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1 **D1, but not D2, dopamine receptor regulates steroid levels during the final**
2 **stages of pikeperch gametogenesis**

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16

17 Short title: Dopamine receptors affect pikeperch reproduction

18

19 **Abstract**

20 In pikeperch, *Sander lucioperca*, aquaculture hormonal treatment is usually applied
21 to synchronize ovulation. However, the effect of dopamine (**DA**) receptor antagonists,
22 in particular those blocking the D1 DA receptors, remains unknown. Thus, the aim of
23 the present study was to investigate and compare the effects of D1 and D2 DA
24 receptor antagonists on the sex-steroid production and reproductive performance of
25 the species. Two experiments were performed during which mature pikeperch

26 females were injected with different molecules: NaCl 0.9 % (negative control) or hCG
27 500 IU/kg (positive control) in both experiments, metoclopramide (a D2 receptor
28 antagonist; 4 mg/kg or 20 mg/kg) or SCH23390 (a D1 receptor antagonist; 0.8 mg/kg
29 or 4 mg/kg) alone (experiment one) or in combination with a salmon-GnRH analogue
30 (**sGnRHa** at 25 µg/kg; experiment two). In experiment two, fish were also injected
31 with sGnRHa (25 µg/kg) as positive control. Samplings of oocytes and blood were
32 performed on the day of injection and after 24 h (both experiments), after 48 h
33 (experiment two) and at the time of ovulation (both experiments). In non-ovulating
34 fish, samplings were performed seven days (experiment one) or fourteen days
35 (experiment two) after injection. In experiment two, various zootechnical parameters
36 of fertilized eggs were recorded (survival, hatching and malformation rates). The two
37 antagonists alone were ineffective in inducing the final stages and regulating sex-
38 steroid (testosterone, 11 ketotestosterone, 17β estradiol and 17,20β -dihydroxy-4-
39 pregnen-3-one) production. When administered with sGnRHa, both SCH23390 and
40 metoclopramide induced the final stages. However, only SCH23390 stimulated
41 testosterone (4 mg/kg) and 17β estradiol (0.8 mg/kg) production compared to
42 sGnRHa alone. None of the treatments affected the survival, hatching or
43 malformation rates. This is the first report suggesting that in pikeperch the D1, but not
44 the D2, DA receptor antagonist would be involved in the testosterone and 17β
45 estradiol production as a potentiator of the sGnRHa effect.

46

47 **Keywords:** pikeperch, ovulation, dopamine, antagonist, sex-steroid

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51 **Implications**

52 This work provides information about the effects of some hormonal treatments on the
53 induction of pikeperch reproduction in order to produce high quality offspring in a
54 synchronous and predictable way for aquaculture. This work will allow optimizing
55 pikeperch spawning and therefore improving production.

56

57 **Introduction**

58 Pikeperch, *Sander lucioperca*, is a species of interest for aquaculture diversification.

59 It is a highly valuable economic and commercial species partly thanks to its flesh
60 quality and recreational value (Kestemont *et al.*, 2015). Despite an increase in inland
61 aquaculture production over the past decades, declining captures remain today the
62 main supply to the increasing market demand (Food and Agriculture Organization of
63 the United Nations, 2017). Consequently, great efforts are made to develop
64 pikeperch aquaculture in order to respond to consumer demand. To achieve this
65 objective, controlling the production of high quality offspring in a synchronous and
66 predictable way, year after year, is a crucial step in the culture process (Zarski *et al.*,
67 2015).

68 Controlled fish spawning is usually supported by the application of hormonal
69 treatment, which allows synchronizing the ovulation process and optimizing egg
70 collection while minimizing the handling and stress to the fish (Zohar and Mylonas,
71 2001). In pikeperch, various hormonal treatments have already been tested. The
72 most widely used, human chorionic gonadotropin (**hCG**), was found to be highly
73 effective in triggering ovulation (Zarski *et al.*, 2015). However, the spawning
74 effectiveness observed in this species was highly variable, with frequently reported
75 ovulation rates between 75% and 100% and embryonic survival rates between 50%

76 and 90% (Zarski *et al.*, 2015). Besides, some authors demonstrated that application
77 of hCG induces immune (Zohar and Mylonas, 2001) and stress responses
78 (Falahatkar and Poursaeid, 2014), which could alter the subsequent reproductive
79 operations (i.e. need to use higher doses of hCG or ineffectiveness of the treatment)
80 (Zohar and Mylonas, 2001). For these reasons, analogues of the gonadotropin-
81 releasing hormone (**GnRHa**) were applied as an alternative treatment in finfish
82 reproduction (Mylonas *et al.*, 2010; Zarski *et al.*, 2015). GnRH acts directly at the
83 pituitary level of the hypothalamic-pituitary-gonadal axis stimulating release of
84 gonadotropin (LH), sex-steroid secretion and finally progression of the final stages of
85 gametogenesis (oocyte meiotic maturation and ovulation). However, both basal and
86 GnRH-stimulated LH secretion are under dopaminergic inhibition (Yaron and Levavi-
87 Sivan, 2011). Thus, the combination of dopamine (**DA**) receptor antagonists with
88 GnRHa is usually used (Mylonas *et al.*, 2010) partly because antagonists would
89 enhance the reproductive effectiveness of GnRHa therapy (Zarski *et al.*, 2015).
90 Usually, the D2 DA receptor antagonists (metoclopramide **MCP**, domperidone or
91 pimozone) have been used. The application of D2 DA receptor antagonists alone had
92 positive effects on spawning in crucian carp, *Carassius carassius* (Cejko and
93 Kucharczyk, 2015). By contrast, in Senegalese sole, *Solea senegalensis*, these
94 treatments were found to be ineffective (Guzman *et al.*, 2011). In some perciforms,
95 the effects of these antagonists on the reproductive effectiveness are highly
96 contradictory and reduce the significance of the DA effect in this fish order (Dufour *et*
97 *al.*, 2010; Zarski *et al.*, 2015). Thus, the effectiveness of DA antagonists seems to be
98 species-specific and its use should be verified for each species separately.
99 In vertebrates, DA effects are mediated through the binding to two receptor families:
100 D1 and D2 receptor families (Cardinaud *et al.*, 1997; Dufour *et al.*, 2010).

101 Interestingly, unlike D2 receptors, there is a lack of data concerning the existence
102 and the role of D1 receptors in fish reproduction, although they were suggested to be
103 involved in the control of GnRH release (Yu and Peter, 1992; Kapsimali *et al.*, 2000).
104 In addition, *in vivo* studies showed that the DA/D1 receptor complex regulates the
105 decrease in serum LH levels and aromatase B transcript levels in the hypothalamus
106 of the goldfish, *Carassius auratus* (Popesku *et al.*, 2010 and 2012). These data
107 suggest that species specificity of DA may be associated with different involvement
108 of D1 and D2 receptors in the overall dopamine-related processes. The use of DA
109 antagonists specific to D1 or D2 receptor family alone has never been tested in
110 pikeperch. In addition, the combination of the D1 receptor antagonist with GnRHa
111 has never been studied to date. This, together with the unclear role of DA in
112 perciform reproduction (Dufour *et al.*, 2010; Zarski *et al.*, 2015), creates the need for
113 a detailed investigation of this mechanism which could form the basis for pikeperch-
114 specific hormonal treatment protocols.

115 Given the lack of data about the effects of dopamine receptor antagonists alone (D1
116 and D2) or in combination with GnRHa (D1) and the unknown involvement of
117 receptors D1 in pikeperch reproduction, we aimed to investigate the *in vivo*
118 physiological (sex-steroid) and zootechnical responses (gonado-somatic index (**GSI**),
119 progress of the oocyte meiotic maturation, ovulation rate, latency time, survival,
120 hatching and malformation rates) to D1 and D2 DA receptor antagonists in pikeperch.

121

122 **Material and methods**

123 This study was split into two independent *in vivo* experiments. The first experiment
124 was performed as a preliminary study to describe the effects of two DA antagonists
125 specific to D1 or D2 receptor, SCH23390 (**SCH**) or MCP, respectively, applied alone

126 on pikeperch reproduction. The second experiment was dedicated to point out the
127 effects of the two antagonists combined with a GnRHa on pikeperch reproduction.
128 Both experiments were performed according to the European and France legislation
129 for fish welfare and approved by the institutional Ethics Committee (APAFIS3073-
130 2016022913149909). Fish were handled after anaesthesia by immersion in a bath
131 containing 150 mg/l of Ethyl 3-aminobenzoate (MS-222; Sigma-Aldrich, Lyon,
132 France).

133

134 *Broodstock management*

135 *Experiment one.* On 1 May 2015, 36 mature females (origin: production pond,
136 Fishery Nove Hradky Ltd, Czech Republic; age: 3 to 4 years old; mean body weight:
137 1.07 ± 0.06 kg) were transported to an outdoor recirculating system (La Bouzule,
138 Laneuvelotte, France). On 4 May 2015, all the fish were individually tagged (ID-100A
139 Microtransponder; Dorset Group BV, Aalten, The Netherlands). Fish were maintained
140 in sub-squared tanks (3000 l, 1 m deep), fed to satiation with forage fish and exposed
141 to natural photoperiod (Nancy, France) and temperature conditions (mean
142 temperature: 17.3 ± 2.7 °C) throughout the two weeks of experiment. Once a week,
143 pH, and ammonia and nitrite concentrations in the water were measured using a
144 WTW 340i pH meter and a CARY I spectrophotometer, respectively. All values
145 remained above 7.5 for pH and below 1 mg/l for nitrites and ammonia.

146

147 *Experiment two.* On 15 January 2016, 47 mature females reared in captivity (origin:
148 Czech Republic; age: 4 to 5 years old; mean body weight: 2.39 ± 0.48 kg) were used
149 in the facilities of the fish farm Asialor (Pierrevillers, France). Tagged fish (FDX-B
150 transponder; Biolog-ID, Bernay, France), were maintained in two 8000 l tanks in a

151 recirculating aquaculture system under 20 lx of light intensity at the water surface.
152 Prior to the experiment, all fish were subjected to an increase in photoperiod and
153 temperature to reach 14L:10D and 12.5 °C, respectively. Throughout the experiment,
154 fish were kept under automatically controlled photoperiod (from 14L:10D to 15L:9D)
155 and temperature (12.9 ± 0.14 °C) mimicking the environmental conditions prevailing
156 during spawning. Dissolved oxygen (> 6 mg/l) and pH (7.8 ± 0.2) were monitored
157 daily. Ammonia and nitrite concentrations in the water were measured using a
158 colourimetric method once a week and remained below 0.5 mg/l.

159

160 *Evaluation of oocyte maturation stages*

161 For both experiments, each female was catheterized for evaluation of the oocyte
162 maturation stages at different sampling times (as described in the sampling strategy)
163 according to the classification by Zarski *et al.* (2012). Briefly, oocytes were sampled
164 using a catheter (CH06; 1.2 mm internal and 2 mm external diameter) and placed in
165 Serra's solution (ethanol/formalin/glacial acetic acid, 6:3:1 v/v/v). After mixing slowly
166 oocytes in Serra's solution and waiting (about 5 min) until the cytoplasm of the oocyte
167 will become clarified, the oocyte maturation stage was evaluated under binocular
168 microscope, magnification $\times 4$ (Motic® SFC-11 Series). In pikeperch, the final stages
169 of maturation were divided into seven morphological stages, from stage I to stage VII
170 (ovulation; Zarski *et al.*, 2012). This allowed following the progression of the oocyte
171 meiotic maturation [hereinafter termed final oocyte maturation (**FOM**)] until ovulation.

172

173 *Hormonal treatments*

174 *Experiment one.* On 4 May 2015, oocyte maturation stages were evaluated for each
175 female. Females between stages II and IV were randomized, sampled for blood (0 h)

176 and injected intraperitoneally with one of the following treatments: (1) negative
177 control with saline solution, the vehicle of all molecules (NaCl 0.9%, n = 5); (2)
178 positive control with hCG (500 IU/kg, n = 5; Sigma-Aldrich); a D2 DA receptor
179 antagonist, (3) **MCP 4** (4 mg/kg, n = 6; Sigma-Aldrich) or (4) **MCP 20** (20 mg/kg, n =
180 7); a D1 DA receptor antagonist, (5) **SCH 0.8** (0.8 mg/kg, n = 6; Abcam, Paris,
181 France) or (6) **SCH 4** (4 mg/kg, n = 7). The doses applied for hCG and MCP 20 were
182 the most commonly used in controlled reproduction of percids (Zarski *et al.*, 2015).
183 For SCH, the doses applied were chosen to be consistent with the literature and to
184 get a common dose between MCP and SCH.

185

186 *Experiment two.* On 15 January 2016, after determination of the oocyte maturation
187 stage, all the females at stage I were randomized and injected intraperitoneally with
188 one of the following treatments: (1) negative control with NaCl (0.9%, n = 9); (2) a
189 first positive control with hCG (500 IU/kg, n = 7); (3) a second positive control with a
190 salmon-GnRHa [25 µg/kg (**sGnRHa**), n = 7; Pyr-His-Trp-Ser-Tyr-D-Arg-Trp-Leu-Pro-
191 NHEt acetate salt; Syndel Laboratories Ltd, Nanaimo, Canada]; (4) MCP 4 or (5)
192 MCP 20 in combination with sGnRHa (25 µg/kg, n = 6 and n = 5, respectively); (6)
193 SCH 0.8 or (7) SCH 4 in combination with sGnRHa (25 µg/kg, n = 7 and n = 6,
194 respectively). Before injection, 10 females were randomly sampled for blood (0 h).
195 The doses applied in both positive control groups were the most commonly used in
196 controlled reproduction of percids (Zarski *et al.*, 2015).

197

198 *Sampling strategy*

199 *Experiment one.* Blood and oocytes were sampled 0 and 24 h after injection and at
200 the time of ovulation or 7 days after injection (Final sampling time, if the female did

201 not ovulate). Blood was sampled from the caudal vein and placed in tubes containing
202 heparin (28 mg/ml; 100 kU Sigma-Aldrich). Plasma was obtained by centrifugation
203 (15 min at 10 000 rpm) and stored at -80 °C until further steroid hormone analysis. At
204 the final sampling time, the fish were killed by overexposure to anaesthetics MS-222
205 (240 mg/l) and the whole gonads were cut out and weighed.

206 The following parameters were recorded: the progression of FOM (= number of
207 oocyte meiotic maturation stages between injection and the time of ovulation (or the
208 end of the experiment); the GSI (= $100 \times \text{gonad weight} / \text{total fish weight}$); the
209 ovulation rate for each treatment group (= $100 \times \text{number of ovulating females} / \text{total}$
210 number of females); and the latency time (= time interval between injection and
211 ovulation).

212

213 *Experiment two.* Blood and oocytes were sampled 0, 24 and 48 h after injection and
214 at the time of ovulation or 14 days after injection (Final sampling time). Then, plasma
215 was recovered and stored at -80 °C until further steroid hormone analysis. From 48
216 h, if the females did not reach stage VI, oocyte maturation stages were determined
217 every 2 days. At stage VI, the genital papilla was sewn (as described by Zarski *et al.*,
218 2015) in order to prevent spontaneous releasing of eggs into the tank. From stage VI,
219 ovulation control was performed every 6 h by gentle massage of the abdomen. At the
220 time of ovulation, eggs were collected in dry plastic containers, weighed and then
221 kept tightly covered at 11 °C for no longer than 30 min until fertilization.

222 For in vitro fertilization, sperm was collected from 30 males of pikeperch (origin:
223 Czech Republic; age: 4 to 5 years old; mean body weight: 2.39 ± 0.36 kg). For each
224 spawn, sperm from three males, injected with hCG (250 IU/kg) at 0 h, was collected
225 in a dry syringe. For each fertilization procedure, only freshly collected sperm (15 min

226 maximum prior to fertilization), with a motility rate above 80% evaluated under a light
227 microscope (magnification \times 400; Motic® B3 Series) (Cejko *et al.*, 2010) was used.
228 For each female, three egg samples (approximately 50-100 eggs each) were placed
229 in three glass Petri dishes containing 5 ml of hatchery water. Simultaneously, 50 μ l of
230 pooled sperm was added in each dish. After vigorous agitation for 15 s, each dish
231 was incubated in plastic cups containing 500 ml of water at 12 °C.
232 The progression of FOM, the ovulation rate and the latency time were recorded. The
233 GSI was not calculated due to the necessity to keep the fish alive. Additional
234 zootechnical parameters were recorded: the embryo survival rate at 72 h post
235 fertilization ($= 100 \times \text{number of viable eggs} / \text{total number of eggs}$), the hatching rate
236 ($= 100 \times \text{number of larvae} / \text{total number of viable eggs}$) and the malformation rate [$=$
237 $100 \times \text{number of larvae showing malformations (lordosis, cardiac oedema, kyphosis,$
238 $\text{fragmentation of oil droplet, yolk sac oedema, spinal curvature, scoliosis, C-shaped}$
239 $\text{larvae}) / \text{total number of larvae}$].

240

241 *Levels of sex steroid hormones in blood plasma*

242 For both experiments, sex-steroid hormones [17β estradiol (**E₂**), testosterone (**T**), 11
243 ketotestosterone (**11KT**) and 17,20 β -dihydroxy-4-pregnen-3-one (**DHP**)] were
244 measured in plasma using commercially available competitive ELISA kits. The kits for
245 E₂ (KAP0621) and T (KAPD1559) were obtained from Diasource (Louvain-La-Neuve,
246 Belgium), the kit for 11KT (582751) from Cayman Chemical (Ann Arbor, USA) and
247 the kit for DHP (MBS2602842) from MyBiosource (San Diego, USA). The sensitivity
248 limit, and the intra- and interassay CV were respectively 0.005 ng/ml (range: 0 -
249 0.935 ng/ml), < 4% and < 5% for E₂; 0.083 ng/ml (range: 0 - 16 ng/ml), < 10% and <

250 9% for T; 1.3 pg/ml (range: 0.78 - 100 pg/ml), < 9% and < 13% for 11KT; and 0.06
251 ng/ml (range: 0.312 - 20 ng/ml), < 9% and < 13% for DHP.

252

253 *Statistical analysis*

254 Statistical analyses were performed using the free software R version 3.3.1. For all
255 dependent variables, homogeneity of variances was tested using Levene test
256 (leveneTest, package 'car', Fox and Weisberg, 2011). For sex-steroids, GSI, latency
257 time, survival rate, hatching rate and malformation rate, data were analysed by a
258 linear mixed model (lmer, package 'lme4', Bates *et al.*, 2015) with hormonal
259 treatment and sampling time as fixed effects, and either the fish and the maturation
260 stage at P0 (experiment one) or only the fish (experiment two) as random effects:
261 model=lmer(Y~treatment*sampling_time+(1|fish)+(1|maturation_stage) with Y:
262 dependent variable. For model validation, residuals were tested for homogeneity and
263 normality using residual vs fitted value and sample vs theoretical quantile (Q-Q)
264 plots, respectively (plotresid, package 'RVAideMemoire', Hervé, 2016). If necessary,
265 data were log transformed, root square transformed or arcsin root square
266 transformed (only for data expressed in percentage). When the model was validated,
267 an anova table was performed to calculate F-tests (Anova, package 'car', Fox and
268 Weisberg, 2011) followed by a Least-squares means (predicted marginal means)
269 multiple comparison between treatments, sampling times and/or their interaction as
270 post-hoc test (lsmeans, package 'lsmeans', Lenth, 2016). When data, even
271 transformed, did not meet the assumptions for the linear mixed model, we used the
272 aligned rank transformation for nonparametric factorial analysis
273 (aligned.rank.transform, package 'ART', Villacorta, 2015) followed by a pairwise
274 comparison using Dunn test (posthoc.kruskal.dunn.test, package 'PMCMR', Pohlert,

275 2016). For the ovulation rate, data were analysed with a chi-square test (chisq.test,
276 package 'MASS', Venables and Ripley, 2002). Data are expressed as mean \pm SEM.
277 The level of significance used in all tests was $P < 0.05$.

278

279 **Results**

280 *Experiment one*

281 *Effect of dopamine receptor antagonists alone on reproductive performance.* In fish
282 treated with hCG, 100% of ovulation was recorded associated with higher GSI and
283 progression of FOM (3 - 5 stages) than in the other groups. In the latter, similar GSI
284 and progression of FOM (0 - 3) were noted. Consequently, MCP and SCH failed to
285 induce ovulation and did not trigger a significant progression of FOM (Table 1).

286

287 *Effect of dopamine receptor antagonists alone on plasma steroid concentrations.*

288 Plasma T and 11KT levels varied significantly as a function of the interaction
289 between hormonal treatments and sampling times ($P < 0.05$; Figure 1). Only females
290 injected with hCG showed a significant decrease in T and 11KT concentrations
291 between 24 h and the final sampling time ($P < 0.001$). However, no significant
292 difference in plasma androgen levels was observed between the groups at each
293 sampling time.

294 Only a time effect was monitored for E_2 with a drop observed at the final sampling
295 time compared to 0 h and 24 h ($P < 0.01$; Figure 2a).

296 No significant difference related to hormonal treatments and sampling times was
297 obtained for plasma DHP levels (Figure 2b).

298 For all steroid levels, high variability was observed.

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Experiment two

Effect of dopamine receptor antagonists in combination with salmon-GnRH analogue on reproductive performance. Only the negative control group did not promote the progression of FOM and ovulation. All the other treatments triggered 100% of ovulation. The latency time and zootechnical parameters (survival rate, hatching rate, malformation rate) were similar in all the groups (Table 2).

Effect of dopamine receptor antagonists in combination with salmon-GnRH analogue on plasma steroid concentrations. Plasma T concentrations showed significant differences related to the interaction between hormonal treatments and sampling times ($P < 0.001$; Figure 3a). All hormonal treatments induced a marked increase in T compared to the negative control at 24 h ($P < 0.05$). At 48 h, the SCH 0.8, SCH 4, MCP 4 and hCG treatments were still effective in maintaining higher T levels compared to the negative control ($P < 0.05$). At the final sampling time, all hormonal treatments reduced the T concentrations ($P < 0.05$). The comparison between sGnRHa alone and the combination of DA receptor antagonists with sGnRHa showed that T levels increased with SCH 4 at 48 h ($P < 0.01$).

Concerning 11KT, significant differences were observed related to the interaction between hormonal treatments and sampling times ($P < 0.001$; Figure 3b). All hormonal treatments induced an increase in 11KT levels at 24 h and 48 h ($P < 0.001$), while at the final sampling time they were significantly abolished but only after SCH and MCP 20 treatments ($P < 0.001$). However, for each sampling time, the DA

324 receptor antagonist failed to change the 11KT level compared to the sGnRHa
325 treatment alone.

326 For E₂, concentrations varied significantly as a function of the interaction between
327 hormonal treatments and sampling times ($P < 0.001$; Figure 4a). All hormonal
328 treatments induced an increase in E₂ levels at 24 h and 48 h ($P < 0.05$) except for
329 MCP 4 and SCH 4 at 24 h, and sGnRHa and MCP 4 at 48 h. At the final sampling
330 time, all treatments decreased E₂ levels ($P < 0.01$). Here also, there was not any
331 significant difference between sGnRHa alone and the combination of DA receptor
332 antagonists with sGnRHa except with SCH 0.8 at 48 h which increased E₂ levels ($P <$
333 0.01).

334 No significant difference related to hormonal treatments and sampling times was
335 obtained for plasma DHP levels (Figure 4b).

336 Mild variability was observed for all steroid levels.

337

338 **Discussion**

339 This study investigated, for the first time, the potential regulatory effects of dopamine
340 receptor antagonists alone on reproductive mechanisms in pikeperch. Our results
341 first confirmed the high effectiveness of hCG in inducing the progression of FOM and
342 ovulation in this species (Zarski *et al.*, 2015). During FOM, oocytes undergo a
343 phenomenon of hydration inducing, in turn, an increase in follicle weight (Mañanós *et*
344 *al.*, 2008). After hCG treatment, the rise in GSI may thus stem from the hydration
345 process even if the latter remains to be demonstrated in pikeperch. Contrary to this
346 positive control, no antagonist treatments were found to induce either GSI increase
347 or ovulation. The latter finding is in accordance with previous reports on the
348 Senegalese sole (Guzman *et al.*, 2011) and the common tench, *Tinca tinca*

349 (Podhorec *et al.*, 2016). In contrast, Cejko and Kucharczyk (2015) showed that the
350 injection of metoclopramide induces ovulation in the crucian carp. This inter-species
351 difference may be due to variable potency of the DA inhibition as already suggested
352 (Mañanós *et al.*, 2008; Zarski *et al.*, 2015). Consequently, we hypothesize that DA
353 receptor antagonists applied alone would be ineffective in inducing ovulation in
354 pikeperch because of a weakness in the DA inhibition during the spontaneous
355 progression of FOM. Alternatively, we might speculate that the doses chosen were
356 not sufficient to generate a GnRH and/or LH endogenous surge. However, when
357 combined with sGnRH α , these DA receptor antagonists do not prevent sGnRH α from
358 triggering ovulation. They do not thus appear as inhibitors of the final stages of
359 gametogenesis in pikeperch. Conversely, DA receptor antagonists would not by
360 themselves allow the reproductive performance to be improved or impaired.

361
362 The DA receptor antagonists alone did not induce significant changes in the sex-
363 steroid levels. These results are in accordance with prior studies in striped bass,
364 *Morone saxatilis* (King *et al.*, 1994), and in Senegalese sole (Guzman *et al.*, 2011) in
365 which application of the D2 DA receptor antagonist did not modify the T and E₂ levels
366 in plasma. The attempt to block the D1 receptor family did not succeed in altering
367 those hormonal levels as well. The ineffectiveness of DA receptor antagonists alone
368 in changing the sex-steroid secretion is consistent with the absence of effect on the
369 ovulation and progression in oocyte maturation. Additionally, this would indicate
370 absence of or weakness in the dopaminergic inhibition by the application of D1 or D2
371 receptor antagonist at the tested doses during the non-hormonally manipulated
372 oocyte maturation process in pikeperch.

373 Interestingly, we observed that the hCG treatment was unequally effective in inducing
374 changes in the sex-steroid levels. These results are quite surprising considering that
375 this molecule is a common substitute for natural (endogenous) fish LH, which
376 induces fish spawning by direct action on gonads and sex-steroid levels (Mylonas *et*
377 *al.*, 2010). Considering our results from experiment one, we noticed that at the same
378 sampling time (e.g. 24 h), fish exhibited different stages of oocyte maturation,
379 contrary to the synchronous stages in experiment two. This high inter-individual
380 variability may have induced a larger range in steroid concentrations among the fish,
381 which could potentially explain statistically irrelevant endocrine response following
382 hCG treatment. However, other differences between the two populations (e.g.
383 environmental conditions, stress status, final number of individuals) may also explain
384 this difference in sensitivity to hCG. In contrast, our experimental setup, that is the
385 presence of two experiments, the number of modalities, the common number of fish
386 per group (King *et al.*, 1994; Barry *et al.*, 1995; Guzman *et al.*, 2011), the number of
387 sampling times and the *P*-values observed lead us to think that we have satisfactory
388 conditions to have a sufficient statistical power for observing potential effects.

389 The sGnRHa treatment stimulated the production of sex-steroids confirming the
390 widely observed activation of the gonadotropic axis in finfishes after such a treatment
391 (Yaron and Levavi-Sivan, 2011). Interestingly, T and E₂ production was stimulated
392 when sGnRHa was complemented with SCH depending on the dose. These results
393 lead us to hypothesize that SCH would boost the sGnRHa effect on T and E₂
394 secretion. To our knowledge, this is the first report of plasma sex-steroid change after
395 exposure to D1 receptor family antagonist in fish. Either this antagonist would directly
396 block the D1 receptors at the gonad level as shown in rats (Venegas-Meneses *et al.*,
397 2015), which in turn would stimulate the ovarian steroidogenesis. Or, the blockage of

398 those receptors in the brain would disrupt the aromatase activity and the further
399 metabolism of T and E₂ (Marsh *et al.*, 2006; Popesku *et al.*, 2012). Also, the blockage
400 of D1 receptors could induce a surge in blood LH (Popesku *et al.*, 2010) which would
401 stimulate the sex-steroid production. Finally, we cannot rule out some indirect effects
402 of SCH through other metabolic factors. Further studies would be needed to check
403 presence of those receptors in the ovary and to test these regulations by
404 investigating the aromatase expression and activity in brain and oocytes as well as
405 LH in the blood plasma.

406

407 Plasma DHP concentrations remained basal and stable over time in all the
408 treatments. In many teleosts, the steroid DHP is the maturation-inducing steroid
409 (**MIS**) (Nagahama and Yamashita, 2008). Progression of FOM was found to be linked
410 to a significant increase in DHP levels in walleye, *Sander vitreus* (Barry *et al.*, 1995),
411 and in striped bass (Mylonas *et al.*, 1997). However, in our study, even after
412 application of hCG or sGnRHa with which all the fish ovulated, no peak of DHP
413 concentration was observed. Surprisingly, these results are not consistent with GnRH
414 commonly known effects on the gonadotrope axis (Yaron and Levavi-Sivan, 2011)
415 and with a prior study in which hCG induced an increase in plasma DHP levels
416 during the progression of FOM in white perch, *Morone americana*, and white bass,
417 *Morone chrysops* (King *et al.*, 1995). Several hypotheses may explain these findings.
418 First, as reported in walleye, the DHP could be rapidly removed from the plasma after
419 its conjugation to a non-immunodetectable molecule such as 17,20-P-sulphate or
420 17,20-P-glucuronate (Scott and Canario, 1992). Second, in a closely related species,
421 the Eurasian perch, *Perca fluviatilis*, Migaud *et al.* (2003) found very low DHP levels
422 in plasma and suggested that these levels may be higher in the early morning than at

423 other times. Due to experimental conditions, our sampling times were performed at
424 the beginning of the afternoon, so we may have missed the peak of detectable DHP
425 in the blood plasma. Third, a close hormone, the $17\alpha,20\beta,21$ -trihydroxy-4-pregnen-3-
426 one (**20 β -S** or 17,20,21-P), identified as MIS in other perciforms (Nagahama and
427 Yamashita, 2008) could also play this role in pikeperch. That would explain the mild
428 level of DHP in this species, even after injection with hCG or sGnRH α . Indeed,
429 although Barry *et al.* (1995) demonstrated that 20 β -S was not detectable in walleye,
430 others studied in an other perciform, the European sea bass, *Dicentrarchus labrax*,
431 suggested that DHP and 20 β -S could be both considered as MIS (Sorbera *et al.*,
432 1999; Asturiano *et al.*, 2000). The DHP would be involved in the initiation of the
433 maturation, just before our first sampling time, explaining the absence of detection in
434 our study, and the 20 β -S just before the ovulation. This indicates that both
435 methodical and physiological studies on MIS in pikeperch should be reconsidered in
436 the future. In any case, by considering DHP as the primary MIS in pikeperch, the lack
437 of DA receptor antagonist effects (positive or negative) on FOM progression would
438 support the absence of DHP regulation by these treatments.

439

440 In conclusion, this study, in the current experimental conditions, showed the
441 ineffectiveness of DA receptor antagonist treatments alone, whatever the receptor
442 family (D1 or D2), in inducing sex-steroid changes, FOM and ovulation in pikeperch.
443 Combined with sGnRH α , these DA receptor antagonists did not prevent sGnRH α
444 from triggering ovulation. However, in this combination with sGnRH α , SCH but not
445 MCP proved efficiency to increase sGnRH α -stimulated steroid levels. Thus, only
446 SCH would be involved in the regulation of sex-steroids indicating a putative
447 potentiator effect of sGnRH α through D1 DA receptor blockage. The use of *in vitro*

448 biological tests of organ culture (e.g. brain, pituitary and ovary) could be useful in the
449 future to pinpoint these endocrine mechanisms.

450

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456

457 **References**

- 458 Asturiano JF, Sorbera LA, Ramos J, Kime DE, Carillo M and Zanuy S 2000. Hormonal
459 regulation of the European sea bass (*Dicentrarchus labrax*, L.) reproductive cycle: an
460 individualized female approach. *Journal of Fish Biology* 56, 1155-1172.
- 461 Barry TP, Malison JA, Lapp AF and Procarione LS 1995. Effects of selected hormones and
462 male cohorts on final oocyte maturation, ovulation, and steroid production in walleye
463 (*Stizostedion vitreum*). *Aquaculture* 138, 331-347.
- 464 Bates D, Machler M, Bolker B and Walker S 2015. Fitting linear mixed-effects models using
465 lme4. *Journal of Statistical Software* 67, 1-48.
- 466 Cardinaud B, Sugamori KS, Coudouel S, Vincent JD, Niznik HB and Vernier P 1997. Early
467 emergence of three dopamine D1 receptor subtypes in vertebrates. *The Journal of*
468 *Biology Chemistry* 272, 2778-2787.
- 469 Cejko BI, Kowalski RK, Kucharczyk D, Targonska K, Krejszef S, Zarski D and Glogowski J
470 2010. Influence of the length of time after hormonal stimulation on selected parameters
471 of milt of ide *Leuciscus idus* L. *Aquaculture Research* 41, 804-813.
- 472 Cejko BI and Kucharczyk D 2015. Application of dopaminergic antagonist: metoclopramide,
473 in reproduction of crucian carp *Carassius carassius* (L.) under controlled conditions.
474 *Animal Reproduction Science* 160, 74-81.

475 Dufour S, Sebert ME, Weltzien FA, Rousseau K and Pasqualini C 2010. Neuroendocrine
476 control by dopamine of teleost reproduction. *Journal of Fish Biology* 76,129-160.

477 Falahatkar B and Poursaeid S 2014. Effects of hormonal manipulation on stress responses
478 in male and female broodstocks of pikeperch *Sander lucioperca*. *Aquaculture*
479 *International* 22, 235-244.

480 Food and Agriculture Organization of the United Nations 2017. Cultured aquatic species
481 information programme. *Sander lucioperca* (Linnaeus, 1758). Retrieved on 10 July
482 2017 from http://www.fao.org/fishery/culturedspecies/Sander_lucioperca/en

483 Fox J and Weisberg S 2011. An R companion to applied regression, 2nd edition. Sage
484 Publications, Inc.

485 Guzman JM, Cal R, Garcia-Lopez A, Chereguini O, Kight K, Olmedo M, Sarasquete C,
486 Mylonas CC, Peleteiro JB, Zohar Y and Mañanós EL 2011. Effects of *in vivo* treatment
487 with the dopamine antagonist pimozide and gonadotropin-releasing hormone agonist
488 (GnRHa) on the reproductive axis of Senegalese sole (*Solea senegalensis*).
489 *Comparative Biochemistry and Physiology, Part A* 158, 235-245.

490 Hervé M 2016. RVAideMemoire: diverse basic statistical and graphical functions. R package
491 version 0.9-61.

492 Kapsimali M, Vidal B, Gonzalez A, Dufour S and Vernier P 2000. Distribution of the mRNA
493 encoding the four dopamine D1 receptor subtypes in the brain of the European eel
494 (*Anguilla anguilla*): comparative approach to the function of D1 receptors in
495 vertebrates. *The Journal of Comparative Neurology* 419, 320-343.

496 Kestemont P, Dabrowski K and Summerfelt RC 2015. *Biology and culture of percid fishes:*
497 *principles and practices*, Springer, Dordrecht, Netherlands.

498 King W, Thomas P, Harrell RM, Hodson RG and Sullivan CV 1994. Plasma levels of gonadal
499 steroids during final oocyte maturation of striped bass, *Morone saxatilis* L. *General and*
500 *Comparative Endocrinology* 95, 178-191.

501 King W, Berlinsky DL and Sullivan CV 1995. Involvement of gonadal steroids in final oocyte
502 maturation of white perch (*Morone americana*) and white bass (*M. chrysops*): *in vivo*
503 and *in vitro* studies. *Fish Physiology and Biochemistry* 14, 489-500.

504 Lenth R 2016. Least-squares means: the R package lsmeans. *Journal of Statistical Software*
505 69:1–33.

506 Mañanós EL, Duncan N and Mylonas CC 2008. Reproduction and control of ovulation,
507 spermiation and spawning in cultured fish. In *Methods in reproductive aquaculture:*
508 *marine and freshwater species* (ed. E Cabrita, V Robles and MP Herraéz), pp. 3-80.
509 CRC Press, Taylor and Francis Group, Boca Raton, FL, USA.

510 Marsh KE, Creutz LM, Hawkins MB and Godwin J 2006. Aromatase immunoreactivity in the
511 bluehead wrasse brain, *Thalassoma bifasciatum* : immunolocalization and co-
512 regionalization with arginine vasotocin and tyrosine hydroxylase. *Brain Research* 1126,
513 91-101.

514 Migaud H, Mandiki R, Gardeur JN, Fostier A, Kestemont P and Fontaine P 2003. Synthesis
515 of sex steroids in final oocyte maturation and induced ovulation in female Eurasian
516 perch, *Perca fluviatilis*. *Aquatic Living Resources* 16, 380-388.

517 Mylonas CC, Scott AP and Zohar Y 1997. Plasma gonadotropin II, sex steroids, and thyroid
518 hormones in wild striped bass (*Morone saxatilis*) during spermiation and final oocyte
519 maturation. *General and Comparative Endocrinology* 108, 223-236.

520 Mylonas CC, Fostier A and Zanuy S 2010. Broodstock management and hormonal
521 manipulations of fish reproduction. *General and Comparative Endocrinology* 165, 516-
522 534.

523 Nagahama Y and Yamashita M 2008. Regulation of oocyte maturation in fish. *Development*
524 *Growth and Differentiation* 50, 195-219.

525 Podhorec P, Socha M, Ben Ammar I, Sokolowska-Mikolajczyk M, Brzuska E, Milla S,
526 Gosiewski G, Stejskal V, Simko M and Kouril J 2016. The effects of GnRHa with and
527 without dopamine antagonist on reproductive hormone levels and ovum viability in
528 tench *Tinca tinca*. *Aquaculture* 465, 158-163.

529 Pohlert T 2016. The Pairwise Multiple Comparisons of Mean Ranks package (PMCMR), R
530 package.

531 Popesku JT, Martyniuk CJ, Denslow ND and Trudeau VL 2010. Rapid dopaminergic
532 modulation of the fish hypothalamic transcriptome and proteome. PLoS ONE 5,
533 e12338.

534 Popesku JT, Martyniuk CJ and Trudeau VL 2012. Meta-type analysis of dopaminergic effects
535 on gene expression in the neuroendocrine brain of female goldfish. Frontiers in
536 Endocrinology 3: 1-24.

537 Scott AP and Canario AVM 1992. $17\alpha,20\beta$ -Dihydroxy-4-pregnen-3-one 20-sulphate: a major
538 new metabolite of the teleost oocyte maturation-inducing steroid. General and
539 Comparative Endocrinology 85, 91-100.

540 Sorbera LA, Asturiano JF, Carillo M, Cerda J, Kime DE and Zanuy S 1999. *In vitro* oocyte
541 maturation in the sea bass: effects of hCG, pituitary extract and steroids. Journal of
542 Fish Biology 55, 9-25.

543 Venables WN and Ripley BD 2002. Modern applied statistics with S. Fourth edition, Springer,
544 New York, NY, USA

545 Venegas-Meneses B, Padilla JF, Juarez CE, Moran JL, Moran C, Rosas-Murrieta NH,
546 Handal A and Dominguez R 2015. Effects of ovarian dopaminergic receptors on
547 ovulation. Endocrine 50, 783-796.

548 Villacorta PJ 2015. ART: Aligned rank transform for nonparametric factorial analysis, R
549 package version 1.0. 1–5.

550 Yaron Z and Levavi-Sivan B 2011. Endocrine regulation of fish reproduction. In Encyclopedia
551 of fish physiology: from genome to environment (ed. AP Farrell), pp. 1500-1508.
552 Elsevier Inc. San Diego, CA, USA.

553 Yu KL and Peter RE 1992. Adrenergic and dopaminergic regulation of gonadotropin-
554 releasing hormone release from goldfish preoptic-anterior hypothalamus and pituitary
555 *in vitro*. General and Comparative Endocrinology 85,138-146.

556 Zarski D, Kucharczyk D, Targonska K, Palinska K, Kupren K, Fontaine P and Kestemont P
557 2012. A new classification of pre-ovulatory oocyte maturation stages in pikeperch,
558 *Sander lucioperca* (L.), and its application during artificial reproduction. *Aquaculture*
559 *Research* 43, 713-721.

560 Zarski D, Horvath A, Held JA and Kucharczyk D 2015. Artificial reproduction of percid fishes.
561 In *Biology and culture of percid fishes* (ed. Kestemont P, Dabrowski K, and Summerfelt
562 RC), pp. 123-161. Springer, Dordrecht, Netherlands.

563 Zohar Y and Mylonas CC 2001. Endocrine manipulations of spawning in cultured fish: from
564 hormones to genes. *Aquaculture* 197, 99-136.

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577 **Table 1** Effect of hormonal treatments on reproductive performance¹ in pikeperch females.

Parameters	Treatments												P-value
	Controls ²				Antagonist groups ³								
	NaCl	SEM	hCG	SEM	MCP 4	SEM	MCP 20	SEM	SCH 0.8	SEM	SCH 4	SEM	
GSI (%)	13.5 ^A	0.3	19.5 ^B	1.3	11.8 ^A	0.8	11.6 ^A	1.4	11.5 ^A	0.8	10.4 ^A	0.4	< 0.001
FOM prog	0 - 1		3 - 5		0 - 1		0 - 3		0 - 1		0 - 1		
OR (%)	0 ^A		100 ^B		0 ^A		17 ^A		0 ^A		0 ^A		< 0.001
LT (h)			53.4		6.2		47.1						

578 GSI = gonado-somatic index; FOM prog = final oocyte maturation progression; OR = ovulation rate; LT = latency time (time between injection and ovulation);

579 NaCl = saline solution; hCG = human chorionic gonadotropin; MCP 4 or MCP 20 = metoclopramide at 4 mg/kg or 20 mg/kg; SCH 0.8 or SCH 4 = SCH23390

580 at 0.8 mg/kg or 4 mg/kg

581 ¹ Reproductive performance parameters are GSI, FOM prog, OR and LT

582 ² Controls include negative control (NaCl) and positive control (hCG)

583 ³ Antagonist groups are treatments with one dopamine receptor antagonist (SCH or MCP)

584 ^{A,B} Means within the same row with different superscripts differ significantly at $P < 0.001$.

585 **Table 2** Effect of hormonal treatments on reproductive performance¹ in pikeperch females.

Parameters	Treatments														P-value
	Controls ²					Antagonist groups with GnRH ³									
	NaCl	hCG	SEM	sGnRHa	SEM	MCP 4	SEM	MCP 20	SEM	SCH 0.8	SEM	SCH 4	SEM		
FOM prog	0 - 1	6		6		6		6		6		6			
OR (%)	0 ^A	100 ^B		100 ^B		100 ^B		100 ^B		100 ^B		100 ^B		< 0.001	
LT (h)		129.	5.4	136.9	20.0	168.5	32.6	208.3	17.0	187.8	27.1	159.7	37.6	> 0.05	
		7													
SR (%)		46.8	8.1	15.0	10.7	28.3	7.7	44.0	15.2	41.5	10.2	24.7	18.0	> 0.05	
HR (%)		32.0	8.3	11.8	10.5	21.0	7.0	37.0	13.4	36.5	12.8	9.0	5.5	> 0.05	
MR (%)		22.6	4.1	40.7	26.9	34.5	15.5	14.3	3.2	35.5	3.9	38.5	28.5	> 0.05	

586 FOM prog = final oocyte maturation progression; OR = ovulation rate; LT = latency time (time between injection and ovulation); SR = survival rate; HR =
587 hatching rate; MR = malformation rate; NaCl = saline solution; hCG = human chorionic gonadotropin; sGnRHa = analogue of gonadotropin releasing hormone;
588 MCP 4 or MCP 20 = metoclopramide at 4 mg/kg or 20 mg/kg; SCH 0.8 or SCH 4 = SCH23390 at 0.8 mg/kg or 4 mg/kg

589 ¹ Reproductive performance parameters are FOM prog, OR, LT, SR, HR and MR

590 ² Controls include negative control (NaCl) and positive controls (hCG and sGnRHa)

591 ³ Antagonist groups with sGnRHa are treatments combining GnRH with one dopamine receptor antagonist (SCH or MCP)

592 ^{A,B} Means within the same row with different superscripts differ significantly at $P < 0.001$.

593

594

595 **Figure captions**

596

597 **Figure 1 Effect of hormonal treatments on plasmatic concentrations of (a)**
598 **testosterone (T) and (b) 11 ketotestosterone (11KT) in pikeperch.** Fish were
599 injected with NaCl (0.9%, n = 5), hCG (500 IU/kg, n = 5), MCP 4 or MCP 20
600 [metoclopramide at 4 mg/kg (n = 6) or 20 mg/kg (n = 7), respectively] and SCH 0.8 or
601 SCH 4 [(SCH23390 at 0.8 mg/kg (n = 6) or 4 mg/kg (n = 7), respectively)]. Blood was
602 sampled at three sampling times: 0 and 24 h after injection and at the time of
603 ovulation or 7 days after injection (if ovulation did not occur; Final). Values are means
604 ± SEM. Different lowercase letters indicate significant differences for the interaction
605 between hormonal treatments and sampling times ($P < 0.05$).

606

607 **Figure 2 Effect of hormonal treatments on plasmatic concentrations of (a) 17β**
608 **estradiol (E₂) and (b) 17,20β-dihydroxy-4-pregnen-3-one (DHP) in pikeperch.**
609 Fish were injected with NaCl (0.9%, n = 5), hCG (500 IU/kg, n = 5), MCP 4 or MCP 20
610 [metoclopramide at 4 mg/kg (n = 6) or 20 mg/kg (n = 7), respectively] and SCH 0.8 or
611 SCH 4 [(SCH23390 at 0.8 mg/kg (n = 6) or 4 mg/kg (n = 7), respectively)]. Blood was
612 sampled at three sampling times: 0 h and 24 h after injection and at the time of
613 ovulation or 7 days after injection (if ovulation did not occur; Final). Values are means
614 ± SEM. Different lowercase letters indicate significant differences between sampling
615 times ($P < 0.05$).

616

617 **Figure 3 Effect of hormonal treatments on plasmatic concentrations of (a)**
618 **testosterone (T) and (b) 11 ketotestosterone (11KT) in pikeperch.** Fish were
619 injected with NaCl (0.9%, n = 9), hCG (500 IU/kg, n = 7), sGnRHa (25 µg/kg, n = 7),

620 sGnRHa (25 µg/kg) in combination with MCP 4 or MCP 20 [metoclopramide at 4
621 mg/kg (n = 6) or 20 mg/kg (n = 5), respectively] or with SCH 0.8 or SCH 4
622 [(SCH23390 at 0.8 mg/kg (n = 7) or 4 mg/kg (n = 6), respectively)]. Blood was
623 sampled at four sampling times: 0, 24 and 48 h after injection and at the time of
624 ovulation or 14 days after injection (if ovulation did not occur; Final). Values are
625 means ± SEM. Different capital letters indicate significant differences between
626 hormonal treatments for the same sampling time ($P < 0.05$). Different lowercase
627 letters indicate significant differences between sampling times for the same hormonal
628 treatment ($P < 0.05$).

629

630 **Figure 4 Effect of hormonal treatments on plasmatic concentrations of (a) 17β**
631 **estradiol (E₂) and (b) 17,20β-dihydroxy-4-pregnen-3-one (DHP) in pikeperch.**

632 Fish were injected with NaCl (0.9%, n = 9), hCG (500 IU/kg, n = 7), sGnRHa (25
633 µg/kg, n = 7), sGnRHa (25 µg/kg) in combination with MCP 4 or MCP 20
634 [metoclopramide at 4 mg/kg (n = 6) or 20 mg/kg (n = 5), respectively] or with SCH 0.8
635 or SCH 4 [(SCH23390 at 0.8 mg/kg (n = 7) or 4 mg/kg (n = 6), respectively)]. Blood
636 was sampled at four sampling times: 0, 24 and 48 h after injection and at the time of
637 ovulation or 14 days after injection (if ovulation did not occur; Final). Values are
638 means ± SEM. Different capital letters indicate significant differences between
639 hormonal treatments for the same sampling time ($P < 0.05$). Different lowercase
640 letters indicate significant differences between sampling times for the same hormonal
641 treatment ($P < 0.05$)