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Seasonality of the reproductive cycle of female *Heterobranchus longifilis* in tropical pond culture

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Abstract

The aim of this study was to monitor the reproductive cycle of female *Heterobranchus longifilis* under tropical pond conditions in order to optimize controlled spawning. Three experimental groups were set up: The first group (I) consisted of adult females transferred from their natural habitat to ponds. The second experimental group (II) consisted of breeders that adapted to husbandry conditions for at least one year. The third set of females (III) was produced under hatchery conditions. Females of the natural population served as controls. Over a one year period blood samples were taken from the individually marked females on a biweekly basis. If ripe eggs were found at the sampling time, females were stripped, the eggs were fertilized and hatched artificially. None of the feral breeders that were transferred to ponds during their natural breeding season could be stripped during that year. The breeding season of both groups II and III started in February. Seasonal changes in egg output showed a maximum between April and July. Egg production of group III was generally higher. The time interval between two strippings of the same female was 6 weeks. Viable eggs were obtained from the adapted feral breeders until August, whereas the breeding season of the hatchery-raised females extended to October. Two thirds of the hatchery-raised females were stripped four or more consecutive times. Throughout the entire season high hatching rates of normal larvae were obtained. Seasonal profiles of the gonadal steroids testosterone and estradiol-17β reflect the annual changes in sexual activity of female *Heterobranchus longifilis* in tropical pond culture, exhibiting maximum values during the breeding times of each group and being lowest during resting periods.

Keywords: Reproduction, seasonality, *Heterobranchus longifilis*, pond culture, steroids, sex hormones, spawning.

Cycle reproducteur chez la femelle Heterobranchus longifilis *élevée en bassin, en zone tropicale.*

Résumé

Cette étude est réalisée en vue d'analyser le cycle reproducteur chez la femelle *Heterobranchus longifilis* élevée en bassin et en conditions de température tropicale, en vue d'optimiser le contrôle de la ponte. Trois groupes expérimentaux ont été testés: le 1er groupe (I) consiste en femelles adultes sauvages transférées de leur habitat naturel en bassins d'élevage; le 2^{e} groupe expérimental (II) consiste en reproducteurs adaptés aux conditions d'élevage depuis au moins 1 an; le 3^{e} groupe de femelles (III) a été produit en écloserie. Les femelles de la population naturelle ont servi de témoins. Durant un an, des échantillons de sang ont été prélevés sur des femelles marquées individuellement deux fois par mois. Dans le cas où lors des prélèvements, des œufs arrivés à maturité sont trouvés, ces œufs sont alors fertilisés et éclos artificiellement. Aucun des reproducteurs sauvages, qui ont été transférés dans les bassins durant leur saison de reproduction naturelle, ne s'est pas reproduit durant l'année. La saison de reproduction des groupes II et III a commencé en février. Les changements saisonniers pour la production d'oeufs ont été les plus importants entre avril et juillet. La production d'œufs du groupe III a été généralement plus forte. L'intervalle de temps entre deux prélèvements d'œufs a été de 6 semaines pour une même femelle. Les œufs viables ont été obtenus des reproducteurs acclimatés jusqu'en août tandis que la saison de reproduction des femelles élevées en écloserie s'est étendue jusqu'en octobre. Les deux tiers des femelles élevées en écloserie ont été échantillonnés au moins 4 fois consécutivement.

Durant toute la saison, des taux d'éclosion importants de larves normales ont été obtenus. Les profils saisonniers de testostérone et d'estradiol-17β reflètent les changements annuels dans l'activité sexuelle de la femelle *Heterobranchus longifilis* en élevage tropical avec des valeurs maximales durant les périodes de reproduction de chaque groupe et des valeurs plus faibles durant les périodes de repos.

Mots-clés : Reproduction, hormones, stéroïdes, élevage, *Heterobranchus longifilis*.

INTRODUCTION

Due to the rising interest in *Heterobranchus longifilis* for tropical aquaculture the demand for fry and fingerling production of this species is increasing. To overcome the severe shortage in fry supply a fish farm with hatchery and pond facilities was established at the Institute of Oceanography of the University of Calabar, Nigeria. Together with mass production of catfish fry research has been started on several aspects of the biology of *H. longifilis*.

A central problem for continuous fry production is the seasonal character of gonadal activity of female broodstock kept in tropical ponds. This phenomenon has been described for other catfish species as well. Micha (1974) and Hogendoorn (1975) observed discontinuous oogenesis for female *Clarias gariepinus* under tropical pond conditions using breeding stock originating from wild populations. They found induction of gametogenesis outside the natural breeding season (July-October) to be almost impossible. Trying to solve similar problems, Sundararaj and Vasal (1976) did not succeed in overcoming the natural seasonality of the female reproductive cycle of feral Indian catfish by applying light and temperature programs. Richter *et al.* (1987) reported comparable results for female *Clarias gariepinus*: feral broodfish kept under subtropical light periodicity and constant high temperature exhibited a substantially extended, yet still interrupted breeding period. The discontinuity of the ovarian cycle of *Clarias gariepinus* could be prevented by using females that were hatched and reared under constant temperature conditions (Richter *et al.*, 1987). Legendre (1986) obtained viable eggs throughout the year from *Heterobranchus longifilis* breeders reproduced from a wild population under hatchery conditions, although sexual activity decreased distinctively at the beginning of the dry season. This seasonal variations were related to changes in water temperature (Legendre, 1986).

The aim of the present study was to compare the reproductive activity of female *Heterobranchus longifilis*, that were taken from the Cross River in southern Nigeria, with female breeders, hatched and reared in ponds. With the aim to optimize broodstock management and fry production seasonal changes in spawning activity and egg output and corresponding plasma levels of the sex steroids testosterone and estradiol-17β were investigated.

MATERIALS AND METHODS

The pond experiments were carried out between January and December 1993 at the Fish Farm Complex of the Institute of Oceanography, University of Calabar. Calabar is located in the Southwest of Nigeria, at 5° of latitude. Two major climatic seasons determine the weather situation: a rainy season from May to November with a more or less pronounced break in August, and a dry season from December to April. The hormone assays were established and conducted at the Institute of Animal Husbandry and Genetics, University of Göttingen, Germany.

Experimental groups

Mature female *Heterobranchus longifilis*, a freshwater, air-breathing catfish, were divided into three experimental groups: The first group (I) consisted of five adults originating from their natural habitat, the Cross River Delta in southern Nigeria. They were transferred to ponds during their breeding season between March and May 1993 (initial average body weight: 2000 g). The second experimental group (II) consisted of 14 broodfish adapted to husbandry conditions for at least one year. A third set of 15 females (group III) was hatched under artificial conditions and reached sexual maturity in captivity at an age of 17 months. The initial average body weight of the fish in groups II and III was 1750 g and 750 g, respectively. All fish were individually marked. Feral females of the Cross River population served as controls.

Maintenance

Each experimental group was kept together with 3-5 males in a pond of $10 \times 4 \times 1$ m. Water was supplied by a farm-owned borehole. The fish were fed locally compounded feed (12% crude protein) at a daily ration of 3% of their wet body weight.

Sampling of plasma and selection of broodfish

Over a one year period every female was checked for its sexual state on a biweekly basis. If few ripe eggs could easily be stripped by gently pressing the abdomen, the female was chosen for artificial reproduction. At the same time blood samples were

taken by caudal puncture. Blood was mixed with tri-sodium citrate to a final dilution of 0.5%, centrifuged at 1 300 g for 10 minutes. Plasma was stored at −20°C until analysis.

Induced breeding of *Heterobranchus longifilis*

The selected females were transferred to indoor tanks and kept individually at appr. 27°C. Fish were injected with a single dose of 4 mg acetone-dried carp pituitary per kg body weight. Twelve hours after injection the females were stripped. A male (appr. 300 g body weight) with well developed genital papilla was sacrified and the testes were dissected for sperm collection. One ml of sperm was diluted with 4 ml of a 0.9% sodium chloride solution. Three hundred g of eggs were mixed with 5 ml of diluted sperm. Eggs were fertilized by adding water and shaking gently for 2-3 minutes. Fertilized eggs were spread on a matrix of plastic fibres placed in incubation aquaria. They were incubated in a flow-through system at 27°C. Hatching started 24 h after fertilization.

Two hundred-250 eggs of each female served as control to calculate the hatching rate of normal larvae.

The pseudogonadosomatic index (PGSI = weight of egg mass collected by stripping × 100/body weight before stripping, Richter *et al.*, 1987) and hatching percentage (no. of hatched normal larvae × 100/no. of fertilized eggs) were calculated.

Steroid analyses

Microtiter-plate enzyme immunoassays were used to determine plasma levels of testosterone (T) and estradiol-17β (E2). Both assays are competitive assays based on a sensitive double antibody technique (Meyer and Güven, 1986). Details of assay and antiserum characteristics have been published elsewhere (Meyer *et al.*, 1990; Dhesprasith, 1995). Before the testosterone analysis, plasma samples were diluted 1/1 with assay buffer and heated to 75°C for 30 min. The estradiol-17β assay was performed with plasma samples diluted 20 fold in assay buffer, pH 7.2, containing 20% NaCl and heated to 75°C for 30 min. The sensitivity of the assays was 2 pg/ml plasma for testosterone and 30 pg/ml plasma for estradiol-17β, resp. Both these assays were validated by showing that plasma samples dilute parallel to standards and that added hormones were recovered in the correct amount.

Statistical analysis

Data for all parameters are expressed as means ± SE. Statistical comparisons were made using the *t*-test to determine significant differences between data ($p<0.01$).

RESULTS

Seasonal changes in egg production

Female *Heterobranchus longifilis* breeders of group I that had been transferred to ponds during their natural breeding season (April and May) did not reach advanced stages of sexual maturity in pond culture. They could not be stripped during that year and did not show elevated plasma levels of testosterone and estradiol-17β. For this reason only results obtained from females of groups II and III are discussed in this paper.

In female *Heterobranchus longifilis*, caught in Cross River as adult broodfish and kept in outdoor ponds for more than one year (group II), the breeding season started in February/March. At that time four out of 14 females had ripe eggs and responded to carp pituitary injection. Following the season, the PGSI as well as the percentage of ripe females was increasing. The pseudogonadosomatic index reached a maximum in April (5.5 ± 3.1%, *fig.* 1*c*), whereas the number of broodfish sensitive to pituitary treatment was highest in May/June (12 out of 14 fish). The last batch of viable eggs was obtained in August by stripping five females. They produced a considerably lower egg mass per kg body weight. The average time interval between two strippings of the same female ranged from 6 weeks at the beginning and peak of the breeding season to 8 weeks towards its end. One female could be stripped 4 times, five females 3 times, and eight females could be selected only once or twice for induced breeding. No ripe females were found between September and December. Hatching rates of normal larvae after artificial fertilization varied between 67 and 87% and did not exhibit a seasonal pattern.

The breeding season of females hatched and reared under artificial rearing conditions (group III), also began in February/March. In contrast to group II all females reached sexual maturity and 14 out of 15 responded to pituitary induction. Eight weeks later all females could be stripped once more, and after another 6 weeks almost all brooders were gravid again (*fig.* 2*c*). The PGSI was highest in June (8.3 ± 2.5%). Thereafter the PGSI as well as the percentage of females responding decreased until late October. Females failed to have ripe eggs in November and December. During the entire spawning season the females of this group always reached a higher PGSI compared to the adapted feral breeders of group II. Ten out of 15 broodfish could be stripped four or more consecutive times with an average cycle duration of 6 weeks. Throughout the season high percentages of normal larvae (75 ± 7%) were obtained.

Seasonal changes in plasma testosterone and estradiol-17β levels

Measurement of plasma testosterone concentrations revealed an increase in females of both groups II

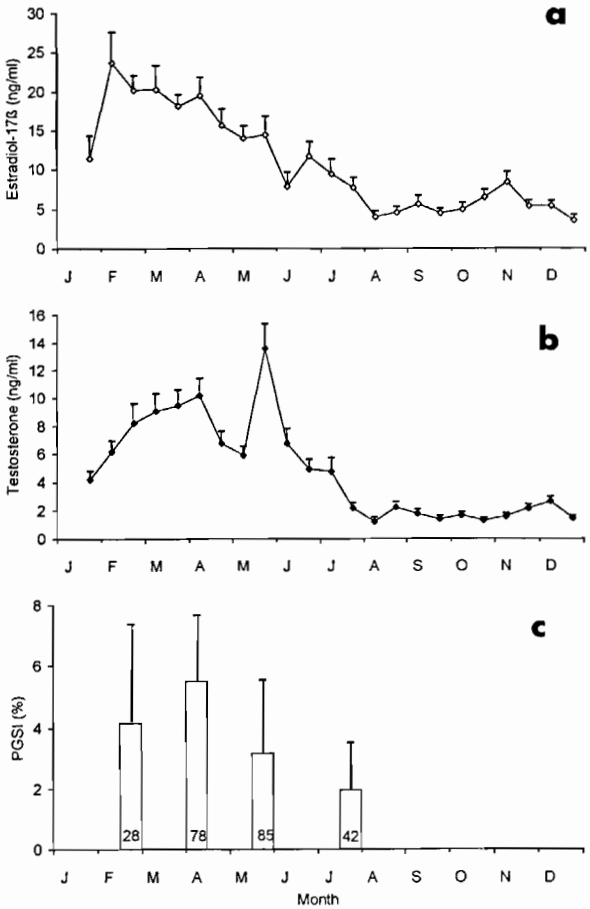

Figure 1. – Seasonal changes in pseudogonadosomatic index (PGSI, c) in relation to the seasonal pattern of plasma concentrations of testosterone (b) and estradiol-17β (a) in adapted feral breeders of female *Heterobranchus longifilis* (group II). Mean values for PGSI are calculated and summarized corresponding to the average cycle duration. The percentage of fish that could be reproduced is given inside the PGSI bars (Mean + SE).

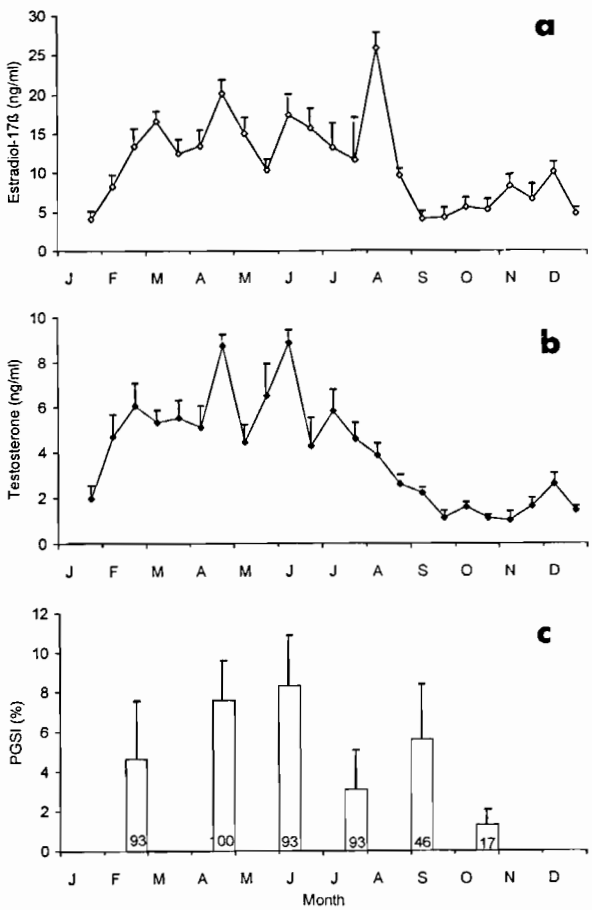

Figure 2. – Seasonal changes in pseudogonadosomatic index (PGSI, c) in relation to the seasonal profile of plasma concentrations of testosterone (b) and estradiol-17β (a) in hatchery-raised females of *Heterobranchus longifilis* (group III). Mean values for PGSI are calculated and summarized corresponding to the average cycle duration. The percentage of females that could be reproduced is indicated inside the PGSI bars (Mean + SE).

and III at the beginning of the year associated with the onset of the breeding season. During the main breeding season between March and July the testosterone values in the adapted feral breeders (group II) were significantly higher than during the resting period between September and December ($p<0.01$, *fig.* 1*b*). Maximum testosterone concentrations coincided with the maximum breeding activity in this group, *i.e.* with the highest PGSI and the highest percentage of females that responded to carp pituitary injection. After the maximum in May, which was also the highest testosterone level reached (13.6 ng/ml), the concentration was decreasing to low levels (<2 ng T/ml plasma) in August and thereafter.

Cultivated females of group III exhibited a pattern of plasma testosterone that was similar to that of the females of group II (*fig.* 2*b*). Again, the testosterone concentrations were significantly higher during the main breeding season between February and August than during the resting phase from late October till December with one exception in early December ($p<0.01$). Maximum testosterone levels were reached in late April (8.7 ng/ml) and early June (8.9 ng/ml), which are associated with the reproductive activity. Plasma testosterone concentrations decreased gradually to basal levels after August, reaching 2 ng/ml or less in October, although the brooders still showed considerable breeding activity in August and late October.

Females of both groups II and III exhibited a slight increase in testosterone concentrations in December.

Plasma estradiol-17β concentrations indicated a seasonal pattern in both groups. E2-levels in adapted breeders were highest at the beginning of the breeding season in February/March (24 ng E2/ml), thereafter until August a steady decline in estradiol values was observed (levels <5 ng/ml, *fig.* 1*a*). A different profile of estradiol-17β was evident in females hatched and reared in captivity (group III, *fig.* 2*a*). Levels

rose from January to April and remained high until August. Afterwards they decreased to basal level. There appeared to be a slight elevation of estradiol concentrations in December in both groups II and III.

In some females a remarkable temporal synchrony of circannual variation in plasma levels of the two gonadal steroids was observed (see for example *fig.* 3). Peaks of testosterone and estradiol-17β occurred simultaneously and coincided with the time of artificial reproduction. However, a correlation between steroid values and the pseudogonadosomatic index was not found.

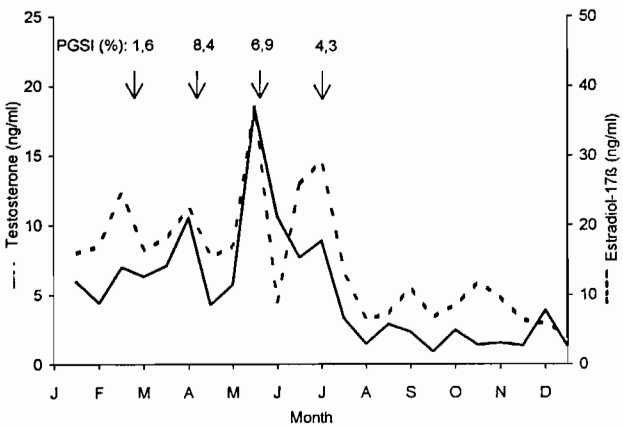

Figure 3. – Seasonal changes in concentrations of testosterone and estradiol-17β in a typical *Heterobranchus longifilis* female (group II, adapted feral breeders). Arrows indicate times of induced spawning. The pseudogonadosomatic index (PGSI) for each spawning time is given above the arrows.

DISCUSSION

Females of *Heterobranchus longifilis* maintained in pond culture a distinct seasonality in their reproductive activity. Circannual variations seemed to be correlated with climatic changes, *i.e.* rainy and dry season.

Oocytes of good quality were first obtained in early March. Both adapted feral and cultivated breeders reached their maximum breeding activity during the main rainy season in May-July. This timing is similar to the natural annual variation found in other catfish species. Gonadal growth in *Heteropneustes fossilis* is completed at least a month before the normal flood season (Vasal and Sundararaj, 1976), which also applies to clariids that mature well in advance of their usual spawning time (Willoughby and Tweddle, 1978). At lower latitudes, *Clarias gariepinus* and other African Clarias sp. spawn mainly in flooded areas during the rainy season (Bruton, 1979). The rainy season seems to be the primary reproductive season for *H. longifilis*, too. In the Cross River, fully matured females were found only in June after a rise in water level (data not shown). Similar to the findings of Micha (1976) the breeding season of the adjusted feral breeders was terminated in August after a decline in both the number of females responding to carp pituitary induction and the quantity of eggs collected. Unlike the adjusted breeders, spawning of the cultivated females could be successfully induced until late October. In contrast to the results of Legendre (1986) no viable eggs were obtained during the dry season in November, December and January. Thus, *H. longifilis* females showed an ovarian resting period, regardless of their origin. The ovarian cycle seems to be regulated by a circannual rhythm which also applies to fish kept in pond culture. However, postponing the ovarian regression phase by using cultivated females increases the productivity of the brood stock due to several repeated spawnings. Up to six spawns per female and year were obtained. The quick recovery of oogenesis after hormonal treatment (about six weeks) is comparable to results published earlier for *H. longifilis* by Legendre (1986) (8 weeks), *H. fossilis* by Sundararaj and Vasal (1976) (4 weeks) and *C. gariepinus* by Hogendoorn and Vismans (1980) and Richter *et al.* (1987) (3-6 weeks). Continuous repeated artificial induction of spawning had no effect on egg production or egg quality.

Plasma concentrations of the two steroid hormones measured in this study showed corresponding seasonal profiles. The observed maximum plasma testosterone values coincided with the times of induced spawning on the one hand and with the higher serum estradiol titres on the other hand. The role of testosterone as a possible precursor of estradiol-17β (Lambert *et al.*, 1971) and its function in maintaining sexual behavior during ovulation and spawning (Scott *et al.*, 1983; Lamba *et al.*, 1983) explains this pattern. Estrogens are implicated in the stimulation of vitellogenesis in the liver (van Bohemen *et al.*, 1981). Therefore a preovulatory estradiol peak is evident in many teleosts. This peak is followed by decreasing E2-levels towards the final stages of maturation, possibly providing the stimulus for rapid increase in gonadotropin (Singh and Singh, 1983). It is this GTH surge which seems to be required for oocyte maturation and ovulation under natural conditions (Donaldson and Hunter, 1983). Elevated levels of plasma estradiol-17β during the breeding season of *Heterobranchus longifilis* could be an indication for repeated vitellogenesis in the females, connected with consecutive ovarian cycles and repeated induced spawning. Estradiol maximum occurred mostly concomitantly with maximum testosterone levels. Absence of an E2-decrease prior to spawning might be an indication of the lack of ability of farmed *H. longifilis* to mature and ovulate spontaneously. In connection with their extended breeding season cultivated breeders maintain slightly higher testosterone concentrations and elevated levels of plasma estradiol in July and August, but not until the end of the reproductive season. MacKenzie *et al.* (1989) reported decreasing T-concentrations in prespawning pond-cultured channel catfish (*Ictalurus punctatus*). Burke *et al.* (1984) found elevated

E2-values after peak serum testosterone levels in spawning brown bullhead (*Ictalurus nebulosus*).

This study provides new information on the reproductive biology of female *Heterobranchus longifilis* in pond culture. Seasonal profiles of the gonadal steroids testosterone and estradiol-17β reflect the annual changes in egg production. A resting period could not be prevented by using hatchery reared broodstock. However, due to the possibility to induce several spawns per year and good hatching rates high fecundity may be achieved in cultivated females. Throughout the entire season more than 1 million healthy larvae could be produced with a relatively small-sized broodstock.

REFERENCES

Bruton M. N. 1979. The breeding and early development of *Clarias gariepinus* (Pisces, Clariidae) in Lake Sibaya, South Africa, with a review of breeding in species of the subgenus *Clarias* (*Clarias*). *Trans. Zool. Soc. London* **35**, 1-45.

Burke M. G., J. F. Leatherland, J. P. Sumpter, 1984. Seasonal changes in serum testosterone, 11-ketotestosterone, and estradiol-17β in the brown bullhead, *Ictalurus nebulosus* Lesueur. *Can. J. Zool.* **62**, 1195-1199.

Dhesprasith D. 1995. Plasma oestradiol-17β and testosterone patterns of female rainbow trout (*Oncorhynchus mykiss*) exposed to different photoperiods. Ph.D. thesis, University of Göttingen, Germany (in press, in English).

Donaldson E. M., G. A. Hunter 1983. Induced final maturation, ovulation and spermiation in cultured fish. In: Fish Physiology. Vol. IXB, Reproduction. W. S. Hoar, D. J. Randall, E. M. Donaldson ed. Academic Press, New York, 351-403.

Hogendoorn H., M. M. Vismans 1980. Controlled propagation of the African catfish, *Clarias lazera* (C. & V.). II. Artificial reproduction. *Aquaculture* **21**, 39-53.

Hogendoorn H., R. Wieme 1975. Preliminary results concerning the culture of *Clarias lazera* in Cameroon. CIFA/75/SE10, FAO/CIFA Symposium on Aquaculture in Africa.

Lamba V. J., S. V. Goswami, B. I. Sundararaj 1983. Circannual and circadian variations in plasma levels of steroids (cortisol, estradiol-17β, and testosterone) correlated with the annual gonadal cycle in the catfish, *Heterobranchus fossilis* (Bloch). *Gen. Comp. Endocrinol.* **50**, 205-225.

Lambert J. G. D., J. H. M. Thijssen, E. V. Eylath 1971. Conversion of ^{3}H-androstendione and ^{3}H-testosterone to ^{3}H-oestradiol-17β *in vitro* by ovary of the guppy, *Poecilia reticulata. Proc. K. Ned. Akad. Wet.* **74**, 52-59.

Legendre M. 1986. Seasonal changes in sexual maturity and fecundity, and HCG-induced breeding of the catfish, *Heterobranchus longifilis* Val. (Clariidae), reared in Ebrie Lagoon (Ivory Coast). *Aquaculture* **55**, 201-213.

MacKenzie D. S., P. Thomas, S. M. Farrar 1989. Seasonal changes in thyroid and reproductive steroid hormones in female channel catfish (*Ictalurus punctatus*) in pond culture. *Aquaculture* **78**, 63-80.

Meyer H. H. D., H. Sauerwein, B. M. Mutayoba 1990. Immunoaffinity chromatography and a biotin-streptavidin amplified enzyme immunoassay for sensitive and specific estimation of estradiol-17β. *J. Steroid Biochem.* **35**, 263-269.

Meyer H. H. D., B. Güven 1986. Improvement of microtitration plate enzyme immunoassays for steroid determination by a second antibody technique. *J. Steroid Biochem.* **25** (Suppl.), 139-141.

Micha J. C. 1974. Fish populations study of Ubangui River: trying local wild species for fish culture. *Aquaculture* **4**, 85-87.

Richter C. J. J., W. J. A. R. Viveen, E. H. Eding, M. Sukkel, A. J. Rothuis, M. F. P. M. van Hoof, F. G. J. van den Berg, P. G. W. J. van Oordt 1987. The significance of photoperiodicity, water temperature and an inherent endogenous rhythm for the production of viable eggs by the African catfish, *Clarias gariepinus*, kept in subtropical ponds in Israel and under Israeli and Dutch hatchery conditions. *Aquaculture* **63**, 169-185.

Scott A. P., J. P. Sumpter, P.A. Hardiman 1983. Hormone changes during ovulation in the rainbow trout, *Salmo gairdneri* R. *Gen. Comp. Endocrinol.* **49**, 128-134.

Singh I. J., T. P. Singh 1983. Annual changes in the total gonadotropic potency in relation to gonadal activity in the freshwater catfish, *Clarias batrachus* (L.). *J. Interdiscipl. Cycle Res.* **14**, 227-239.

Sundararaj B. I., S. Vasal 1976. Photoperiod and temperature control in the regulation of reproduction in the female catfish, *Heterobranchus fossilis. J. Fish. Res. Board Can.* **33**, 959-973.

Vasal S., B. I. Sundararaj 1976. Response of the ovary of the catfish, *Heteropneustes fossilis* (Bloch), to various combinations of photoperiod and temperature. *J. Exp. Zool.* **197**, 247-264.

Van Bohemen C. G., J. G. D. Lambert 1981. Estrogen synthesis in relation to estrone, estradiol, and vitellogenin plasma levels during the reproductive cycle of the female rainbow trout, *Salmo gairdneri. Gen. Comp. Endocrinol.* **45**, 105-114.

Willoughby N. G., D. Tweddle 1978. The ecology of the catfish *Clarias gariepinus* and *Clarias ngamensis* in the Shire Valley, Malawi. *J. Zool.* **186**, 507-514.