

# Domestication may affect the maternal mRNA profile in unfertilized eggs, potentially impacting the embryonic development of Eurasian perch (*Perca fluviatilis*)

Taina Rocha de Almeida, Maud Alix, Aurélie Le Cam, Christophe Klopp, Jérôme Montfort, Lola Toomey, Yannick Ledore, Julien Bobe, Dominique Chardard, Bérénice Schaerlinger, et al.

## ► To cite this version:

Taina Rocha de Almeida, Maud Alix, Aurélie Le Cam, Christophe Klopp, Jérôme Montfort, et al.. Domestication may affect the maternal mRNA profile in unfertilized eggs, potentially impacting the embryonic development of Eurasian perch (*Perca fluviatilis*). PLoS ONE, Public Library of Science, 2019, 14 (12), pp.1-25. 10.1371/journal.pone.0226878 . hal-02627554

HAL Id: hal-02627554

<https://hal.inrae.fr/hal-02627554>

Submitted on 8 Apr 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Domestication may affect the maternal mRNA profile in unfertilized**  
2 **eggs, potentially impacting the embryonic development of Eurasian**  
3 **perch (*Perca fluviatilis*)**

4  
5 Tainá Rocha de Almeida<sup>1,•¶</sup>, Maud Alix<sup>1,•¶</sup>, Aurélie Le Cam<sup>2</sup>, Christophe Klopp<sup>3</sup>, Jérôme  
6 Montfort<sup>2</sup>, Lola Toomey<sup>1</sup>, Yannick Ledoré<sup>1</sup>, Julien Bobe<sup>2</sup>, Dominique Chardard<sup>1</sup>, Bérénice  
7 Schaerlinger<sup>1\*</sup> and Pascal Fontaine<sup>1</sup>

8  
9  
10 <sup>1</sup>UR AFPA, University of Lorraine, INRA, F-54000 Nancy, France

11 <sup>2</sup>LPGP, UR1037 Fish Physiology and Genomics, INRA, Rennes, France

12 <sup>3</sup>INRA, Sigenae, MIAT, CS 52627, Castanet-Tolosan, Toulouse, France

13  
14  
15 \*Corresponding author

16 E-mail: [berenice.schaerlinger@univ-lorraine.fr](mailto:berenice.schaerlinger@univ-lorraine.fr) (BS)

17  
18 ¶ • Both authors contributed equally to this work and are thus co-first authors

## 26 **Abstract**

27           Domestication is an evolutionary process during which we expect populations to  
28 progressively adapt to an environment controlled by humans. It is accompanied by genetic and  
29 presumably epigenetic changes potentially leading to modifications in the transcriptomic profile  
30 in various tissues. Reproduction is a key function often affected by this process in numerous  
31 species, regardless of the mechanism. The maternal mRNA in fish eggs is crucial for the proper  
32 embryogenesis. Our working hypothesis is that modifications of maternal mRNAs may reflect  
33 potential genetic and/or epigenetic modifications occurring during domestication and could have  
34 consequences during embryogenesis. Consequently, we investigated the transcriptomic profile of  
35 unfertilized eggs from two populations of Eurasian perch. These two populations differed by their  
36 domestication histories (F1 vs. F7+ – at least seven generations of reproduction in captivity) and  
37 were genetically differentiated ( $F_{ST}=0.1055$ ,  $p<0.05$ ). A broad follow up of the oogenesis  
38 progression failed to show significant differences during oogenesis between populations.  
39 However, the F1 population spawned earlier with embryos presenting an overall higher  
40 survivorship than those from the F7+ population. The transcriptomic profile of unfertilized eggs  
41 showed 358 differentially expressed genes between populations. In conclusion, our data suggests  
42 that the domestication process may influence the regulation of the maternal transcripts in fish eggs,  
43 which could in turn explain differences of developmental success.

44 **Keywords:** microarray; egg content; fish populations; reproductive cycle; gonadogenesis; egg  
45 quality.

46

47

48

## 50 **Introduction**

51           Domestication is an evolutionary and continuous process enabling wild animals to adapt to  
52 humans and anthropogenic environments [1,2]. It involves the combination of several genetic and  
53 potentially epigenetic modifications driving gene expression and phenotypic changes. Differences  
54 in gene expression due to rapid adaptation to new environments were already reported from the  
55 first generation of reproduction in captivity in steelhead trout (*Oncorhynchus mykiss*) [3]. Thus,  
56 the regulation of genes expression may change early during domestication. The main genetic  
57 mechanisms involved are inbreeding, genetic drift and natural, relaxed and artificial selections  
58 [1,2]. The two first mechanisms have important consequences when founder populations are small  
59 because they rapidly lead to important changes in allelic frequencies [4]. The relaxed selection  
60 consists of a reduction of selection pressure on traits which are not necessary anymore in captive  
61 conditions. The artificial selection results from the selection of breeders according to phenotypes  
62 chosen by humans. Finally, the natural selection occurs and usually eliminates animals which are  
63 not adapted to anthropogenic environments [5]. Studies suggest a relationship between epigenetic  
64 modifications and phenotypic plasticity in response to the environment in some animal and plant  
65 species [6]. In fish, the current knowledge shows a modification of the epigenetic signature in  
66 individuals reared in hatcheries in comparison to their wild counterparts in salmonids and the  
67 European sea bass (*Dicentrarchus labrax*) [7–10]. These modifications affect somatic and  
68 germinal cells [8] and may play a role during the first steps of domestication [10]. However, the  
69 relationships and timeline between genetic and epigenetic modifications remain unclear [6].

70           All these mechanisms depend greatly on the breeding practices. Indeed, independent trials  
71 of domestication may lead potentially to various types of modifications that may have either

72 beneficial (adaptation) or deleterious (maladaptation) effects on various biological functions of the  
73 new domesticated populations. The artificial selection, for one or several phenotypes is specific to  
74 the domestication process and has consequences that may not be predicted, since morphological,  
75 behavioral and physiological traits of animals are intrinsically related [11]. The consequences of  
76 modifications of the balance between these traits are not yet properly understood. Indeed, it appears  
77 that the artificial selection of specific phenotypes may have deleterious effects on other biological  
78 traits because most of resource intakes are dedicated to the selected traits. This imbalance often  
79 leads to a decrease of reproduction abilities [5,12], commonly seen in numerous terrestrial and  
80 aquatic species. A recent meta-analysis investigating the effect of birth-origin (captive vs. wild)  
81 on the reproductive success of animals reared in different anthropogenic environments was  
82 performed. For all of the 44 analyzed species, the offspring survival success was decreased in  
83 “captive-born” animals compared to their “wild-born” counterparts [13]. It usually involves  
84 developmental failures characterized by fertilization issues, embryonic lethalties or the occurrence  
85 of deformities. They often ensue from defects of incorporation or synthesis of the eggs’ molecular  
86 content. Indeed, the abundance of these molecules, controlling embryogenesis process after  
87 fertilization, can directly be affected by modifications of extrinsic or intrinsic factors faced by  
88 females during oogenesis [14]. Among them, the maternal mRNA expression profile may thus  
89 result from genetic and epigenetic changes in the breeders’ ancestors during the domestication  
90 process. It could potentially help to make the link between mechanisms described above and the  
91 reproductive success of captive populations in comparison to their wild counterparts.

92         There are two main ways to study the domestication process [5]. First, longitudinal studies  
93 allowing a continuous follow up of a population throughout the domestication process across  
94 generations. This method is the most efficient to understand phenotypic and molecular

95 modifications occurring at each step of the domestication process. However, it is long and difficult  
96 to perform logistically. The second way, which is commonly used, corresponds to a comparison  
97 between wild and domesticated populations. Such method has been previously used and  
98 successfully highlighted differences in several fish species, such as steelhead trout (*O. mykiss*) [3],  
99 Atlantic salmon (*Salmo salar*) [15–17], Atlantic cod (*Gadus morhua*) [18,19], greater amberjack  
100 (*Seriola dumerili*) [20] and Eurasian perch (*Perca fluviatilis*) [21,22]. However, numerous pieces  
101 of information are often lacking (genetic relatedness between populations, rearing conditions and  
102 history of the domestication process) and thus prevent drawing accurate conclusions. Indeed, as  
103 previously explained, phenotypic modifications potentially lead by genetic/epigenetic  
104 modifications, depend on rearing practices. Such information is not always tracked properly by  
105 farmers. For example, for several domesticated fish stocks, wild breeders are introduced to keep a  
106 sufficiently high genetic diversity [23] without keeping track of these introductions leading to  
107 incomplete traceability [24]. Today, with the increasing knowledge accuracy and the development  
108 of molecular tools, such information becomes important to draw proper conclusions. One way to  
109 overcome this issue consists in investigating the genetic differentiation between wild and farmed  
110 studied populations. This preliminary step would help understanding differences between  
111 populations.

112 In the context of fish production diversification, numerous efforts are done to domesticate  
113 a large number of new species [23]. However, the lack of knowledge on biological and  
114 physiological needs of some species may lead to inadequate domestication attempts with  
115 deleterious consequences on the biological traits described above. The Eurasian perch (*P.*  
116 *fluviatilis*) is a promising species in aquaculture for the production diversification. It is a freshwater  
117 fish species widely distributed in Europe and in the Northern part of Asia [25]. It has a niche

118 market with a traditional demand in several European countries [26,27]. The Eurasian perch is a  
119 synchronous early spring spawner and its oogenesis induction and progression are mostly  
120 controlled by temperature and photoperiod variations [28–33]. Consequently, manipulation of  
121 these two factors allowed defining a photothermal program largely used in Eurasian perch farms  
122 for out-of season reproduction in recirculating aquaculture systems (RAS) [32]. Despite this  
123 successful control of its reproductive cycle allowing out-of-season spawning, the reproduction  
124 success remains variable even if the same rearing conditions are applied to the broodstock [34]. It  
125 is probably due to the lack of knowledge on potential intrinsic and/or extrinsic modulating factors,  
126 including the history and details of the domestication progression experienced by populations.

127 In the present study, we chose to compare two Eurasian perch populations, reared in the  
128 same conditions but with different histories of domestication. We hypothesized that the level of  
129 domestication may modulate the accumulation of maternal mRNA in eggs during oogenesis,  
130 potentially impacting the embryos early development after fertilization.

131

## 132 **Material and Methods**

133

### 134 **Origin of fish and broodstock management**

135 Fish were handled in accordance with national and international guidelines for animal  
136 welfare protection (*Directive* 2010/63/EU. Agreement number: APAFIS#1390-  
137 2018031516387833 v2 accepted by the Lorraine Ethic Committee for Animal Experimentation  
138 (CELMEA) and the French Ministry of Research). Two populations of three years old Eurasian  
139 perch originating from artificial reproductions in November 2011 were used. They correspond to

140 (i) fish at an advanced stage of the domestication process coming from breeders reared in RAS for  
141 at least seven generations (F7+ population) and (ii) Eurasian perch originating from breeders  
142 collected in the Geneva Lake at the embryonic stage and reared in RAS conditions (F1 population).  
143 All animals came from the fish farm “Lucas Perches” (Hampont, France), which provided us the  
144 information of the presumable number of generations of the F7+ population and that their ancestors  
145 had supposedly been caught in Geneva Lake. Fish were transferred to our indoor facilities after  
146 weaning period in February 2012 (mean weight of  $3.78 \pm 1.07$  g). Both populations were reared  
147 separately in different tanks but under the same RAS conditions (constant photoperiod (L:D -  
148 16:8), 300 lux at the water surface during the lighting period and at 20-21°C) to avoid oogenesis  
149 stimulation [32,35] until they reached mean weight of  $287 \pm 89$  g.

150 About two months before the experiment began (May 2014), 654 Eurasian perch (313 from  
151 the F7+ and 341 from the F1 populations) were transferred to the Aquaculture Experimental  
152 Platform (AEP, registration number for animal experimentation C54-547-18) belonging to the  
153 URAPA lab and located at the Faculty of Sciences and Technologies of the University of Lorraine  
154 (France). They were divided into six independent groups (three per population with an equivalent  
155 number of fish in each group). Fish were put into six identical rooms consisting of independent  
156 RAS with 3000 liters tanks. Temperature, photoperiod and light intensity were accurately  
157 controlled in each room using dedicated software. Environmental conditions were the same during  
158 acclimation phase and growing period. In order to induce gonadogenesis, breeders were submitted  
159 to a photothermal program allowing effective induction and control of the reproduction cycle [28]  
160 from August 18<sup>th</sup> 2014 to June 22<sup>nd</sup> 2015 (day 1 to day 309, Fig 1). Water levels of dissolved  
161 oxygen ( $9.53 \pm 0.07$  mg/L and  $9.73 \pm 0.12$  mg/L), pH ( $7.55 \pm 0.10$  and  $7.47 \pm 0.00$ ), nitrite ( $0.29$   
162  $\pm 0.14$  mg/L and  $0.09 \pm 0.01$  mg/L) and ammonium ( $0.67 \pm 0.22$  mg/L and  $0.27 \pm 0.02$  mg/L) were



163 monitored twice a week and kept under the respective thresholds in breeders' tanks (for F1 and  
164 F7+ populations, respectively). At the beginning of the experiment, all animals were individually  
165 tagged with P.I.T. tags (Transponder ISO 2 x 12 mm, Biolog-id) to monitor individuals all along  
166 the experiment. All fish were fed twice a day to satiation five days a week. In alternation, they  
167 were fed three days with commercial pellets (Sturgeon Grower N°5, Le Gouessant) and two days  
168 with frozen squids and shrimps (Pomona). On Saturdays and Sundays, they were fed once with  
169 commercial pellets to satiety.

170

171 **Fig 1. Graphic summary of the experimental design.** The photothermal program was used to  
172 control each step of the reproductive cycle and spawning of Eurasian perch. Fish sampling was  
173 performed at T0, T31, T87, T154 and T253 days after the beginning of the photophase initial  
174 decrease (induction of the reproductive cycle). For each sampling point, sexual steroids and  
175 Vitellogenin levels were measured in the plasma and a histological follow up of the oogenesis  
176 progression was performed. During spawning, each spawn was split into two parts. The first one  
177 was not fertilized and frozen for further molecular analyses while the other part was fertilized to  
178 assess the developmental progression. All details are given in the method paragraph.

179

## 180 **Experimental design, tissue sampling and morphometric measures**

181 In order to follow the oogenesis process, five sampling points of females were performed  
182 throughout the reproduction cycle: T0 at the beginning of the experiment allowed us to determine  
183 the initial status of breeders, T31, T87 and T154 sampling points allowed us to check the oogenesis  
184 along its progression and T253 the final status of the gonads before the spawning season (Fig 1).  
185 Four to five females per tank were collected at each sampling point. Firstly, fish were anesthetized

186 by immersion into a Tricaine methanesulfonate solution (120 mg/L; Sigma-Aldrich) for five  
187 minutes to collect blood from the caudal vein. Blood was centrifuged at 8000 rpm for 10 minutes  
188 in previously heparinized (4.5 mg heparin sodium salt from porcine intestinal mucosa 100KU,  
189 Sigma-Aldrich) microtubes. Plasma aliquots were conserved at -80°C for further evaluation of  
190 sexual steroids and Vitellogenin concentrations measurements.

191 After blood sampling, fish were killed using an overdose of Tricaine methanesulfonate  
192 (240 mg/L; Sigma-Aldrich) in accordance to European Ethical guidelines (Directive 2010/63/UE).  
193 Total weight was measured before collecting the gonads which were weighted to calculate the  
194 gonado-somatic index ( $GSI = \text{gonad weight} / \text{total weight} * 100$ ) and fixed as described below for  
195 histological studies.

196

## 197 **Evaluation of Steroids and Vitellogenin concentrations in the plasma**

198 The 17- $\beta$ -estradiol (E2, ng/mL) and testosterone (T, ng/mL) assays were performed on 50  
199  $\mu$ L of plasma of each sampled female for all sampling points (around 15 females per  
200 population/sampling point) using the DIAsource E2-ELISA kit (KAP0621, DIAsource) and the  
201 DIAsource Testosterone ELISA kit (KAPD1559, DIAsource), respectively. Samples were diluted  
202 from 1/1 to 1/50 for E2 and from 1/1 to 1/10 for T measurements depending on the oogenesis  
203 developmental stage. The E2 assay sensitivity was  $5 \pm 2$  pg/mL and the range of use was from 0 to  
204 880 pg/mL with an intra assays CV varying from 4 to 17% and an inter assay CV of about 27%.  
205 Concerning T, the sensitivity was 0.083 ng/mL and the range of use was from 0 to 16 ng/mL. The  
206 intra assays CV varied from 7 to 19 % and the inter assay CV was about 16%.

207 The Vitellogenin plasmatic concentration was indirectly estimated in 80  $\mu$ l of plasma by  
208 measuring concentrations of the alkali-labile phosphate level as described in [36].

209

## 210 **Gonads histology**

211 Female gonads from all sampling points were fixed in Bouin's solution for one week before  
212 being washed in 70% alcohol. Samples were then dehydrated in ascending series of ethanol (70-  
213 100%) before being embedded in paraffin with an orientation allowing transversal cuts. Five  
214 micrometer sections were performed with a Leitz Wetzlar microtome and collected on glass slides.  
215 Masson's trichrome staining was done according to a protocol adapted from [37] as follows:  
216 Hematoxylin solution modified according to Gill III (Merck) was used from five to ten minutes;  
217 Phloxine (VWR) was diluted in water at 0.5% and used for five minutes; Light Green (Sigma) was  
218 diluted in water at 0.5% and used from three to five minutes. Stained sections were examined,  
219 photographed and analyzed using a light upright optical microscope (Nikon Eclipse Ni-U)  
220 associated with a DS-Fi1 digital camera and the software NIS BR (Nikon France, Champigny-sur-  
221 Marne, France) at low magnification (x2 and x4).

222 Oocytes stages were determined according to [38] and classified into six classes: primary  
223 growth (PG), early cortical alveoli stage (ECA), late cortical alveoli stage (LCA), early  
224 vitellogenesis (EV), late vitellogenesis (LV) and atresia (A). Oogonia (O) stages were also  
225 identified.

226 The gonadic maturation state was determined by counting all oocytes of each class on one  
227 complete and representative transversal stained sections of the ovary for T0-T154 and three  
228 representative transversal sections for T253 because the gonads were then too large to be laid on  
229 one slide.

230

## 231 **Gamete collection and fertilization**

232 Before the spawning season, all females were caught to take some oocytes using a catheter  
233 and determine their oocytes maturation according to [39]. On May 13<sup>rd</sup> and 15<sup>th</sup> 2015, females  
234 from all tanks were examined and were allocated to separate tanks for the spawning season,  
235 according to their oocyte maturation stage and regardless of their original population. Thus, one  
236 tank contained the females having oocytes at stages I and II, another tank contained the females at  
237 stages III and IV (which were all coming from the F1 population), and another tank contained  
238 females that could not be staged reliably. Males from F7+ and F1 populations were kept apart in  
239 two tanks depending on their origin. When one female spawned, all females of this tank were  
240 monitored daily to identify ovulation and collect the eggs by stripping them. This procedure was  
241 always performed between 4am and 5am and each spawn was treated individually. The first spawn  
242 observed in each tank was not considered for the experiment because once in the water, the eggs  
243 are activated and their ability to be fertilized decrease rapidly. The spawning season took place  
244 from May 29<sup>th</sup> to June 22<sup>nd</sup> 2015 and each female stripped was identified as to its original  
245 population by its P.I.T. tag. Eggs were fertilized as described in [40] with sperm from three males  
246 (total volume of 100  $\mu$ l sperm/g dry eggs). Eggs stripped in the same day were fertilized using the  
247 same pool of sperm and at the end of the spawning season no day effect was seen in the  
248 reproductive performance. All males used to perform fertilization came from the F1 population,  
249 that presented a higher quality (motility estimated under a light upright optical microscope (Nikon  
250 Eclipse Ni-U)). The other part of each spawn (18 individual samples of about 200mg) was  
251 immediately frozen in liquid nitrogen and kept at -80°C for subsequent transcriptomic analysis.

252

## 253 **Study of reproductive performance**

254 In total, 32 spawn, corresponding to 32 females (13 from F7+ population and 19 from the  
255 F1), were treated as described above. A previous study performed in our team showed that cell  
256 cleavage timetable can be highly variable between embryos even within the same spawn [41].  
257 Thus, we choose to perform our first evaluation of the embryonic survival at 24 hours post  
258 fertilization (hpf) because it was the earliest stage to establish a relevant estimation of survival.  
259 We were thus not able to make a distinction between non-fertilized eggs and dead embryos.  
260 Eurasian perch eggs are surrounded by a jelly coat that protects embryos from the outside  
261 environment [29]. This jelly coat prevents to sort the eggs depending on their survival at each stage  
262 as it is currently performed with other fish species for which eggs are not attached. Thus, we  
263 performed the following protocol to evaluate the survival in the same ribbon samples at several  
264 timepoints.

265 Just after fertilization, three ribbon fragments (samples) of around 500 eggs were randomly  
266 cut from each spawn/ribbon and incubated to determine the percentage of embryos alive at  
267 different timepoints. We considered that the borders of each fragment should be avoided since  
268 embryonic development in this region could be impacted by the cutting. At each specific timepoint,  
269 around 100 embryos were counted and, among them, the number of alive embryos (those reaching  
270 the proper developmental stage) was counted to evaluate the percentage of alive embryos. These  
271 evaluations were performed in the middle of each fragment at 24, 48, 72 and 120 hpf to avoid  
272 potential border effects described above. The percentage of embryos alive was estimated using the  
273 following formula: (number of alive embryos at one stage/total number of embryos studied at that  
274 stage) x 100. In addition, three other fertilized samples of around 100 eggs/sample were kept apart,  
275 without manipulation, until the hatching period. They were used to determine the percentage of  
276 embryos hatching using the following formula: (number of hatched embryos/total number of

277 embryos in the fragment) x 100 and deformities rates according to [42]. The global deformities  
278 rate (Dr) and specific deformities rates allowed studying defects in the axis (Ad), yolk (Yd),  
279 cardiac (Cd), mouth (Md), eyes (Ed) and others (Od) as described in [42].

280

## 281 **RNA extraction**

282 Total RNA was extracted from unfertilized frozen eggs from the 32 spawn (mean weight  
283 of 100 mg, 10-15 eggs) using TRIzol reagent (Life Technologies) at a ratio of 100mg per mL of  
284 reagent and following the manufacturer's instructions with some modifications. Indeed, a milling  
285 step was added during the homogenization step to get rid more easily of the gelatinous envelope  
286 and chorion surrounding eggs. To do so, a bullet blender (Next Advance) and zirconium oxide  
287 beads 1.0 mm were used. In addition, a supplementary centrifugation (4°C, 30min. 13000 rpm)  
288 was performed before the addition of the chloroform to remove the lipid content in eggs. A  
289 NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies) was used to evaluate the  
290 quantity of total RNA and an Agilent 2100 Bioanalyzer (Agilent Technologies) was used to  
291 evaluate the integrity of the RNA extracted. Samples exhibited an integrity score higher than 7 and  
292 were used for the microarray analysis.

293

## 294 **Microarray analysis**

295 The Eurasian perch array (SurePrint G3 Custom Gene Expression Microarray, 8x60K -  
296 Agilent Technologies) contains 48,986 non-redundant probes previously identified and available  
297 from the PhyloFish Database [43]. The “One-Color Microarray-Based Gene Expression Analysis  
298 (Low Input Quick Amp Labeling) Protocol” was followed for samples preparation, hybridization,  
299 washing and scanning of slides and data extractions. Briefly, 150 ng of total RNA were used for

300 the amplification/Cy3-labeling step. After this step, samples were purified (RNeasy mini kit,  
301 Qiagen) and quantified using a NanoDrop ND-1000 Spectrophotometer (NanoDrop  
302 Technologies). Samples exhibiting a yield higher than 1.03  $\mu\text{g}$  of cRNA and a specific activity  
303 higher than 10.30 pmol of Cy3/ $\mu\text{g}$  of cRNA were fragmented and used to hybridize arrays (one  
304 sample failed the labeling step and was excluded from the experiment). Samples (600 ng of Cy3-  
305 cRNA) were randomly distributed onto four slides. After 17h of hybridization at 65°C, slides were  
306 washed, dried and scanned with an Agilent Technologies Scanner (G2505C). Scanned images  
307 were extracted with Agilent Feature Extraction software.

308 Data extracted from scanned images were normalized and  $\log(2)$  transformed for statistical  
309 analyses (all data are available in the Gene Expression Omnibus database under the accession code  
310 GSE119802). Using the GeneSpring software, an unpaired t-test followed by a Benjamini-  
311 Hochberg correction was used to identify genes differentially expressed (DEG) between the two  
312 populations ( $p < 0.05$ ). Then, a hierarchical clustering analysis (unsupervised average linkage) was  
313 performed using Cluster 3.0 software (version 1.52). Clusters were visualized using TreeView  
314 software (version 1.1.6r4).

315

## 316 **Gene ontology analyses**

317 In order to give an overview of gene ontology (GO) terms represented among differentially  
318 expressed genes between F1 and F7+ populations, they were functionally annotated and classified  
319 using Blast2GOv4.1.9 software [44]. Default parameters were used for blastx and GO annotations.

320 In the following, we performed a GO overrepresentation analysis in which we compared  
321 the list of DEG to a reference list corresponding to all expressed genes in the microarray. To  
322 retrieve this reference list, we first filtered all genes present in the array. Genes were, considered

323 as expressed when they presented a signal above background in at least 75% of the samples and in  
324 at least one of the populations. In a second time, as the Eurasian perch genome is not yet available  
325 in any databases allowing performing GO analyses, we chose to retrieve human orthologs  
326 identifiers for the reference and DEG lists. It allowed us to perform the analysis. In order to find  
327 these identifiers, we aligned probes designed for the array corresponding to each sequence in the  
328 lists against the Eurasian perch transcriptome extracted from the genome, provided by [45], with  
329 minimap2 (version 2.7 with -m 20 parameters). Because the gene prediction file had small UTRs,  
330 we extended the prediction on both transcript sides by 2kb. If a probe had a unique alignment, it  
331 was assigned to the corresponding transcript. If the probe had two corresponding transcripts  
332 located one after the other on the genome, we assigned it to the transcript having the match closest  
333 to its center. Probes with no match or over two matches were not assigned to a transcript. We then  
334 retrieved the human orthologs identifiers from the annotation file provided by [45]. The genes still  
335 missing identifiers in the DEG list were manually annotated using UniProt accessions. In total,  
336 among the 358 DEG, 265 human orthologs identifiers were found. The GO overrepresentation  
337 analysis was performed using Panther14.0 [46]. Parameters were set at default, meaning that a  
338 Fisher's exact test and a Benjamini-Hochberg correction were applied. We asked for GO-Slim  
339 Biological Processes (BP) and Pathways and only corrected p-values  $<0.05$  were considered as  
340 significant.

341

## 342 **Real-time PCR analysis**

343 Genes presenting a  $\log(2)$  fold change ( $\log(2)FC$ )  $> 4$  in microarray were additionally  
344 studied by real-time qPCR in all samples previously used for the microarray. After RNA  
345 extraction, a DNase treatment (DNase I, RNase free - Thermo Scientific) was applied to 5  $\mu\text{g}$  of



346 all samples (n=32) following the manufacturer's protocol. The reverse transcription was performed  
347 in a final volume of 20 µl using a M-MLV Reverse Transcriptase (Sigma-Aldrich), 1 µg of RNA  
348 and random nonamers (2.5 µM - Sigma-Aldrich) and following the manufacturer's protocol.  
349 Reverse transcript products were diluted 1:27 and 5 µl were used for the real-time PCR, using  
350 PerfeCTa SYBR Green SuperMix (Quanta Bioscience) and 5 pmol of each primer. Primers were  
351 designed using Primer3Plus or Primer design Tool-NCBI software (S1 Table). The real-time qPCR  
352 was performed using a Step One Plus system (Applied Biosystems, Foster City, USA). The PCR  
353 program consists of a first step at 95°C for two minutes followed by 40 cycles consisting of a  
354 denaturation step at 94°C for 15s, an annealing step at 50-58°C depending on the primers pairs for  
355 15s and an elongation step at 72°C for 30s. The amplification was followed by a melting curve  
356 stage, according to manufacturer's parameters in order to check the primers specificity. The  
357 abundance of the target cDNA in each sample was calculated using a serial dilution of a pool of  
358 all cDNA samples using the StepOne Software (Applied Biosystems, version 2.1). This dilution  
359 curve was used to certify the reaction efficiency (80-120%). All samples were analyzed in  
360 duplicate and the geometric mean of the expression level data of the *adenosine kinase-like*,  
361 *RNAI8s*, *TATA-box binding* and *ELAV-like protein 1-like* (S1 Table) was used as reference to  
362 normalize the data obtained. These genes were found as stable in microarray and preliminary real-  
363 time qPCR analyses in all samples. The values obtained after normalization were analyzed using  
364 a Mann-Whitney test (RStudio software version 1.0.143) to compare differences of gene  
365 expression between populations (p<0.05).

366

## 367 **Genetic variability between populations**

368 Genomic DNA was extracted from 42 fin samples representing the two populations (21 F1  
369 and 21 F7+), using the universal salt-extraction method, according to [47]. Purity and quantity of  
370 genomic DNA were assessed using a NanoDrop ND-1000 Spectrophotometer (NanoDrop  
371 Technologies). Eight microsatellites previously used on *P. fluviatilis* [48] were selected: *PflaL1*,  
372 *PflaL2*, *PflaL4*, *PflaL6* [49], *SviL7* [50], *Svi17* [51], *YP60* and *YP111* [52]. Two multiplex  
373 amplifications were done using fluorescently labeled primers. The first multiplex (A) contained  
374 *PflaL2* (FAM), *PflaL4* (PET), *SviL7* (VIC), *Svi17* (FAM) and *YP111* (PET). The second one (B)  
375 contained *YP60* (FAM), *PflaL1* (VIC) and *PflaL6* (FAM). Polymerase chain reaction was carried  
376 out using the Multiplex TEMPase 2X MasterMix (VWR), 10 pmol of fluorescent primer mix,  
377 genomic DNA and water for a final volume of 30  $\mu$ l. PCR conditions for multiplex A were: 95°C  
378 for five minutes, 28 cycles at 95°C for 30 seconds, 55°C for 90 seconds and 72°C for 30 seconds,  
379 and a final extension of 45 seconds at 60°C. For multiplex B, PCR conditions were: 95°C for five  
380 minutes, six cycles at 95°C for 30s, 48°C for 90s and 72°C for 30s, 22 cycles at 95°C for 30s, 50°C  
381 for 90s and 72°C for 30s, and a final extension at 60°C for 45s. PCR products were diluted (1:151)  
382 with deionized water and added Hi-Di™ Formamide (Applied Biosystems) and GeneScan 600  
383 LIZ Size Standard (Applied Biosystem). The fragment analysis was performed on a 3500 Genetic  
384 Analyzer (Applied Biosystems HITACHI) and alleles were scored with Geneious 11.0.2 [53].

385 Genetic diversity was estimated through calculation of observed ( $H_o$ ) and expected ( $H_e$ )  
386 heterozygosities in GENETIX [54]. Population differentiation was assessed by estimating the  
387 “global”  $F_{ST}$  statistic on populations through an analysis of molecular variance (AMOVA when  
388 considering only one group of populations) performed in Arlequin with 10000 permutations.  
389 Divergence between populations was estimated with a  $F_{ST}$ -pairwise test (10000 permutations).

390

## 391 **Statistical Analysis**

392 Differences in GSI, sexual steroids and Vitellogenin concentrations, oocytes stages  
393 abundance on histological cut and specific deformities were estimated using a non-parametric  
394 Wilcoxon-Mann-Whitney. Normality and homogeneity of variance were tested using respectively  
395 Shapiro-Wilk and Levene's test.

396 In order to investigate possible differences of embryonic development between  
397 populations, percentage of embryos alive at 24, 48, 72 and 120 hpf and at hatching were compared  
398 between populations using a one-way repeated-measures ANOVA. The statistical model included  
399 as fixed effect the percentage of alive embryos for F1 and F7+ populations. It has been controlled  
400 by the introduction of the females as covariate. This model was chosen after comparison with a  
401 second one without a covariate. They were ranked according to their Akaike information criterion  
402 and the one having the lowest criterion was chosen [55]. A TukeyHSD was performed to identify  
403 differences between populations at different times individually.

404 For all tests, a p-value  $\leq 0.05$  was considered statistically significant. All values given are  
405 represented as means  $\pm$  standard error of mean (SEM). All tests were performed using R  
406 (v.1.1.423) [56]. The package *stats* was used to perform Shapiro-Wilk, Wilcoxon-Mann-Whitney,  
407 ANOVA and TukeyHSD tests. While *car* was employed for Levene's test.

408

## 409 **Results**

410 Two Eurasian perch populations (F1 and F7+) were used in the present study. Analysis of  
411 their genetic differentiation using microsatellites revealed a  $F_{ST}$  of 0.1055 ( $p < 0.001$ ). In addition,

412 the F7+ population presented a higher observed heterozygosity (mean=0.440) compared to the F1  
413 (mean=0.348), indicating a larger genetic diversity of the F7+ population.

414

## 415 **Follow up of the gonadogenesis progression reveals few differences** 416 **between populations.**

417 Five sampling points of females were performed as shown in Fig 1. The GSI of both  
418 populations increased progressively all along gonadogenesis to reach close to 13% one month  
419 before the spawning season (S1 FigA). The only difference between populations can be seen at  
420 T31 with a higher GSI ( $p = 0.02$ ) for the F7+ females ( $0.91 \pm 0.05\%$ ) compared to the F1 ones  
421 ( $0.69 \pm 0.06\%$ ). A follow up of the hormonal status of females did not present any significant  
422 difference of the testosterone level between populations. At T253 a higher level was recorded for  
423 the F1 ( $50.35 \pm 5.89$  ng/mL) compared to the F7+ ( $35.81 \pm 6.49$  ng/mL,  $p = 0.06$  – S1 FigB).  
424 Similar data were obtained for the 17- $\beta$ - $\text{estradiol}$  (E2), except for the T253 for which the hormonal  
425 level was higher ( $p = 0.03$ ) for F7+ ( $8.71 \pm 0.34$  ng/mL) than for F1 ( $7.39 \pm 0.44$  ng/mL), no other  
426 statistical difference was found (S1 FigC). The follow up of the Vitellogenin level in the blood did  
427 not present any significant difference between F7+ and F1 populations (S1 FigD).

428 These data suggest that the oogenesis progression for both populations was similar.  
429 However, the histological study of gonads revealed that F1 females presented higher percentage  
430 of late vitellogenesis oocytes ( $57 \pm 6\%$ ) at the end of the oogenesis in comparison to the F7+ ones  
431 ( $41 \pm 4\%$ ; T253, Fig 2A and B), suggesting that oogenesis was slightly more advanced in the F1  
432 than in the F7+.

433

434 **Fig 2. Histological follow up of the oogenesis progression.** (A) Percentage of each cellular stage  
435 in gonads belonging to F7+ and F1 populations during oogenesis period. Stages are represented  
436 as: primary growth and oogonia - PG+O, early cortical alveoli - ECA, late cortical alveoli stage -  
437 LCA, early vitellogenesis - EV, late vitellogenesis - LV and atresia - A. Differences between the  
438 two populations were tested using non-parametric Wilcoxon-Mann-Whitney test ( $p < 0.05$ ).  
439 Significance levels are represented as follows: \*,  $p = 0.05-0.01$ ; \*\*,  $p = 0.01-0.001$ ; \*\*\*,  $p =$   
440  $0.001-0.0001$ ; and \*\*\*\*,  $p < 0.0001$ . (B) Histological section of gonads representative of F7+ and  
441 F1 ovaries at T253. Arrows points to LV stages and arrowheads indicate the PG+O stages. Scale  
442 bars represent 1000  $\mu\text{m}$ .

443

#### 444 **The embryonic survival is higher in F1 than in F7+ spawn**

445 Following the observation of a slightly faster oocytes development in F1 population, the  
446 first spawning was more precocious for F1 than for F7+ fish. F1 females, coming from all three  
447 original replicate tanks, spawned between thirteen and seven days earlier than F7+ first spawner  
448 (Fig 3).

449 The main effect for population yielded an F ratio of  $F(1,30) = 4.266$ ,  $p = 0.0476$ , indicating  
450 a significance difference on the number of embryos alive depending on the population.

451 Since an overall population effect on the percentage of embryos alive was observed,  
452 TukeyHSD tests were performed between populations and revealed significant differences  
453 between them at 48 ( $p = 0.02$ ), 72 ( $p = 0.03$ ) and 120 hpf ( $p = 0.05$ ) and at hatching ( $p = 0.03$ ).  
454 Consequently, embryonic survivorship was significantly higher in F1 than in F7+ population from  
455 48 hpf (Fig 4A and S2 Table). Interestingly, more heterogeneity of survivorship is seen in F7+  
456 population at all timepoints (coefficient of variation (CV) = 62%, 88%, 95%, 90% and 121% at

457 24, 48, 72 and 120 hpf and at hatching, respectively) in comparison to F1 in the same stages (CV  
458 = 51%, 56%, 56%, 59% and 64% at 24, 48, 72 and 120 hpf and at hatching, respectively). The  
459 overall occurrence of deformities and that of specific deformities were comparable between  
460 populations and did not present any statistical difference (Fig 4B and S3 Table).

461

462 **Fig 3. Timetable of the spawn obtained for both populations during the spawning season in**  
463 **relation to temperature and photophase increase at the end of the photothermal program.**

464 Bars with numbers correspond to the number of spawn obtained each day for F1 and F7+ females.

465

466 **Fig 4. Evaluation of the embryonic developmental success in F1 and F7+ populations. (A)**

467 Percentage of embryos surviving at 24, 48, 72 and 120 hours post-fertilization (hpf) and at hatching

468 in F1 (light gray dots) and F7+ (black dots) populations estimated based on the total number of

469 studied embryos (about 100 embryos). In (A), dots represent population means  $\pm$  SEM at each

470 observed time (n= 19 and 13 for F1 and F7+, respectively). P-values presented represent

471 comparisons between populations at each time of observation obtained using a TukeyHSD

472 preceded by a significant one-way ANOVA in repeated measures ( $p \leq 0.05$  were considered as

473 significant). (B) Total deformities rates (Dr) and specific rates in various tissues at hatching in F1

474 and F7+ populations (Ad - Axis, Cd - Cardiac, Yd - Yolk, Md - Mouth, Od - Other, Ed - Eyes).

475 No significant difference has been identified between populations using non-parametric Wilcoxon-

476 Mann-Whitney test ( $p < 0.05$ ; n=23).

477

478 **Eggs transcriptomic analysis**

479 A large scale analysis was performed on 31 spawn to compare the maternal transcriptomic  
480 profiles of non fertilized eggs. The statistical analysis revealed 358 differentially expressed genes  
481 (DEG) between populations (S4 Table). An unsupervised average linkage clustering analysis was  
482 performed using the expression data of these 358 DEG. It allowed splitting apart both populations  
483 revealing that 172 genes were over-expressed in the F7+ population and 186 genes were over-  
484 expressed in the F1 population (Fig 5A and S5 Table).

485

486 **Fig 5. Heatmaps showing differentially expressed genes between F1 and F7+ populations. (A)**

487 Unsupervised hierarchical cluster analysis of 358 differentially expressed genes ( $p < 0.05$ ) between  
488 populations. The dendrogram on the left represents gene correlation distances between the genes.

489 (B) Unsupervised hierarchical cluster analysis of the 10 most differentially expressed genes ( $p <$   
490  $0.05$ ) with a  $\log(2)FC > 4$ , between populations. Gene abbreviated name and  $\log(2)FC$  are shown  
491 between parentheses on the right. In both parts of the figure, red color indicates over-expression,  
492 and green color indicates under-expression while black is used for median expression. Top bar  
493 indicates the origin of the samples: black for F7+ samples and gray for F1 ones. Node similarity  
494 score bars represents the similarity between tree branches. It ranges from 1 (identical) to -1  
495 (opposites), while 0 means they are completely uncorrelated.

496

497 A functional annotation of these 172 and 186 over-expressed genes allowed mapping 86  
498 genes for each population, among which 73 and 76 genes containing specific GO annotations were  
499 identified for F7+ and F1 populations, respectively (S6 and S7 Tables).

500 Using Panther software, no Pathways was enriched and the term “immune system” was  
501 overrepresented (FDR  $p$ -value = 0.00251; S8 Table). Four of the genes represented belong to the

502 butyrophilin or the butyrophilin-like family but two of them were up-regulated in the F1 population  
503 while the others were down-regulated in this population. For three of these genes, the  $\log(2)FC$   
504 was  $>2$  and were thus among the 16% most differentially expressed genes between the two  
505 populations.

506 Indeed, among the 358 DEG only 60 presented a  $\log(2)FC > 2$  between the two populations.  
507 Fifty DEG showed a  $\log(2)FC$  between 2 and 3-fold (S4 Table) and 10 genes had a  $\log(2)FC$  higher  
508 than 4-fold (5 over-expressed in the F7+ population and 5 in the F1; Fig 5B). We choose to check  
509 the expression level of the genes having a  $\log(2)FC > 4$  by real-time qPCR. Among them, three  
510 sequences could not be identified because the probes actually recognized contigs grouping  
511 numerous unidentified genes in the Eurasian perch transcriptomic database (Fig 5B). Among the  
512 remaining genes, four were more expressed in the F7+ population: *mex3b* ( $\log(2)FC = 8.26$ ),  
513 *bloc1s1* ( $\log(2)FC = 4.91$ ), an *uncharacterized protein* ( $\log(2)FC = 4.57$ ) and *hace1* ( $\log(2)FC =$   
514  $5.53$ ). In the same way, *per2* ( $\log(2)FC = 5.68$ ), *nibl1* ( $\log(2)FC = 4.04$ ) and *iyd* ( $\log(2)FC = 4.62$ )  
515 were more abundant in the F1 population.

516 Expression level differences were confirmed for *mex3b* ( $\log(2)FC = 7.26$  for the RT-  
517 qPCR), the *uncharacterized protein* ( $\log(2)FC = 1.65$  for the RT-qPCR), *hace1* ( $\log(2)FC = 1.65$   
518 for the RT-qPCR) and *per2* ( $\log(2)FC = 3.13$  for the RT-qPCR; Fig 6). However, for *bloc1s1* and  
519 *nibl1* the expression levels between both populations were not significantly different by RT-qPCR  
520 even if they followed the same profile than in the microarray (Fig 6). In addition, concerning *iyd*  
521 not only no significantly different expression was observed, but also the profile observed by RT-  
522 qPCR was in favor to a higher expression in the F7+ population, which is in contradiction with the  
523 microarray data.

524



525 **Fig 6. Compared analysis of seven genes among the most differentially expressed**  
526 **(log(2)FC >4) between microarray and RT-qPCR experiments.** The same egg samples were  
527 used to perform both techniques for F7+ (n=13) and F1 (n=18 for microarray and n=19 for RT-  
528 qPCR) populations. An unpaired t-test followed by a multiple testing correction Benjamini-  
529 Hochberg was applied on microarray results while a Mann-Whitney-Wilcoxon test was used on  
530 RT-qPCR results. Bars correspond to the means  $\pm$  standard error. Significance levels are  
531 represented as follows: \*, p = 0.05–0.01; \*\*, p = 0.01–0.001; \*\*\*, p = 0.001–0.0001; and \*\*\*\*, p  
532 < 0.0001.

533

## 534 **Discussion**

535 The Differentiation Index ( $F_{ST}$ ) illustrates a genetic divergence between populations  
536 ranging from 0 (gene flow between populations) to 1 (isolated populations without any gene flow).  
537 In the literature, a lower  $F_{ST}$  (0.002, p < 0.0023) was found between various samples of Eurasian  
538 perch all around the Geneva Lake [48] and authors considered that there was only one population  
539 in the lake. The same authors showed a minimum  $F_{ST}$  of 0.45 (p < 0.0001) between a wild and  
540 nine farmed populations of Eurasian perch that were all supposed to originate from the same  
541 geographic location [24]. They were considered as distinct populations. In comparison, our  $F_{ST}$   
542 (0.1055, p < 0.0001) is in between the above mentioned examples. The interpretation of significant  
543 differences when using highly variable loci, such as microsatellites, has to be very carefully  
544 analyzed since its biological meaning can often be weak [57]. In the present study, we selected the  
545 most variable microsatellites according to literature on Eurasian perch microsatellite  
546 differentiation. In addition, the higher heterozygosity index for the F7+ (0.440) compared to the  
547 F1 (0.348) shows a higher heterogeneity in the F7+ population compared to the F1. Our F7+

548 population came from a partner fish farmer but first originated from the transfer from another fish  
549 farm. The initial stock was supposedly from Geneva lake but it was difficult to obtain a reliable  
550 traceability. Consequently, it is possible that some individuals from different geographic locations  
551 may have been introduced in the F7+ stock, which is a common practice in aquaculture [24].  
552 Alternatively, the random independent sampling process to create each population stock (F1 and  
553 F7+) could have led to different degrees of genetic drift in each stock. In our conditions, none of  
554 these hypotheses can be ruled out.

555

## 556 **Control of the reproductive cycle**

557 The oogenesis progression was similar between populations even if females from the F1  
558 population seemed to respond faster to the photothermal stimulation and kept a slight non-  
559 significant advance all along the process. This advance became significant one month before the  
560 spawning with a higher proportion of late vitellogenesis oocytes and may contribute to explain the  
561 early onset of the spawning season of F1 females (thirteen to seven days before the F7+ ones). For  
562 technical reasons it has not been possible to determine the end of the spawning season of F7+ fish.  
563 The short spawning season of F7+ population in comparison to F1 is thus not relevant. It is worthy  
564 to note that at the end of the experimental period all remaining females that did not spawn  
565 presented developed gonads, once they were all slaughtered at the end of the experiment.

566 In this study, sexual steroids and Vitellogenin levels were either slightly or not different  
567 during the reproduction cycle between populations. The same pattern has been observed for the  
568 GSI. Differences found may either be mediated by yet unknown mechanisms or very subtle  
569 variations of hormone levels controlling the oogenesis progression. From the current knowledge,

570 it remains difficult to precisely point which of these parameters imposed these modulations  
571 between the two populations.

572         Concerning the developmental success, results showed that F1 population presented an  
573 embryonic survival higher than the F7+ one with differences ranging from 16 to 26% of survival  
574 depending on the timepoint. This difference is the lowest at 24 hpf (16%), leading to a non-  
575 significant result at this timepoint. In any case, differences between populations may be due to egg  
576 quality issues that led either to fertilization impairments or higher mortality occurrence. These  
577 results enable us to conclude that the overall egg quality was higher in F1 than in F7+ population.  
578 Egg quality relies greatly upon its intrinsic content and some molecules may have very early and  
579 essential effect during embryogenesis, including pronuclear congregation and mitotic spindle  
580 assembly [58]. In addition, only few exchanges occur between the developing embryos and the  
581 environment [34] reinforcing the importance of the egg content during early embryogenesis.  
582 Moreover, the F7+ population shows more heterogeneous survivorship. It is potentially related to  
583 individuals' history in the tank during the oogenesis (e.g. behavioral difference). In any case this  
584 difference of egg quality may be explained by the resource allocation theory [12]. Even if  
585 domestication of Eurasian perch begun 20 years ago, numerous questions remain to properly  
586 achieve their breeding in recirculating aquaculture systems. Since no selective program has begun  
587 yet for this species [23], the artificial selection driven by farmers remains empirical. In addition,  
588 rearing conditions are not fully optimized and fish farmers continue to make changes in their  
589 practices. Consequently, an imbalance between biological functions may occur in this species  
590 because resource intakes are allocated toward certain traits at the expense of other functions, such  
591 as reproduction. No zootechnical practices may be undertaken to compensate the lack of intake  
592 that may occur at each generation. This data is in accordance with numerous studies showing a

593 decrease of the reproductive success in domesticated populations. Indeed, lower fertilization and  
594 hatching rates in farmed Atlantic cod (*G. morhua*) [18,19], lower survival rate at the eyed stage in  
595 farmed Atlantic salmon (*S. salar*) [59], lower hatching rate in cultured common sole (*Solea solea*)  
596 [60] and oogenesis impairments in captive-reared greater amberjack (*S. dumerili*) [20] were  
597 described in comparison with their respective wild counterparts. Therefore, this problem is widely  
598 observed in fish species and further studies are needed to test the hypotheses proposed in the  
599 resource allocation theory during fish domestication. If these hypotheses are confirmed, it means  
600 that a more accurate knowledge of metabolic needs for each biological function at each step of the  
601 life cycle is required before starting any selection program. Farmers should then take this  
602 information into account in order to implement a compensation program when the resource  
603 allocation is imbalanced. In this manner, all biological functions may benefit from an increase  
604 of the global resource intake. In addition, the choice of selected biological traits in addition to the  
605 follow up of the fitness of individuals should be assessed properly in order to detect early any  
606 deviation.

607         Concerning the Eurasian perch (*P. fluviatilis*), other studies showed that wild populations  
608 had higher reproductive performance than captive ones but with even more drastic differences than  
609 in the present work [21,22]. Differences between our study and these ones may be due to  
610 accumulation of the domestication effect on oogenesis and spermatogenesis, since in those studies  
611 developmental performance of embryos was evaluated from pure crosses from each population. In  
612 the present study, our goal was to investigate exclusively the effect of the domestication on  
613 females' performance. Consequently, we chose to reduce the potential effect of sperm quality and  
614 fertilized all spawn with sperm from F1 males. Another explanation could be that rearing

615 conditions using in other studies were different (tank size and colour, first spawn or not...) from  
616 ours and influenced differently wild populations in the different studies.

617 Variations of gene expression profiles between wild and “domesticated” populations have  
618 been demonstrated in whole larvae and embryos of Atlantic salmon (*S. salar*) [15,61], fertilized  
619 eggs of Atlantic cod (*G. morhua*) [18] and whole larvae of steelhead trout (*O. mykiss*) [3] but no  
620 study investigated the maternal mRNA profile. These observations strengthen the necessity to  
621 better understand the contribution of maternal mRNA to the embryonic early development in our  
622 conditions.

623

## 624 **Eggs transcriptome**

625 Our working hypothesis was that the domestication process may impact the maternal  
626 mRNA content of the eggs and thus the transcriptomic profile of unfertilized eggs coming from  
627 F1 and F7+ populations. In total, 358 genes presented a significant difference of expression  
628 between populations.

629 The GO analysis revealed one biological process term overrepresented among the DEG list  
630 that we analyzed, suggesting that this function may be affected by the domestication process. This  
631 biological process corresponds to the “immune system” grouping proteins usually involved in the  
632 regulation of the adaptative immune response such as activation and proliferation of effective T  
633 cells and cytokine production [62], controlling inflammation. However, these genes can be  
634 expressed in several tissues and may thus be involved in other biological functions. In fish, the T  
635 cell receptor signaling pathway seems to be as complex as in mammals but remains yet to be  
636 understood [63,64]. Some transcriptomic studies in eggs, embryos and early larvae showed that  
637 genes related with the immune system are often differentially expressed depending on egg quality

638 or the domestication level. Concerning studies on the egg quality, genes representing the immune  
639 system are mostly down-regulated in eggs presenting lower potential to properly develop [65,66].  
640 Concerning domestication comparisons, several genes involved in the immune system were down-  
641 regulated in a domesticated population of Atlantic salmon (*S. salar*) compared to their wild  
642 counterparts [15,16]. In the latter case, the populations used were selected for their growth abilities  
643 and authors proposed the existence of a trade-off between growth and immune response during the  
644 selection process. In another study performed in the steelhead trout (*O. mykiss*) [3], authors  
645 proposed that it could simply reflect an adaptation of individuals to the captive environment,  
646 implying an up-regulation of genes involved in this function in domesticated fish. However, no  
647 information on the direction of perturbations (up- or down-regulation of genes in domesticated or  
648 wild populations) was given. In our study, the term “immune system” involved genes that were  
649 up-regulated either in the F1 population (five genes) or in the F7+ population (four genes).  
650 Additionally, most of the highlighted genes code for Butyrophilin proteins involved in several  
651 biological functions, on top of their role in the immune system. More particularly, they regulate  
652 the oil droplets secretion in the milk produced by mammals [67]. Eurasian perch eggs contain a  
653 large oil droplet necessary for proper embryonic development since impairments in its formation  
654 have been correlated to embryogenesis defects [68]. It would be interesting to further investigate  
655 a potential role of these proteins in the formation of oil droplets in fish eggs.

656 In addition, some of the genes presenting the wider variations of expression between  
657 populations are known to be involved during the early embryogenesis and may partly explain  
658 developmental defects leading to early lethalties observed in the F7+ population. The *period2*  
659 gene (*per2*) codes for a protein belonging to the basic-helix-loop-helix-PAS (bHLH-PAS) protein  
660 family and belongs to the clock genes controlling many developmental and physiological events

661 [69]. However, in several mammalian species, numerous clock genes, including *per2*, have been  
662 observed in the developing oocytes and eggs as maternal mRNA. These mRNA are not controlled  
663 by circadian rhythms and disappear after the MZT [70,71]. Authors proposed that *per2* could be  
664 involved in the control of meiosis but, up to now, no proof has corroborated this hypothesis and  
665 further investigations are needed to study their role in this tissue. Similar data have been observed  
666 in the developing spermatogonia in mice in which clock genes are expressed without any link with  
667 the circadian rhythm. In this study, authors made the hypothesis that this gene is mainly linked to  
668 the differentiation process [72]. Our study shows for the first time that a *per2* gene is expressed in  
669 fish eggs, suggesting similarities with mammals. In addition, it is highly expressed in eggs of the  
670 F1 population. Our study does not allow making any hypothesis about the role of *per2* or if it is  
671 dependent or independent of the circadian rhythm. However, it suggests that *per2* role during the  
672 gonadogenesis may be evolutionary conserved.

673         The gene *hace1* codes for the HECT domain and Ankyrin repeat Containing E3 ubiquitin-  
674 protein ligase 1. It was first identified as a potential tumor suppressor in humans [73]. Later,  
675 absence or mutation of this gene was related with developmental issues such as some  
676 neurodevelopmental syndromes in humans [74], a shortening of the body axis, an inhibition of eye  
677 pigments formation and a delay in neural tube closure in xenopus [75]. In this last study, authors  
678 compared their data with another work performed with zebrafish and showing no clear phenotype  
679 in this species. They argued that the zebrafish study revealed the role of HACE1 by using splice-  
680 sites morpholino efficient to repress zygotic but not maternal RNA [76] while their study involved  
681 translational blocking morpholinos blocking both maternal and zygotic RNA. Thus they suggest  
682 that phenotypes in xenopus could be linked to the maternal pool of RNA. Recently, a study  
683 demonstrated that HACE1 is involved in the normal development and proper function of the heart

684 in zebrafish [77]. However, authors used splice-site morpholinos suggesting that this cardiac  
685 phenotype could be due to later zygotic expression of HACE1. Consequently, even if no maternal  
686 role of HACE1 has been studied in the zebrafish, studies performed on xenopus suggest that a  
687 maternal expression of these mRNA have consequences on the neural tube development. Up to  
688 now, two main targets of Hace1 have been identified: Rac1 involved in the cell cycle control and  
689 NADPH oxidase regulating the reactive oxygen species production. A previous study showed that  
690 some enzymes involved in the control of the oxidative stress present a variation of expression  
691 depending on oocytes quality in Eurasian perch [78]. In some marine invertebrate species, a  
692 modification of redox homeostasis may help to avoid the polyspermy during fertilization [79]. In  
693 our study, the mRNA abundance of *hace1* is higher in the F7+ population, potentially accelerating  
694 NADPH oxydase degradation and thus influencing fertilization. With the current knowledge, no  
695 transduction pathway could be favored.

696 Similarly, the mRNA abundance of *mex3b* mRNA was higher in the domesticated  
697 population. *Mex3b*, or *muscle excess 3*, codes for an evolutionary conserved RNA-binding protein  
698 involved in post-transcriptional regulations [80]. It is associated to proper embryonic development  
699 by establishing antero-posterior patterning in *Tribolium* [81], *Caenorhabditis elegans* [82] and  
700 *Xenopus laevis* [83]. As a whole, embryonic patterning is regulated by expression and spatial  
701 distribution of many transcripts. In xenopus, *mex3b* mRNA presents a long conserved untranslated  
702 3'UTR involved in its auto regulation [83]. In turn, the protein is involved in the mRNA stability  
703 and regulates the abundance of several genes, involved in diverse cellular functions [83]. In the  
704 present work, the maternal expression of *mex3b* mRNA presents the highest variation of  
705 expression level between the two populations ( $\log_2FC > 7$  in the RT-qPCR). Consequently, the



706 high expression of this transcript in the F7+ eggs may be linked to fine tuning of numerous  
707 molecular functions in the embryo and lead to diverse phenotypes.

708 Finally, a mRNA coding for an *uncharacterized* protein was found to be significantly more  
709 expressed in the eggs laid by F7+ females. BLAST analyses against the Uniprot and NCBI  
710 databases showed that they are highly homologous to other uncharacterized proteins in other fish  
711 species presenting homologies with some domains of ADP ribosylation enzymes. However, these  
712 sequences have not been identified yet.

713

## 714 **Conclusions**

715 Our study showed that reproductive performance of Eurasian perch females may be  
716 influenced by the domestication process which is probably closely related to the rearing practices  
717 potentially leading to several genetic and/or epigenetic modifications. This study revealed that  
718 even if the breeders of two Eurasian perch populations (F1 vs. F7+) were reared and induced in  
719 the same conditions, the F1 population started to spawn earlier than the F7+ during the spawning  
720 season. In other words, it shows that, in our conditions, the domestication process may influence  
721 the responsiveness of females to the reproductive environmental stimuli in captive environment.

722 The F1 population produced eggs having a better potential to develop properly until  
723 hatching compared to eggs from the F7+ population. These differences in egg quality may be  
724 linked with the important variation in the eggs transcriptomic content between populations. The  
725 identification of several genes presenting distinct expression between the two populations could  
726 open new paths of investigation to understand their role and mechanism of regulation during  
727 embryogenesis and depending on the domestication level.

728           Finally, the genetic differentiation analysis between studied populations did not allow us  
729 to isolate the domestication as the only factor explaining our data. It reinforces the necessity of  
730 studying populations presenting a clearer life history to further understand the dynamic of  
731 modifications occurring during the domestication process. It is particularly important in the  
732 perspective of a growing pressure toward fish farmers and scientists to initiate selection programs  
733 for several fish species.

734

## 735 **Acknowledgements**

736 We thank, Paul Georges and Franck Bertrand for their involvement in some experiments as  
737 trainees, Alain Iuretig for the care given to the breeders during the growing period and Yves Le  
738 Roux for the assistance with the survival statistical analysis. We are also grateful to Mikhail  
739 Ozerov and Freed Ahmad from University of Turku for kindly providing the annotated Eurasian  
740 perch genome used in GO analysis and the members of the UR AFPA DAC and LPGP Rennes for  
741 their support.

742

## 743 **References**

- 744 1. Price EO. Behavioral Aspects of Animal Domestication. *Q Rev Biol.* 1984;59(1):1–32.
- 745 2. Price EO. Behavioral development in animals undergoing domestication. *Appl Anim Behav*  
746 *Sci.* 1999 Dec;65(3):245–71.
- 747 3. Christie MR, Marine ML, Fox SE, French RA, Blouin MS. A single generation of  
748 domestication heritably alters the expression of hundreds of genes. *Nat Commun.* 2016 Feb  
749 17;7:10676.
- 750 4. Beaumont C, Roussot O, Marissal-Avry, N, Mormede P, Prunet P, Roubertoux P.  
751 Génétique et adaptation des animaux d'élevage : introduction. *Inra Prod Anim.*  
752 2002;15(5):343–8.

- 753 5. Mignon-Grasteau S, Boissy A, Bouix J, Faure J-M, Fisher AD, Hinch GN, et al. Genetics of  
754 adaptation and domestication in livestock. *Livest Prod Sci.* 2005 Apr 1;93(1):3–14.
- 755 6. Vogt G. Facilitation of environmental adaptation and evolution by epigenetic phenotype  
756 variation: insights from clonal, invasive, polyploid, and domesticated animals. *Environ*  
757 *Epigenetics* [Internet]. 2017 Mar 29 [cited 2019 May 28];3(1). Available from:  
758 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5804542/>
- 759 7. Luyer JL, Laporte M, Beacham TD, Kaukinen KH, Withler RE, Leong JS, et al. Parallel  
760 epigenetic modifications induced by hatchery rearing in a Pacific salmon. *Proc Natl Acad*  
761 *Sci.* 2017 Dec 5;114(49):12964–9.
- 762 8. Gavery MR, Nichols KM, Goetz GW, Middleton MA, Swanson P. Characterization of  
763 Genetic and Epigenetic Variation in Sperm and Red Blood Cells from Adult Hatchery and  
764 Natural-Origin Steelhead, *Oncorhynchus mykiss*. *G3 Genes Genomes Genet.* 2018 Nov  
765 1;8(11):3723–36.
- 766 9. Rodriguez Barreto D, Garcia de Leaniz C, Verspoor E, Sobolewska H, Coulson M,  
767 Consuegra S. DNA Methylation Changes in the Sperm of Captive-Reared Fish: A Route to  
768 Epigenetic Introgression in Wild Populations. *Mol Biol Evol* [Internet]. [cited 2019 Jul 26];  
769 Available from: [https://academic.oup.com/mbe/advance-](https://academic.oup.com/mbe/advance-article/doi/10.1093/molbev/msz135/5513369)  
770 [article/doi/10.1093/molbev/msz135/5513369](https://academic.oup.com/mbe/advance-article/doi/10.1093/molbev/msz135/5513369)
- 771 10. Anastasiadi D, Piferrer F. Epimutations in developmental genes underlie the onset of  
772 domestication in farmed European sea bass. Wittkopp P, editor. *Mol Biol Evol.* 2019 Jul  
773 10;msz153.
- 774 11. Dobney K, Larson G. Genetics and animal domestication: new windows on an elusive  
775 process. *J Zool.* 2006;269(2):261–71.
- 776 12. Waaij VD, H E. A resource allocation model describing consequences of artificial selection  
777 under metabolic stress. *J Anim Sci.* 2004 Apr 1;82(4):973–81.
- 778 13. Farquharson KA, Hogg CJ, Grueber CE. A meta-analysis of birth-origin effects on  
779 reproduction in diverse captive environments. *Nat Commun.* 2018 Mar 13;9(1):1055.
- 780 14. Pelegri F. Maternal factors in zebrafish development. *Dev Dyn.* 2003 Nov;228(3):535–54.
- 781 15. Bicskei B, Bron JE, Glover KA, Taggart JB. A comparison of gene transcription profiles of  
782 domesticated and wild Atlantic salmon (*Salmo salar* L.) at early life stages, reared under  
783 controlled conditions. *Bmc Genomics.* 2014;15(1):884.
- 784 16. Bicskei B, Taggart JB, Glover KA, Bron JE. Comparing the transcriptomes of embryos  
785 from domesticated and wild Atlantic salmon (*Salmo salar* L.) stocks and examining factors  
786 that influence heritability of gene expression. *Genet Sel Evol* [Internet]. 2016 Dec [cited  
787 2018 Mar 6];48(1). Available from: <http://www.gsejournal.org/content/48/1/20>

- 788 17. Yeates SE, Einum S, Fleming IA, Holt WV, Gage MJG. Assessing risks of invasion  
789 through gamete performance: farm Atlantic salmon sperm and eggs show equivalence in  
790 function, fertility, compatibility and competitiveness to wild Atlantic salmon. *Evol Appl.*  
791 2014 Apr 1;7(4):493–505.
- 792 18. Lanes CFC, Bizuayehu TT, de Oliveira Fernandes JM, Kiron V, Babiak I. Transcriptome of  
793 Atlantic Cod (*Gadus morhua* L.) Early Embryos from Farmed and Wild Broodstocks. *Mar*  
794 *Biotechnol.* 2013 Dec;15(6):677–94.
- 795 19. Lanes CFC, Bizuayehu TT, Bolla S, Martins C, de Oliveira Fernandes JM, Bianchini A, et  
796 al. Biochemical composition and performance of Atlantic cod (*Gadus morhua* L.) eggs and  
797 larvae obtained from farmed and wild broodstocks. *Aquaculture.* 2012 Jan;324–325:267–  
798 75.
- 799 20. Zupa R, Rodríguez C, Mylonas CC, Rosenfeld H, Fakriadis I, Papadaki M, et al.  
800 Comparative Study of Reproductive Development in Wild and Captive-Reared Greater  
801 Amberjack *Seriola dumerili* (Risso, 1810). Qiu G-F, editor. *PLOS ONE.* 2017 Jan  
802 5;12(1):e0169645.
- 803 21. Khendek A, Alix M, Viot S, Ledoré Y, Rousseau C, Mandiki R, et al. How does a  
804 domestication process modulate oogenesis and reproduction performance in Eurasian  
805 perch? *Aquaculture.* 2017 Apr;473:206–14.
- 806 22. Křišť'an J, Stejskal V, Policar T. Comparison of reproduction characteristics and  
807 broodstock mortality in farmed and wild eurasian perch (*Perca fluviatilis* L.) females during  
808 spawning season under controlled conditions. *Turk J Fish Aquat Sci.* 2012;12(2):191–197.
- 809 23. Teletchea F, Fontaine P. Levels of domestication in fish: implications for the sustainable  
810 future of aquaculture. *Fish Fish.* 2014 Jun 1;15(2):181–95.
- 811 24. Ben Khadher S, Fontaine P, Milla S, Agnèse J-F, Teletchea F. Genetic characterization and  
812 relatedness of wild and farmed Eurasian perch (*Perca fluviatilis*): Possible implications for  
813 aquaculture practices. *Aquac Rep.* 2016 May 1;3:136–46.
- 814 25. Stepien CA, Haponski AE. Taxonomy, Distribution, and Evolution of the Percidae. In:  
815 Kestemont P, Dabrowski K, Summerfelt RC, editors. *Biology and Culture of Percid Fishes*  
816 [Internet]. Dordrecht: Springer Netherlands; 2015 [cited 2018 Mar 12]. p. 3–60. Available  
817 from: [http://link.springer.com/10.1007/978-94-017-7227-3\\_1](http://link.springer.com/10.1007/978-94-017-7227-3_1)
- 818 26. Overton JL, Toner D, Policar T, Kucharczyk D. Commercial Production: Factors for  
819 Success and Limitations in European Percid Fish Culture. In: Kestemont P, Dabrowski K,  
820 Summerfelt RC, editors. *Biology and Culture of Percid Fishes: Principles and Practices*  
821 [Internet]. Dordrecht: Springer Netherlands; 2015 [cited 2019 May 29]. p. 881–90.  
822 Available from: [https://doi.org/10.1007/978-94-017-7227-3\\_35](https://doi.org/10.1007/978-94-017-7227-3_35)
- 823 27. Steinfeldt S, Fontaine P, Overton JL, Policar T, Toner D, Falahatkar B, et al. Current Status  
824 of Eurasian Percid Fishes Aquaculture. In: Kestemont P, Dabrowski K, Summerfelt RC,  
825 editors. *Biology and Culture of Percid Fishes: Principles and Practices* [Internet].

- 826 Dordrecht: Springer Netherlands; 2015 [cited 2019 May 29]. p. 817–41. Available from:  
827 [https://doi.org/10.1007/978-94-017-7227-3\\_32](https://doi.org/10.1007/978-94-017-7227-3_32)
- 828 28. Fontaine P, Wang N, Hermelink B. Broodstock Management and Control of the  
829 Reproductive Cycle. In: Kestemont P, Dabrowski K, Summerfelt RC, editors. *Biology and*  
830 *Culture of Percid Fishes* [Internet]. Springer Netherlands; 2015 [cited 2017 Mar 20]. p.  
831 103–22. Available from: [http://link.springer.com/bases-doc.univ-](http://link.springer.com/bases-doc.univ-lorraine.fr/chapter/10.1007/978-94-017-7227-3_3)  
832 [lorraine.fr/chapter/10.1007/978-94-017-7227-3\\_3](http://link.springer.com/bases-doc.univ-lorraine.fr/chapter/10.1007/978-94-017-7227-3_3)
- 833 29. Gillet C, Dubois JP. A survey of the spawning of perch (*Perca fluviatilis*), pike (*Esox*  
834 *lucius*), and roach (*Rutilus rutilus*), using artificial spawning substrates in lakes.  
835 *Hydrobiologia*. 1995;300(1):409–415.
- 836 30. Wang N, Teletchea F, Kestemont P, Milla S, Fontaine P. Photothermal control of the  
837 reproductive cycle in temperate fishes: Photothermal control of reproduction. *Rev Aquac.*  
838 2010 Dec;2(4):209–22.
- 839 31. Migaud H, Bell G, Cabrita E, McAndrew B, Davie A, Bobe J, et al. Gamete quality and  
840 broodstock management in temperate fish. *Rev Aquac.* 2013 May;5:S194–223.
- 841 32. Abdulfatah A, Fontaine P, Kestemont P, Gardeur J-N, Marie M. Effects of photothermal  
842 kinetics and amplitude of photoperiod decrease on the induction of the reproduction cycle  
843 in female Eurasian perch *Perca fluviatilis*. *Aquaculture*. 2011 Dec;322–323:169–76.
- 844 33. Sulistyio I. Reproductive cycle and plasma levels of sex steroids in female Eurasian perch  
845 *Perca fluviatilis*. *Aquat Living Resour.* 1998;11(2):101–10.
- 846 34. Schaerlinger B, Źarski D. Evaluation and Improvements of Egg and Larval Quality in  
847 Percid Fishes. In: Kestemont P, Dabrowski K, Summerfelt RC, editors. *Biology and*  
848 *Culture of Percid Fishes* [Internet]. Dordrecht: Springer Netherlands; 2015 [cited 2017 Mar  
849 20]. p. 193–223. Available from: [http://link.springer.com/10.1007/978-94-017-7227-3\\_6](http://link.springer.com/10.1007/978-94-017-7227-3_6)
- 850 35. Migaud H, Fontaine P, Kestemont P, Wang N, Brun-Bellut J. Influence of photoperiod on  
851 the onset of gonadogenesis in Eurasian perch *Perca fluviatilis*. *Aquaculture*. 2004  
852 Nov;241(1–4):561–74.
- 853 36. Ben Ammar I, Teletchea F, Milla S, Ndiaye WN, Ledoré Y, Missaoui H, et al. Continuous  
854 lighting inhibits the onset of reproductive cycle in pikeperch males and females. *Fish*  
855 *Physiol Biochem.* 2015 Apr;41(2):345–56.
- 856 37. Gabe M. *Techniques histologiques*. Paris: Masson; 1968. vi, 1113.
- 857 38. Rinchar J, Kestemont P. Comparative study of reproductive biology in single- and  
858 multiple-spawner cyprinid fish. I. Morphological and histological features. *J Fish Biol.*  
859 1996;49(5):883–94.
- 860 39. Źarski D, Bokor Z, Kotrik L, Urbanyi B, Horváth A, Targońska K, et al. A new  
861 classification of a preovulatory oocyte maturation stage suitable for the synchronization of

- 862 ovulation in controlled reproduction of Eurasian perch, *Perca fluviatilis* L. *Reprod Biol.*  
863 2011 Nov 1;11(3):194–209.
- 864 40. Żarski D, Horváth á., Kotrik L, Targońska K, Palińska K, Krejszeff S, et al. Effect of  
865 different activating solutions on the fertilization ability of Eurasian perch, *Perca fluviatilis*  
866 L., eggs. *J Appl Ichthyol.* 2012 Dec;28(6):967–72.
- 867 41. Alix M, Chardard D, Ledoré Y, Fontaine P, Schaerlinger B. An alternative developmental  
868 table to describe non-model fish species embryogenesis: application to the description of  
869 the Eurasian perch (*Perca fluviatilis* L. 1758) development. *EvoDevo* [Internet]. 2015 Dec  
870 [cited 2016 Sep 23];6(1). Available from: <http://www.evodevojournal.com/content/6/1/39>
- 871 42. Alix M, Zarski D, Chardard D, Fontaine P, Schaerlinger B. Deformities in newly hatched  
872 embryos of Eurasian perch populations originating from two different rearing systems. *J*  
873 *Zool.* 2017 Jun;302(2):126–37.
- 874 43. Pasquier J, Cabau C, Nguyen T, Jouanno E, Severac D, Braasch I, et al. Gene evolution and  
875 gene expression after whole genome duplication in fish: the PhyloFish database. *BMC*  
876 *Genomics* [Internet]. 2016 Dec [cited 2017 Mar 20];17(1). Available from:  
877 <http://bmcbgenomics.biomedcentral.com/articles/10.1186/s12864-016-2709-z>
- 878 44. Gotz S, Garcia-Gomez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, et al. High-  
879 throughput functional annotation and data mining with the Blast2GO suite. *Nucleic Acids*  
880 *Res.* 2008 Apr 15;36(10):3420–35.
- 881 45. Ozerov MY, Ahmad F, Gross R, Pukk L, Kahar S, Kisand V, et al. Highly Continuous  
882 Genome Assembly of Eurasian Perch (*Perca fluviatilis*) Using Linked-Read Sequencing.  
883 *G3 Genes Genomes Genet.* 2018 Dec 1;8(12):3737–43.
- 884 46. Mi H, Muruganujan A, Huang X, Ebert D, Mills C, Guo X, et al. Protocol Update for large-  
885 scale genome and gene function analysis with the PANTHER classification system (v.14.0).  
886 *Nat Protoc.* 2019 Mar;14(3):703.
- 887 47. Aljanabi S. Universal and rapid salt-extraction of high quality genomic DNA for PCR-  
888 based techniques. *Nucleic Acids Res.* 1997 Nov 15;25(22):4692–3.
- 889 48. Ben Khadher S, Agnès J-F, Milla S, Teletchea F, Fontaine P. Patterns of genetic structure  
890 of Eurasian perch (*Perca fluviatilis* L.) in Lake Geneva at the end of the spawning season. *J*  
891 *Gt Lakes Res.* 2015 Sep;41(3):846–52.
- 892 49. Leclerc D, Wirth T, Bernatchez L. Isolation and characterization of microsatellite loci in the  
893 yellow perch (*Perca flavescens*), and cross-species amplification within the family  
894 Percidae. *Mol Ecol.* 2000 Jul;9(7):995–7.
- 895 50. Wirth T, Saint-Laurent R, Bernatchez L. Isolation and characterization of microsatellite loci  
896 in the walleye (*Stizostedion vitreum*), and cross-species amplification within the family  
897 Percidae. *Mol Ecol.* 1999 Nov;8(11):1960–2.

- 898 51. Borer SO, Miller LM, Kapuscinski AR. Microsatellites in walleye *Stizostedion vitreum*.  
899 Mol Ecol. 1999;8(2):336–8.
- 900 52. Li L, Wang HP, Givens C, Czesny S, Brown B. Isolation and characterization of  
901 microsatellites in yellow perch (*Perca flavescens*). Mol Ecol Notes. 2007;7(4):600–3.
- 902 53. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious  
903 Basic: An integrated and extendable desktop software platform for the organization and  
904 analysis of sequence data. Bioinformatics. 2012 Jun 15;28(12):1647–9.
- 905 54. Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F, Chikli L, et al. GENETIX 4.05,  
906 logiciel sous Windows TM pour la génétique des populations. Montpellier, France:  
907 Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de  
908 Montpellier II; 2004.
- 909 55. Cavanaugh JE, Neath AA. The Akaike information criterion: Background, derivation,  
910 properties, application, interpretation, and refinements. WIREs Comput Stat.  
911 2019;11(3):e1460.
- 912 56. R Core Team (2017). R: A language and environment for statistical computing. R  
913 Foundation for Statistical Computing. [Internet]. Vienna, Austria.; Available from: URL  
914 <https://www.R-project.org/>.
- 915 57. Hedrick PW. Perspective: Highly Variable Loci and Their Interpretation in Evolution and  
916 Conservation. Evolution. 1999;53(2):313–8.
- 917 58. Dekens MPS, Pelegri FJ, Maischein H-M, Nüsslein-Volhard C. The maternal-effect gene  
918 futile cycle is essential for pronuclear congression and mitotic spindle assembly in the  
919 zebrafish zygote. Dev Camb Engl. 2003 Sep;130(17):3907–16.
- 920 59. McGinnity P, Prodohl P, Ferguson A, Hynes R, Maoileidigh N o., Baker N, et al. Fitness  
921 reduction and potential extinction of wild populations of Atlantic salmon, *Salmo salar*, as a  
922 result of interactions with escaped farm salmon. Proc R Soc B Biol Sci. 2003 Dec  
923 7;270(1532):2443–50.
- 924 60. Lund I, Steinfeldt SJ, Suhr KI, Hansen BW. A comparison of fatty acid composition and  
925 quality aspects of eggs and larvae from cultured and wild broodstock of common sole  
926 (*Solea solea* L. ). Aquac Nutr. 2008 Dec;14(6):544–55.
- 927 61. Bicskei B, Taggart JB, Glover KA, Bron JE. Comparing the transcriptomes of embryos  
928 from domesticated and wild Atlantic salmon (*Salmo salar* L.) stocks and examining factors  
929 that influence heritability of gene expression. Genet Sel Evol [Internet]. 2016 Dec [cited  
930 2018 Mar 6];48(1). Available from: <http://www.gsejournal.org/content/48/1/20>
- 931 62. Huang Y, Wange RL. T Cell Receptor Signaling: Beyond Complex Complexes. J Biol  
932 Chem. 2004 Jul 9;279(28):28827–30.

- 933 63. Partula S. Surface markers of fish T-cells. *Fish Shellfish Immunol.* 1999 May 1;9(4):241–  
934 57.
- 935 64. Laing KJ, Hansen JD. Fish T cells: Recent advances through genomics. *Dev Comp*  
936 *Immunol.* 2011 Dec 1;35(12):1282–95.
- 937 65. Żarski D, Nguyen T, Le Cam A, Montfort J, Dutto G, Vidal MO, et al. Transcriptomic  
938 Profiling of Egg Quality in Sea Bass (*Dicentrarchus labrax*) Sheds Light on Genes Involved  
939 in Ubiquitination and Translation. *Mar Biotechnol.* 2017 Feb;19(1):102–15.
- 940 66. Mommens M, Fernandes JM, Tollefsen KE, Johnston IA, Babiak I. Profiling of the  
941 embryonic Atlantic halibut (*Hippoglossus hippoglossus* L.) transcriptome reveals maternal  
942 transcripts as potential markers of embryo quality. *BMC Genomics.* 2014 Sep 30;15(1):829.
- 943 67. Robenek H, Hofnagel O, Buers I, Lorkowski S, Schnoor M, Robenek MJ, et al.  
944 Butyrophilin controls milk fat globule secretion. *Proc Natl Acad Sci.* 2006 Jul  
945 5;103(27):10385–90.
- 946 68. Żarski D, Palińska K, Targońska K, Bokor Z, Kotrik L, Krejszeff S, et al. Oocyte quality  
947 indicators in Eurasian perch, *Perca fluviatilis* L., during reproduction under controlled  
948 conditions. *Aquaculture.* 2011 Mar;313(1–4):84–91.
- 949 69. Crews ST. Control of cell lineage-specific development and transcription by bHLH–PAS  
950 proteins. *Genes Dev.* 1998 Mar 1;12(5):607–20.
- 951 70. Amano T, Tokunaga K, Kakegawa R, Yanagisawa A, Takemoto A, Tatemizo A, et al.  
952 Expression analysis of circadian genes in oocytes and preimplantation embryos of cattle  
953 and rabbits. *Anim Reprod Sci.* 2010 Sep;121(3–4):225–35.
- 954 71. Amano T, Matsushita A, Hatanaka Y, Watanabe T, Oishi K, Ishida N, et al. Expression and  
955 Functional Analyses of Circadian Genes in Mouse Oocytes and Preimplantation Embryos:  
956 *Cry1* Is Involved in the Meiotic Process Independently of Circadian Clock Regulation1.  
957 *Biol Reprod.* 2009 Mar 1;80(3):473–83.
- 958 72. Alvarez JD, Chen D, Storer E, Sehgal A. Non-cyclic and Developmental Stage-Specific  
959 Expression of Circadian Clock Proteins During Murine Spermatogenesis1. *Biol Reprod.*  
960 2003 Jul 1;69(1):81–91.
- 961 73. Anglesio MS, Evdokimova V, Melnyk N, Zhang L, Fernandez CV, Grundy PE, et al.  
962 Differential expression of a novel ankyrin containing E3 ubiquitin-protein ligase, *Hace1*, in  
963 sporadic Wilms' tumor versus normal kidney. *Hum Mol Genet.* 2004 Sep 15;13(18):2061–  
964 74.
- 965 74. Hollstein R, Parry DA, Nalbach L, Logan CV, Strom TM, Hartill VL, et al. *HACE1*  
966 deficiency causes an autosomal recessive neurodevelopmental syndrome. *J Med Genet.*  
967 2015 Dec;52(12):797–803.



- 968 75. Iimura A, Yamazaki F, Suzuki T, Endo T, Nishida E, Kusakabe M. The E3 ubiquitin ligase  
969 Hace1 is required for early embryonic development in *Xenopus laevis*. *BMC Dev Biol.*  
970 2016 Sep 21;16:31.
- 971 76. Daugaard M, Nitsch R, Razaghi B, McDonald L, Jarrar A, Torrino S, et al. Hace1 controls  
972 ROS generation of vertebrate Rac1-dependent NADPH oxidase complexes. *Nat Commun*  
973 [Internet]. 2013 Jul 17 [cited 2018 Apr 3];4. Available from:  
974 <http://www.nature.com/doi/10.1038/ncomms3180>
- 975 77. Razaghi B, Steele SL, Prykhozhiy SV, Stoyek MR, Hill JA, Cooper MD, et al. hace1  
976 Influences zebrafish cardiac development via ROS-dependent mechanisms. *Dev Dyn.* 2018  
977 Feb 1;247(2):289–303.
- 978 78. Castets M-D, Schaerlinger B, Silvestre F, Gardeur J-N, Dieu M, Corbier C, et al. Combined  
979 analysis of *Perca fluviatilis* reproductive performance and oocyte proteomic profile.  
980 *Theriogenology.* 2012 Jul;78(2):432-442.e13.
- 981 79. Schomer B, Epel D. Redox Changes during Fertilization and Maturation of Marine  
982 Invertebrate Eggs. *Dev Biol.* 1998 Nov;203(1):1–11.
- 983 80. Buchet-Poyau K, Courchet J, Hir HL, Séraphin B, Scoazec J-Y, Duret L, et al.  
984 Identification and characterization of human Mex-3 proteins, a novel family of  
985 evolutionarily conserved RNA-binding proteins differentially localized to processing  
986 bodies. *Nucleic Acids Res.* 2007 Feb 1;35(4):1289–300.
- 987 81. Kimelman D, Martin BL. Anterior–posterior patterning in early development: three  
988 strategies. *Wiley Interdiscip Rev Dev Biol.* 2012 Mar 1;1(2):253–66.
- 989 82. Draper BW, Mello CC, Bowerman B, Hardin J, Priess JR. MEX-3 Is a KH Domain Protein  
990 That Regulates Blastomere Identity in Early *C. elegans* Embryos. *Cell.* 1996 Oct  
991 18;87(2):205–16.
- 992 83. Takada H, Kawana T, Ito Y, Kikuno RF, Mamada H, Araki T, et al. The RNA-binding  
993 protein Mex3b has a fine-tuning system for mRNA regulation in early *Xenopus*  
994 development. *Development.* 2009 Jul 15;136(14):2413–22.

995

## 996 **Supporting information**

997 **S1 Fig. Study of oogenesis progression.** Evolution of (A) gonado somatic index - GSI and  
998 plasmatic levels of (B) 17- $\beta$ - $\alpha$ estradiol - E2, (C) testosterone and (D) Vitellogenin during oogenesis  
999 on the populations F1 and F7+. Bars correspond to mean values  $\pm$  standard error. Asterisks indicate

1000 significant differences between populations at  $p < 0.05$  using non-parametric Wilcoxon-Mann-  
1001 Whitney test. Significance levels are represented as follows: \*,  $p = 0.05-0.01$ .

1002

1003 **S1 Table. Primer sequences used for real-time PCR experiment of the 7 most expressed genes**  
1004 **in the two populations and reference genes used for data normalization.**

1005

1006 **S2 Table. Survivorship at 24, 48, 72 and 120 hours post-fertilization (hpf) and at hatching in**  
1007 **F1 and F7+ populations.** Columns 2 and 3 correspond to data presented in the Fig 4A. Different  
1008 letters mean significant differences between populations an ANOVA one-way repeated measures  
1009 followed by a TukeyHSD ( $p \leq 0.05$ ;  $n = 19$  and  $13$  for F1 and F7+, respectively). Population means  
1010  $\pm$  SEM are presented.

1011

1012 **S3 Table. Deformities rates (Dr) and specific rates in various tissues at hatching in F1 and**  
1013 **F7+ populations (Ad - Axis, Cd - Cardiac, Yd - Yolk, Md - Mouth, Od - Other, Ed - Eyes).**

1014 No significant differences have been identified between the two populations using non-parametric  
1015 Wilcoxon-Mann-Whitney test ( $p < 0.05$ ;  $n = 23$ ). Population means  $\pm$  SEM are presented.

1016

1017 **S4 Table. Differentially expressed genes list.** PhyloFish gene ID, gene description, False  
1018 Discovery Rate (p-value) and  $\log_2$ FC of the 358 differentially expressed genes on eggs from the  
1019 F7+ and F1 populations.

1020

1021 **S5 Table. Differentially expressed genes listed in the same order as they appear in the**  
1022 **clustering analysis.** PhyloFish gene ID is shown for each gene and the numbers 1-358 correspond  
1023 to their position in the figure resulting from clustering analysis.

1024

1025 **S6 Table. F7+ Gene Ontology list.** PhyloFish gene ID, gene description, Gene Ontology  
1026 Category, ID and Terms represented in the eggs transcriptome of the F7+ population. GO  
1027 Categories are: P = Biological Process, F = Molecular Function and C = Cellular Component.

1028

1029 **S7 Table. F1 Gene Ontology list.** PhyloFish gene ID, gene description, Gene Ontology Category,  
1030 ID and Terms represented in the eggs transcriptome of the F1 population. GO Categories are: P =  
1031 Biological Process, F = Molecular Function and C = Cellular Component.

1032

1033 **S8 Table. Genes belonging to the enriched term found in the overrepresentation analysis.**  
1034 PhyloFish gene ID, gene description, Uniprot protein and gene names, Uniprot Acession Number,  
1035 False Discovery Rate (p-value), Regulation and log(2)FC of the genes represented in enriched  
1036 function affected in the transcriptome of the eggs belonging to F7+ and F1 population.

1037