Reproductive cycle and plasma levels of sex steroids in female Eurasian perch *Perca fluviatilis*

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Abstract - From April 1995 to April 1996, the annual reproductive cycle of the Eurasian perch *Perca fluviatilis* was studied at the Fishfarming Lindre Center (Moselle, France). At monthly intervals (or at intervals of 10 days during the periovulatory period), 5 females were caught and dissected. From sampled organs, the gonado-, hepato- and viscerosomatic indexes (GSI, HSI, VSI) were calculated, oocyte diameters (OD) and the plasma levels of testosterone (T), 17β-estradiol (E2), 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P) and protein-phosphorus (PPP) were measured. After the sexual resting period observed from May to August (GSI < 1 %, OD < 200 µm, VSI = 4-6 %), oogenesis began in September when the water temperature decreased from 26.4 to 14.1 °C. The GSI increased progressively until mid March (15 %), then rapidly until spawning (25 %, OD = 850 µm) which occurred in April (14-15 °C). The plasma levels of T, E2, 17,20β-P and PPP were low during the sexual resting period. E2 and PPP levels increased significantly at the onset of the oogenesis in September, then the E2 level raised abruptly in November (3-4 ng . mL-1). In December, the T level increased rapidly to 15-20 ng . mL-1. The testosterone, E2 and PPP levels remained very high until spawning, indicating the existence of active vitellogenesis. The highest HSI (2.1-2.2 %) recorded in winter confirmed this. During the periovulatory period, a peak of E2 (4 ng . mL-1) appeared, whereas T level diminished. In this study, 17,20β-P levels remained low (0.2-0.6 ng . mL-1) and relatively constant. No 17,20β-P peak was observed during the periovulatory period. Sampling at 10 day intervals was probably inadequate to specify the hormonal variations related to the final oocyte maturation and the ovulation. © Ifremer-Elsevier, Paris.

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1. INTRODUCTION

Eurasian perch \textit{Perca fluviatilis} is a likely candidate species for development and diversification of European freshwater aquaculture [11] and has generated increased interest during recent years. Among the different aptitudes of perch to intensive culture, several advantages can be highlighted. Animal husbandry aspects of \textit{P. fluviatilis} larvae and post-larvae have recently been reviewed Kestemont et al. [21], indicating that fish can be weaned and accept an artificial dry diet at an early life stage [22]. Moreover, survival and growth performances in various culture systems (flow through or recirculating tank culture, floating cages) are suitable for intensive fish farming [10, 43], fish tolerating high stocking densities [34] in spite of a marked tendency to growth heterogeneity and sibling cannibalism in larval and juvenile stages [32, 33]. Likewise the dietary protein requirements of Eurasian perch have been specified recently Fiogbé et al. [8].

Many aspects of \textit{P. fluviatilis} reproductive biology have been studied e.g. maturation, fecundity and egg production [1, 5, 14, 17, 19, 36], seasonal cycle and histology of gametes [26, 45] and spawning behaviour [14, 20, 44]. Nevertheless, the hormonal aspects have received little attention, compared to the yellow perch \textit{Perca flavescens} [4, 6, 7, 15, 28]. In preliminary experiments, Tamazouzt et al. [42] obtained maturing females with a gonadosomatic index (GSI) of 10.8 ± 1.8 % (mean ± SD) after a 7 month photothermal conditioning. Considering the importance of hormonal mechanisms in the reproductive process, a certain knowledge of them is required to achieve control of reproduction of Eurasian perch via artificial final maturation and spawning induction (e.g. photo-thermal regimes, hormonal injections, etc.).

Therefore, the present study aims to describe the seasonal changes in the condition factors, ovarian development and sexual plasma steroid levels in the Eurasian perch \textit{Perca fluviatilis}, thus completing the information on its reproductive processes.

2. MATERIAL AND METHODS

2.1. Study site and fish capture

Perch were sampled from the Lindre pond, Moselle, France (surface area 620 ha, volume \(12 \times 10^6 \text{ m}^3\), altitude 210 m) from April 1995 to April 1996. Five females were caught at each sampling date, except in August 1995 and September 1995 (only 2 or 3 females). From April 1995 to September 1995, Eurasian perch were sampled using rods and lines and immediately used for experimental purposes. In the first week of October, a perch broodstock (100 kg) was caught using a seine-net and then stocked together with excess forage fish (5-6 cm cyprinids) in a concrete raceway tank (30 m\(^3\)) supplied with running water from the Lindre pond. From October (Oct. 24\(^{th}\)) until the end of the study, perch were taken from this broodstock with a landing-net and immediately used for experimental purposes. From April 1995 to February 1996, perch were sampled monthly and in order to specify their biometrical and physiological changes prior to spawning, 10 day interval samplings were conducted between March and April 1996.

Seasonal variations in the physico-chemical parameters of the pond have been described by Tamazouzt et al. [43]. During the present study, water temperature varied between 0.8 and 26.4 °C (figure 1).

2.2. Fish, organ and plasma samples

Mature females were selected by size (19-24 cm total length TL, 100-200 g body weight) to avoid interferences with size or age of fish. Considering that in Moselle, the sexual maturity of female perch occurs during the second year and that a 19-24 cm size (TL) corresponds to 3-4 year old fish [9], it is highly probable that the females sampled in this study were in their second or third reproductive cycle. Organ samplings were always made early in the morning (8:00-9:00 am). Immediately after samplings, fish were anaesthetized with a 2-phenoxyethanol solution (0.3 mL - L\(^{-1}\)), weighed (body weight BW ± 0.1 g), measured for standard length (SL ± 0.1 cm) and dissected. A 1 mL blood sample was withdrawn from heart using heparinized syringe and the plasma was separated (4 000 rpm, 25 min) and stored at -25 °C until assayed. Gonads, liver and viscera including mesenteric fat were removed entirely and weighed (± 0.01 g) in situ. The gonadosomatic (GSI), hepatosomatic (HSI) and viscero-somatic (VSI) indexes were calculated as GSI (%) = \((GW \times 100)/BW\), HSI (%) = \((LW \times 100)/BW\) and VSI (%) = \((VW \times 100)/BW\), where GW = weight of the ovary (g), LW = weight of the liver (g) and VW = weight of the viscera (g). To determine the respective importance of somatic and gonad development in fish conditions, condition factors were calculated with \((K_1)\) and without \((K_2)\) gonad weight, as follows \(K_1 = 100 \times BW/SL^3\) and \(K_2 = 100 \times (BW-GW)/SL^3\), where SL = standard length (cm) [19, 26].

2.3. Histological study

From each female, a fragment of ovary was removed and fixed in Bouin’s Holland solution and embedded in paraplast for histological examination. The tissues were subsequently cut at a 6 \(\mu\)m thickness and stained with a trichrome: hemaluin, phloxine and light green [25]. For the oocyte development (histological examination, \textit{table I}), the nomenclature of Rinchard and Kestemont [39] was used. The diameter of twenty oocytes which were in the most advanced stage from each ovary, was measured (± 1.0 \(\mu\)) using D.I.G software. Only oocytes which were cut through the nucleus were measured.
2.4. Steroid and plasma protein phosphorus

Plasma concentrations of testosterone (T), 17β-estradiol (E2) and 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P) were measured by radioimmunoassay (RIA) analysis after two extractions with cyclohexane/ethyl acetate according to Fostier and Jalabert [12]. Cross reactivities of antisera with a variety of common steroids have been previously described Prat et al. [37]. Six standards of different hormone concentrations were run in each assay and were used as quality controls for estimating intra- and interassay coefficients of variation (CV). For the three steroids, the sensitivity was 5 pg·mL⁻¹. For T, E2 and 17,20β-P, the interassay CVs (n = 2) were 11.5, 9.6 and 8.5 %, respectively. The intra-assay CVs (n = 2) were 9.9, 2.0 and 7.5 %, respectively.

Plasma vitellogenin levels were indirectly determined by measuring colorimetrically, in 100 μL of plasma, protein-phosphorus contents (PPP) according to the technique of Martin and Doty [31] modified by Wallace and Jared [46].

2.5. Statistical analysis

Statistical analysis of these data were performed using the SAS system with general linear model (GLM) procedures for unbalanced one-way analysis of variance (ANOVA) (SAS-Stat User's Guide, vol. 2, version 6, 4th edn.). All quantitative data are expressed as means ± SD (standard deviation). The experimental units were fish sampled on different dates. This date effect was considered as a simple fixed effect. The mean differences between data were analyzed using Student-Newman-Keuls (SNK) test. Before statistical analysis, values of E2 and T were ln-transformed due to the abnormality of distribution. Likewise some data were deleted due to doubtful residues.

3. RESULTS

For the years 1995 and 1996, perch spawned during the two last weeks of April, when the water temperature increased from 10-11 °C to 14-15 °C and photoperiod had increased to 14 h day length (figure 1).

3.1. Gonado-, hepato- and viscerosomatic indexes

Just before spawning, perch showed the highest GSI (P<0.05) which reached 25 % in April (figure 2). On the other hand, lowest GSIs (<1 %) were recorded at the end of spring and summer which corresponds to the post-spawning period (P<0.05). The onset of ovarian development occurred between August and September, indicated by the early accumulation of yolk vesicles and the beginning of endogenous vitellogenesis. This period corresponded to a marked drop in temperature (figure 1). From August up to 18 March, the female GSI increased progressively [GSI (%) = 0.648 + 0.073 x (x: time in days), n = 9, r = 0.99], although between December and February (lowest temperatures – figure 1), GSI grew slowly. At the beginning of spring, the GSI rapidly reached its maximum values coinciding with the rising water temperature and photoperiod (figure 2).

Highest HSIs (P<0.05) were recorded during January and February, reaching 2.1-2.2 % of body weight. From early spring, HSI dropped rapidly and after spawning in 1996 the values did not differ significantly from those of the previous year.

After spawning, VSI increased to its maximum values (4.6 %, P<0.05). It generally diminished from September to April (spawning period), although a transitory increase was recorded between January and March. Condition factors K1 showed minimum levels (2.0-2.3) during the post-spawning and quiescence periods. From January to April, the highest K1 values (2.4-2.6) were obtained, while condition factors calculated by excluding gonad weight K2 (1.8 ± 0.2) were lowest during the spawning season. This level
increased significantly during summer. In October, K2 rose to 2.3 ± 0.1 and remained beyond 2.0 until next spawning in April 1996.

3.2. Oocyte diameter (OD) and development

As some shrinkage must have occurred in histological sections, the reported diameters are probably underestimated. As presented in figure 2, at spawning period in April 1995, oocytes measured 847 ± 38 μm. After spawning the remaining oocytes were principally primary oocytes (the ovary contained empty follicles and disorganized ovigerous lamellae). Previtellogenic oocytes with diameters ranging from 102 ± 5 to 142 ± 15 μm (figure 3A) were observed in summer. In September, OD increased significantly to 428 ± 70 μm (P<0.05). After a recovery period during summer, the appearance of cortical alveoli (yolk vesicles, figure 3B) in the cytoplasm was observed. Growth of oocytes can be divided into 2 major periods (figure 2). The first period, from July to December, during which OD grew rapidly to reach 757 ± 7 μm, according to the following linear regression: OD (μm) = -270 + 4.14 x (x: time in days), n = 6, r = 0.99. The second period, lasting from December to spawning, during which OD grew more slowly: OD (μm) = 480 + 0.99 x (x: time in days), n = 9, r = 0.82. In December and March, exogenous vitellogenic oocytes (accumulation of yolk globules: figure 3C, D) were observed. The maximum average diameter of 862 ± 39 μm was recorded on 28 March (figure 2). Figure 3E shows a mature oocyte with migrating germinal vesicle prior to spawning. The diameter of oocytes in the post-spawning period (25 April 1996, figure 3F) was 140 ± 10 μm which did not significantly differ (P<0.05) from those observed from May to July 1995.

3.3. Plasma sex steroid and protein phosphorus levels

Plasma E2 concentration began to rise in September and highly increased to 1.9 ng · mL⁻¹ in November. This level was maintained high until spawning (figure 4A). From September to February, PPP levels varied from 0.5 to 0.8 μg · mL⁻¹, then abruptly rose to 1.8-2.4 μg · mL⁻¹ (figure 4B) at the beginning of March. This level was maintained until spawning after which it dropped. From spawning to November, plasma T levels were less than 0.5 ng · mL⁻¹ and then increased suddenly to 16.3 ± 3.7 ng · mL⁻¹ (figure 5A) from December onwards, when water temperature and day length decreased to 4 °C and 8 h light, respectively. After spawning in 1995, plasma levels of 17,20β-P were relatively constant, varying between 0.20 ± 0.08 and 0.62 ± 0.17 ng · mL⁻¹ (figure 5B). About two weeks before spawning in 1996, plasma

Figure 1. Variations of water temperature (°C) and day length (h) in Lindre pond during the study.
17,20β-P levels doubled and rapidly decreased ten days after spawning.

4. DISCUSSION

The present study complements existing data on morpho-anatomical indexes, condition factors and oocyte growth of Eurasian perch *Perca fluviatilis* [5, 19, 27, 45] and provides new data on biochemical and sexual endocrinological changes of this species.

GSI was comparable to that reported by Treasurer and Holliday [45] on this species in Loch Kinord and Loch Davan (Scotland) and, Jamet and Desmolles [19] in Lake Aydat (France). Ovarian development could be related to the oocyte diameter and maturity stages. From July to December, a rapid increase in oocyte diameter was due to an accumulation of yolk. Treasurer and Holliday [45] found a comparable range of oocyte diameters varying between 200-700 μm. A slower oocyte growth, from winter to early spring, was recorded while yolk accumulations continued resulting in an increase in gonadosomatic index (sharply) and oocyte diameter. The largest oocytes were also observed during this period by Treasurer and Holliday [45]. It must be noted that the oocyte diameters were probably underestimated due to histological treatment.

The histological examination of the oocytes during the present study does not allow the precise prediction of the date of spawning in Lindre pond since oocytes in final maturation stage (germinal vesicle breakdown - GVBD) were not found. From the last sampling carried out on 25 April 1996, the gonadosomatic index sharply diminished and the ovaries only contained germ cells (oogonia and previtellogenic oocytes). The ovaries of one female showed preovulatory atretic stage, with most of the vitellogenic oocytes in regression while ovaries of other females contained many empty follicular sacs and some residual oocytes undergoing resorption, indicating that spawning occurred.

According to Henderson et al. [16] in walleye *Stizostedion vitreum* and Makarova [27] in Eurasian perch, a maximum HSI was observed in February and March, respectively. They suggest that, during this period, the liver was probably increasingly active in the elaboration of constituents deposited in the gonads. In the present study, a similar pattern of dynamic liver weight was observed, a marked depletion of HSI was recorded prior to spawning.
Figure 3. Sequence of oocyte development stages in female Eurasian perch *P. fluviatilis* during an annual reproductive cycle, according to Rinchard and Kestemont's histological terminology (1996). A. Previtellogenic oocytes (stage 1) in fish sampled on July 21, 1995; gv: germinal vesicle; scale bar: 28 µm. B. Endogenous vitellogenic oocytes (stage 2) in fish sampled on September 29, 1995; yv: yolk vesicle; scale bar: 75 µm. C. Early exogenous vitellogenic oocyte (stage 4) in fish sampled on December 22, 1995; yg: yolk globule; scale bar: 102 µm. D. Late exogenous vitellogenic oocyte (stage 4) in fish sampled on March 28, 1996; zr: zona radiata; l: lipid droplets; scale bar: 91 µm. E. Mature oocyte (stage 5) in fish sampled on April 15, 1996; gv_m: migrating germinal vesicle; l: lipid coalescence; scale bar: 82 µm. F. Postovulatory atretic oocyte (at, stage 6) with disorganized theca layer and primary oocytes (po) in fish sampled on April 25, 1996; scale bar: 82 µm.
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The high VSI observed during summer corresponded to an active feeding period. As gonadal recrudescence took place by late summer, viscera lost mesenteric fat resulting in VSI decline until spawning season. Makarova [27] and Kirillov and Akhremenko [24] confirmed that P. fluviatilis accumulates mesenteric fat deposits in July and August and loses them from October until spawning in April.

Variations in condition factors (K₁ and K₂) were similar to those described by Le Cren [26] and Jamet and Desmolles [19]. As suggested by these authors, some sacrifice in general body weight is made towards the build up of the ova indicated by low level of the condition-minus-gonad through winter.

Beside morpho-anatomical changes, fluctuations of sexual steroids occurred in the annual reproductive cycle of yellow perch [6]. Compared with other species (Cyprinids, Salmonids), Eurasian perch showed low levels. All plasma sex steroids and protein phosphorous in the present study showed lower levels during the sexual resting phase. When gonadosomatic index increased in September, changes in plasma E₂ and PPP concentrations were noted. Then, E₂ level sharply rose in November which corresponded to HSI increase. At this period, Eurasian perch shows E₂ levels (3.4 ng·mL⁻¹) similar to those of walleye [30] and higher than the 0.6-0.8 ng·mL⁻¹ recorded in yellow perch [6]. E₂ is synthetized in the granulosa cells of ovarian follicles and acts as an inducer of hepatic vitellogenin synthesis Wallace and Selman [47] and Santos et al. [41]. Vitellogenin (expressed as PPP levels) was delivered from the liver and explained the ovarian growth from December onwards. Over winter, when E₂ synthesis and vitellogenesis intensified, the E₂ levels were stable and a marked rise in PPP concentration was observed in early spring. Inversely, hepato-somatic index diminished suggesting that the hepatic products were intensively depleted. A similar increase in PPP concentrations was also observed in rainbow trout Oncorhynchus mykiss Whitehead et al. [48] during the breeding season. Compared to walleye [30], E₂ level evolved differently in Eurasian perch over winter and early spring. In walleye, vitellogenesis was completed in early winter, that explained a progressive decrease of E₂ level until spawning. On the other hand, in Eurasian perch, an active vitellogenesis was maintained until spawning and high levels of E₂ and PPP were recorded during this period. Like in gudgeon Gobio gobio [38], E₂ fluctuations were in harmony with GSI and oocyte diameter variations, and followed the pattern observed.

Figure 4. Variations in plasma concentrations (means ± standard deviation) of estradiol-17β (A) and protein phosphorous (B) in female Eurasian perch P. fluviatilis L. during an annual reproductive cycle.

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in white perch *Morone americana* [18]. Finally, an increase of E2 level during the periovulatory period was likewise noted by Barry et al. [2] in walleye, Dabrowski et al. [6] in yellow perch, Jackson and Sullivan [18] and King et al. [23] in white perch and white bass *Morone chrysops*, Prat et al. [37] in sea bass *Dicentrarchus labrax* and Rinchard et al. [38, 40] in gudgeon and roach *Rutilus rutilus*. In fact, this second surge in E2 would occur a few days before ovulation and would be related to the recovery of oocyte growth [40]. E2 level remained high during the oocyte maturation (germinal vesicle migration - germinal vesicle breakdown) and decreased with oocyte hydration and ovulation [23, 40]. In the present study, a 10 d-interval sampling did not allow a precise determination of E2 fluctuations during the periovulatory period. The increase of E2 levels during the prespawning period is however surprising and requires further investigations.

A very significant increase in T levels was observed in December (15-20 ng · mL⁻¹). High levels of T were maintained, although a slight decrease was observed for three weeks before spawning. Changes in plasma T concentrations observed during the present study differed from those in other percid fish like walleye and yellow perch. In these species, changes in plasma T levels showed two peaks, firstly in October-November coinciding with E2 peak level and secondly in April just before spawning [6, 30]. During these periods, Eurasian perch offered higher levels (20-30 ng · mL⁻¹) than walleye and yellow perch (1-5 ng · mL⁻¹). In harmony with E2 fluctuations, variations of T, a precursor of E2 which acts on hepatic vitellogenesis, confirmed that vitellogenesis remained active until spawning in Eurasian perch. Likewise, the high testosterone levels observed during the periovulatory period could play a part in the pre-spawning GtH surge and the effects of endogenous LHRH on the pituitary [30].

Immediately before spawning, the level of 17,20β-P increased to 0.6 ng · mL⁻¹. It may act as a maturation-inducing steroid in carp *Cyprinus carpio* [41, 49], yellow perch [6, 15], walleye [29], white bass and white perch [23]. In the present study, 17,20β-P plasma levels figured a rather smooth profile since they varied between 0.2 and 0.6 ng · mL⁻¹. As reported by Barry et al. [2], percids may require only low levels of 17,20β-P to induce germinal vesicle breakdown (GVBD) and 17,20β-P may be rapidly conjugated and removed from blood. In vitro, 17,20β-P appeared as a most effective
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