1998

Reproductive Cycle and Plasma Levels of Sex Steroids in Female Eurasian Perch Perca Fluviatilis

Isdy Sulistyo

Jacques Rinchard
The College at Brockport, jrinchar@brockport.edu

Pascal Fontaine

Jean-Noel Gardeur

Bruno Capdeville

See next page for additional authors

Aquat. Living Resour. 11 (2) (1998) 101-110 / © Ifremer-Elsevier, Paris

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

Isdy Sulistyo [1], Jacques Rinchard [2], Pascal Fontaine [1,*], Jean-Noël Gardeur [3], Bruno Capdeville [4], Patrick Kestemont [2]

[1] *Laboratoire de biologie appliquée, université Henri-Poincaré-Nancy 1, 34 rue Sainte-Catherine, 54000 Nancy, France.*

[2] *Unité de recherches en biologie des organismes, facultés universitaires Notre-Dame-de-la-Paix, 61, rue de Bruxelles, B-5000 Namur, Belgium.*
e-mail: patrick.kestemont@cc.fundp.ac.be.

[3] *Laboratoire des sciences animales, Ensaia-INPL, 2 ,avenue de la Forêt-de-Haye, 54500 Vandoeuvre-lès-Nancy, France.*
e-mail: gardeur@mais.ensaia.u-nancy.fr.

[4] *Centre piscicole départemental du domaine de Lindre, 57260 Lindre-basse, France.*

Received June 13, 1997; accepted March 23, 1998.

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 (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 (E_2), 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, E_2, 17,20β-P and PPP were low during the sexual resting period. E_2 and PPP levels increased significantly at the onset of the oogenesis in September, then the E_2 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, E_2 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 E_2 (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.

Eurasian perch / reproductive cycle / oogenesis / sexual steroids

Résumé – Cycle de reproduction et dosage dans le plasma des stéroïdes sexuels chez la perche femelle eurasienne *Perca fluviatilis*. Entre avril 1995 et avril 1996, une étude a été conduite au Centre Piscicole Départemental du Domaine de Lindre (Moselle, France) pour étudier le cycle annuel de reproduction de la perche eurasienne *Perca fluviatilis*. Celle-ci s'appuie sur des captures mensuelles (ou tous les 10 jours en période péri-ovulatoire) de 5 femelles, sur lesquelles le prélèvement d'organes a permis le calcul des indices gonado-, hépato- et viscéro-somatique (IGS, IHS, IVS), la mesure du diamètre ovocytaire (DO) et le dosage des teneurs, dans le plasma, en testostérone (T), 17β-estradiol (E_2), 17,20β-dihydroxy-4-pregnen-3-one (17,20β-P) et phosphore protéique (PPP). Après la phase de repos sexuel observée de mai à août (IGS < 1 %, DO < 200 µm, IVS = 4-6 %), l'ovogenèse débute en septembre lorsque la température de l'eau diminue de 26,4 à 14,1 °C. Dès lors, l'IGS augmente progressivement jusqu'à la mi-mars (15 %), puis fortement jusqu'à la ponte (25 %, DO = 850 µm), qui intervient en avril (14-15 °C). Les teneurs en T, E_2, 17,20β-P et PPP sont faibles pendant la phase de repos sexuel. Les taux de E_2 et de PPP augmentent significativement avec le début de l'ovogenèse en septembre, puis la teneur en E_2 s'élève fortement en novembre (3-4 ng · mL^{-1}). En décembre, la concentration en T atteint rapidement des valeurs de 15-20 ng · mL^{-1}. Les taux de T, E_2 et PPP restent très élevés jusqu'à la période de ponte ce qui indique le maintien d'une vitellogenèse active. Le calcul de l'IHS (2,1-2,2 %) plus élevé pour la période hivernale le confirme. Pendant la période péri-ovulatoire, un pic de E_2 (4 ng · mL^{-1}) survient, alors que T diminue. Au cours de cette étude, les teneurs en 17,20β-P restent faibles (0,2-0,6 ng · mL^{-1}) et relativement constantes. Aucun pic de 17,20β-P n'a été observé pendant la période péri-oovulatoire. Un échantillonnage tous les 10 jours s'avère insuffisant pour préciser les fluctuations hormonales liées à la maturation ovocytaire finale et à l'ovulation. © Ifremer-Elsevier, Paris.

***Perca fluviatilis* / cycle de reproduction / ovogenèse / stéroïdes sexuels**

* Corresponding author, fax. (33) 03 83 32 30 16.

1. INTRODUCTION

Eurasian perch *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 *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 *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 *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 *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 12×10^6 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. 24th) 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 anesthetized 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 viscerosomatic (VSI) indexes were calculated as GSI (%) = (GW × 100)/BW, HSI (%) = (LW × 100)/BW and VSI (%) = (VW × 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 $K_2 = 100 \times (BW\text{-}GW)/SL^3$, 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 µm thickness and stained with a trichrome: hemaluin, phloxine and light green [25]. For the oocyte development (histological examination, *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 µm) using DIG software. Only oocytes which were cut through the nucleus were measured.

Table I. Microscopic characteristics for the determination of the maturity stages in the ovary according to Rinchard and Kestemont (1996)

	Ovarian stage	Oocyte stages present in the ovary	Description of the most advanced oocytes
(1)	Previtellogenic	Previtellogenic oocytes	Oocytes with vacuole free cytoplasm
(2)	Onset of endogenous vitellogenesis	Previtellogenic oocytes and oocytes in endogenous vitellogenesis	Appearance of yolk vesicles, occupy 2 or 3 rings in the cytoplasm periphery (early endogenous vitellogenesis)
(3)	Completion of endogenous vitellogenesis	Previtellogenic oocytes and oocytes having complete endogenous vitellogenesis	Oocytes are full of yolk vesicles. Follicular and cellular layers are differentiated (late endogenous vitellogenesis)
(4)	Exogenous vitellogenesis	Previtellogenic oocytes and oocytes at different stages of exogenous vitellogenesis	Oocytes accumulate yolk globules and yolk vesicles are at the periphery of the cytoplasm
(5)	Final maturation	Previtellogenic oocytes and oocytes in final maturation	Appearance of micropyle and migration of the germinal vesicle to the micropyle
(6)	Post-spawning	Previtellogenic oocytes and pre- and postovulatory follicles	The pre- and postovulatory follicles hypertrophy, the yolk substance degenerates

2.4. Steroid and plasma protein phosphorus

Plasma concentrations of testosterone (T), 17β-estradiol (E_2) 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, E_2 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 E_2 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 K_1 showed minimum levels (2.0-2.3) during the post-spawning and quiescence periods. From January to April, the highest K_1 values (2.4-2.6) were obtained, while condition factors calculated by excluding gonad weigth K_2 (1.8 ± 0.2) were lowest during the spawning season. This level

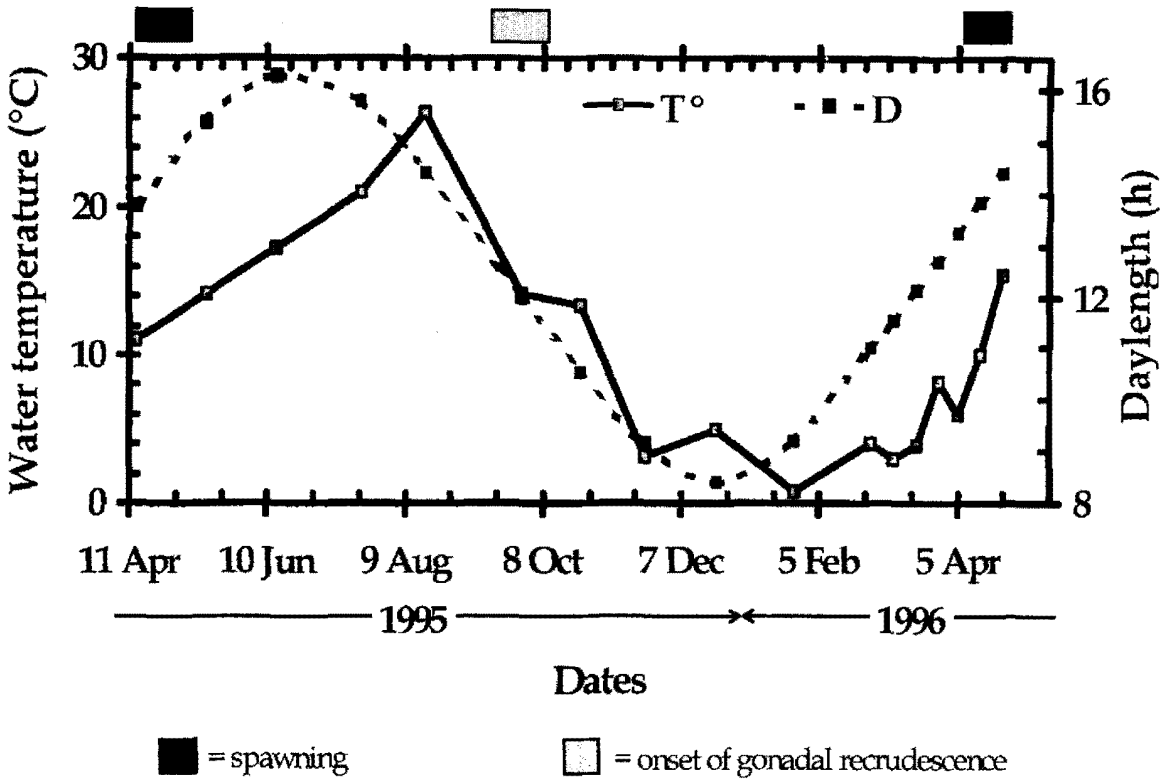

Figure 1. Variations of water temperature (°C) and day length (h) in Lindre pond during the study.

increased significantly during summer. In October, K_2 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 3*A) 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 3*B) 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 3*C, D) were observed. The maximum average diameter of 862 ± 39 µm was recorded on 28 March (*figure 2*). *Figure 3*E shows a mature oocyte with migrating germinal vesicle prior to spawning. The diameter of oocytes in the post-spawning period (25 April 1996, *figure 3*F) was 140 ± 10 µm which did not signi-ficantly differ ($P<0.05$) from those observed from May to July 1995.

3.3. Plasma sex steroid and protein phosphorus levels

Plasma E_2 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 4*A). 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 4*B) 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 5*A) 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 5*B). About two weeks before spawning in 1996, plasma

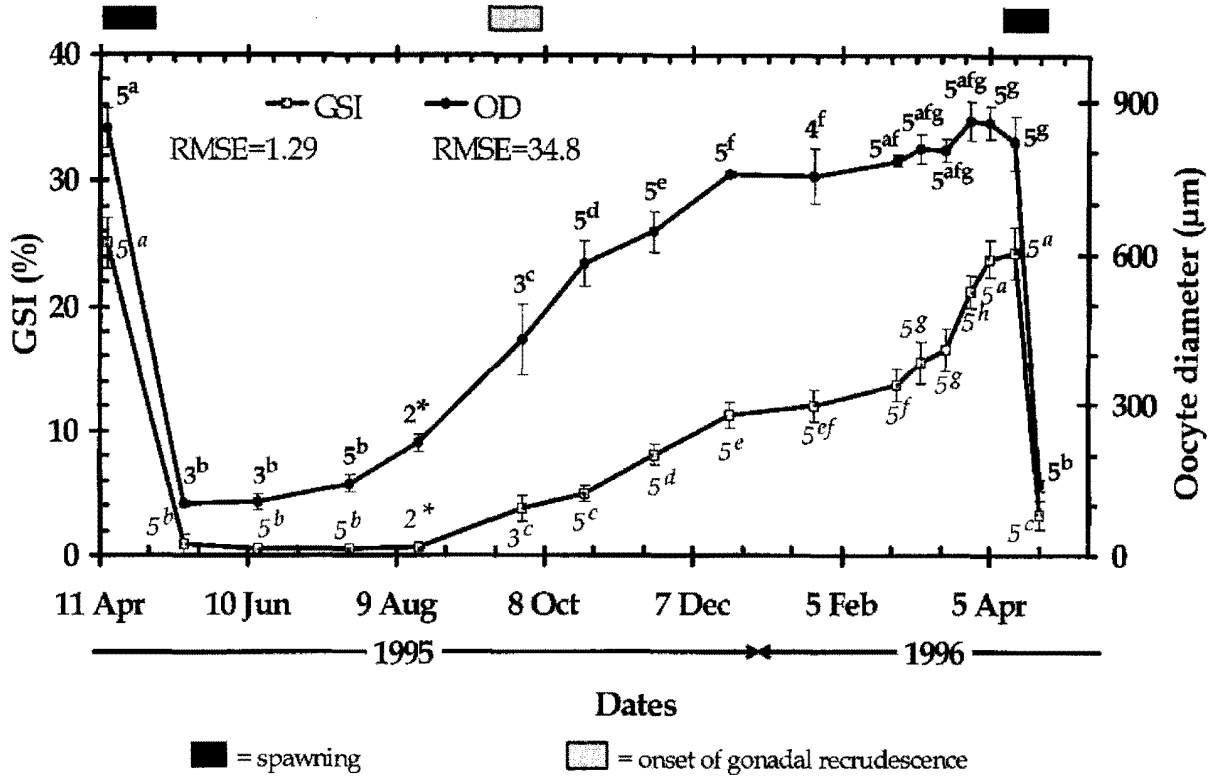

Figure 2. Gonadosomatic indexes and diameter of the most advanced oocytes (µm) in female Eurasian perch *P. fluviatilis* L. during an annual reproductive cycle. Values represent means ± standard deviation and are underestimated owing to the histological method used. Numbers indicate sample size and the numbers with same superscript were not significantly different ($P<0.05$). * = data were not analysed statistically. RMSE = root mean square errors.

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.

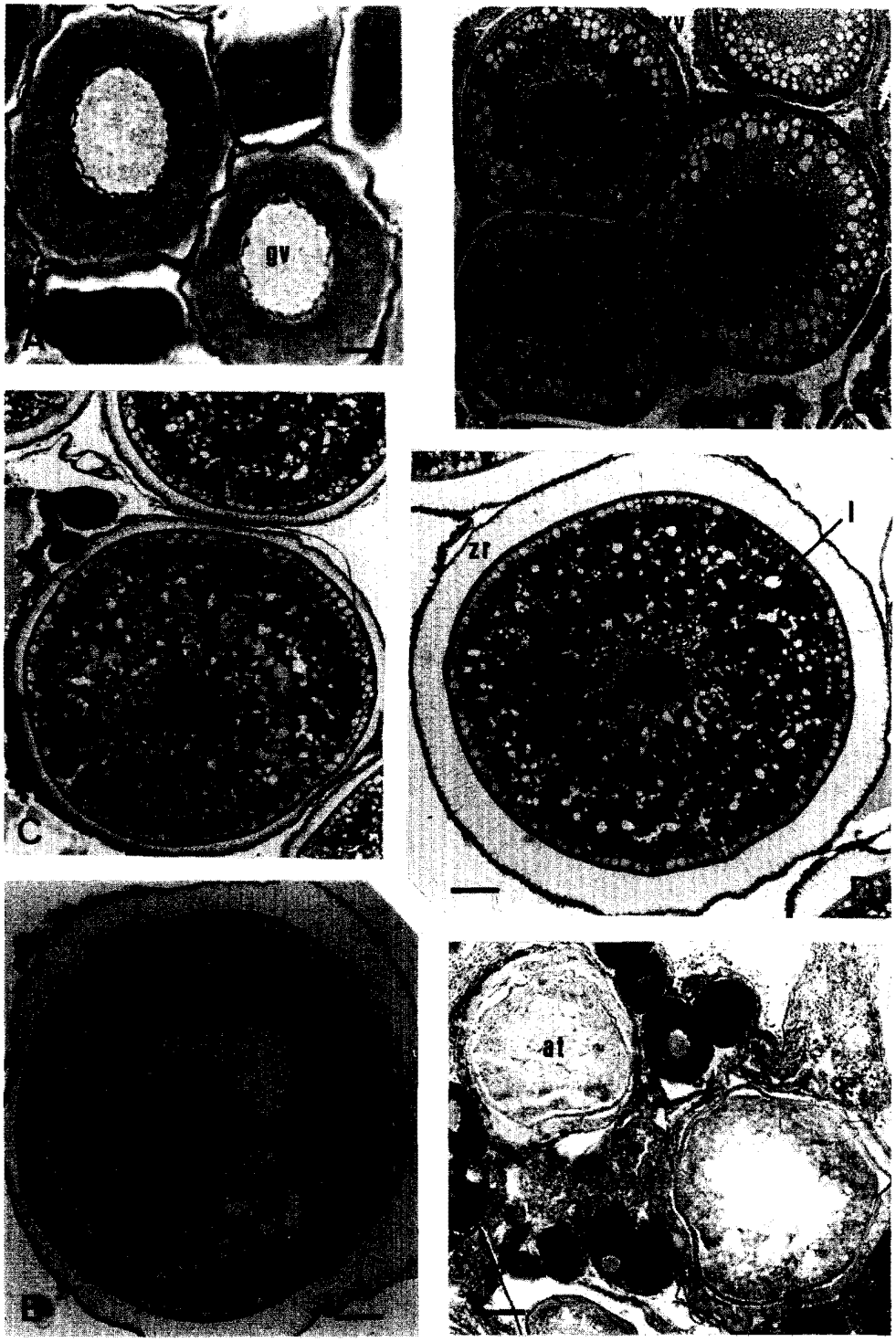

Figure 3. Sequence of oocyte development stages in female Eurasian perch *P. fluviatilis* during an annual reproductive cycle, according to Rinchard and Kestemont's histological terminology (1996). **A**. Previtellogenic oocytes (stage 1) in fish sampled on July 21, 1995; gv: germinal vesicle; scale 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.

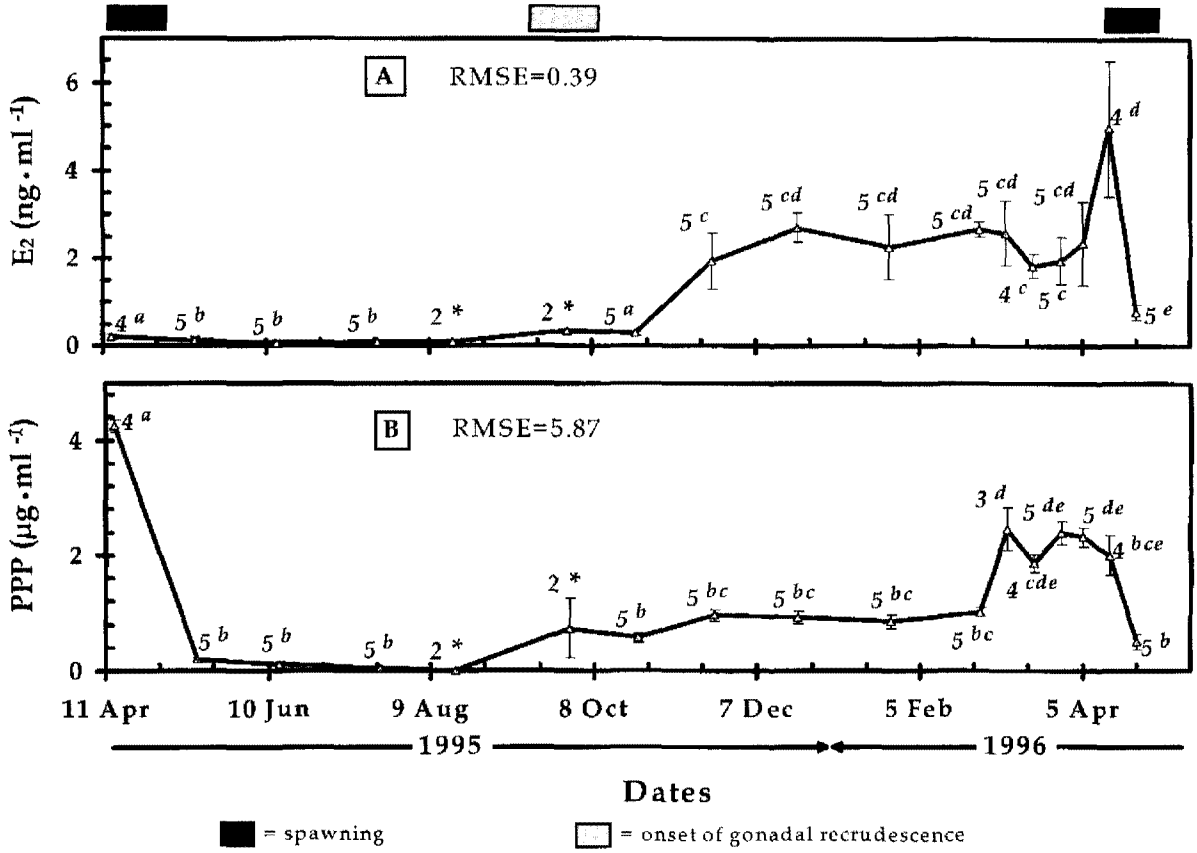

Figure 4. Variations in plasma concentrations (means ± standard deviation) of estradiol-17β (**A**) and protein phophorous (**B**) in female Eurasian perch *P. fluviatilis* L. during an annual reproductive cycle.

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 (K_1 and K_2) 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 phosphorus in the present study showed lower levels during the sexual resting phase. When gonadosomatic index increased in September, changes in plasma E_2 and PPP concentrations were noted. Then, E_2 level sharply rose in November which corresponded to HSI increase. At this period, Eurasian perch shows E_2 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]. E_2 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 E_2 synthesis and vitellogenesis intensified, the E_2 levels were stable and a marked rise in PPP concentration was observed in early spring. Inversely, hepato-somatic index dimi-nished suggesting that the hepatic products were intensively depleted. A similar increase in PPP concen-trations was also observed in rainbow trout *Oncorhynchus mykiss* Whitehead et al. [48] during the breeding season. Compared to walleye [30], E_2 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 E_2 level until spawning. On the other hand, in Eurasian perch, an active vitellogenesis was maintained until spawning and high levels of E_2 and PPP were recorded during this period. Like in gudgeon *Gobio gobio* [38], E_2 fluctuations were in harmony with GSI and oocyte diameter variations, and followed the pattern observed

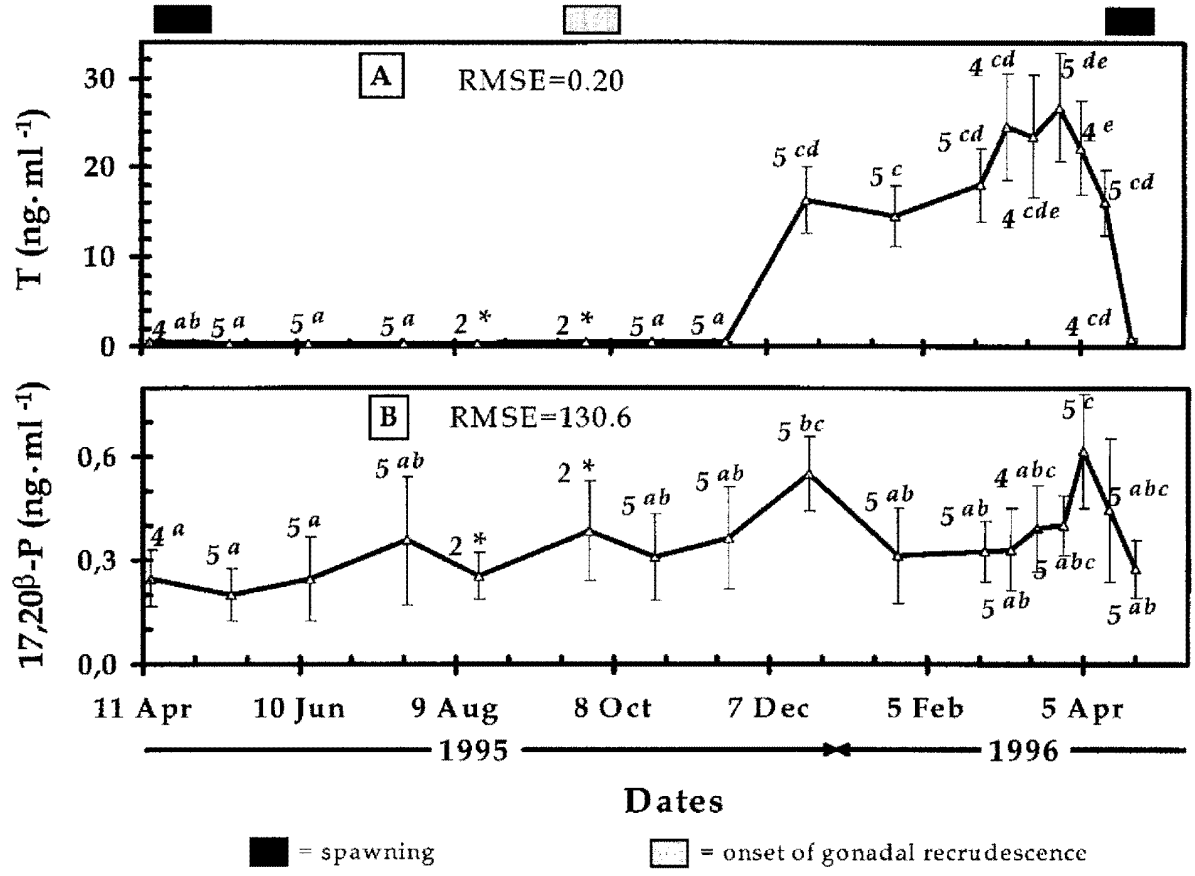

Figure 5. Variations in plasma concentrations (means ± standard deviation) of testosterone (**A**) and 17,20β-dihydroprogesterone (**B**) in female Eurasian perch *P. fluviatilis* during an annual reproductive cycle.

in white perch *Morone americana* [18]. Finally, an increase of E_2 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 E_2 would occur a few days before ovulation and would be related to the recovery of oocyte growth [40]. E_2 level remained high during the oocyte maturation (germinal vesicle migration - germinal vesicle breakdown) and decreased with oocyte hydratation and ovulation [23, 40]. In the present study, a 10 d-interval sampling did not allow a precise determination of E_2 fluctuations during the periovulatory period. The increase of E_2 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 E_2 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 E_2 fluctuations, variations of T, a precursor of E_2 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

inducer of final oocyte maturation and ovulation. Two or three days before spawning, Barry et al. [2] recorded a peak of 17,20β-P (1.2-1.5 ng · mL^{-1}). As for E_2, the 10 d-interval sampling did not allow a precise determination of 17,20β-P fluctuations during the periovulatory period. Another hypothesis is that, as reported in black sea bass (*Centropristis striata*) [3], another maturation inducing steroid could regulate the ovulation process in Eurasian perch. In many perciforms, late final oocyte maturation was associated with elevation in plasma levels of both 17,20β-P and 17,20β,21-trihydroxy-4-pregnen-3-one (17,20β, 21-P) [23]. In these species, the two hormones may have different roles during final oocyte maturation, ovulation and spawning [35]. Their relative importance must be specified in Eurasian perch, 17,20β, 21-P could act as a maturation-inducing steroid.

In conclusion, the sampling at 10 days intervals contributes to the completion of existing data on biological and physiological changes during an annual reproductive cycle of Eurasian perch *Perca fluviatilis*. Nevertheless the 10 days intervals could be shortened to 3 days intervals, especially during the last two weeks before spawning in order to examine, in rather more detail, oocyte and steroid changes, i.e. maturational stages and related dynamic steroids.

Acknowledgements

We express our gratitude to the staff of different teams involved in these study. This work has been partly sustained by the society Gabriel France and the Indonesian Ministry of Education and Culture (HEP/ADB project - Loan IND No 2054). We also thank gratefully Dr A. Fostier (Inra, Rennes, France) for providing the antibodies for radioimmunoassay and Prof. R. Leloup (Unité d'histologie et embryologie, FUNDP, Belgium) for access to his laboratory.

REFERENCES

[1] Alm G., Maturity, mortality and growth of Perch, *Perca fluviatilis* L., grown in ponds, Inst. Freshw. Res. Drottningholm Rep. 35 (1954) 11–20.

[2] Barry T.P., Malison J.A., Lapp A.F., Procarione L.S., Effects of selected hormones and male cohorts on final oocyte maturation, ovulation, and steroid production in walleye (*Stizostedion vitreum*), Aquaculture 138 (1995) 331–347.

[3] Cerda J., Selman K., Wallace R.A., Observations on oocyte maturation and hydration *in vitro* in the black sea bass *Centropristis striata* (Serranidae), Aquat. Living Resour. 9 (1996) 325–335.

[4] Ciereszko A., Ramseyer L., Dabrowski K., Cryopreservation of yellow perch semen, Progress. Fish-Cult. 55 (1993) 261–264.

[5] Craig J.F., The body composition of adult perch, *Perca fluviatilis* in Windermere, with reference to seasonal changes and reproduction, J. Anim. Ecol. 46 (1977) 617–632.

[6] Dabrowski K., Ciereszko R.E., Ciereszko A., Toth G.P., Christ S.A., El-Saidy D., Ottobre J.S., Reproductive physiology of yellow perch (*Perca flavescens*): environmental and endocrinological cues, J. Appl. Ichthyol. 12, (1996) 139–148.

[7] Dabrowski K., Ciereszko A., Ramseyer L., Culver D., Kestemont P., Effects of hormonal treatment on induced spermiation and ovulation in the yellow perch (*Perca flavescens*), Aquaculture 120 (1994) 171–180.

[8] Fiogbé E.D., Kestemont P., Mélard C., Micha J.C., The effects of dietary crude protein on growth of the Eurasian perch *P. fluviatilis*, Aquaculture 144 (1996) 239–249.

[9] Flesch A., Biologie de la perche (*Perca fluviatilis*) dans le réservoir du Mirgenbach (Cattenom, Moselle),Thèse, Université Metz, 1994, 237 p.

[10] Fontaine P., Tamazouzt L., Capdeville B., Growth of the Eurasian perch (*Perca fluviatilis* L.) reared in floating cages and in water recirculated system: first results, J. Appl. Ichthyol. 12 (1996) 181–184.

[11] Fontaine P., Tamazouzt L., Terver D., Georges A., Actual state of production of perch: problems and prospects. 1. Mass rearing potentialities of the common perch under controlled conditions, in: Kestemont P., Billard R. (eds.), Aquaculture of freshwater species (except salmonids), European Aquaculture Society, Spec. Publ. no. 20, 1993, pp. 46–48.

[12] Fostier A., Jalabert B., Steroidogenesis in rainbow trout (*Salmo gairdneri*) at various preovulatory stages: changes in plasma hormone levels and in vivo and in vitro responses of the ovary to salmon gonadotropin, Fish Physiol. Biochem. 2 (1986) 87–99.

[13] Gillet C., Dubois J.P., A survey of the spawning of perch (*Perca fluviatilis*), pike (*Esox lucius*), and roach (*Rutilus rutilus*), using artificial spawning substrates in lakes, Hydrobiologia 300/301 (1995) 409–415.

[14] Gillet C., Dubois J.P., Bonnet S., Influence of temperature and size of females on the timing of spawning of perch, *Perca fluviatilis* in lake Geneva from 1984 to 1993, Env. Biol. Fishes 42 (1995) 355–363.

[15] Goetz F.W., Theofan G., In vitro stimulation of germinal vesicle breakdown and ovulation of yellow perch (*Perca flavescens*) oocytes: Effects of 17α-hydroxy-20β-dihydroprogesteron and prostaglandins, Gen. Comp. Endocrinol. 37 (1979) 273–285.

[16] Henderson B.A., Wong J.L., Nepszy S.J., Reproduction of walleye in lake Erie: allocation of energy, Can. J. Fish. Aquat. Sci. 53 (1996) 127–133.

[17] Houthuijzen R.P., Backx J.J.G.M., Buijse A.D., Exceptionnaly rapid growth and early maturation of perch in a freshwater lake recently converted from an estuary, J. Fish Biol. 43 (1993) 320–324.
[18] Jackson L.F., Sullivan C.V., Reproduction of white perch: the annual gametogenic cycle, Trans. Am. Fish. Soc. 124 (1995) 563–577.
[19] Jamet J.L., Desmolles F., Growth, reproduction and condition of roach (*Rutilus rutilus* L.), perch (*Perca fluviatilis* L.) and ruffe (*Gymnocephalus cernuus* L.) in eutrophic lake Aydat (France), Int. Rev. Ges. Hydrobiol. 79 (1994) 305–322.
[20] Jones D.H., The spawning of perch (*Perca fluviatilis* L.) in Loch Leven, Kinross, Scotland, Fish. Manage. 13 (1982) 139–151.
[21] Kestemont P., Mélard C., Fiogbé E., Vlavonou R., Masson G., Nutritional and animal husbandry aspects of rearing early life stages of eurasian perch *Perca fluviatilis*, J. Appl. Ichthyol. 12 (1996) 157–165.
[22] Kestemont P., Fiogbé E., Parfait O., Micha J.C., Mélard C., Relationship between weaning size, growth, survival and cannibalism in the common perch larvae *Perca fluviatilis* : preliminary data, in: Lavens P., Jaspers E., Roelants I. (eds.), Larvi'95, European Aquaculture Society, Spec. Publ. 24, 1995, pp. 285–288.
[23] King W.V., Berlinsky D.L., Sullivan C.V., Involvement of gonadal steroids in final oocyte maturation of white perch (*Morone americana*) and white bass (*M. chrysops*): in vivo and in vitro studies, Fish Physiol. Biochem. 14 (1995) 489–500.
[24] Kirillov A.F., Akhremenko A.K., Seasonal changes in energy substrate concentration in the tissues of the Vilyui Reservoir perch, *Perca fluviatilis*, J. Ichthyol. 1982, 129–133.
[25] Langeron M., Précis de microscopie: technique-expérimentation-diagnostic, Masson et Cie, Paris, 1942, 1339 p.
[26] Le Cren E.D., The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*), J. Anim. Ecol. 20 (1951) 201–219.
[27] Makarova N.P., Seasonal changes in some of the physiological characteristics of the perch (*Perca fluviatilis* L.) of Ivan'kovo reservoir, J. Ichthyol. 13 (1973) 742–752.
[28] Malison J.A., Best C.D., Kayes T.B., Hormonal control of growth and sexual size dimorphism in yellow perch (*Perca flavescens*), Am. Zool. 22 (1982) 955.
[29] Malison J.A., Held J.A., Reproduction and spawning in walleye (*Stizostedion vitreum*), J. Appl. Ichthyol. 12 (1996) 153–156.
[30] Malison J.A., Procarione L.S., Barry T.P., Kapuscinski A.R., Kayes T.B., Endocrine and gonadal changes during the annual reproductive cycle of the freshwater teleost, *Stizostedion vitreum*, Fish Physiol. Biochem. 13 (1994) 473–484.
[31] Martin J.B., Doty D.M., Determination of inorganic phosphate, Analyt. Chemist. 21 (1949) 965–967.
[32] Mélard C., Baras E., Mary L., Kestemont P., Relationships between stocking density, growth, cannibalism and survival rate in intensively cultured larvae and juveniles of perch (*Perca fluviatilis*), Ann. Zool. Fennici 33 (1996*b*). 643–651.
[33] Mélard C., Kestemont P., Baras E., First results of European perch (*Perca fluviatilis*) intensive rearing in tank: effect of temperature and size grading on growth, Bull. Fr. Pêche Piscic. 336 (1995) 19–27.
[34] Mélard C., Kestemont P., Grignard J.C., Intensive culture of juvenile and adult Eurasian perch (*Perca fluviatilis*): effect of major biotic and abiotic factors on growth, J. Appl. Ichthyol. 12 (1996) 175–180.
[35] Mylonas C.C., Magnus Y., Klebanov Y., Gissis A., Zohar Y., Reproductive biology and endocrine regulation of final oocyte maturation of captive white bass, J. Fish Biol. 51 (1997) 234–250.
[36] Papageorgiou N.K., Fecundity and reproduction of perch (*Perca fluviatilis* L.) in lake Agios Vasilios, Greece, Freshw. Biol. 7 (1977) 559–565.
[37] Prat F., Zanuy S., Carillo M., de Mones A., Fostier A., Seasonal changes in plasma levels of gonadal steroids of sea bass. *Dicentrarchus labrax* L., Gen. Comp. Endocrinol. 78 (1990) 361–373.
[38] Rinchard J., Kestemont P., Kühn E.R., Fostier A., Seasonal changes in plasma levels of steroid hormones in an asynchronous fish the gudgeon *Gobio gobio* L. (Teleostei, Cyprinidae), Gen. Comp. Endocrinol. 92 (1993) 168–178.
[39] Rinchard J., Kestemont P., Comparative study of reproductive biology in single- and multiple-spawner cyprinid fish I. Morphological and histological features, J. Fish Biol. 49 (1996) 883–894.
[40] Rinchard J., Kestemont P., Heine H., Comparative study of reproductive biology in single- and multiple-spawner cyprinid fish. II. Sex steroid and plasma protein phosphorus concentrations, J. Fish Biol. 50 (1997) 169–180.
[41] Santos A.J.G., Furukawa K., Kobayashi M., Bando K., Aida K., I. Hanyu I., Plasma gonadotropin and steroid hormone profiles during ovulation in the carp *Cyprinus carpio*, Bull. Jpn. Soc. Sci. Fish. 52 (1986) 1159–1166.
[42] Tamazouzt L., Fontaine P., Terver D., Décalage de la période de reproduction de la perche commune (*Perca fluviatilis* L.) en eau recyclée, Ichtyophysiol. Acta 17 (1994) 29–40.
[43] Tamazouzt L., Dubois J.P., Fontaine P., Capdeville B., Terver D., Zootechnical performance and body compositon of *P. fluviatilis* pelleted diet in a floating cage: Effect of daily ration, Ann. Zool. Fennici 33 (1996) 635–641.
[44] Treasurer J.W., Some aspects of the reproductive biology of perch *Perca fluviatilis* L. Fecundity, maturation and spawning behaviour, J. Fish Biol. 18 (1981) 729–740.
[45] Treasurer J.W., Holliday F.G.T., Some aspects of the reproductive biology of perch *Perca fluviatilis* L.: A histological description of the reproductive cycle, J. Fish Biol. 18 (1981) 359–376.
[46] Wallace R.A., Jared D.W., Studies on amphibian yolk. VII: Plasma phosphoprotein synthesis by vitellogenic females and estrogen-treated males of *Xenopus laevis*, Can. J. Biochem. 46 (1968) 953–959.
[47] Wallace R.A., Selman K., Cellular and dynamic aspects of oocyte growth in teleosts, Am. Zool. 21 (1981) 325–343.
[48] Whitehead C., Bromage N.R., Breton B., Matty A.J., Effect of altered photoperiod on plasma gonadotropin and testosterone levels in male rainbow trout, J. Endocrinol. 81 (1979) 139–140.
[49] Yaron Z., Endocrine control of gametogenesis and spawning induction in the carp, Aquaculture 129 (1995) 49–73.