Cyclic changes of steroid production activity in the ovary and estradiol levels in the blood plasma of the mudfish, *Labeo capensis*

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The annual reproductive cycle of female Labeo capensis was investigated using histochemical and endocrinological parameters. It was found that steroid production occurred in three localities in the ovary, namely, special theca cells, granulosa cells and interstitial cells. The 3β-HSD activity (steroid activity) in the granulosa cells and estradiol-17β concentration in the plasma reached a peak during exogenous vitellogenesis. During the oocyte maturation phase the 3β-HSD activity in the special theca cells and the GSI reached a maximum. Possible functions of steroids secreted by special theca cells and granulosa cells are discussed.

Die jaarlikse broeisiklus van vroulike *Labeo capensis* is met behulp van endokrinologiese en histochemiese parameters ondersoek. Steroïedproduksie is in drie lokaliteite in die ovarium aangetref, te wete die spesiale teka-selle, granulosa-selle en interstisiële selle. Steroïedaktiwiteit (3β-HSD-aktiwiteit) in die granulosa-selle en estradiol-17β-konsentrasie in die bloedplasma het 'n maksimum tydens eksogene vitellogenese bereik. Gedurende oösietrypwording het 3β-HSD-aktiwiteit in die spesiale teka-selle asook die GSI-waarde 'n maksimum getoon. Die moontlike funksie van die steroïede wat deur die spesiale teka- en granulosa-selle geproduseer word, word bespreek.

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Labeo capensis is an indigenous freshwater fish which is abundant in the Orange-Vaal River system and can probably be harvested on a commercial scale (Jubb 1967). It is, however, essential that the reproductive cycle of *Labeo capensis* be determined under natural conditions before attempts are made to artificially spawn this species. Aspects of the reproductive cycle, such as the histochemical features of the gonads and the estradiol levels in the plasma which are still lacking, need to be investigated.

Histochemical research showed that steroid hormones which are important for reproduction are formed in the gonads of freshwater fish species such as *Carassius auratus* and *Salmo gairdneri* (Jalabert 1976; van den Hurk & Peute 1979). Steroid production in the ovary was observed in the granulosa cells and/or theca cells of developing and mature oocytes. Post ovulatory follicles as well as interstitial cells are also sources of sex steroids in the ovary (Lofts & Bern 1972; Guraya 1976; Nagahama, Chan & Hoar 1976). The occurrence of steroid production in different cells of the ovary can be related to the different phases of oocyte development (van den Hurk & Peute 1979). Differentiation in gonad development can be monitored in a seasonal study under natural conditions by using parameters such as oocyte development, fluctuation in estradiol-17β concentration, GSI and mean 3β-HSD acitivity in the ovary. The aim of the present investigation was to establish whether an increase in the sex steroid production coincides with gonadal maturation under natural conditions.

Materials and methods

Female mudfish (*Labeo capensis*) were collected in the Vaal River (Transvaal, S.A.) during consecutive months between June 1982 (winter) and May 1983 (autumn). By using gill nets of 110 mm mesh size, only 2,7 to 4-year-old fish were captured. Blood samples from the heart of each individual were immediately taken after capture and centrifuged to obtain the plasma. The plasma was kept on ice for determination of estradiol concentration. The body as well as the gonad mass of each fish were determined after blood sampling was completed. The abovementioned variables were used to determine the gonosomatic index (GSI). The following formula was employed in the calculation of the GSI (%).

$$\frac{\text{gonad mass}}{\text{body mass}} \times 100$$

Radio immuno assay techniques were employed to determine estradiol-17β (E_2) concentration in the blood plasma. A Radioeir Isotopen Service, I125-Estradiol ER 155 test kit, developed for use with human serum, was used. The validity of this technique was confirmed by little or no difference found between the values obtained with the abovementioned kit and the method described by Yaron, Terkatin-Shimony, Shaham & Salzer (1977). The latter was evaluated for cross-reactions with other substances in the plasma of *L. capensis* with no evidence of interference with the specific anti E produced, according to the method of Yaron *et al.* (1977). Histological and histochemical samples for analysis were also taken from the gonads. Samples for histochemical analysis were immediately frozen at -70°C and prepared for determination of 3β-hidroxy steroid dehydrogenase (3β-HSD). Cryo sections (10 μm) were obtained by employing a Reichert-Jung (Cryocut E) cryostat. The Baillie, Calman, Ferguson & Mck. Hart (1966) method, modified by van den Hurk (1973), was used to localize 3β-HSD activity in the ovary. Steroid (3β-HSD) activity in the ovary was quantified in order to establish a

mean numerical value for each month. An activity scale (0–3) of the combined activity within the granulosa, special theca and interstitial cells was employed to quantify 3β-HSD activity.

3β-HSD activity scale (0–3)

0 — No activity; no 3β-HSD activity observed.

1 — Low activity

Few interstitial (ISC) and special theca cells (STC) indicate low to moderate 3β-HSD activity.

2 — Moderate activity

The number of 3β-HSD active special theca and interstitial cells increase. Especially the STC indicate strong activity. The granulosa cells indicate moderate 3β-HSD activity.

3 — Strong activity

Large numbers of the granulosa cells indicate moderate to strong 3β-HSD activity. Strong 3β-HSD activity are indicated in the STC. The colour reaction within the STC is more intense than within the granulosa cells. Rat adrenal gland is used as an indicator for strong 3β-HSD activity (Figure 2A).

The abovementioned activity scale is based on the separate arbitrary grading of 3β-HSD activity in the stroma and granulosa cells of *Salmo gairdneri* (van den Hurk & Peute 1979).

Results

The mean value of the GSI reached a peak of 15,4% during September (Spring). During October and November the GSI remained at a high level after a significant increase from August to September ($P < 0,005$). The GSI value decreased in the summer to a minimum of 1,4% during January ($P < 0,05$). The GSI value gradually increased during autumn and winter (Figure 1).

The estradiol-17β (E_2) concentration reached a minimum of 68,2 pg/ml during December and then gradually increased to 350,8 pg/ml during July with a further distinct increase during August to a maximum value of 633,5 pg/ml ($P < 0,05$). It then decreased considerably ($P < 0,05$) in spring before the minimum value was recorded during December (Figure 1).

During January the lowest mean 3β-HSD activity was observed as 0,7 (activity scale : 0–3). A gradual increase was observed in autumn and winter. The mean 3β-HSD activity reached a peak during August (2,5; activity scale: 0–3) after which a gradual decrease was observed (Figure 1).

Steroid activity was prominent at three localities in the ovary, namely, interstitial cells, special theca cells and granulosa cells. Steroid activity in the interstitial cells was observed throughout the year (Figure 2B). The intensity of 3β-HSD activity in the interstitial cells did not fluctuate much, but the number of 3β-HSD active interstitial cells increased during early oocyte development. The majority of 3β-HSD active interstitial cells were observed during summer and autumn when stages I, II and III oocytes were present in the ovaries in large numbers. Oocyte developmental stages of *L. capensis* were identified according to the stages described for *Ophiocephalus punctatus* by Malhotra, Jyoti & Gupta (1978). Results concerning the stages of oocyte development and the corresponding stage of ovary recrudescence during the different seasons of the breeding cycle will be reported in a separate paper.

In special theca cells 3β-HSD activity was evident throughout the year. The intensity of the steroid activity increased during oocyte development in winter. During early oocyte development, single 3β-HSD active STC were observed but groups of 3β-HSD active STC occurred during late oocyte development (Figures 2D, E and F).

Steroid activity in the granulosa cells was observed at stage IV, V and VI oocytes (Malhotra *et al.* 1978). More granulosa cells showed increasing 3β-HSD activity during late oocyte development. 3β-HSD activity was present in the entire granulosa layer during advanced late oocyte development (Figures 2C, E, F and G).

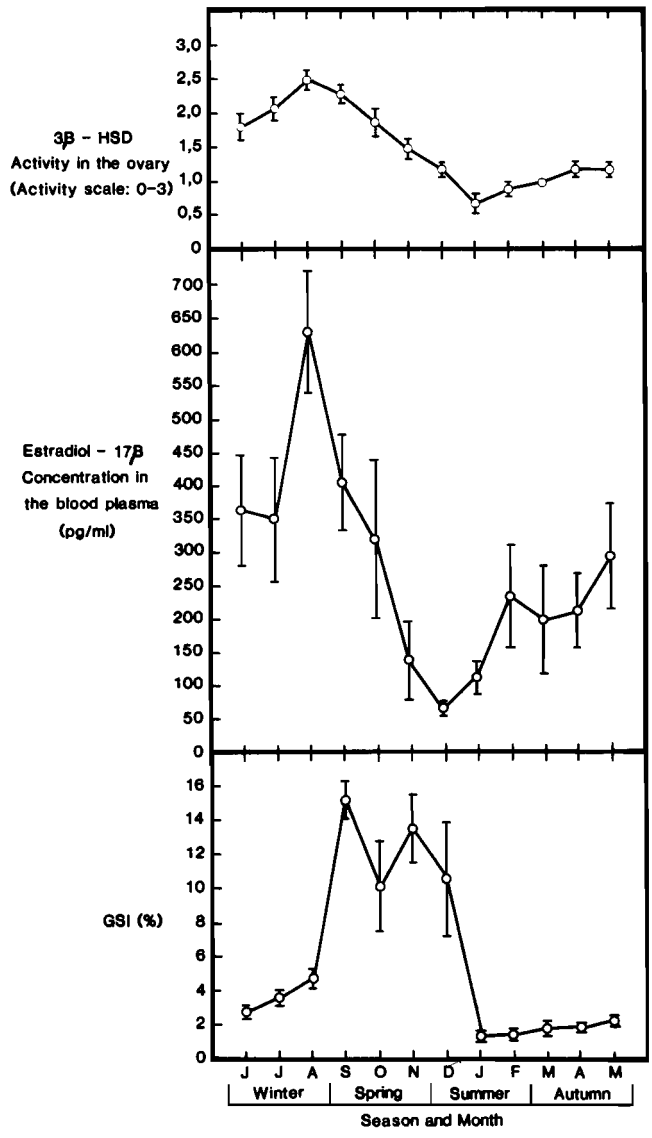

Figure 1 Mean monthly values (± *SE*) for GSI, Estradiol-17β concentration in the blood plasma and 3β-HSD activity in the ovary of *Labeo capensis* ($n = 10$).

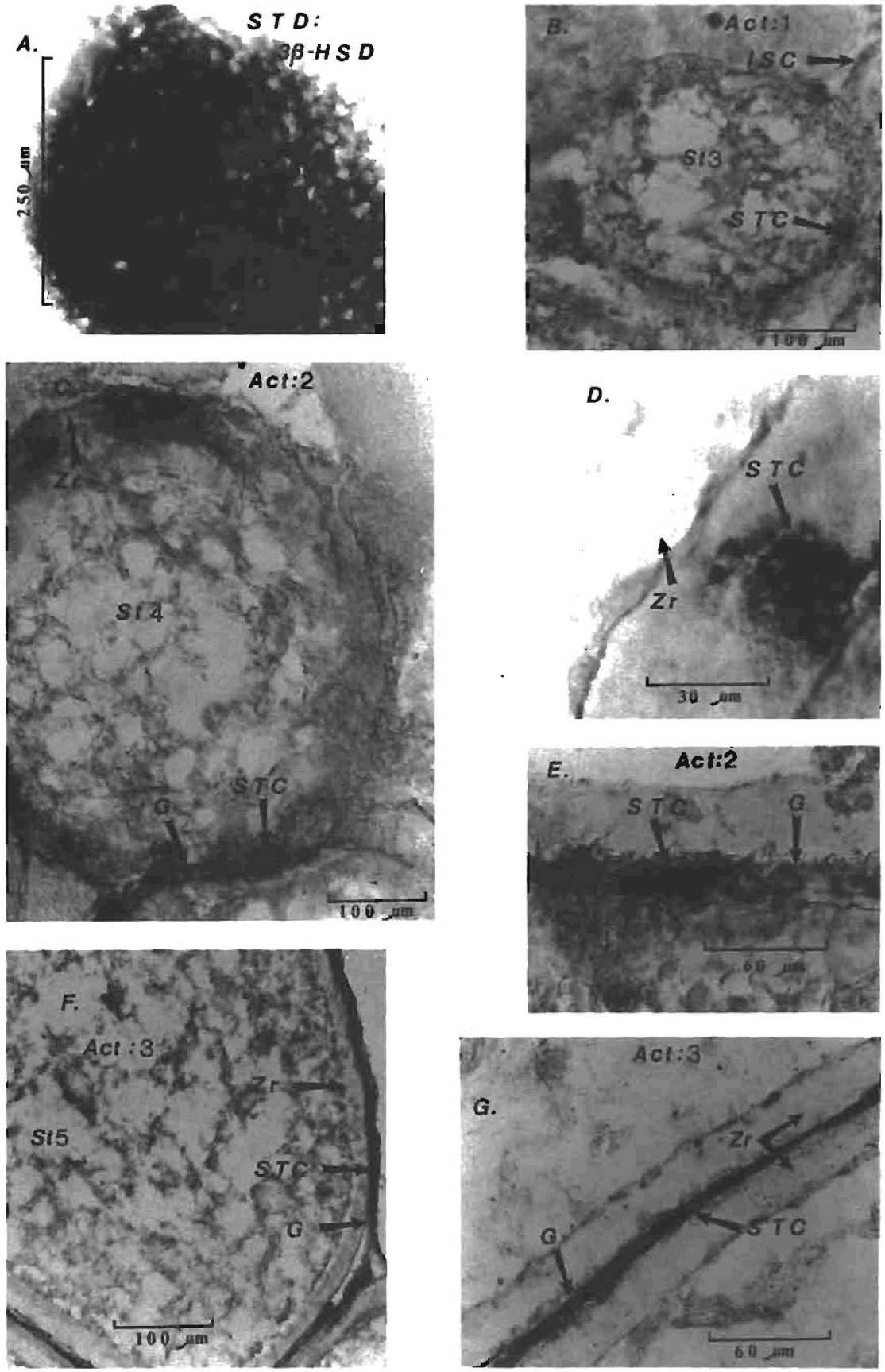

Figure 2 Localizing and quantifying of 3β-HSD activity in the ovary of *Labeo capensis*. **A.** Standard (STD) for high intensity 3β-HSD activity; ×205. 3β-Hydroxy steroid dehydrogenase (3β-HSD) activity in the adrenal gland of the rat. **B.** Activity 1 (Act: 1) ×205. Interstitial cells (ISC); special theca cells (STC); Stage III oocyte (St3). **C.** Activity 2 (Act: 2) ×205. Special theca cells (STC); Granulosa cells (G); Zona radiata (Zr); Stage IV oocyte (St4). **D.** Groups of special theca cells (STC) ×830. Zona radiata (Zr). **E.** Activity 2 (Act: 2) ×410. Special theca cells (STC); Granulosa cells (G). **F.** Activity 3 (Act: 3) ×205. Special theca cells (STC); Granulosa cells (G); Zona radiata (Zr); Stage V oocyte (St5). **G.** Activity 3 (Act: 3) ×410. Special theca cells (STC); Granulosa cells (G); Zona radiata (Zr).

Discussion

Steroid production observed in the granulosa layer, special theca and interstitial cells of the ovary of *L. capensis* clearly proved through intensity variation that steroid production increased or decreased according to the stage of ovarian development. The intensity of 3β-HSD activity (steroid production) in the interstitial cells did not fluctuate much but the number of 3β-HSD active interstitial cells increased during summer and autumn. This serves as an indication that the number of interstitial cells is related to maturation of oocytes. This implies that interstitial cells might produce a steroid which stimulates oocyte maturation (van den Hurk & Peute 1979).

The technique used to establish the localities of steroid production was not developed to quantify steroid concentration or to identify different steroids. Radio immuno assay techniques should be used in further experiments to establish the nature of the steroid observed in each of the described layers. The present results only state that oocyte development as well as ovarian recrudescence takes place under control or in the presence of sex steroids. Therefore, the fluctuations in the estradiol levels, GSI and 3β-HSD activity, presented in the present study (Figure 1) prove that these variables can be used to determine the onset, duration and termination of ovarian development.

The mean 3β-HSD activity reached a minimum value during January (summer), which coincided with the end of the breeding season. It also confirmed that the next breeding phase had not started despite the fact that the interstitial cells already showed increased 3β-HSD activity. The gradual increase in 3β-HSD activity in the special theca cells during autumn and winter occurred in correlation with the increase in GSI and thus ovarian development (Figure 1). The presence of single 3β-HSD active special theca cells during early oocyte development (summer and autumn) in contrast to the groups of 3β-HSD active special theca cells during oocyte maturation leaves the impression that these cells have a specific endocrine function in the final maturation of ova (Figure 2D). This phenomenon was also observed in the ovaries of *Oncorhynchus kisutch* and *Oncorhynchus gorbuscha* (Nagahama, Clarke & Hoar 1978) as well as *S. gairdneri* (van den Hurk & Peute 1979). Special theca cells in the ovary of *S. gairdneri* showed an intensive reaction during the final maturation phase of the oocytes (van den Hurk & Peute 1979).

The GSI value (Figure 1) reached a peak during September while the mean 3β-HSD activity reached a peak during August whereafter a decrease is noticeable. This is due to the fact that final maturation was achieved and spawning occurred. During the spawning phase of the breeding cycle a high concentration of steroids is not necessary with the decrease in 3β-HSD activity in the granulosa cells as a result. Yaron (1971) observed decreasing 3β-HSD activity in the granulosa cells during the last phases of vitellogenesis in *Tilapia nilotica* and *Acanthobrama terrae-sanctae*. In *C. auratus* the 3β-HSD activity in the granulosa cells reached a peak just before vitellogenesis commenced (Khoo 1975). However, steroid production was also observed in the granulosa cells of mature oocytes of *Oryzias latipes* (Iwasaki 1973). This proves that steroid production in oocytes during the breeding cycle is species specific.

Nagahama, Kagawa & Young (1982) suggested that estrogen precursors are produced in the theca cells and are then converted to estradiol-17β in the granulosa layer. In the special theca cells of *L. capensis* intensive 3β-HSD activity occurred in winter and spring which relate to the final maturation phase. It seems that the special theca cells provide the granulosa layer with steroid precursors during vitellogenesis but also produce maturation induction steroids during the final maturation phase.

Van Bohemen & Lambert (1978) found that the increased 3β-HSD activity in the granulosa layer of *S. gairdneri* during vitellogenesis are related to the increase of the estradiol-17β concentration in the blood plasma. A similar increase in estradiol-17β concentration in the blood plasma of *L. capensis* was observed. The highest 3β-HSD activity in the granulosa layer as well as the highest mean 3β-HSD activity occurred during August when the estradiol-17β concentration reached a peak and extensive exogenous yolk (vitellogenin) incorporation occurred. The estradiol-17β concentration and 3β-HSD activity in the granulosa layer decreased during September when the oocytes reached the final maturation phase. Abovementioned simultaneous increase and decrease of estradiol-17β concentration and granulosa layer 3β-HSD activity leads us to conclude that this steroid is probably produced within the granulosa layer of the ovary. Further research is needed to confirm this.

References

BAILLIE, A.H., CALMAN, K.C., FERGUSON, M.M. & Mck.HART D. 1966. Developments in steroid histochemistry. Academic Press, London and New York.

GURAYA, S.S. 1976. Recent advances in the morphology, histo-chemistry and biochemistry of steroid-synthesizing cellular sites in the nonmammalian vertebrate ovary. *Int. Rev. Cytol.* 44: 365–409.

IWASAKI, Y. 1973. Histochemical detection of -3β hydroxy-steroid dehydrogenase in the ovary of medaka, *Oryzias latipes*, during annual reproductive cycle. *Bull. Fac. Fish. Hokkaido Univ.* 23: 177–184.

JALABERT, B. 1976. *In vitro* oocyte maturation and ovulation in rainbow trout (*Salmo gairdneri*), northern pike (*Esox lucius*) and goldfish (*Carassius auratus*). *J. Fish Res. Bd. Can.* 33: 974–988.

JUBB, R.A. 1967. Freshwater fishes of Southern Africa. p. 123. Gothic Printing Company, Cape Town.

KHOO, K.H. 1975. The corpus luteum of goldfish (*Carassius auratus* L.) and its functions. *Can. J. Zool.*, 53: 1306–1323.

LOFTS, B. & BERN, H.A. 1972. The functional morphology of steroidogenic tissues. In: Steroids in non-mammalian vertebrates (ed.) Idler, D.R., Academic Press, New York and London.

MALHOTRA, Y.R., JYOTI, M.K. & GUPTA, K. 1978. Ovarian cycle and spawning season of *Ophiocephalus punctatus*, inhabiting Jammu Waters, India. *Jpn. J. Ichthyol.* 25(3): 190–195.

NAGAHAMA, Y., CHAN, K. & HOAR, W.S. 1976. Histochemistry and ultrastructure of pre- and postovulatory follicles in the ovary of the goldfish, *Carassius auratus*. *Can J. Zool.* 54: 1128–1139.

NAGAHAMA, Y., CLARKE, W.C. & HOAR, W.S. 1978. Ultrastructure of putative steroid-producing cells in the gonads of coho (*Oncorhynchus kisutch*) and pink salmon (*Oncorhynchus gorbuscha*). *Can. J. Zool.* 56: 2508–2519.

NAGAHAMA, Y., KAGAWA. H & YOUNG, G. 1982. Cellular sources of sex steroids in teleost gonads. *Can. J. Fish. Aquat. Sci.* 39: 56–64.

VAN BOHEMEN, Ch. G. & LAMBERT, J.G.D. 1978. Steroidogenesis in the ovary of the rainbow trout, *Salmo gairdneri*. *J. Endocrinol.* 80: 37–38.

VAN DEN HURK, R. 1973. The localization of steroidogenesis in the testes of oviparous and viviparous teleosts. *Proc. Kon. Ned. Acad. Wet. Ser.* C76: 270–279.

VAN DEN HURK, R. & PEUTE, J. 1979. Cyclic changes in the ovary of the rainbow trout, *Salmo gairdneri*, with special reference to sites of steroidogenesis. *Cell Tissue Res.* 199: 289–306.

YARON, Z. 1971. Observations on the granulosa cells of *Acanthobrama terrae-sanctae* and *Tilapia nilotica* (Teleostei). *Gen. Comp. Endocrinol.* 17: 247–252.

YARON, Z., TERKATIN-SHIMONY,A., SHAHAM, Y. & SALZER, H. 1977. Occurrence and biological activity of Estradiol-17β in the intact and ovariectomised *Tilapia aurea* (Cichlidae, Teleostei). *Gen. Comp. Endocrinol.* 33: 45–52.