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Synthesis of sex steroids in final oocyte maturation and induced ovulation in female Eurasian perch, *Perca fluviatilis*

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Abstract

This study was designed to specify the testosterone (T), estradiol-17β (E_2) and 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P) variations during the pre-ovulatory period in female Eurasian perch, *Perca fluviatilis*, and also examine the ovarian synthesis of 17,20β-P and 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S). Five females (100–300 g) were collected from a concrete pond from the Lindre Center (Moselle, France) between March and April 1999, three times a week, and sex steroids measured. Two females were also collected from the Lindre pond on three dates (18 February, 14 and 23 March) for an in vitro study. Significant plasma 17,20β-P peaks were measured for the first time in this species reaching 3.5 ng ml^{-1}. However, the synthesis and release of 17,20β-P in blood seems to not be synchronized with the final maturation and ovulation stages. The in vitro study showed that ovaries at the pre-ovulatory stage produce some 17,20β-P but no 20β-S from a tritiated precursor (17-P-^{3}H). 17,20β-P is produced after 30 min and is still present in the medium after 2 h of incubation, but is no longer detectable after 4 h. Some other non-identified compounds were synthesized at this stage suggesting that 17,20β-P was quickly metabolized. Moreover, the plasma level increases, observed in spawned females suggest that this steroid might be involved in the oviposition process. These results suggest a role for 17,20β-P in the final stages of oocyte development.

Résumé

Synthèse des stéroïdes sexuels au cours de la maturation finale des ovocytes et l'induction de l'ovulation chez la perche commune femelle, *Perca fluviatilis*. Une étude a été conduite dans le but de préciser les variations de testostérone (T), d'œstradiol (E_2) et de 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P) au cours de la période pré-ovulatoire chez des femelles de perche commune *Perca fluviatilis* d'une part et d'examiner in vitro la synthèse ovarienne de 17,20β-P et 17,20β, 21-trihydroxy-4-pregnen-3-one (20β-S) d'autre part. Trois fois par semaine, 5 femelles (100–300 g) ont été capturées dans l'étang de Lindre (Moselle, France) sur la période de mars–avril 1999 et leurs teneurs en hormones stéroïdes sexuelles dosées. Deux femelles supplémentaires ont été capturées, les 18 février, 14 et 23 mars, pour l'étude in vitro. Dans le plasma, des pics significatifs de la teneur en 17,20β-P (3,5 ng ml^{-1}) ont été mesurés pour la première fois. Cependant, la synthèse et la libération de 17,20β-P dans le sang ne semblent pas synchrones avec les stades de la maturation ovocytaire et l'ovulation. L'étude in vitro montre que les ovaires en période pré-ovulatoire produisent l'hormone 17,20β-P, mais pas de 20β-S à partir d'un précurseur tritié (17-P-^{3}H). La 17,20β-P est produite au bout d'une demi-heure et est encore présente dans le milieu après 2 h d'incubation, mais n'est plus détectée après 4 h d'incubation. D'autres composés non identifiés sont synthétisés à ce stade ce qui suppose que la 17,20β-P est rapidement métabolisée. Chez les femelles qui ont pondu, la teneur en 17,20β-P dans le plasma est élevée ; par conséquent, ce stéroïde pourrait être impliqué dans le processus de l'oviposition. Ces résultats confirment le rôle de la 17,20β-P dans les phases finales du développement ovocytaire.

Keywords: Ovarian steroidogenesis; Maturation-inducing steroid; Eurasian perch

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1. Introduction

Reproduction of Eurasian perch, *Perca fluviatilis*, has received little attention contrary to other percids such as yellow perch, *Perca flavescens*, (Dabrowski et al., 1996) and walleye *Stizostedion vitreum* (Malison et al., 1994; Malison and Held, 1996). Limited data exists concerning the endocrine control of the reproductive cycle of this species, especially during the final maturation and ovulation stages. There is only limited data relative to body composition variations (Craig, 1977), sexual maturation (Treasurer and Holliday, 1981), variation of the gonadosomatic index (Le Cren, 1951; Jamet and Desmolles, 1994), global seasonal changes of plasma sex steroids in the natural habitat (Sulistyo et al., 1998, 2000), and out-of-season conditions (Migaud et al., 2002).

As for the pre-ovulatory changes in teleosts with group synchronous ovarian development, the plasma estradiol-17β (E_2) and testosterone (T) levels, high during vitellogenesis, decrease when the maturation-inducing steroid (MIS), such as 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P), increases just after the gonadotropin surge (Fostier et al., 1978, 1981; Breton et al., 1983; Kime, 1993; Mylonas et al., 1998). Besides, a T peak was sometimes reported during the oocyte maturation period (Kime, 1993; Borg, 1994; Mylonas et al., 1998). The E_2 concentration is low at hydratation and ovulation stages among several perciforms such as white perch, *Morone americana*, (King et al., 1995), white bass, *Morone chrysops* (Berlinsky et al., 1995; Mylonas et al., 1998), striped bass, *Morone saxatilis* (Berlinsky and Specker, 1991; Mylonas et al., 1997a) and sea bass, *Dicentrarchus labrax* (Prat et al., 1990; Asturiano et al., 2000). As far as Eurasian perch is concerned, Sulistyo et al. (1998) showed that plasma E_2 and T increased prior to spawning whereas the plasma 17,20β-P profile did not display any significant peak with levels ranging from 0.2 to 0.5 ng ml^{-1}. These results suggest that either another MIS in Eurasian perch could be involved, or the peaks would be so pulsatory that they would have not been detected during sampling.

Two types of steroids have been identified in different species as MISs: 17,20β-P in several species, such as in rainbow trout, *Oncorhynchus mykiss* (Fostier et al., 1973; Jalabert et al., 1977; Fostier and Jalabert, 1986), amago salmon, *Oncorhynchus rhodurus* (Patino and Thomas, 1990), yellow perch (Goetz and Bergman, 1978; Goetz and Theofan, 1979), plaice, *Pleuronectes platessa* (Scott and Canario, 1992) and tilapia, *Oreochromis mossambicus* (Rocha and Ries-Henriques, 1998), and 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S) in species such as spotted seatrout, *Cynoscion nebulosus* (Thomas and Trant, 1989; Pinter and Thomas, 1999), white bass (King et al., 1995), turbot, *Psetta maxima* (Mugnier et al., 1997) and Atlantic croaker, *Micropogonias undulates* (Trant et al., 1986; Patino and Thomas, 1990). Several in vitro experiments using ovarian follicles from various teleost species showed that these two steroids are effective inductors of final oocyte maturation (Jalabert, 1976; Goetz and Bergman, 1978; Scott and Canario, 1987; Pinter and Thomas, 1999). Studies on moronid fish, such as *M. americana* (King et al., 1995) or *M. chrysops* (Mylonas et al., 1997b), showed that the plasma levels of both 17,20β-P and 20β-S increased during the final maturational stages, suggesting a role for both steroids during stage 4 (peripheral germinal vesicle, GV), ovulation and spawning, whereas plasma E_2 and T levels decreased. The same phenomenon was also observed in in vitro experiments with ovarian fragments. Furthermore, studies on other teleost species such as black sea bass, *Centropristis striata*, suggested that these two MISs (17,20β-P and 20β-S) could not be involved in the final maturational process (Cerda et al., 1996).

In Eurasian perch, sex steroid variations during the pre-ovulatory period and the hormonal control of final oocyte maturation, ovulation and spawning are little documented. The MIS is still unknown in this species but is likely to be 17,20β-P, as it has been already shown in the yellow perch (same genus). This study focused on the hormonal control of the last stages of the reproductive cycle in female Eurasian perch and was performed (1) so as to clarify the androgen and estrogen (T and E_2) variations during the pre-ovulatory period and (2) to examine the ovarian potential synthesis of 17,20β-P and 20β-S during the pre-ovulatory stages.

2. Materials and methods

2.1. Fish

Fish (100 kg of fish in total) were harvested from the Lindre pond (surface area 62×10^5 m^2, volume 12×10^6 m^3, altitude 210 m, latitude 48°49'N, longitude 6°43'E), in Moselle, France, and were maintained in an external outside concrete pond. Female perch were collected at 10:00 h three times a week at 2-day intervals, from early March to late April 1999. Four to seven females were caught at each sampling date (154 females sampled in total), and only maturing females, recognized by swollen abdomen and genital papilla, were considered. Further sampling was carried out between March and April 2000 to investigate the in vitro synthesis of 17,20β-P and 20β-S by ovaries. Six females were collected at the Lindre center at three different dates (two at each date, 18 February, 14 and 23 March) for an in vitro study. First spawning was observed on 10 April.

2.2. Plasma sampling

Immediately after capture, fish were anaesthetized in a 2-phenoxyethanol solution (1 ml l^{-1}, SIGMA), then individually weighed (body weight BW ± 0.1 g), measured for standard length (SL ± 1 mm), blood sampled and dissected. Fish averaged 175 ± 72 g (n = 5, from 77 to 392 g) in weight and 223 ± 26 mm in length (mean ± S.D.). A 1 ml-blood sample was taken from the heart using a heparinized syringe. Blood was then centrifuged at 4000 rpm (3400 × g) for 25 min and

plasma stored in vials at –25 °C until assay. The gonads, liver and viscera were entirely removed and weighed (± 0.01 g).

For the in vitro study, the gonads were removed and approximately 1 g of each ovary, taken from the middle, was placed in 20 ml glass flasks containing 5 ml of an incubation medium, L15 medium Leibovitz (with L-glutamine, SIGMA).

The gonadosomatic index, I_G (%) was calculated as follows: $I_G = 100 \times (GW/BW)$, where GW is weight of ovary and BW is body weight in gram (g).

2.3. Ovarian histology

A fragment from the ovary of each female was removed and fixed in Bouin Holland's solution and embedded in Paraplast for histological examination. The tissues were subsequently cut to a 5 µm thickness, and stained with a trichrome method according to Rinchard et al. (1996): Regaud's haematoxyline at 57 °C, phloxine and light green. The development stages of the oocyte were determined on a single ovarian section per fish, according to Malservisi and Magnin (1968) and Treasurer and Holliday (1981). Oocytes percentage at each stage were not determined, as oocyte development in perch is synchronous. However, two different oocyte stages can coexist at some stage and a female was, therefore, assigned to a category depending on the most abundant stage observed. Seven different oocyte stages were observed as follows:

- Stage 1: ovary containing previtellogenic oocytes.
- Stage 2: ovary with vitellogenic oocytes characterized by the yolk globules, and peripheral yolk vesicles. The nucleus is in a central position.
- Stage 3: yolk globules form a homogenous mass with some lipid droplets inside the cytoplasm. The nucleus, i.e. GV starts to migrate from its central position to the periphery (pre-migrating GV, Fig. 1a).
- Stage 4: GV completes its migration towards the periphery (animal pole) and a large lipid droplet (or sometimes several) is located at the vegetative pole (Fig. 1b).
- Stage 5: GV breaks down (Fig. 1c).
- Stage 6: ovulation stage characterized by vitellus and a large lipid droplet (Fig. 1d).
- Stage 7: oviposition, the emptied ovary contains primary oocytes.

2.4. Radioimmunoassays

Plasma concentrations of T, E_2 and 17,20β-P were determined using radioimmunoassay (RIA) analysis according to Fostier and Jalabert (1986), following two extractions with cyclohexane/ethylacetate (v/v). The extractions were performed on 50 µl of plasma for each steroid. Samples were assayed in duplicate and the steroid standards in triplicate. Fostier and Jalabert (1986) described the cross reactivity of antiserum with a variety of common steroids. T, E_2 and 17,20β-P intra-assay coefficients of variation were 5.9, 7.5 and 9.6% (n = 14), respectively. All samples were analyzed in a single RIA for each steroid. The detection limits were 7.5 pg ml^{-1} for T, E_2 and 17,20β-P.

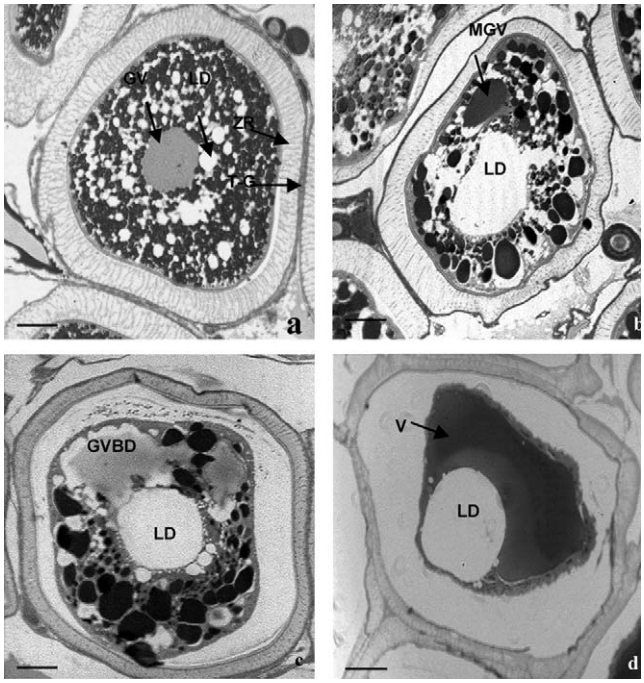

Fig. 1. Ova development stages in female Eurasian perch *P. fluviatilis* during the pre-ovulatory period (March–April 1999). **a**: specimen sampled on 7 April (×100): stage 3; **b**: 14 April (×100): stage 4; **c**: 21 April (×100): stage 5; **d**: 23 April (×100): stage 6. Scale = 100 µm. GV: germinal vesicle; LD: lipid droplet; T-G: thecal and granulosal walls; ZR: zona radiata (chorion with micro-villosity); mGV: migrated germinal vesicle; GVBD: germinal vesicle breakdown; V: vitellus.

2.5. In vitro steroid production

Incubations (5 ml) were initiated by addition of 370 kBq of [1,2,6,7-^{3}H]17-hydroxy-4-pregnen-3-one as the tritiated precursor (17-P-^{3}H Amersham Pharmacia Biotech, s.a. = 2.25 TBq mmol^{-1}). After 30 min, 2 and 4 h of incubation at 9–11 °C, the metabolism of the tritiated precursor was stopped by adding 40 µg of the non-labeled precursor in 100 µl of ethanol. The medium and the tissue were immediately frozen and stored at –20 °C until assay.

The steroids were extracted from the media three times with 4 ml of cyclohexane; 20 µg of standard steroids, 17,20β-P and 17,20β,21-P were added to the organic phase. The organic phases removed from the extractions were vacuum dried and the extracts dissolved in 200 µl of ethanol. Ethanol extracts were chromatographed by thin layer chromatography (TLC) using Merck 620 F_{254} 20-cm silica plates (Merck ref. 10583) with concentration zones. Plates were developed twice in a saturated atmosphere of solvent, benzene:acetone (80:20, v/v) at room temperature (20–22 °C). Standard steroids were visualized with the help of a UV lamp (λ = 254 nm) and radioactive peaks were detected with a mono-dimensional gas ionization scanner (Packard 7222).

The migration zones corresponding to the two reference steroids, 17, 20β-P and 17,20β,21-P, were eluted twice from

the bands of the silica plates with 5 ml of dichloromethane:methanol (90:10). Following centrifugation 1000 × *g* for 10 min, the supernatants were pooled and vacuum dried before finally being dissolved in 200 µl of ethanol. Extracts were then analyzed through reverse-phase HPLC (High Performance Liquid Chromatography, Constametric 3000, LDC) using a 5 µC18 Nucleosil 100 column at a flow rate of 1 ml min^{-1}. A fraction of each sample was diverted to a radiochemical detector. The resulting signals (spectrophotometric and radiochemical) were recorded and analyzed with the help of a computer (Flo-one program, Packard).

2.6. Statistical analysis

Statistical analyses were performed using the mixed procedure with repeated time, as data between sampling points were related (SAS software, Littell et al., 1996), for unbalanced analysis of variance (ANOVA) without any treatment effects. The individual 17,20β-P levels at various oocyte stages were analyzed using an unbalanced one-way ANOVA (SAS, Mixed procedure). The minimum level of significance was set at $P \leq 0.05$ and the coefficient of variation of the root mean square error (CV RMSE) is indicated for each graph. All quantitative data are expressed as Lsmeans (adjusted means) ± standard error (S.E.) and were compared to the adjusted Bonferroni test. NS and S stand, respectively, for not significant and significant.

3. Results

3.1. Ovarian development

In 1999, the I_G rose gradually from March until the spawning period in late April, when it reached its maximum of 23.8 ± 1.2% (mean ± S.E., Fig. 2). The sudden decrease observed on 26 April corresponded to the beginning of the spawning period. The temperature increased progressively from 5.9 to 12.5 °C on 26 April, when the perch started to spawn. In 2000, the I_G averaged 16.9 ± 0.5, 17.8 ± 0.4 and 21.8 ± 0.4% (mean ± S.E.) for the three sampling dates (in vitro incubation), respectively.

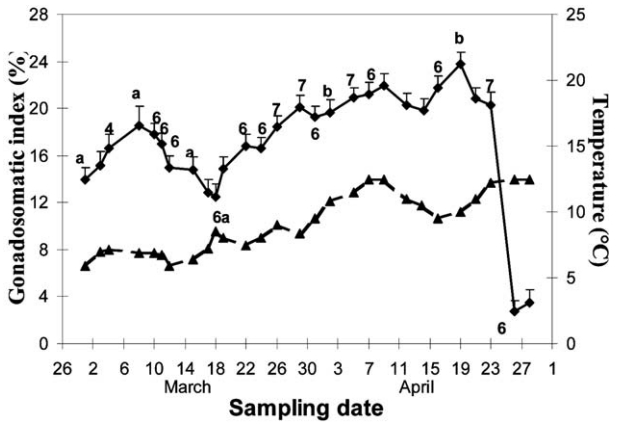

Fig. 2. Water temperature (▲, dashed line) in Lindre pond and gonadosomatic index (I_G, ◆, mean + S.E., n = 5) in *P. fluviatilis* during the pre-ovulatory period. Numbers indicate sample size (when different to 5) and the different letter indicate mean values significantly different ($P < 0.05$, only the main differences were indicated). CV RMSE = 14%.

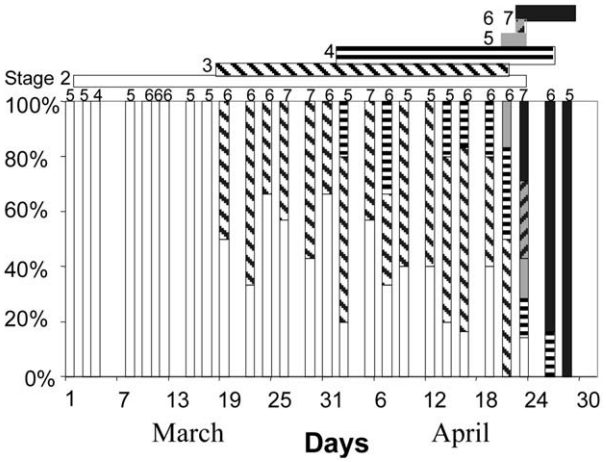

Fig. 3. Oogenesis stages observed in *P. fluviatilis* during the pre-ovulatory period. Numbers above each element indicate sample size. Stage 2: ovaries with vitellogenic oocytes; stage 3: pre-migrating GV; stage 4: oocytes with peripheral GV; stage 5: GVBD; stage 6: ovulation; stage 7: spawning.

The immature ovaries sampled with previtellogenic oocytes were not considered in this study. In March, all the sampled ovaries contained only vitellogenic oocytes. The first oocytes in stage 3 (Fig. 1a) were observed from 19 March (Fig. 3). Then, the first oocytes with a peripheral GV (Fig. 1b) were sampled on 2 April (Fig. 2) and among the four females sampled, three of them presented migrated GV. From 2 to 18 April, broodstock at stages 2–4 were observed with a higher proportion of fish at the late vitellogenesis stage (stage 3: pre-migrating GV), which represent 33–70% of the stages observed (Fig. 3). The germinal vesicle break down (GVBD) stage (Figs. 1c and 3) was observed for the first time in ovaries sampled on 21 April and the ovulation stage on 23 April (Figs. 1d and 3). However, the occurrence of these stages was very low among the different samples. The first oviposition (stage 7) was recorded on 23 April (Fig. 3).

3.2. Plasma steroid levels

3.2.1. Estradiol

The plasma E_2 levels began to rise in mid-March (Fig. 4a) and one significant peak was detected throughout the sampling period in females sampled on 19 April (3.6 ± 0.6 ng ml^{-1}, Lsmean, $P < 0.05$); another peak appeared on 19 March (3.3 ± 0.6 ng ml^{-1}) but not significant. The mean plasma levels of E_2 at various stages of development (Fig. 5a) ranged from detection to 1.2 ± 0.2 ng ml^{-1}, but no significant differences between the several stages were observed because of some very high variation and different sample sizes. Nevertheless, the plasma levels as well as the intra-stage variability seemed to decrease the more the oocytes were at an advanced stage, but the size of the samples decreased. The highest

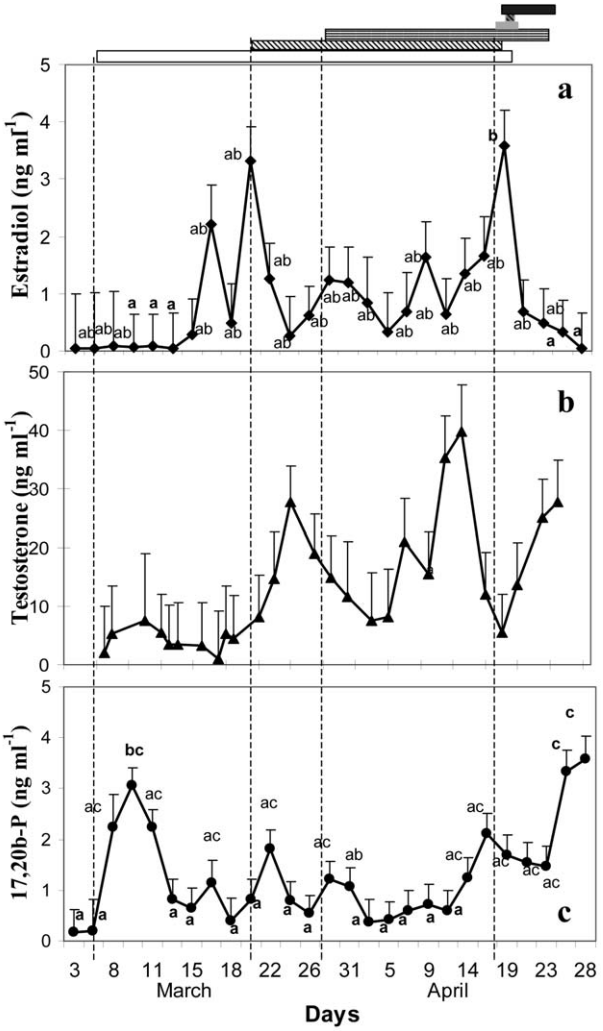

Fig. 4. Plasma levels (mean + S.E.) of estradiol-17β (a), testosterone (b) and 17,20β-P (c) in *P. fluviatilis* during the pre-ovulatory period. CV RMSE = 155%, 40% and 69%, respectively, for E_2, T and 17,20β-P.

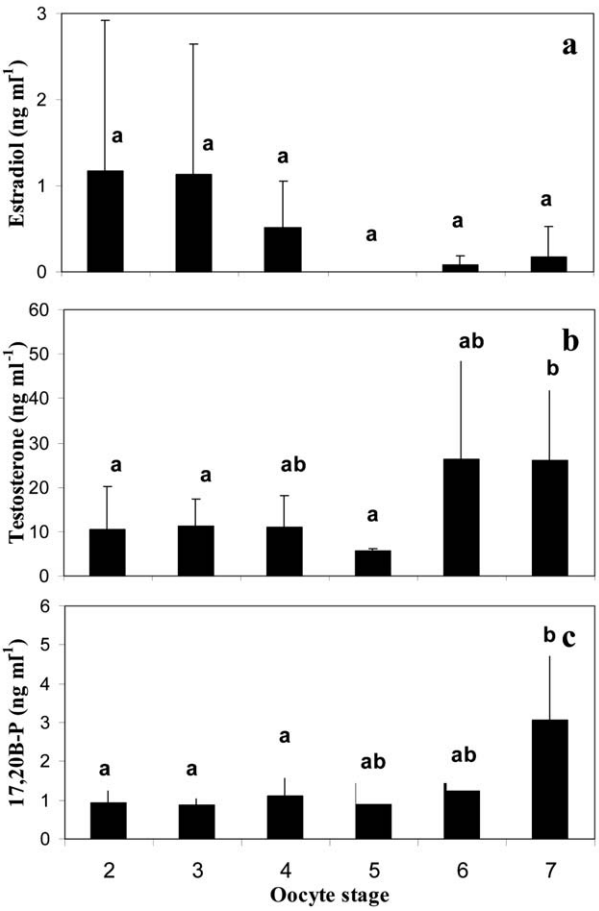

Fig. 5. Plasma levels (mean + S.E.) of estradiol-17β (a), testosterone (b) and 17,20β-P (c) in *P. fluviatilis* during the pre-ovulatory period at various stages of development. Stage 2 (n = 43), vitellogenic oocytes; stage 3 (n = 22), pre-migrating GV; stage 4 (n = 5), oocytes with peripheral GV; stage 5 (n = 2), GVBD; stage 6 (n = 2), ovulation; stage 7 (n = 13), spawning. The different letter indicates mean values significantly different ($P < 0.05$). CV RMSE = 291%, 70% and 77%, respectively, for E_2, T and 17,20β-P.

individual values were observed in fish with oocytes in stage 2 or 3 (1.2 ± 0.2 and 1.1 ± 0.3 ng ml^{-1}, respectively).

3.2.2. Testosterone

Plasma T levels (Fig. 4b) were observed to follow the same pattern as E_2, although no statistically significant variations were shown, with low values up to 22 March, ranging from 2.8 to 7.8 ng ml^{-1}. Levels appear to be higher in late March (27.7 ± 6.1 ng ml^{-1}) and mid April (39.4 ± 8.1 ng ml^{-1}); the first "peak" was slightly delayed and the second "peak" advanced, compared to those observed for E_2. However, high variability between females sampled at the same period concealed the observation of significant differences. Fig. 5b shows the mean plasma concentrations for each stage of oocyte development. Mean plasma T levels display large variations between the different stages observed. The concentrations are similar at stages 2–4 (12.9 ± 2.6, 13.8 ± 2.9 and 11.0 ± 5.0 ng ml^{-1}, respectively). The T level is significantly higher at stage 7 (24.2 ± 8.0 ng ml^{-1}) than at stages 2, 3 and 5. Levels measured at stage 6 are similar to those observed at stage 7, but not significantly different when compared to previous stages.

3.2.3. 17,20β-P

As far as 17,20β-P is concerned, the mean plasma levels (Fig. 4c) displayed two significant peaks. The first peak occurred in late winter with values of 3.1 ± 0.4 ng ml^{-1} on 10 March. From 12 March to 2 April, plasma concentrations fluctuated slightly and dropped to a basic level of approximately 0.6 ng ml^{-1}. Just before the spawning period, the 17,20β-P levels increased significantly from 12 April to 27 and 28 April, and reached mean values of 3.4 ± 0.4 and 3.6 ± 0.5 ng ml^{-1}, respectively. These levels are significantly different from those measured from 10 March to 12 April, except for 22 March. The mean plasma levels of 17,20β-P at various stages of development (Fig. 5c) ranged from 0.9 ± 0.2 (stage 3) to 3.0 ± 0.2 ng ml^{-1} (stage 7). Plasma levels observed at stage 7 are significantly higher than those measured at stages 2–4, although individual values are very

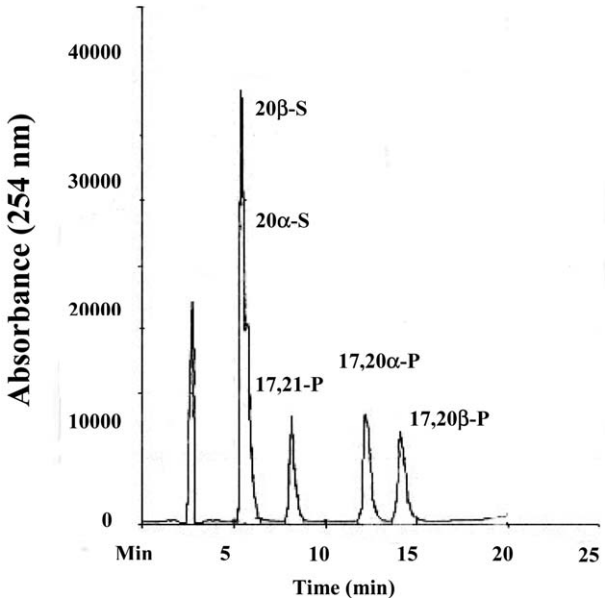

Fig. 6. HPLC chromatograms of standards steroids separated on a C-18 column and eluted with water:acetonitrile:methanol at a flow rate of 0.5 ml min^{-1}. The retention times are as follow: 5.5 min (17,20α,21-P and 17,20β), 8.0 min (17,21-P), 12.5 min (17,20α-P) and 14.5 min (17,20β-P).

variable, ranging from 1 to almost 7 ng ml^{-1}. Concentrations at stages 5 and 6 are similar (1.4 ng ml^{-1}, NS, $P > 0.05$).

3.3. In vitro steroid production

First spawning occurred on 10 April. Broodstock collected at the first sampling date (18 February) were at stage 2 whereas those collected at the two following sampling dates (14 and 23 March) were at stages 3 and 4, respectively. The HPLC chromatograms showed that among the peaks identified corresponding to the five references, 17,20β-P, only corresponded to a radioactive peak (Figs. 6 and 7). Other radioactive peaks were observed in both TLC migration zones analyzed, but were not investigated any further. The chromatograms concern the first female sampled at the last sampling point (end of March), whose gonad fragments were incubated for 2 h (Fig. 7). The same patterns were observed for the other sampling dates and the second female sampled. The three different incubation periods tested showed that 17,20β-P is produced after 30 min and is still present in the medium following 2 h of incubation, but is no longer visible after 4 h. However, no radioactive peak corresponding to the 20β-S reference was observed in the chromatograms (Fig. 7a) regardless of sampling date or incubation time. No significant variations were observed between the amounts of 17,20β-P produced at the three different sampling dates. Regardless of sample analyzed, the different amounts of radioactivity found in the media were low compared to the activity of the added.

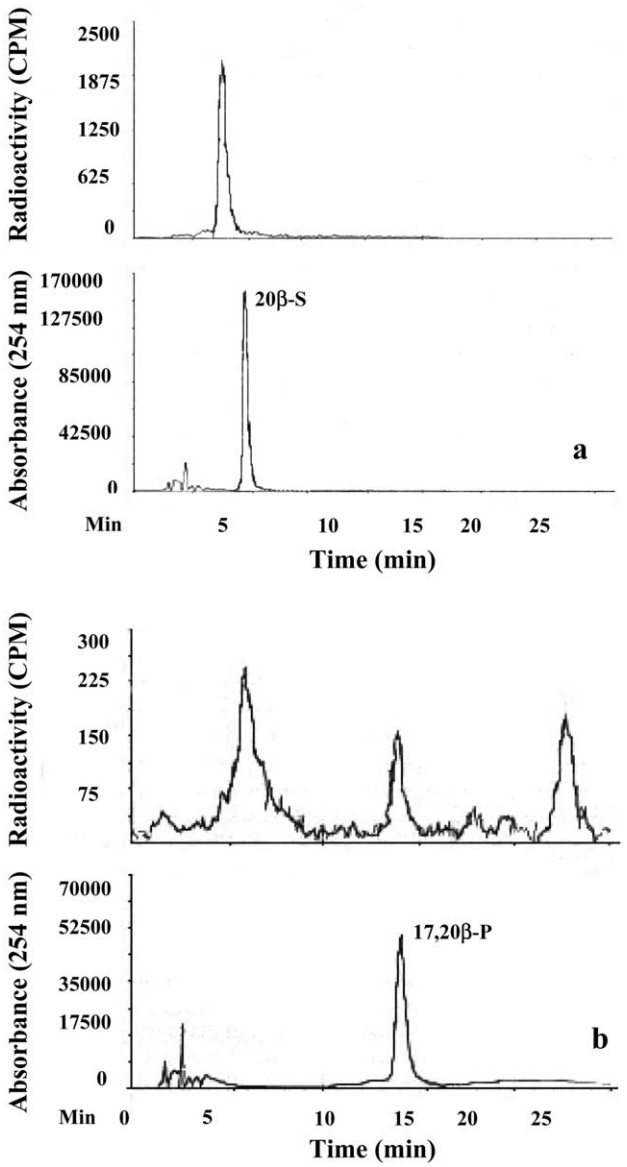

Fig. 7. HPLC radio-chromatogram of steroid metabolites synthesized by perch ovarian tissues from a fish sampled at the end of March incubated for 2 h with [^{3}H]-17-P. Extract of incubate has been subjected to TLC and the zones corresponding to 20β-S (a) and 17,20β-P (b), respectively, have been eluted for HPLC.

4. Discussion

According to Marza (1938), oogenesis in Eurasian perch is synchronous. However, in the current study, ovarian development is not synchronous between the different broodstock. This could explain the high variability observed in plasma steroid concentrations. Even though hormonal studies on animals are usually characterized by large variability, variability observed in this study was higher than that previously reported by Sulistyo et al. (1998). This is probably due to the difference of temperature profile between these two studies. Indeed, a cooling period occurred in April 1999 (<10 °C)

during the normal onset of the spawning season, possibly delaying ovarian development up to ovulation. Moreover, the individual sensitivity of the broodstock to environmental changes might be different. With respect to 17,20β-P, the basic levels measured in this study were similar to those previously reported by Sulistyo et al. (1998), averaging 0.3–0.5 ng ml^{-1}. However, for the first time, some significant peaks (7 ng ml^{-1}) were assayed with individual plasma levels and averages of 3 ng ml^{-1}. These levels are similar to those observed among white bass at the GVBD stage (King et al., 1995; Mylonas et al., 1997b) and slightly higher than among striped bass (Berlinsky and Specker, 1991). The sampling protocol at 2-day intervals enabled the detection of 17,20β-P peaks. The first significant increase occurred on 10 March while the histological observations had not shown any oocyte in the final maturational stage or with migrated GV, but oocytes still at late vitellogenesis stage. The first pre-maturational oocytes corresponding to the appearance of larger lipid droplets and the beginning of the GV migration occurred on 1 April, i.e. 21 days after the first 17,20β-P peak. No direct significant relation could be shown between the plasma steroid levels and the oocyte stage of development, with exception to the last stage, which showed significantly higher levels than stages 2 and 3. Thus, there would seem to be some delay between the plasma 17,20β-P increases and the effects of this steroid on the maturation of oocytes. Some high plasma 17,20β-P contents were measured over 21 days with some high variability among the fish. This period of 21 days may correspond to the duration of the spawning period, although the 21-day delay between the first high plasma 17,20β-P levels and the histological changes (first migrating oocytes) seem to be long to be able to reach any conclusion about the role of 17,20β-P in the final oocyte maturation. Considering the fact that the first females with oocytes at stage 4 (peripheral GV stage) might not have been sampled, our results suggest that 17,20β-P could be the MIS in Eurasian perch. As it was suggested by Haddy and Pankhurst (1999), stress exerts an inhibitory effect on gonadal steroidogenesis in fish and was shown to increase 17,20β-P levels in blood. Confinement and sampling could have stressed the fish resulting in higher plasma 17,20β-P levels at the beginning of the experiment.

In *Morone* species such as white bass or white perch, the MIS surge occurs few hours prior to the GVBD stage and the sex steroids are being quickly eliminated (King et al., 1995). In the present study, the in vitro experiment also confirmed this hypothesis, since the perch ovaries metabolize 17-P as a precursor into a small amount of 17,20β-P, and not into 20β-S, during the pre-ovulatory period. Barry et al. (1995) observed the same pattern among walleye. As far as yellow perch is concerned, plasma 17,20β-P levels are higher in the oocytes at the GVBD stage than at the migrating GV stage (Barry et al., 1995). But 17,20β-P levels are lower during the spawning season in percid fish compared to some salmonid fish species. In walleye, for example, higher 17,20β-P levels are related to stage 4 (peripheral GV), but the peaks observed are 10–100 times lower than those measured in salmonid and cyprinid fish (Scott and Canario, 1987; Malison et al., 1994). Also, in percid fish, according to the results of in vitro experiments among walleye (Barry at al., 1995), the 17,20β-P requirements to induce GVBD might be low. Thus, the low levels observed at stage 5, corresponding to the GVBD stage, could be sufficient to trigger final oocyte maturation. Furthermore, as it was suggested in walleye (Scott and Canario, 1987), the 17,20β-P might be quickly converted in non-immuno-detectable compounds such as 17,20-P-sulphate or 17,20-P-glucuronate. Also, the three different incubation periods tested showed that 17,20β-P is produced after only half-an-hour and is still present in the medium following 2 h of incubation, but no longer detectable after 4 h. Variations of the plasma levels may also be observed depending on the time of day at which the blood-samples were taken. A preliminary study suggested that plasma 17,20β-P levels are likely to be higher in the early morning than in the rest of the day (Migaud, unpublished data). Finally, the hypothesis would suggest that another steroid could be involved in final oocyte maturation. As far as 20β-S is concerned, this in vitro study showed that its synthesis was not detectable at the three different durations of incubation tested, as it was observed among walleye (Barry et al., 1995). The plasma 17,20β-P concentrations strongly increase during the 2 weeks prior to the beginning of the spawning period. These levels are significantly higher than those measured in early April. Moreover, the levels at oviposition are significantly higher than those among stages 2–4, which would suggest another role for this steroid. Firstly, it might also be involved in spawning release and synchronization and thus may act as a pheromone, the same way in which it was demonstrated in goldfish. Secondly, the 17,20β-P might also be involved in ovulation through prostaglandins (Goetz et al., 1989). In fact, the first ovulation stages were observed on 23 April while the plasma 17,20β-P levels increase from 12 April.

The plasma E_2 profile observed in this study during the pre-ovulatory period, confirmed the results from Sulistyo et al. (1998). In fact, the very low plasma levels of E_2 (less than 80 pg ml^{-1}) and T (less than 5 ng ml^{-1}) during the first half of March, may be related to the end of the vitellogenic stage. Nevertheless, the comparison between plasma levels at the different stages did not show any significant differences for both steroids. Different peaks occurred over a 1-month period for both steroids, the same as for the 17,20β-P profile. This 1-month period corresponded approximately to the spawning period. Moreover, it was shown that T stimulates the effects of luteinizing hormone (LH) on 17,20β-P synthesis in trout ovarian follicles (Jalabert and Fostier, 1984). T could play a role in the protection or the preservation of viability of the oocytes when vitellogenesis is completed, and before stage 4 (peripheral GV) (Kime, 1993).

In conclusion, these results strongly suggest a role for 17,20β-P in the final stages of ovarian development in Eurasian perch. However, the highest plasma levels were de-

tected before any sign of oocyte maturation and following spawning. In our opinion, either the increase of 17,20β-P in blood would not be synchronized with the peripheral GV or GVBD stages, or that low levels could trigger final maturation and ovulation. Therefore, it would be interesting to study 17,20β-P changes inside the ovary. Besides, the highest plasma levels found at the oviposition suggest a pheromonal role. Moreover, the in vitro study showed that ovaries do not produce any 20β-S before the maturation stages, but 17,20β-P. Unfortunately, we were not able to directly measure 20β-S in perch blood as no label and antiserum were available. The peak levels of E_2 and T were confirmed in spite of a strong variability, probably in relation to an unusual temperature profile.

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