention should be paid by the farmers to the choice of renewing  
493 broodstock in particular when they maintain fish coming from several generations of  
494 domestication in their facilities,

495

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500

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502 and wrote the main part of the manuscript, and performed the gene expression analysis.  
503 Sylvain Milla, Pascal Fontaine and Tomas Policar initiated the research and/or managed the  
504 work and gave final approval for submission. Sandrine Viot and Camille Rousseau  
505 performed the plasma steroid assays and histological analysis, Maud Alix managed the  
506 spawning season and compared the reproductive performance of both populations, Yannick  
507 Ledoré took care of fish daily and managed the samplings; SNM Mandiki and Patrick  
508 Kestemont performed the enzymatic activity assay and RIA assay for aromatase activity and  
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510

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649

650 **Legends**

651

652 **Fig. 1. The photothermal program (adapted from Fontaine et al. 2015).** The photothermal  
653 program was used to induce perch reproduction. The dark curve corresponds to the  
654 photoperiod variations. The light curve corresponds to the temperature variations. T0, T35,  
655 T157, T295 are the four sampling times which represent days 0, 35, 157 and 295 after the  
656 start of the experiment.

657

658 **Fig. 2. GSI changes (%) in F1 and domesticated females.** (9 females per sampling period +  
659 Standard deviation). Sampling times T0, T35, T157 and T295 correspond respectively to days  
660 0, 35, 157 and 295 after the beginning of the photothermal program. The asterisk represents a  
661 significant difference between the 2 fish populations ( $p < 0.05$ ).

662

663 **Fig. 3. Different developmental stages observed in domesticated and F1 perch female**  
664 **ovaries.** (9 females per sampling time + Standard deviation). D and F1 refer to Domesticated  
665 and F1 respectively. Sampling periods T0, T35, T157 and T295 correspond respectively to  
666 days 0, 35, 157 and 295 after the beginning of the photothermal program.

667

668 **Fig. 4. Plasma sexual steroids and vitellogenin levels variation. (A) E2 levels variation**  
669 **(ng/mL) in F1 and domesticated females. (B) Testosterone levels variation (ng/mL) in**  
670 **F1 and domesticated females. (C) Vitellogenin levels variation ( $\mu\text{g ALP/mL}$ ) in F1 and**  
671 **domesticated females. (D) 11KT variations (pg/mL) in F1 and domesticated females.** (9  
672 females per time per population + standard deviation). Sampling periods T0, T35, T157 and  
673 T295 correspond respectively to days 0, 35, 157 and 295 after the beginning of the  
674 photothermal program. The asterisk (\*) represents a significant difference ( $p < 0.05$ ) and (\*\*)  
675 ( $p < 0.001$ ).

676

677 **Fig.5. Plasma DHP and aromatase activity. (A) Plasma DHP levels (ng/mL) variation in**  
678 **domesticated and F1 perch females. (B) Aromatase activity variation (fmol/min.mg) in**  
679 **domesticated and F1 perch females.** (9 females per time per population + standard  
680 deviation). Sampling periods T0, T35, T157 and T295 correspond respectively to days 0, 35,  
681 157 and 295 after the beginning of the photothermal program.

682

683 **Fig. 6. GnRH 3 and gonadotropins gene expression quantification in the brain and**  
684 **pituitary. (A) GnRH 3 mRNA levels in F1 and domesticated female brains. (B) GnRH 3**  
685 **mRNA levels in F1 and domesticated female pituitaries. (C)  $\beta$ FSH mRNA levels in F1**  
686 **and domesticated female brains. (D)  $\beta$ FSH mRNA levels in F1 and domesticated female**  
687 **pituitaries. (E)  $\beta$ LH mRNA levels in F1 and domesticated female brains.. (F)  $\beta$ LH**  
688 **mRNA levels in F1 and domesticated female pituitaries.** (9 females per time per population  
689 + standard deviation). Sampling periods T0, T35, T157 and T295 correspond respectively to  
690 days 0, 35, 157 and 295 after the beginning of the photothermal program. mRNA levels have  
691 been quantified by q-PCR. The asterisk (\*) represents a significant difference ( $p < 0.05$ ) and  
692 (\*\*) ( $p < 0.001$ ).

693

694 **Fig. 7. Cortisol levels variation (ng/mL) in F1 and domesticated females.** (9 females per  
695 time per population + standard deviation). Sampling times T0, T35, T157 and T295  
696 correspond respectively to days 0, 35, 157 and 295 after the beginning of the photothermal  
697 program. The asterisk (\*) represents a significant difference ( $p < 0.05$ ).

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