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

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Abstract — From April 1995 to April 1996, the annual reproductive cycle of male Eurasian perch *Perca fluviatilis* was studied at the Lindre Center (Moselle, France). At monthly intervals, five males (mean body weight of 133 ± 43 g and total length of 175 ± 9 mm) were caught. From sampled organs, the gonadosomatic (GSI), hepatosomatic (HSI) and viscerosomatic indexes (VSI) were calculated and plasma testosterone (T) and 11-ketotestosterone (11KT) levels were measured. After the spawning period in mid-April, GSI and HSI dropped and VSI increased to 3.8 ± 0.1 %. In September, GSI reached its maximum (8.5 ± 1.8 %). At this time, spermatocytes, spermatids and spermatozoa were abundant, whereas in June only spermatogonia were observed. During winter, GSI was stable at approximately 5 %, HSI reached its maximum (1.9 ± 0.3 %) and VSI was low (2.6 ± 0.2 %). From April to November 1995, plasma T and 11KT concentrations were low (< 0.5 ng·mL^{-1}). Plasma T levels increased significantly in December and reached peak levels (12.3 ± 2.1 ng·mL^{-1}) in January, then decreased in February and increased again until spawning in April (6.8 ± 2.1 ng·mL^{-1}). This second elevation could be related to the beginning of a new spermatogenic cycle. Plasma levels of 11KT increased significantly from October to February 1996 (4.9 ± 1.1 ng·mL^{-1}). From February to the spawning period (April 1996), plasma 11KT decreased significantly, but values were significantly higher than those measured in fall. Males were spermiating from January to spawning in April. © 2000 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

Reproductive cycle / sex hormones / steroids / male / *Perca fluviatilis*

Résumé — Cycle de reproduction et teneurs en stéroïdes du plasma chez la perche eurasienne mâle *Perca fluviatilis*. Le cycle annuel de reproduction de la perche eurasienne mâle, *Perca fluviatilis*, est étudié au Domaine de Lindre (Moselle, France) d'avril 1995 à avril 1996. Cinq mâles (poids moyen de 133 ± 43 g, longueur totale moyenne de 175 ± 9 mm) sont examinés mensuellement. Les indices gonadosomatique (IGS), hépatosomatique (IHS) et viscérosomatique (IVS) sont calculés, les teneurs en testostérone (T) et 11-kétotestostérone (11KT) mesurées. Après la période de ponte (mi-avril 1995), IGS et IHS diminuent et l'IVS augmente (3,8 ± 0,1 %). En septembre, l'IGS atteint son maximum (8,5 ± 1,8 %) et les testicules présentent des spermatocytes, des spermatides et des spermatozoïdes, alors qu'au mois de juin, il n'y a que des spermatogonies. Pendant l'hiver, l'IGS reste stable (environ 5 %), l'IHS atteint son maximum (1,9 ± 0,3 %) et l'IVS reste faible (2,6 ± 0,2 %). D'avril à novembre 1995, les teneurs en testostérone et 11KT du plasma sont faibles (< 0,5 ng·mL^{-1}). La teneur en T du plasma augmente fortement en décembre pour atteindre un maximum en janvier (12,3 ± 2,1 ng·mL^{-1}). Elle diminue en février, puis s'élève à nouveau jusqu'à la ponte en avril (6,8 ± 2,4 ng·mL^{-1}). Cette seconde élévation pourrait coïncider avec le démarrage d'un nouveau cycle de spermatogenèse. La teneur en 11KT augmente significativement d'octobre à février 1996 (4,9 ± 1,1 ng·mL^{-1}), puis elle diminue de février jusqu'à la ponte en avril 1996, mais reste supérieure à celles dosées à l'automne. De janvier jusqu'à la période de ponte, les mâles sont spermiants. © 2000 Ifremer/Cnrs/Inra/Ird/Cemagref/Éditions scientifiques et médicales Elsevier SAS

Cycle de reproduction / hormones sexuelles / stéroïdes / mâle / *Perca fluviatilis*

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1. INTRODUCTION

Recently, the reproductive cycle and variations in plasma levels of sex steroids have been described in female Eurasian perch, *Perca fluviatilis* [41]. Considering the male reproductive cycle, data exist on body composition variations [7], sexual maturation [15, 35], variation of the gonadosomatic index (GSI) [17] and sperm and spermatozoa characteristics [21, 36]. However, there is no study on sexual hormone fluctuations. Testosterone (T) and 11-ketotestosterone (11KT) have been established as major sex steroids involved in spermatogenesis and spermiation during the reproductive cycle of freshwater and marine fish [2, 3, 5, 8, 10, 19, 24, 30, 32, 37, 46]. Kime and Hews [18] have studied in vitro the androgen steroid synthesis in pike, *Esox lucius*, and Eurasian perch and have shown that both species testes produce 11-oxygenated androgens, especially 11KT, a biosynthetic pattern that appears to be typical of teleost testes. 11-Oxygenated androgens would be more effective than T in stimulating spermatogenesis, reproductive behaviour and secondary sexual characters [3, 18]. In Japanese eel, *Anguilla japonica*, 11KT was found to induce all stages of spermatogenesis and to stimulate the Sertoli cells in testes, in vitro [11]. Among Percids and Moronids, works dealing with the variations of the androgen steroids (T and 11KT) during a reproductive cycle have been conducted in yellow perch, *Perca flavescens* [6, 8], walleye, *Stizostedion vitreum* [26, 27], white bass, *Morone chrysops* [1, 31] and striped bass *Morone saxatilis* [32, 47]. A multitude of androgen profiles was observed in fish [3]. Generally, peak androgen levels are reached with the onset of spermiation. Plasma androgens in most teleosts, although high during prespawning and/or spawning season, remained relatively low during the rest of the reproductive cycle [3, 11].

In yellow perch and walleye, the onset of the gonadal development coincides with the decrease in water temperature and photoperiod in autumn (September–October), whereas reproduction occurs in spring when water temperature and photoperiod increase [8, 27]. Photoperiod variations may not be crucial during the late stages of spermatozoa maturation and/or storage [6]. Water temperature fluctuations seem therefore to be the main environmental factor controlling the annual reproductive cycle of Percids, especially in males.

Thus, the present study examined the plasma T and 11KT patterns, in relation to testis development and environmental factor variations, in wild male Eurasian perch during an annual reproductive cycle, and compared these patterns with those found in other teleost fish.

2. MATERIALS AND METHODS

2.1. Fish capture and experimental site

Eurasian perch males were sampled monthly with hook and lines from Lindre pond (surface area 62 × 10^5 m^2, volume 12 × 10^6 m^3, altitude 210 m), in Moselle, France, from April 1995 to April 1996. Five males were caught at each sampling date [41], except in July and August 1995, due to problems of capture during this period. The water temperature varied from 0.8 °C in February to 26.4 °C in August (*figure 1*). The morphometric characteristics of Lindre pond and the water physico-chemical quality have been described by Tamazouzt et al. [42].

2.2. Organ and plasma sampling

Immediately after capture, fish were anaesthetized in a 2-phenoxyethanol solution (0.3 $mL \cdot L^{-1}$), then individually weighed (body weight BW ± 0.1 g), measured for standard length (SL ± 1 mm), blood-sampled and dissected. Fish averaged 133.0 ± 42.9 g in weight and 175 ± 9 mm in length.

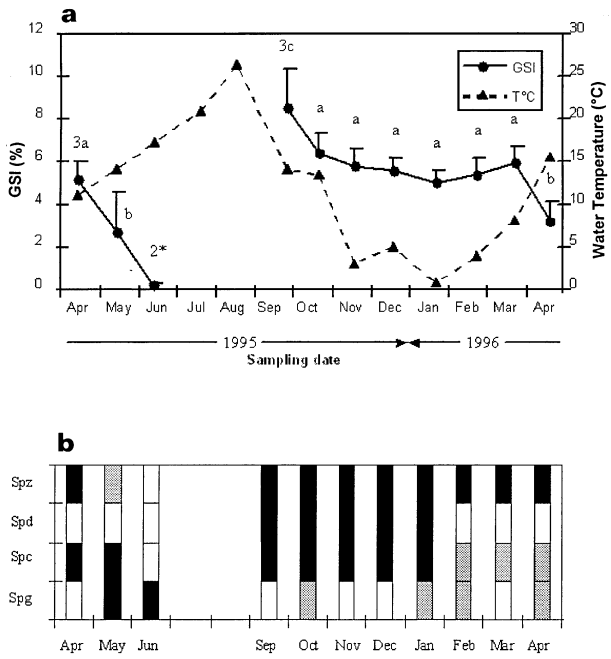

Figure 1. a) Variations in water temperature in Lindre pond and gonadosomatic index (GSI) in male Eurasian perch, *Perca fluviatilis*, during an annual reproductive cycle (April 1995–April 1996). GSI values represent mean ± standard error with $n = 5$. Numbers indicate sample size (when different to 5) and the numbers with the same superscript were not significantly different ($P < 0.05$). * Sample excluded from statistical analysis; RMSE (root mean square error) = 1.06. **b)** Stages of spermatogenesis observed in male Eurasian perch, *Perca fluviatilis*, during an annual reproductive cycle. Black boxes indicate the very abundant stages, the grey boxes the less abundant stages and the white boxes absence. Spg, spermatogonia; Spc, spermatocyte; Spd, spermatid; Spz, spermatozoa.

A 1-mL blood sample was withdrawn from the heart using a heparinized syringe. Blood was centrifuged at 4 000 rpm for 25 min and the plasma was stored in vials at –25 °C until assay. Gonads, liver and viscera including mesenteric fat were entirely removed and weighed (± 0.01 g).

2.3. Morpho-anatomical parameters

The gonadosomatic (GSI), hepatosomatic (HSI) and viscerosomatic (VSI) indexes (%) were calculated as follows:

$$GSI = (GW \times 100)/BW$$

$$HSI = (LW \times 100)/BW$$

$$VSI = (VW \times 100)/BW$$

where GW is the weight of the testis (g), LW is the weight of the liver (g), VW is the weight of the viscera (g) and BW is the weight of the body. To determine the respective importance of somatic and gonad development in fish conditions, condition factors were determined with and without gonad weight, as follows:

$$K_1 = 100 \times BW/L^3,$$
$$K_2 = 100 \times (BW - GW)/L^3$$

where L is the standard length (cm) of fish [15, 23].

2.4. Androgen measurement

Plasma concentrations of T and 11KT were determined using RIA analysis according to Fostier and Jalabert [9], after two extractions with cyclohexane/ethyl acetate (v/v). The extractions were performed on 50 µL of plasma for each steroid. All samples were assayed in duplicate and standards in triplicate. Cross reactivities of antisera with a variety of common steroids have been described by Prat et al. [37]. Six samples at different concentrations were run in each assay and were used as quality controls for estimating inter- and intra-assay coefficients of variation (CV). For the two hormones, the sensitivity of the assay was 5 $pg \cdot mL^{-1}$ and the intra-assay coefficients of variation (n = 2) were 9.9 and 6.2 % for T and 11-KT, respectively. The inter-assay CVs were 11.5 and 8.2 % for T and 11KT, respectively.

2.5. Histological study

From each male, a fragment from the testes was removed and fixed in Bouin's Holland solution and embedded in paraplast for histological examination. The tissues were subsequently cut to 4 µm thick, mounted and stained with a trichrome method: Regaud's haematoxyline at 57 °C, phloxine and light green [22]. Testis development was monitored according to the presence of different sperm cell types, i.e. spermatogonia which were the largest cells, spermatocytes (smaller than spermatogonia) identified by densely staining chromatin, spermatids (single and larger cell than spermatozoa) and spermatozoa recognized by their kidney-shaped cell with light coloured hair-like tails [45].

2.6. Statistical analysis

All quantitative data are expressed as means ± SD (standard deviation). Statistical analysis of this data was 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 ed.). The minimum level of significance was set at $P \leq 0.05$. The mean differences between data were analysed using Student-Newman-Keuls (SNK) test. Before statistical analysis, values of 11KT were transformed, using a logarithmic or square-root transformation, to satisfy tests for normality and homogeneity of variances. Statistic analysis was applied to transformed data, but untransformed data give an idea of plasma steroid concentrations. Normality was checked by Shapiro-Wilk test ($n <$ 2 000). Likewise some data were deleted from statistical analysis because of the abnormality of distribution, even after the logarithmic transformation of the data.

3. RESULTS

3.1. Morpho-anatomical parameters

The lowest GSI (0.2 ± 0.1 %) was recorded in late June, while the highest values (8.5 ± 1.8 %) were observed in September (*figure 1a*). GSI declined sharply in October (6.4 ± 0.9 %, $P < 0.05$), then remained unchanged at about 5 % until March. HSI rose gradually from September (1.1 ± 0.1 %) to February, when it reached its maximum of 1.9 ± 0.3 % and remained high until spawning (*figure 2a*). VSI dropped significantly from 3.9 ± 0.9 % in September to 2.6 ± 0.2 % ($P < 0.05$) in October and then varied slowly until spawning (*figure 2a*). After spawning (April 1996), a marked drop in all indexes was recorded, with GSI averaging 3.2 ± 0.9 %, HSI 1.3 ± 0.2 % and VSI 2.5 ± 0.3 %, respectively. Condition factors peaked significantly (2.8 ± 0.2 and 2.6 ± 0.2 for K_1 and K_2, respectively, $P < 0.05$) in September and remained stable until April.

3.2. Histological observations

Spermatozoa and spermatocytes were very abundant in testes in April, but no spermatids could have been identified in the testes of the three males sampled (*figure 3a*). In May, about 1 month after spawning, testes contained some residual spermatozoa

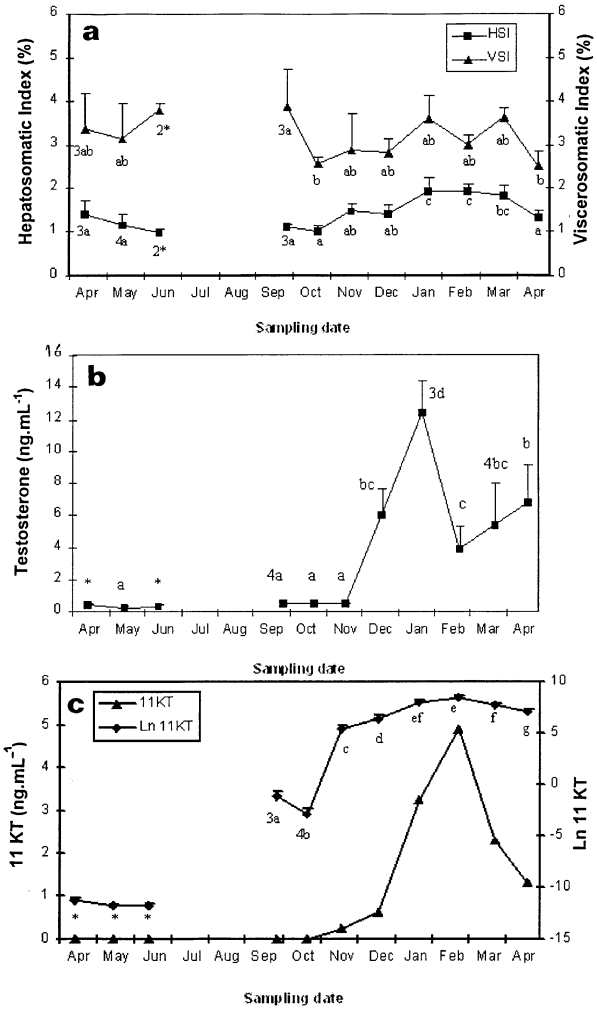

Figure 2. a) Variations in hepatosomatic (HSI) and viscero-somatic (VSI) indexes in male Eurasian perch, *Perca fluviatilis*, during an annual reproductive cycle (April 1995–April 1996). See *figure 1* for captions. RMSE (root mean square error) = 1.06. **b, c)** Changes in plasma levels (mean ± standard deviation) of testosterone (b) and 11-ketotestosterone (c) in male Eurasian perch, *Perca fluviatilis*, during an annual reproductive cycle. See *figure 1* for captions. Root means square error are 1.49 and 0.33 for testosterone and 11-ketotestosterone, respectively.

surrounded by empty spaces, as well as stem cells, spermatogonia and spermatocytes (*figures 1b* and *3b*). Only spermatogonia were found in males sampled in June (*figure 3c*). Testes from fish caught in September, contained spermatocytes, spermatids and spermatozoa in equal proportions (*figures 1b* and *3d*). Spermatogonia were observed in the periphery of the tissue wall. Spermatozoa increased in numbers in December (*figure 3e*). At that time, all sampled males were spermiating with a small amount of milt flowing out during handling. Two months before spawning, in February, spermatozoa were dominant (*figure 3f*) and only a few spermatids were observed.

3.3. Plasma steroid concentrations

From April to November, plasma levels of T and 11KT were very low, below 0.5 ng·mL^{-1}, and often undetectable for 11KT (*figure 2*). Plasma T concentration increased significantly in December and reached a peak in January (12.3 ± 2.1 ng·mL^{-1}, $P < 0.05$), then it dropped sharply in February and increased again towards spawning in April (6.8 ± 2.4 ng·mL^{-1}). Plasma 11KT levels began to rise in November and a peak was detected in February (4.9 ± 1.1 ng·mL^{-1}, $P < 0.05$). Then, the plasma 11KT concentration was significantly reduced prior to the spawning period in April 1996 (1.3 ± 0.4 ng·mL^{-1}, $P < 0.05$).

4. DISCUSSION

The failure to capture perch males in July and August does not allow us to determine precisely the onset of testis recrudescence. However, several authors reported that the GSI of yellow perch [13, 43, 45] and Eurasian perch [44, 45] remains low throughout the summer period. It is highly probable that, in the Eurasian perch, the onset of testis recrudescence occurred at the end of August, and progressed rapidly, coinciding with the drop in water temperature. According to the observations of Treasurer et al. [44] and Turner [45], the highest GSI was recorded in September and resulted from an active spermatogonia transformation to form advanced cells as spermatocytes. Similarly in the gudgeon, *Gobio gobio* [16], the spermatocyte division led to an abrupt elevation of GSI. In other studies with Eurasian perch, the development of the testes occurred within about 6 weeks, GSI was reported to rise in August and peak in October [23, 44]. The increase in GSI would be related to the appearance of spermatocytes in September in the testis. In yellow perch, a maximum GSI was observed either in November [43] or in January [13, 45]. In fact, GSI was low at the end of August, but markedly increased within 6 weeks. Our data only differed from those of Jamet and Desmolles [15] who reported in Lake Aydat (France) a GSI peak in March, 1 month before spawning.

HSI rose gradually from September to February, and reached its maximum, remained high until spawning and then decreased just prior to spawning. According to Makarova [25], highest HSI are observed in June in Eurasian perch of Ivan'Kovo reservoir (Russia) and are related to the beginning of intensive feeding activity. The highest VSI values, which occurred in summer, suggested that Eurasian perch broodstock develop visceral energy stores by an active feeding activity during summer. These stores were later depleted due to gonadal recrudescence.

As in rainbow trout *Oncorhynchus mykiss* [39, 40] and brown trout *Salmo trutta* [19], the plasma T

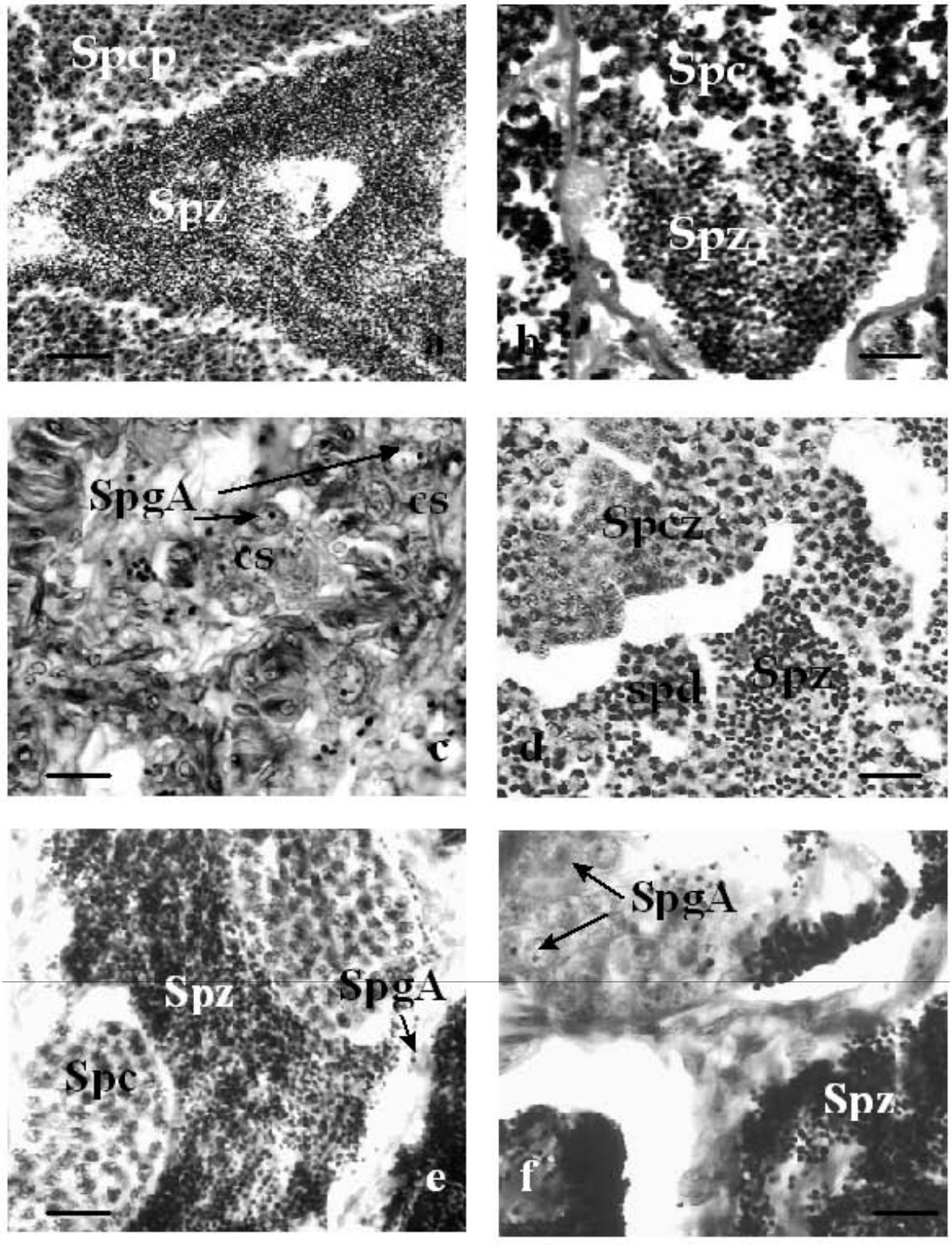

Figure 3. Sequence of testis development stages in male Eurasian perch, *Perca fluviatilis*, during an annual reproductive cycle. **a)** Specimen sampled on 14 April 1995; **b)** specimen sampled on 15 May 1995; **c)** specimen sampled on 14 June 1995; **d)** specimen sampled on 29 September 1995, × 250; **e)** specimen sampled on 22 December 1995; **f)** specimen sampled on 27 February 1996. Bars = 100 µm for (a) and 40 µm for (b)-(f). Spg, spermatogonia; Spc, spermatocyte (z, stage zygoten, p, stage pachyten); Spd, spermatid; Spz spermatozoa; Cs: Sertoli cells.

concentrations reached maximum values 1 month before 11KT, which suggests a differential role for these two steroids. Kime and Manning [19] concluded that T may be involved in spermiogenesis, and 11KT in the initiation of spermiation. In Eurasian perch, T would be a biosynthetic precursor to 11KT [3, 34]. The two androgens covaried in male Eurasian perch as in white perch, *Morone americana* [14]. In the striped bass, *M. saxatilis*, declines in the plasma levels of both androgens (T and 11KT) were related to the progression of spermiation in males [32]. Therefore, androgens are probably involved mainly in early spermatogenesis and spermiogenesis and to a lesser extent in spermiation (production of expressible milt), since data for other fishes indicate that total expressible milt remained unchanged in the presence of declining plasma T and 11KT [29]. However, a study on white perch [14] showed that T and 11KT levels were low at the beginning of the spermiation period, then increased throughout that period, and declined before the end of the spermiation period. It is difficult to make a correlation between plasma androgen concentrations and the main phases of the reproductive cycle, because intratesticular steroid levels might be sufficient to mediate changes in the spermatogenetic cycle before levels are high enough to be detected in the serum.

While androgens are involved in the early part of the spermatogenetic cycle in fish, spermatozoa maturation and spermiation could be controlled by progestogens [4, 32, 33]. Some authors reported that progestogens, as 17, 20β-dihydroxy-4-pregnen-3-one (17,20-P), which are responsible for final oocyte maturation in the female of many fishes, could also play an important role in regulating spermiation in fish [33], while the effects of 11KT on spermiation are not considered important in most fishes [3]. However, in some teleosts, 17,20-P does not correlate with spermiation, whereas plasma 11KT levels do [31, 38]. An increase of 11KT has been shown during the first half of the spermiation period in several members of the Moronidae family as in the European sea bass [37], the white bass [31] and the white perch [14].

The presence of spermatozoa in the testes sampled in September, while plasma T levels remained low and GSI was maximal, is not compatible with Kime and Manning's hypothesis [19]. Two possible suggestions could therefore be proposed. First, a very slight increase in plasma T concentration was enough to induce the onset of testis development. Second, spermatozoa observed in testis that were sampled in September, were produced several months ago during the previous reproductive cycle. As testes in September present different stages of spermatogenesis (*figure 3d*) and phagocytosis of spermatozoa by Sertoli cells, which is common in salmonids [39], was not observed in perch, the first hypothesis seems to be more convincing. Some spermatocytes, spermatids or spermatozoa were observed in the two males caught in June. Nevertheless, these two hypotheses must be verified by performing an appropriate sampling strategy, in order to complete the summer data. The second increase in T, observed between February and April 1996, could be related to the beginning of a new spermatogenesis cycle for further reproduction. It would also support the role of T in spermiogenesis in Eurasian perch.

The peak of plasma T detected in our study was higher than the one found in snapper (1.0 ng·mL^{-1}) [5], walleye (< 3 ng·mL^{-1}) [33] and Japanese flounder (0.5 ng·mL^{-1}) [28], but lower than in brown trout (33.2 ng·mL^{-1}) [19], rainbow trout (80 ng·mL^{-1}) [39], bluegill (26 ng·mL^{-1}) [20] and winter flounder (> 25 ng·mL^{-1}) [12]. When plasma T levels peaked in January, spermatozoa, spermatids and spermatocytes were abundant, whereas in February spermatozoa increased in testis and only a few spermatocytes and spermatogonia were observed. This observation could support the role of T in intensifying cellular division and in producing other steroids as a precursor, such as 11KT. Kime and Hews [18] indicated that, in vitro, Eurasian perch testes were able to produce 11-oxygenated androgens (11-oxo and 11β-hydroxytestosterone) from testosterone. Miura et al. [29] reported that 11KT alone is capable of regulating the entire spermatogenetic cycle in Japanese eel (*Anguilla japonica*), and that T has no role other than providing substrate for the production of 11KT.

In February, plasma level of 11KT was maximum, spermatozoa were abundant and almost all sampled fish were spermiating. These data lend support to the hypothesis that 11KT plays a role in completing spermatogenesis [48] but also suggests another role of this androgen in maintaining spermatozoa viability from this period until spawning [27]. A similar profile of plasma 11KT levels was found in walleye, but with a higher peak (30 ng·mL^{-1}) [27]. A greater 11KT peak was also detected in rainbow trout (250 ng·mL^{-1}) [40], bluegill (56 ng·mL^{-1}) [20] and winter flounder (300 ng·mL^{-1}) [12]. However, in Eurasian perch, this peak was two times greater than in yellow perch (< 1.5 ng·mL^{-1}) [8]. For each species, there could be a different threshold value under which steroid has no effect on the reproductive cycle.

The role of 11KT in promoting secondary sexual characteristics, as in bluegill [20] or salmonids [40], could not be verified in Eurasian perch which do not display any sexual dimorphism in external appearance.

5. CONCLUSION

Gonadal recrudescence in male Eurasian perch coincided with the decline in water temperature, and the gonadosomatic index increased very rapidly. Testes development occurred while the levels of T and 11KT in plasma were low or undetectable. Plasma T concentration was maximum in January and spermatozoa became more abundant in February. Plasma 11KT concentrations peaked 1 month later, when all males were spermiating. Male Eurasian perch expressed

flowing milt from January to the spawning period in April. This study confirms the role of androgens in early spermatogenesis and spermiogenesis but cannot make any statement on the role of these two steroids in spermiation, since 17,20-P was not measured. The pre-spawning increase in the T level could be related to a new spermatogenesis cycle in prospect of the further reproductive cycle.

Male Eurasian perch, in contrast to females who need a long chilling phase to develop properly their gonads, are spermiating very early in the annual reproductive cycle as spermatozoa are abundant in September. From an aquaculture perspective, water temperature manipulations could be used to obtain spermiating males, which have previously been reared in stable conditions.

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