

Seasonal Changes in Steroid Metabolism in the Male Reproductive Organ-System of the African Catfish, *Clarias gariepinus*

J.W. RESINK, W.G.E.J. SCHOONEN, R. VAN DEN HURK*, W.J.A.R. VIVEEN¹ and J.G.D. LAMBERT

Department of Experimental Zoology, Research Group for Comparative Endocrinology, University of Utrecht, P.O. Box 80.058, 3508 TB Utrecht (The Netherlands)

¹*Department of Fish Culture and Fisheries, Agricultural University, P.O. Box 338, 6700 AH Wageningen (The Netherlands)*

(Accepted 8 December 1986)

ABSTRACT

Resink, J.W., Schoonen, W.G.E.J., Van Den Hurk, R., Viveen, W.J.A.R. and Lambert, J.G.D., 1987. Seasonal changes in steroid metabolism in the male reproductive organ-system of the African catfish, *Clarias gariepinus*. *Aquaculture*, 63: 59–76.

Steroid and steroid glucuronide synthesis in feral male African catfish was investigated in vitro by incubating testes with [³H]-pregnenolone and seminal vesicles with [³H]-androstenedione. In testes, the capacity to form progestins, androgens, especially 11-oxygenated ones, and steroid glucuronides increased enormously in the periods of full spermatogenesis and breeding, with 5 β -pregnane-3 α ,17 α ,20 α -triol and 5 β -pregnane-3 α ,17 α -diol-20-one glucuronide as the main polar products. In the same periods, seminal vesicles are able to form 11-oxygenated androgens from [³H]-androstenedione and show an increased capacity to form testosterone. Their ability to produce testosterone glucuronide is strongly enhanced in the period of full spermatogenesis and further increases in the breeding period. In this latter period, the capacity to form 5 β -androstane-3 α ,17 α -diol glucuronide is also stimulated. The function of the main steroids and steroid glucuronides is discussed, with special reference to the water-soluble compounds that may serve as sex pheromones.

INTRODUCTION

Nowadays, there is an increasing interest in the role of pheromones in fish reproduction, simple manipulation of reproductive processes such as ovulation and sperm release forming an important motive. Formerly, investigations were restricted mainly to the source of pheromones. Most reports dealing with this topic reveal that in many fish species sex pheromones are emitted by the gonads,

*To whom correspondence should be addressed.

whereas in other species accessory glands are involved (for reviews, see Liley, 1982; Liley and Stacey, 1983; Stacey et al., 1986). In recent years, attention has been directed to the nature of the sex pheromones. Colombo et al. (1980, 1982) were the first to point to steroid glucuronides as chemical messengers for fish. They found that etiocholanolone glucuronide, produced in the mesorchial gland of black goby, *Gobius joso*, had an attractive effect on ovulated females. Van Den Hurk and Lambert (1983) demonstrated that male zebrafish, *Brachydanio rerio*, were attracted by a mixture of conspecific ovarian steroid glucuronides. In this species, ovulation could be induced by a conspecific testicular fraction containing steroid glucuronides, and was absent after β -glucuronidase treatment of this fraction (Lambert et al., 1986; Van Den Hurk et al., 1987b).

In captivity African catfish, *Clarias gariepinus*, do show gametogenesis and production of gonadal steroids, but they neither ovulate nor show spontaneous spawning. Moreover, Schoonen and Lambert (1986a,b) and Schoonen et al. (1987a,b,c) found differences between the steroidogenic capacity of testes and seminal vesicles of feral and pond catfish during the breeding period. Indeed, the absence of spontaneous reproduction under husbandry conditions may be due to the absence of suitable steroidal sex pheromones. On the other hand, recent results of Henken et al. (1987) indicate a possible role of male sex pheromones in stimulating vitellogenesis in laboratory-reared female catfish. Likewise, under experimental conditions male sex pheromones have been found to attract female conspecifics shortly after ovulation; the pheromone in all probability is produced by the seminal vesicle and excreted with the seminal vesicle fluid (Resink et al., 1987a). Thus, it seems that the male gonads, and particularly the seminal vesicles, can produce sex pheromones at various stages of the reproductive cycle.

Looking for the nature of the male pheromones, it is necessary to know the steroidogenic capacity of the testes and the seminal vesicle of feral fish during gonadal recrudescence and the ensuing breeding period (Van Den Hurk et al., 1986; Van Oordt et al., 1987). Enzyme-histochemical studies by Resink et al. (1987b) demonstrated that both in testis and seminal vesicle, steroids are produced by interstitial cells. In vitro experiments, carried out by Schoonen and Lambert (1986a,b) and Schoonen et al. (1987a,b,c) with labeled steroid precursors, have shown that progestins, androgens, including 11-oxygenated ones, and 5β -reduced C21- and C19-steroids are synthesized in testes as well as in seminal vesicle, and that both organs are able to form steroid glucuronides. In the latter respect, the seminal vesicle seemed more important because of its greater capacity to synthesize steroid glucuronides and the greater diversity of glucuronides produced by it.

In the present studies, in vitro incubations were carried out to determine the capacity of testes and seminal vesicles of feral *Clarias gariepinus* to synthesize steroids, and particularly steroid glucuronides, during the annual reproductive

cycle. Based on the results of previous experiments by Schoonen and Lambert (1986a,b) and Schoonen et al. (1987a,b,c), [^3H]-pregnenolone was used as a steroid precursor in incubating testicular tissue, and [^3H]-androstenedione in incubating tissue fragments of seminal vesicles. Material was collected during three different stages of the reproductive cycle, i.e. the resting period, the period of full spermatogenesis and the breeding period (Van Den Hurk et al., 1986; Van Oordt et al., 1987). Histological examination of reproductive organs and measurement of the plasma gonadotropin concentration were carried out to interpret the biochemical data.

MATERIALS AND METHODS

Materials

[7- ^3H]-pregnenolone (9.4 Ci/mmol) and [7- ^3H]-androstenedione (9.2 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, and purity was checked by thin-layer chromatography. Reference steroids were obtained from Steraloids and Makor. Hepes and β -glucuronidase of *E. coli* were obtained from Boehringer, and Leibovitz L-15 medium from Serva. All chemicals and solvents (Baker) were of analytical grade.

Animals

Adult male African catfish, *Clarias gariepinus*, average weight 1.5–2 kg, were caught with a gill net from deep areas of the swamps in the Hula Nature Reserve in Northern Israel in 1984 on 21 January ($n=3$), 7 February ($n=3$), 27 February ($n=3$), 21 March ($n=3$), 10 April ($n=5$), 1 May ($n=3$) and 15 May ($n=3$) and transported to the nearby laboratory. The fish were immediately anaesthetized with 0.1% 2-phenoxy-ethanol and weighed. Blood samples were taken by puncturing the caudal vasculature, using heparinized syringes. After centrifugation (10 min at 800g, 4°C) the plasma was stored at -20°C until gonadotropin (GTH) measurement. After decapitation, testes and seminal vesicles were removed and weighed to determine the gonadosomatic index (GSI) and seminal vesicle somatic index (SVSI), i.e.: $\text{GSI} = (\text{gonadal weight/body weight}) \times 100$; $\text{SVSI} = (\text{seminal vesicle weight/body weight}) \times 100$. Mid-parts of testes and seminal vesicles were used for histological studies and in vitro incubations.

Histological study

Testis and seminal vesicle tissue was fixed in Bouin-Hollande and formol-calcium, respectively. After dehydration, the fixed material was embedded in

paraffin and sectioned at 5 μm . Sections were stained with haemalum-eosin (Burck, 1981).

Incubation procedure

From each fish 0.5 g of minced testes tissue was incubated with 2.1 μCi [^3H]-pregnenolone and 2.0 g of minced seminal vesicle tissue with 2.1 μCi [^3H]-androstenedione. The tissue was added to 2.0 ml Leibovitz-15 medium fortified with 15 mM Hepes (pH 7.6), containing the steroid precursor dissolved in 70 μl propyleneglycol. No co-factors were added. The incubations were carried out at 30°C under continuous shaking for 24 h in the presence of air and stopped by adding 10 ml ethanol.

Extraction

Before extraction, 25 μg of each of the following carriers were added. For testes incubations: pregnenolone, progesterone, 17 α -hydroxyprogesterone, 17 α ,20 β -dihydroxy-4-pregnen-3-one, 17 α ,20 α -dihydroxy-4-pregnen-3-one, 5 β -pregnane-3 α ,17 α -diol-20-one, 5 β -pregnane-3 α ,17 α ,20 α -triol, androstenedione, testosterone, 11 β -hydroxyandrostenedione, 11 β -hydroxytestosterone, 11-ketotestosterone, etiocholanolone, 5 β -androstane-3 α ,17 β -diol, 5 β -androstane-3 β ,17 β -diol, 5 β -dihydrotestosterone, 5 α -androstane-3 α ,17 β -diol; for seminal vesicle incubations: androstenedione, testosterone, 11 β -hydroxyandrostenedione, 11 β -hydroxytestosterone, 11-ketoandrostenedione, 11-ketotestosterone, 5 β -androstane-3,17-dione, etiocholanolone, 5 β -androstane-3 α ,17 β -diol, 5 β -androstane-3 β ,17 β -diol, 5 β -dihydrotestosterone. The free steroids and steroid conjugates were extracted from the tissue by ethanol (3 \times 10 ml). The ethanol-medium mixture was evaporated and the residue was redissolved in water (2.5 ml). Dichloromethane was then added (4 \times 10 ml) to extract the free steroids from the water. The combined dichloromethane extracts were evaporated and subjected to TLC. The water fraction was evaporated and the residue dissolved in 2.0 ml sodium acetate buffer (0.1 M, pH 6.5) and treated with 100 μl β -glucuronidase (100 U/ml) overnight at 37°C under continuous shaking in the presence of air. After addition of the carrier steroids, the steroid moieties of the hydrolyzed steroid glucuronides were extracted with dichloromethane (4 \times 10 ml), evaporated and subjected to TLC.

Identification and quantification

Biosynthesized steroids were separated and identified by TLC in several systems, before and after acetylation (Table 1). The following systems were used: I toluene-cyclohexane (1:1), II benzene-ethylacetate (3:1), III chloroform-ethanol (95:5), and IV ethylacetate-hexane-acetic acid (75:20:5). The

TABLE 1

Identification of products from incubations of testis and seminal vesicle of African catfish

Compound TLC systems ^a	
pregnenolone	I (3×) II (4×) III (2×)
progesterone	I (3×) II (4×)
17 α -hydroxyprogesterone	I (3×) II (4×) III (2×) A ⁻ II (2×)
17 α ,20 β -dihydroxy-4-pregnen-3-one	I (3×) II (4×) III (2×)
17 α ,20 α -dihydroxy-4-pregnen-3-one	I (3×) II (4×) IV (1×)
5 β -pregnane-3 α ,17 α -diol-20-one	I (3×) II (4×) III (2×)
5 β -pregnane-3 α ,17 α ,20 α -triol	I (3×) II (4×) III (2×)
androstenedione	I (3×) II (4×) III (2×)
testosterone	I (3×) II (4×) III (2×) A ⁺ II (2×)
11 β -hydroxyandrostenedione	I (3×) II (4×)
11 β -hydroxytestosterone	I (3×) II (4×) III (2×)
11-ketoandrostenedione	I (3×) II (4×) III (2×) A ⁻ II (2×)
11-ketotestosterone	I (3×) II (4×) IV (1×)
5 β -androstane-3,17-dione	I (3×) II (4×)
etiocholanolone	I (3×) II (4×) III (2×) A ⁺ II (2×)
5 β -androstane-3 α ,17 β -diol	I (3×) II (4×) III (2×)
5 β -androstane-3 β ,17 β -diol	I (3×) II (4×) III (2×) A ⁺ II (2×)
5 β -dihydrotestosterone	I (3×) II (4×) III (2×) A ⁺ II (2×)
5 α -androstane-3 α ,17 β -diol	I (3×) II (4×) III (2×) A ⁺ II (2×)
5 β -pregnane-3 α ,17 α -diol-20-one glucuronide	I (3×) II (4×) III (2×)
testosterone glucuronide	I (3×) II (4×) III (2×) A ⁺ II (2×)
etiocholanolone glucuronide	I (3×) II (4×) III (2×) A ⁺ II (2×)
5 β -androstane-3 α ,17 β -diol glucuronide	I (3×) II (4×) III (2×) A ⁺ II (2×)
5 β -androstane-3 β ,17 β -diol glucuronide	I (3×) II (4×) III (2×) A ⁺ II (2×)
5 β -dihydrotestosterone glucuronide	I (3×) II (4×) III (2×) A ⁺ II (2×)

^aFor explanation of Roman numerals, see text; A⁺ positive acetylation; A⁻ negative acetylation.

final identification was carried out by recrystallization to constant specific activity in earlier studies by Schoonen and Lambert (1986a,b) and Schoonen et al. (1987b,c).

Radioactivity in the different fractions was assayed by using a Searle Analytic 92 Scintillator counter with Xylofluor (organic samples) or Hydroluma (aqueous samples). Quantification of the radioactive areas on the TLC plates was carried out by means of a Berthold thin-layer chromatogram scanner. The final calculation of total yield (pmol/kg body weight) of testicular compounds, corrected for changes in testes weight, was carried out as follows: total yield = (% yield \times incubated total radioactivity \times testes weight) / (incubated testis weight \times spec. act. \times body weight). For total yield of compounds in the seminal vesicle: total yield = (% yield \times incubated total radioactivity \times seminal vesicle weight) / (incubated seminal vesicle weight \times spec. act. \times body weight).

GTH measurement

The gonadotropin (GTH) content in the blood plasma was measured using a homologous radioimmunoassay for catfish GTH according to the method of Goos et al. (1986). The most important characteristics of this assay are its accuracy of 4–8% and its range of 0.4–6.3 ng GTH/ml plasma for 100- μ l samples.

Statistical analysis

The means of individual values are given with the standard error of the mean (SEM). Student's *t*-test was used in comparing the GTH values. Total yield, percentage yield, GSI and SVSI values in the three different periods were analyzed with a Kruskal-Wallis ANOVA. When this ANOVA indicated significant differences between the periods, values were compared with those of fish in the preceding period using a Mann-Whitney U-test (Sokal and Rohlf, 1969).

RESULTS

Morphology and plasma GTH levels

During the resting period, from January to March, mean GTH, GSI and SVSI values were low (Fig. 1). The testes contained small seminiferous tubules with primary spermatogonia and clusters of old sperm cells (Fig. 2A). The seminal vesicles tubules, lined with cylindrical epithelium, had collapsed and contained little secretion (Fig. 3A).

During the period of full spermatogenesis, in April, mean GTH, GSI and SVSI values were significantly higher than in the resting period (Fig. 1). Seminiferous tubules had swollen and contained cysts with secondary spermatogonia, spermatocytes and spermatids (Fig. 2B). The lumen of some tubules contained a few spermatids and ripe sperm cells. Seminal vesicle tubules were wide and filled with secretion (Fig. 3B). The interstitium was stretched and the appearance of the epithelial cells varied from squamous to cylindrical.

During the breeding period, in May, mean GTH, GSI and SVSI values did not differ significantly from those in the period of full spermatogenesis. Seminiferous tubules predominantly contained cysts with spermatocytes and spermatids; their lumina were filled with spermatids and ripe sperm cells. In addition, sperm cells were stored in the seminal vesicle fluid (Fig. 3C). The epithelium of the wide seminal vesicle tubules varied from squamous to cylindrical.

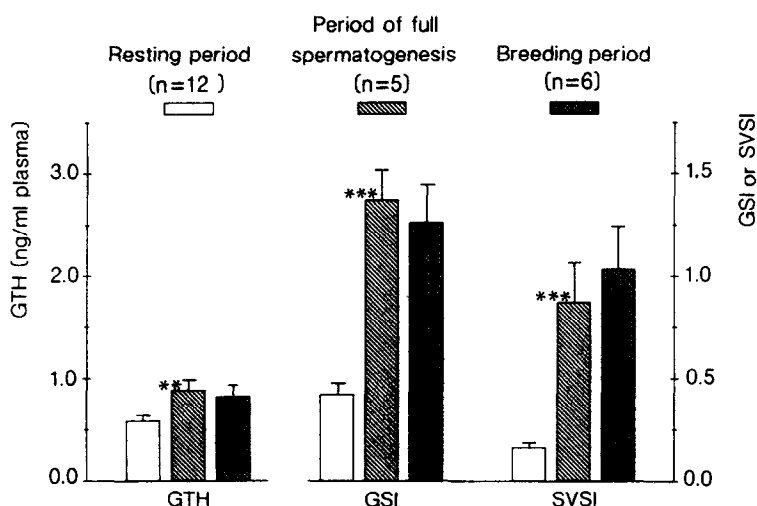


Fig. 1. Gonadotropin level in the plasma (GTH), gonadosomatic index (GSI) and seminal vesicle somatic index (SVSI) of African catfish during the reproductive cycle. Values are means \pm SEM. Data were compared with those of fish in the preceding period. ** $P < 0.01$, *** $P < 0.005$.

Steroidogenesis in the testes

Fourteen steroids and three steroid glucuronides were identified after incubation of testicular tissue with [^3H]-pregnenolone. The percentage yields of these compounds are summarized in Table 2. Our main interest was in the end products which could be considered as physiologically important. Therefore only the compounds synthesized in an amount of more than 3% were taken into consideration. Because of the long incubation time of 24 h and the excess of precursor, the products with a percentage yield less than 3% are considered to be of minor physiological importance or could be intermediates.

From the products with a percentage yield of more than 3%, progesterone, 17α -hydroxyprogesterone, 5β -pregnane- $3\alpha,17\alpha$ -diol-20-one, androstenedione and 5β -pregnane- $3\alpha,17\alpha$ -diol-20-one glucuronide reached their maximal percentage yield in the period of full spermatogenesis. The percentage yields of the latter three compounds remained high during the breeding period, whereas those of progesterone and 17α -hydroxyprogesterone decreased. Yields of 5β -pregnane- $3\alpha,17\alpha,20\alpha$ -triol, 11β -hydroxyandrostenedione and 11β -hydroxytestosterone decreased in the period of full spermatogenesis. The conversion of pregnenolone appeared significantly higher in the breeding period than in the other periods.

The total yields of compounds with a percentage yield of more than 3% are given in Fig. 4. In the resting period, total yields of all products were low; the most important compounds were 5β -pregnane- $3\alpha,17\alpha,20\alpha$ -triol and 11β -

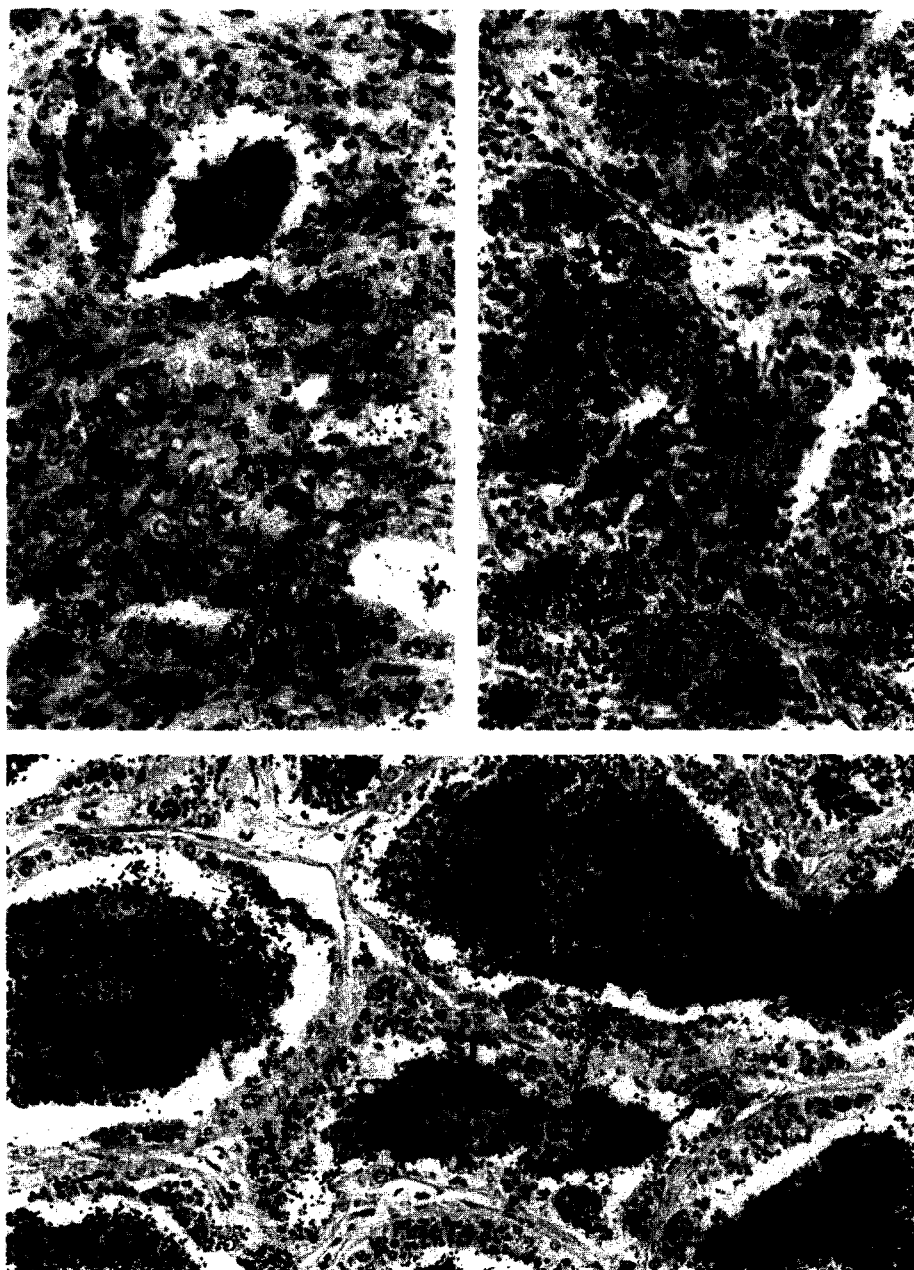


Fig. 2. Cross sections through testes of African catfish. A, resting period; B, period of full spermatogenesis and C, breeding period. Note the narrow seminiferous tubules containing primary spermatogonia (PS) and old sperm cells (OS) in A, the swollen tubules lined with cysts containing secondary spermatogonia (SS), spermatocytes (SC) or spermatids (ST) in B, and the swollen tubules with lumina containing spermatids and ripe sperm cells (SP) in C ($\times 240$).



Fig. 3. Cross sections through seminal vesicles of African catfish. A, resting period; B, period of full spermatogenesis and C, breeding period. Note the small tubules containing little secretion (S) and lined with cylindrical epithelium (CE) in A, the swollen tubules filled with secretion and lined with squamous epithelium (SE) in B, and the ripe sperm cells (SP) in the lumen of the tubules in C ($\times 240$).

TABLE 2

Percentage yields (means \pm SEM) of steroids and steroid glucuronides obtained by incubating testes tissue of African catfish during the reproductive cycle

	Resting (n = 12)	Full spermato- genesis (n = 5)	Breeding (n = 6)
pregnenolone	6.0 \pm 1.4	10.1 \pm 1.4	4.4 \pm 0.6***
progesterone	0.8 \pm 0.3	4.2 \pm 1.1***	1.6 \pm 0.3***
17 α -hydroxyprogesterone	0.3 \pm 0.1	7.5 \pm 2.1***	1.3 \pm 0.3**
17 α ,20 β -dihydroxy-4-pregnen-3-one	1.1 \pm 0.3	2.3 \pm 0.5*	1.7 \pm 0.4
17 α ,20 α -dihydroxy-4-pregnen-3-one	0.7 \pm 0.2	1.3 \pm 0.4*	1.5 \pm 0.5
5 β -pregnane-3 α ,17 α -diol-20-one	1.4 \pm 0.3	6.3 \pm 0.6***	5.8 \pm 0.7
5 β -pregnane-3 α ,17 α ,20 α -triol	15.9 \pm 2.3	8.2 \pm 3.0*	18.2 \pm 1.5*
androstenedione	0.8 \pm 0.2	6.5 \pm 0.8***	5.0 \pm 1.5
testosterone	0.1 \pm 0.1	1.2 \pm 0.2***	2.4 \pm 0.3**
etiocholanolone	0.2 \pm 0.1	1.5 \pm 0.3***	2.5 \pm 0.5
11 β -hydroxyandrostenedione	25.6 \pm 2.8	14.7 \pm 4.3*	19.5 \pm 2.7
11 β -hydroxytestosterone	6.2 \pm 1.3	2.5 \pm 0.3*	6.9 \pm 1.1***
11-ketotestosterone	1.8 \pm 0.6	1.5 \pm 0.7	1.1 \pm 0.2
5 α -androstane-3 α ,17 β -diol	0.1 \pm 0.1	0.6 \pm 0.1***	0.6 \pm 0.2
5 β -pregnane-3 α ,17 α -diol-20-one glucuronide	3.6 \pm 0.4	5.8 \pm 0.8*	3.7 \pm 0.8
testosterone glucuronide	0.3 \pm 0.1	0.3 \pm 0.2	0.7 \pm 0.4
5 β -androstane-3 α ,17 β -diol glucuronide	0.2 \pm 0.1	0.2 \pm 0.1	1.2 \pm 0.3*

Data were compared with those of fish in the preceding period (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$).

hydroxyandrostenedione. In the following period of full spermatogenesis, total yields of all products had distinctly increased. In the breeding period, a further significant increase of the total yields of 5 β -pregnane-3 α ,17 α ,20 α -triol and 11 β -hydroxytestosterone had taken place. Also, the total yield of 11 β -hydroxyandrostenedione had increased during the breeding period, though not significantly. In this period, total yields of progesterone, 17 α -hydroxyprogesterone and androstenedione had decreased, and 5 β -pregnane-3 α ,17 α -diol-20-one and its glucuronide had not changed significantly.

Steroidogenesis in the seminal vesicle

Nine steroids and five steroid glucuronides were identified after incubation of seminal vesicle tissue with [^3H]-androstenedione. The percentage yields of these compounds are shown in Table 3. The products with a percentage yield less than 3% were considered to be of minor physiological importance or could be intermediates.

The percentage yield of 11 β -hydroxytestosterone, and the conversion of the substrate androstenedione, did not change during the reproductive cycle. In the period of full spermatogenesis the percentage yields of free and glucuronidized testosterone increased. They remained at that level until the breeding

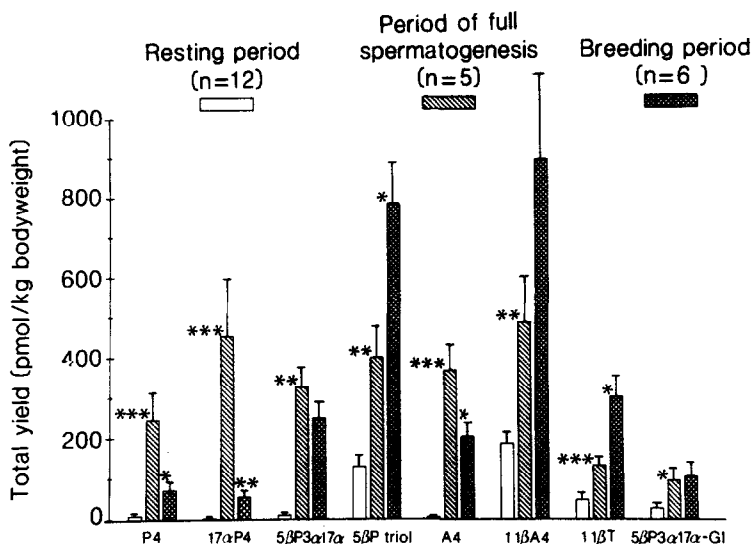


Fig. 4. Total yields of steroids and steroid glucuronides in the resting period, the period of full spermatogenesis and the breeding period, as obtained by incubating testicular tissue of African catfish with [^3H]-pregnenolone. Values are means \pm SEM. Data were compared with those of fish in the preceding period. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$. P4=progesterone, 17 α P4=17 α -hydroxyprogesterone, 5 β P3 α ,17 α =5 β -pregnane-3 α ,17 α -diol-20-one, 5 β P triol=5 β -pregnane-3 α ,17 α ,20 α -triol, A4=androstenedione, 11 β A4=11 β -hydroxyandrostenedione, 11 β T=11 β -hydroxytestosterone, 5 β P3 α ,17 α -Gl=5 β -pregnane-3 α ,17 α -diol-20-one glucuronide.

period. The percentage yields of etiocholanolone and etiocholanolone glucuronide decreased during the period of full spermatogenesis. A continuous decrease from the resting period until the breeding period was also observed for the percentage yields of 11 β -hydroxyandrostenedione and 11-ketotestosterone. The percentage yield of the latter compound was significantly ($P < 0.05$) lower during the resting period as compared with the breeding period. The decrease in percentage yield of 5 β -androstane-3 β ,17 β -diol glucuronide seen during the period of full spermatogenesis was reversed during the breeding period.

The total yields of compounds with a percentage yield of more than 3% are given in Fig. 5. In the resting period, total yields of all products were low. In the period of full spermatogenesis, total yields of testosterone, 11 β -hydroxyandrostenedione, 11 β -hydroxytestosterone, 11-ketotestosterone and testosterone glucuronide had markedly increased, whereas those of etiocholanolone, 5 β -androstane-3 α ,17 β -diol glucuronide, 5 β -androstane-3 β ,17 β -diol glucuronide and etiocholanolone glucuronide had not changed. The most important compounds formed from [^3H]-androstenedione were testosterone and its glucuronide. In the breeding period, a further increase in total yields of these

TABLE 3

Percentage yields (means \pm SEM) of steroids and steroid glucuronides obtained by incubating seminal vesicle tissue of African catfish during the reproductive cycle

	Resting (<i>n</i> = 12)	Full spermato- genesis (<i>n</i> = 5)	Breeding (<i>n</i> = 6)
androstenedione	11.4 \pm 3.8	13.0 \pm 0.6	17.3 \pm 5.3
testosterone	11.1 \pm 2.0	31.0 \pm 2.9***	30.7 \pm 4.6
11 β -hydroxyandrostenedione	9.0 \pm 1.6	7.8 \pm 0.9	2.5 \pm 1.0***
11 β -hydroxytestosterone	3.2 \pm 0.8	3.8 \pm 0.1	3.2 \pm 0.8
11-ketotestosterone	10.1 \pm 1.6	6.1 \pm 1.7	4.5 \pm 1.8
etiocholanolone	5.1 \pm 1.1	1.0 \pm 0.4*	1.4 \pm 0.8
5 β -androstane-3 α ,17 β -diol	2.8 \pm 1.1	0.5 \pm 0.5	2.6 \pm 0.7**
5 β -androstane-3 β ,17 β -diol	1.0 \pm 0.6	0.1 \pm 0.1	0.1 \pm 0.1
5 β -dihydrotestosterone	0.4 \pm 0.2	0.2 \pm 0.2	0.2 \pm 0.1
testosterone glucuronide	7.9 \pm 1.1	14.5 \pm 2.7*	12.9 \pm 2.6
etiocholanolone glucuronide	3.2 \pm 0.5	1.0 \pm 0.3***	0.1 \pm 0.1
5 β -androstane-3 α ,17 β -diol glucuronide	5.7 \pm 0.8	2.4 \pm 0.9*	6.5 \pm 3.5
5 β -androstane-3 β ,17 β -diol glucuronide	3.6 \pm 0.8	0.5 \pm 0.2***	3.0 \pm 2.5
5 β -dihydrotestosterone glucuronide	2.9 \pm 0.6	1.2 \pm 0.2**	1.0 \pm 0.5

Data were compared with those of fish in the preceding period (* P < 0.05, ** P < 0.01, *** P < 0.005).

products and an additional increase in that of 5 β -androstane-3 α ,17 β -diol glucuronide had taken place.

DISCUSSION

In 1984 gonadal recrudescence in male African catfish from the Hula Nature Reserve in Northern Israel started between 21 March and 10 April. The enlargement of the testes during that period, as reflected by an increase in GSI, can be ascribed to a strong spermatogenic activity. The situation corresponds to that described by Van Den Hurk et al. (1986) and Van Oordt et al. (1987) for the same population of catfish. The enlargement of the seminal vesicles was mainly caused by an increase in the amount of fluid in the tubules. Following the period of full spermatogenesis, the African catfish from the Hula Nature Reserve came into breeding condition in May. By that time the testis tubules were filled with large quantities of ripe sperm cells, ready for release during spawning. Sperm cells were also stored within the tubules of the seminal vesicles, a phenomenon recently described by Van Den Hurk et al. (1987a).

In the present study a slight but significant rise in circulating GTH levels was observed in animals collected during the period of full spermatogenesis. This is in agreement with the increase in hormone synthesis, storage and release by the gonadotropic cells during that period, as described by Peute et al. (1986) and Van Oordt et al. (1987). Apparently, this limited rise in GTH output was

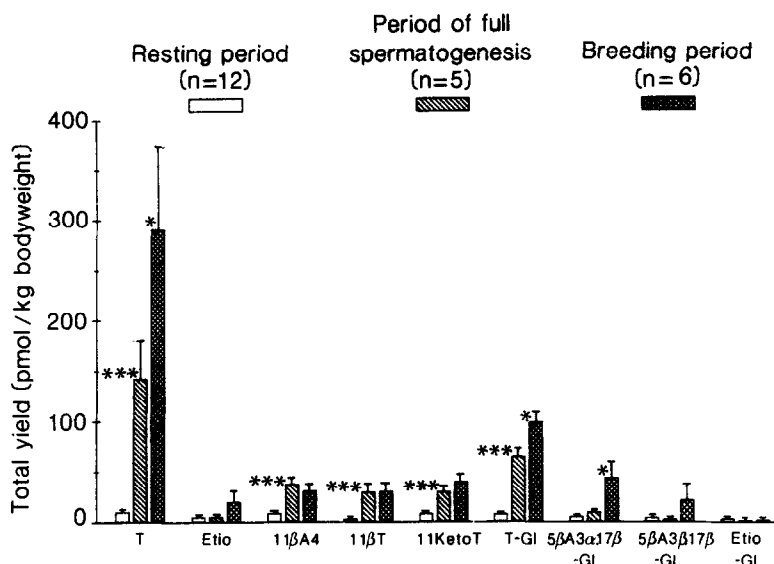


Fig. 5. Total yields of steroids and steroid glucuronides in the resting period, the period of full spermatogenesis and the breeding period, as obtained by incubating seminal vesicle tissue of African catfish with [^3H]-androstenedione. Values are means \pm SEM. Data were compared with those of fish in the preceding period. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$. T = testosterone, Etio = etiocholanolone, 11 β A4 = 11 β -hydroxyandrostenedione, 11 β T = 11 β -hydroxytestosterone, 11KetoT = 11-ketotestosterone, T-Gl = testosterone glucuronide, 5 β A3 α ,17 β -Gl = 5 β -androstane-3 α ,17 β -diol glucuronide, 5 β A3 β ,17 β -Gl = 5 β -androstane-3 β ,17 β -diol glucuronide, Etio-Gl = etiocholanolone glucuronide.

sufficient for the growth of testes and seminal vesicles. Regulation of these processes by gonadotropin has been demonstrated for *Heteropneustes fossilis* (Sundararaj and Nayyar, 1967; Sundararaj et al., 1971; Nayyar et al., 1976). In this catfish species, hypophysectomy resulted in regression of testes and seminal vesicles; replacement therapy with gonadotropins appeared to restore their functional activity.

In the testes of feral African catfish both the percentage yield and the total yield of the progestins progesterone, 17 α -hydroxyprogesterone and 5 β -pregnane-3 α ,17 α -diol-20-one, and of androstenedione strongly increased during the period of full spermatogenesis. The Δ^4 -steroids among them are intermediates in the synthesis of 11-oxygenated androgens. The latter, i.e. 11 β -hydroxyandrostenedione and 11 β -hydroxytestosterone, were seen to increase in total yield during the period of full spermatogenesis and the ensuing breeding period, though not in percentage yield. These biochemical data are in agreement with previous histochemical results by Van Den Hurk et al. (1986), showing a strong increase in steroidogenic activity of the interstitial cells in the testes of *Clarias gariepinus* during the periods of full spermatogenesis and breeding. Although in the testes of the Indian catfish, *Heteropneustes fossilis*,

interstitial cells were not prominent during the period of active spermatogenesis and the ensuing breeding period (Nayyar and Sundararaj, 1970), a correlation between plasma testosterone elevation and growth of testes and seminal vesicles was found by Lamba et al. (1983). A similar correlation was observed in *Ictalurus nebulosus* (Burke et al., 1984). Testosterone, however, is hardly formed in testes of *Clarias gariepinus* during the reproductive cycle. Schoonen and Lambert (1986a) and Schoonen et al. (1987b) also found low yields of testosterone in testes of this species even after incubation with [^3H]-androstenedione. In this catfish, 11 β -hydroxyandrostenedione appears to be one of the main testicular products. When administered intraperitoneally to juvenile male African catfish, it stimulates spermatogenesis and development of the seminal vesicle, whereas testosterone and androstenedione do not have such an effect (Resink and Van Den Hurk, unpublished). 11-Oxygenated androstene derivatives are also particularly important in sustaining the differentiation and early development of the testis in rainbow trout (Van Den Hurk and Lambert, 1982). In adult male sockeye salmon, 11-ketotestosterone appeared to be a potent androgen (Idler et al., 1961).

The capacity to synthesize 11-oxygenated androgens is not restricted to the testes, but was also demonstrated for the seminal vesicles, the total yield of 11 β -hydroxyandrostenedione, 11 β -hydroxytestosterone and 11-ketotestosterone being higher in the periods following the resting period. Unlike the testes, the seminal vesicles showed a strong capacity for the synthesis of testosterone, which continued to increase during the periods of full spermatogenesis and breeding. This hormone together with the testicular androgens may stimulate secondary sex characters and spawning behaviour, and – contrary to the non-aromatizable 11-oxygenated androgens – stimulate gonadotropin synthesis in the pituitary, as demonstrated by De Leeuw et al. (1986) and Van Oordt et al. (1987). The androgens produced by the seminal vesicle might also induce secretory activity of the seminal vesicle epithelium, as suggested by Sundararaj and Nayyar (1969) after treatment of hypophysectomized *Heteropneustes fossilis* with testosterone-propionate. Enlargement of the seminal vesicle after extirpation of the testes in *Clarias gariepinus* (Resink et al., 1987a) may point to an autonomous role of seminal vesicle steroids in this respect.

The rise in progesterone and 17 α -hydroxyprogesterone synthesis was restricted to the period of full spermatogenesis. The two progestins have been demonstrated to stimulate the synthesis of gonadotropin in juvenile rainbow trout (Van Den Hurk, 1982; Van Den Hurk et al., 1984). Possibly these compounds assist in stimulating gonadotropin storage in the pituitary of African catfish and its secretion, as described by Peute et al. (1986) and Van Oordt et al. (1987), for the period of full spermatogenesis. At any rate, in female African catfish 17 α -hydroxyprogesterone can be used to evoke an elevation in plasma gonadotropin levels (Goos et al., 1987).

In agreement with the results of Schoonen and Lambert (1986a,b) and

Schoonen et al. (1987a,b,c), it could be demonstrated that during the breeding period the testes and seminal vesicles of *Clarias gariepinus* show a considerable capacity to synthesize steroid glucuronides. Glucuronidation of steroids has been considered as a deactivation mechanism (Dutton, 1980; Kime, 1982, 1986). Steroid glucuronides, however, have been demonstrated to play an important role as sex pheromones in *Gobiüs jazo* (Colombo et al., 1980, 1982) and the zebrafish, *Brachydanio rerio* (Van Den Hurk and Lambert, 1983; Lambert et al., 1986; Van Den Hurk et al., 1987b). In the African catfish the seminal vesicle has been shown to attract female conspecifics after ovulation (Resink et al., 1987a). The seminal vesicle fluid contains water-soluble steroid conjugates (Schoonen et al., 1987a). This means that such steroid conjugates may be involved in the sex attracting function of the seminal vesicles. Among the steroid glucuronides presently demonstrated in the seminal vesicles, i.e. testosterone glucuronide, 5β -androstane- $3\alpha,17\beta$ -diol glucuronide, 5β -androstane- $3\beta,17\beta$ -diol glucuronide, 5β -dihydrotestosterone glucuronide and etiocholanolone glucuronide, total yield of the first two was markedly stimulated during the spawning season. Thus these two compounds may add to the sex attracting function of the seminal vesicle during this period.

Although the percentage yield of the steroid glucuronides following [^3H]-androstenedione incubation of seminal vesicle tissue was not considerably higher in the breeding period than before that time of year, the total yield of all glucuronides except testosterone glucuronide was very small during the resting period and the period of full spermatogenesis. Thus testosterone glucuronide might contribute to male pheromones during gonadal recrudescence. In this respect, it is of interest that Henken et al. (1987) observed an enhanced vitellogenesis in female *Clarias gariepinus*, cultured together with males, when compared with females from a monosex culture. This may point to the existence of a male "vitellogenic" pheromone. Other constituents of such a vitellogenic pheromone might be the testicular steroid glucuronide 5β -pregnane- $3\alpha,17\alpha$ -diol-20-one glucuronide, and the highly polar steroid 5β -pregnane- $3\alpha,17\alpha,20\alpha$ -triol. A pheromonal role of polar progesterone derivatives has recently been claimed by Stacey and Sorensen (1987) for the goldfish, *Carassius auratus*. The two water-soluble compounds can also be synthesized by the testes during the breeding period and could function as pheromones during that period. Removal of the testes, however, does not seem to diminish the attractiveness of the male for the female after ovulation (Resink et al., 1987a).

ACKNOWLEDGEMENTS

The authors wish to thank Prof. Dr. E.A. Huisman and Prof. Dr. P.G.W.J. Van Oordt for critically reviewing the manuscript, Dr. H.J.Th. Goos for his help in carrying out the GTH radioimmunoassay, Ms. M. van Hattum and Ms. R. van Gelderen for typing the manuscript, the Image Processing and Design

Department of the Subfaculty of Biology for making the drawings, and Dr. M. Gophen, Director of the Kinneret Limnological Laboratory at Tahgba, Israel, for his hospitality.

These investigations were partly supported by the Netherlands Foundation for Technical Research (STW), future Technical Science Branch/Division of the Netherlands Organization for the Advancement of Pure Research (ZWO), and partly by Funds of the Dutch-Israeli *Clarias* Project, granted to Prof. Dr. P.G.W.J. Van Oordt by the Dutch Ministry of Development Cooperation.

REFERENCES

- Burck, H.C., 1981. Histologische Technik-Leitfaden für die Herstellung mikroskopische Präparate in Unterricht und Praxis. Thieme, Stuttgart, New York, NY, 205 pp.
- Burke, M.G., Leatherland, J.F. and Sumpter, J.P., 1984. Seasonal changes in serum testosterone, 11-ketotestosterone and 17 β -estradiol levels in the brown bullhead, *Ictalurus nebulosus* Lesueur. Can. J. Zool., 62: 1195-1199.
- Colombo, L., Marconato, A., Colombo Belvédère, P. and Frisco, C., 1980. Endocrinology of teleost reproduction: a testicular steroid pheromone in the black goby, *Gobius jozo* L. Boll. Zool., 47: 355-364.
- Colombo, L., Colombo Belvédère, P., Marconato, A. and Bentivegna, F., 1982. Pheromones in teleost fish. In: C.J.J. Richter and H.J.Th. Goos (Editors), Proc. Int. Symp. Reproductive Physiology of Fish, Wageningen, The Netherlands, 2-6 August 1982. PUDOC, Wageningen, pp. 84-94.
- De Leeuw, R., Wurth, Y.A., Zandbergen, M.A., Peute, J. and Goos, H.J.Th., 1986. The effects of aromatizable androgens, non-aromatizable androgens and estrogens on gonadotropin release in castrated African catfish, *Clarias gariepinus* (Burchell). A physiological and ultrastructural study. Cell Tissue Res., 243: 587-594.
- De Leeuw, R., Goos, H.J.Th. and Van Oordt, 1987. The regulation of gonadotropin release by neurohormones and gonadal steroids in the African catfish, *Clarias gariepinus*. Aquaculture, 63: 43-58.
- Dutton, G.J., 1980. Glucuronidation of Drugs and other Compounds. CRC Press, Boca Raton, FL, 286 pp.
- Goos, H.J.Th., De Leeuw, R., Burzawa-Gérard, E., Richter, C.J.J. and Terlouw, M., 1986. Purification of gonadotropic hormone from the pituitary of the African catfish, *Clarias gariepinus* (Burchell), and the development of a homologous radioimmunoassay. Gen. Comp. Endocrinol., 62: 162-170.
- Goos, H.J.Th., Richter, C.J.J., Eding, E.H., De Leeuw, R., Scott, A.P. and Van Oordt, P.G.W.J., 1987. The effect of pimoide-LHRHa and 17 α -progesterone on plasma steroid levels and ovulation in the African catfish, *Clarias gariepinus*. Aquaculture, 63: 157-168.
- Henken, A.M., Boon, J.B., Cattel, B.C. and Lobée, H.W.J., 1987. Differences in growth rate and feed utilization between male and female African catfish, *Clarias gariepinus* (Burchell 1822). Aquaculture, 63: 221-232.
- Idler, D.R., Bitners, I.I. and Schmidt, P.J., 1961. 11-Ketotestosterone: an androgen for sockeye salmon. Can. J. Biochem. Physiol., 39: 1737-1742.
- Kime, D.E., 1982. The control of gonadal androgen biosynthesis in fish. In: C.J.J. Richter and H.J. Th. Goos (Editors), Proc. Int. Symp. Reproductive Physiology of Fish, Wageningen, The Netherlands, 2-6 August 1982. PUDOC, Wageningen, pp. 95-98.

- Kime, D.E., 1986. Maturational and temperature effects on steroid hormone production by testes of the carp, *Cyprinus carpio*. *Aquaculture*, 54: 49-55.
- Lamba, V.J., Goswami, S.V. and Sundararaj, B.I., 1983. Circannual and Circadian variations in plasma levels of steroids (cortisol, estradiol-17 β , estrone, and testosterone) correlated with the annual gonadal cycle in the catfish, *Heteropneustes fossilis* (Bloch). *Gen. Comp. Endocrinol.*, 50: 205-225.
- Lambert, J.G.D., Van Den Hurk, R., Schoonen, W.G.E.J., Resink, J.W. and Van Oordt, P.G.W.J., 1986. Gonadal steroidogenesis and the possible role of steroid glucuronides as sex pheromones in two species of teleosts. *Fish Physiol. Biochem.*, 2 (1-4): 101-107.
- Liley, N.R., 1982. Chemical communication in fish. *Can. J. Fish. Aquat. Sci.*, 39: 22-35.
- Liley, N.R. and Stacey, N.E., 1983. Hormones, pheromones and reproductive behavior. In: W.S. Hoar, D.J. Randall, and E.M. Donaldson (Editors), *Fish Physiology*, Vol. IX, Reproduction. Academic Press, New York, NY, pp. 1-63.
- Nayyar, S.K., Keshavanath, P., Sundararaj, B.I. and Donaldson, E.M., 1976. Maintenance of spermatogenesis and seminal vesicles in the hypophysectomized catfish, *Heteropneustes fossilis* (Bloch): effects of ovine and salmon gonadotropin, and testosterone. *Can. J. Zool.*, 54: 285-292.
- Nayyar, S.K. and Sundararaj, B.I., 1970. Seasonal reproductive activity in the testes and seminal vesicles of the catfish, *Heteropneustes fossilis* (Bloch). *J. Morphol.*, 130: 207-226.
- Peute, J., Zandbergen, M.A., Goos, H.J.Th., De Leeuw, R., Pinkas, R., Viveen, W.J.A.R. and Van Oordt, P.G.W.J., 1986. Pituitary gonadotropin contents and ultrastructure of the gonadotrops in the African catfish, *Clarias gariepinus*, during the annual cycle in a natural habitat. *Can. Zool.*, 64: 1718-1726.
- Resink, J.W., Van Den Hurk, R., Groenininx van Zoelen, R.F.O. and Huisman, E.A., 1987a. The seminal vesicle as source of sex attracting substances in the African catfish, *Clarias gariepinus*. *Aquaculture*, 63: 115-127.
- Resink, J.W., Van Den Hurk, R., Voorthuis, P.K., Terlouw, M., De Leeuw, R. and Viveen, W.J.A.R., 1987b. Quantitative enzyme histochemistry of steroid and glucuronide synthesis in testes and seminal vesicle, and its correlation to plasma gonadotropin level in *Clarias gariepinus*. *Aquaculture*, 63: 97-114.
- Schoonen, W.G.E.J. and Lambert, J.G.D., 1986a. Steroid metabolism in the testis of the African catfish, *Clarias gariepinus* (Burchell), during the spawning season, under natural conditions and kept in ponds. *Gen. Comp. Endocrinol.*, 61: 40-52.
- Schoonen, W.G.E.J. and Lambert, J.G.D., 1986b. Steroid metabolism in the seminal vesicles of the African catfish, *Clarias gariepinus* (Burchell) during the spawning season, under natural conditions and kept in ponds. *Gen. Comp. Endocrinol.*, 61: 355-367.
- Schoonen, W.G.E.J., Granneman, J.C.M., Lambert, J.G.D. and Van Oordt, P.G.W.J., 1987a. Steroidogenesis in the testes and seminal vesicles of spawning and non-spawning African catfish, *Clarias gariepinus*. *Aquaculture*, 63: 77-88.
- Schoonen, W.G.E.J., Granneman, J.C.M., Lambert, J.G.D., Viveen, W.J.A.R. and Van Oordt, P.G.W.J., 1987b. Quantitative studies of steroid bioconversions in the seminal vesicles of spawning male African catfish, *Clarias gariepinus* (Burchell), under natural conditions and of non-spawning catfish under natural and fish farm conditions. *Comp. Biochem. Physiol.*, in press.
- Schoonen, W.G.E.J., Lambert, J.G.D., Resink, J.W., Viveen, W.J.A.R. and Van Oordt, P.G.W.J., 1987c. A quantitative study of steroid bioconversions in the testes of the African catfish, *Clarias gariepinus* (Burchell), under natural spawning and natural and cultivated non-spawning conditions. *J. Endocrinol.*, 112: 323-332.
- Sokal, R.R. and Rohlf, F.J., 1969. *Biometry. The Principles and Practice of Statistics in Biological Research*. W.H. Freeman and Company, San Francisco, CA, 776 pp.
- Stacey, N.E. and Sorensen, P.F., 1987. 17 α ,20 β -dihydroxy-4-pregnen-3-one: a steroidal primer pheromone increasing milt volume in the goldfish, *Carassius auratus*. *Can. J. Zool.*, 64: 2412-2417.

- Stacey, N.E., Kyle, A.L. and Liley, N.R., 1986. Fish reproductive pheromones. In: D. Duvall, D. Muller-Schwarze and R.M. Silverstein (Editors), *Chemical Signals in Vertebrates*, Vol. IV, Fourth International Conference on Chemical Signals in Vertebrates, Laramie, WY, 27-30 July 1985. Plenum Press, New York, NY, in press.
- Sundararaj, B.I. and Nayyar, S.K., 1967. Effects of exogenous gonadotrophins and gonadal hormones on the testis and seminal vesicles of hypophysectomized catfish, *Heteropneustes fossilis* (Bloch). *Gen. Comp. Endocrinol.*, 8: 403-416.
- Sundararaj, B.I. and Nayyar, S.K., 1969. Effects of castration and/or hypophysectomy on the seminal vesicles of the catfish, *Heteropneustes fossilis* (Bloch). *J. Exp. Zool.*, 172: 369-384.
- Sundararaj, B.I., Nayyar, S.K., Anand, T.C. and Donaldson, E.M., 1971. Effects of salmon pituitary gonadotropin, ovine luteinizing hormone and testosterone on the testes and seminal vesicles of hypophysectomized catfish, *Heteropneustes fossilis*. *Gen. Comp. Endocrinol.*, 17: 73-82.
- Van Den Hurk, R., 1982. Effects of steroids on gonadotropic (GtH) cells in the pituitary of rainbow trout, *Salmo gairdneri*, shortly after hatching. *Cell Tissue Res.*, 224: 361-368.
- Van Den Hurk, R. and Lambert, J.G.D., 1982. Temperature and steroid effects on gonadal sex differentiation in rainbow trout. In: C.J.J. Richter and H.J.Th. Goos (Editors), *Proc. Int. Symp. Reproductive Physiology of Fish*, Wageningen, The Netherlands, 2-6 August 1982. PUDOC, Wageningen, pp. 69-77.
- Van Den Hurk, R. and Lambert, J.G.D., 1983. Ovarian steroid glucuronides function as sex pheromones for male zebrafish, *Brachydanio rerio*. *Can. J. Zool.*, 61: 2381-2387.
- Van Den Hurk, R., Gielen, J.Th. and Terlouw, M., 1984. Accumulation of glycoprotein gonadotropin in the pituitary of juvenile rainbow trout in response to androgens and C21-steroids, including 11-steroids. *Cell Tissue Res.*, 235: 635-642.
- Van Den Hurk, R., Viveen, W.J.A.R., Pinkas, R. and Van Oordt, P.G.W.J., 1986. The natural gonadal cycle in the African catfish, *Clarias gariepinus*; a basis for applied studies on its reproduction in fish farms. *Isr. J. Zool.*, in press.
- Van Den Hurk, R., Resink, J.W. and Peute, J., 1987a. The seminal vesicle of the African catfish, *Clarias gariepinus*. A histological, enzyme-histochemical, ultrastructural and physiological study. *Cell Tissue Res.*, 247: 573-582.
- Van Den Hurk, R., Van Zoelen, G.A., Schoonen, W.G.E.J., Resink, J.W. and Lambert, J.G.D., 1987b. Do testicular steroid conjugates of zebrafish, *Brachydanio rerio*, evoke ovulation in female conspecifics? *Gen. Comp. Endocrinol.*, 66: 18-19.
- Van Oordt, P.G.W.J., Peute, J., Van Den Hurk, R. and Viveen, W.J.A.R., 1987. Annual correlative changes in gonads and pituitary gonadotropes of feral African catfish, *Clarias gariepinus*. *Aquaculture*, 63: 27-41.