Annual Correlative Changes in Gonads and Pituitary Gonadotropes of Feral African Catfish, *Clarias gariepinus*

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ABSTRACT

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The reproductive cycle of male and female African catfish (*Clarias gariepinus*) can be divided into a breeding period (May-August), a resting period (September-February) and a period of full gametogenesis (March-April). The pituitary gonadotropin (GTH) content and the ultrastructure of the gonadotropes largely parallel the cyclical changes in the gonads. The main characteristics of the pituitary GTH cycle are a prespawning GTH surge and a postspawning regression of the gonadotropes. These phenomena are suppressed when African catfish are kept under favourable husbandry conditions. Under such circumstances the pituitary stores large amounts of GTH and shows a limited, continuous secretion of the hormone, sufficient for a sustained gametogenesis and gonadal steroid production, but not for spontaneous spawning. Under such adequate fish culture conditions, ovulation can be induced and healthy larvae obtained at any time of year.

INTRODUCTION

Studies on reproductive physiology of fish have often been carried out on laboratory animals, kept under standardized, artificial conditions. Recently, at the Symposium on Reproductive Physiology of Fish in Wageningen, The Netherlands, Billard (1982) stated, however, that "there is now a trend toward studying the animal in its natural environment, where it is submitted to a larger variety of environmental factors". In nature these environmental factors largely determine the timing of reproduction and thus the reproductive

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strategy of a species (Fontaine, 1976; Wootton, 1982). Therefore, knowing the ecology of a species is an essential basis for the study of its reproductive physiology (Scott, 1979) as well as for adapting it to fish culture (Billard, 1982).

For the African catfish, *Clarias gariepinus*, the environmental factors have for several years been the subject of ecological studies by Bruton (1979). The reproductive physiology of *Clarias gariepinus*, and especially the endocrine regulation of reproduction, are being investigated by the Utrecht Group for Comparative Endocrinology (Van Oordt, 1986), and the adaptation of the species for fish culture by the Department of Fish Culture and Fisheries at Wageningen (Hogendoorn, 1983; Viveen et al., 1985). As a basis both for the physiological and the applied studies it was necessary to know the cyclical events taking place under natural conditions in the gonads and in the gonadotropic cells of the pituitary, directly stimulating gametogenesis and steroid production in the gonads. To that end, African catfish were collected from their natural habitat in Northern Israel during one year. The gonads were studied histologically and enzyme-cytochemically. In the pituitary the ultrastructure of the gonadotropes was investigated as well as their gonadotropin content. The results have been the subject of two recent publications (Van Den Hurk et al., 1986; Peute et al., 1986). The present report combines these two papers into a review of the data in annual correlative changes in gonads and pituitary gonadotropes of feral African catfish.

MATERIALS AND METHODS

Adult male and female African catfish were obtained at two locations in Northern Israel, i.e. in the Hula Nature Reserve and in Lake Kinneret. From July 1981 up to mid-November 1981 the fish were caught in the Hula Nature Reserve, by means of a gill net that was pulled through the water and through the mud of the shallow ponds. During a dry spell in the last weeks of this period, fish could be collected from these ponds by hand.

From mid-November 1981 to mid-February 1982 fish were obtained from Lake Kinneret, because in that period entering the Nature Reserve would have disturbed the migrating birds visiting the ponds. The fish in the lake were caught by means of a purse seine. From mid-February 1982 up to June 1982 the collection of fish in the Hula ponds was authorized again.

Water temperatures during this annual cycle varied from 11 to 30°C, with the lowest temperatures recorded from November to March (11–15°C in the Hula ponds and 16–19°C in Lake Kinneret). Temperatures in the Hula ponds increased in March (18°), April (20–22°C), May (25°) and June/July/August (27–28°). During the breeding season (May–August) the mean temperatures were 25–28°C. From October onwards (20°C) the temperature decreased markedly to 11°C in January.

At autopsy, length and weight of the fish were measured and external data

on the feeding conditions and the gonads were recorded. After dissection, the gonads were weighed and the gonadosomatic index (GSI = gonad weight in $g \times 100$/body weight in g) was established. Subsequently, mid parts from testes and ovaries were either fixed and embedded in paraffin or quickly frozen with CO_2 (for detailed information, see Van Den Hurk et al., 1986). The paraffin sections were used for studying the histological structure of the gonads; cryosections were used to demonstrate and locate the presence of the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD).

After dissecting the skull, the pituitary was removed and either stored in acetone for measurement of the gonadotropin contents or processed for the electron microscopical study of the gonadotropin-producing cells (for detailed information, see Peute et al., 1986). The gonatropin contents were measured with a homologous radioimmunoassay (RIA) according to Goos et al. (1986).

RESULTS

Periodicity of testicular development

The male reproductive organ, situated in the dorsocaudal region of the body cavity, consists of paired testes, an unpaired seminal vesicle and a sperm duct. Light microscopical studies of the testes made it possible to divide the annual reproductive cycle in male African catfish into three periods, i.e. the breeding period, the resting period and the period of full gametogenesis.

The breeding period lasts from May until August and is characterized by a relatively high but decreasing GSI (Fig. 1). During this period, males can be found in a prespawning, spawning or a postspawning stage of testicular development. The prespawning stage is characterized by a strong spermatogenic activity. The testis tubules are lined with cysts containing spermatogonia B and primary spermatocytes. Often cysts with secondary spermatocytes and spermatids are present at this stage. The lumen of the seminiferous tubules is relatively small and either lacks spermatogenic material or contains a few spermatids or sperm cells (Fig. 2). The Leydig cells show a moderate 3β-HSD activity (Table 1). During the spawning stage, the lumen of the seminiferous tubules is large and mainly contains newly formed sperm cells. The wall of the tubules is lined by a narrow and discontinuous band of spermatogenic cysts, and the 3β-HSD activity in the Leydig cells is at its maximum.

The prespawning and the spawning stages of testicular development seem to alternate, particularly in May and June, the actual spawning period. Therefore, it is not unlikely that the catfish of the Hula Nature Reserve spawn several times during that period.

The postspawning stage is reached at the end of the breeding period, when spermiation and spawning are not followed by the rapid production of new sperm cells. Spermatogenic cysts are lacking, and the wall of the tubules is
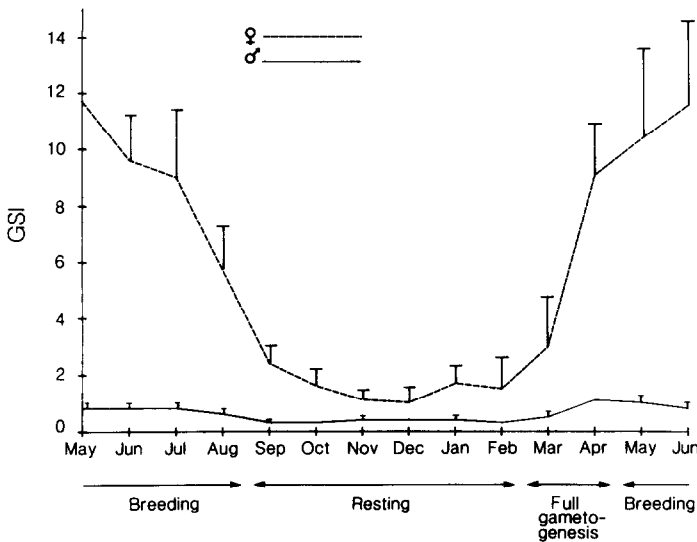
Fig. 1. Gonadosomatic index (GSI = gonadal weight in g × 100/body weight in g) of male and female catfish during one annual cycle.

lined mainly by Sertoli cells and a few spermatogonia A. The Leydig cells have a moderate 3β-HSD activity.

The resting period begins in August and ends in March. During that period the GSI is relatively low (Fig. 1) and the males are in a postspawning or a preparatory stage of testicular development. The postspawning stage was observed in testes collected in late summer and autumn and was accompanied by a decreasing 3β-HSD activity in the Leydig cells. The preparatory stage is characterized by spermatogonial multiplication, leading to an increase in number of spermatogonia A, lining the wall of the testis tubules, and in March to the appearance of small cysts with spermatogonia B. At that stage, the 3β-HSD activity of the Leydig cells is very weak. Fish caught near warm springs in Lake Kinneret during December and January showed testes in the prespawning stage. This precocious development of testicular processes may be due to the temperature of the water, which was some degrees higher than in the Hula Reserve during winter.

The prespawning stage is invariably found in the testes during the period of full gametogenesis, from March to May. This period is characterized by an increase in GSI (Fig. 1) and in spermatogenic activity, and by a moderate 3β-HSD activity of Leydig cells.

Periodicity of ovarian development

The paired ovaries are situated in the dorsal region of the body cavity. They are sac-like structures, consisting of a wall with lamellae penetrating the cen-
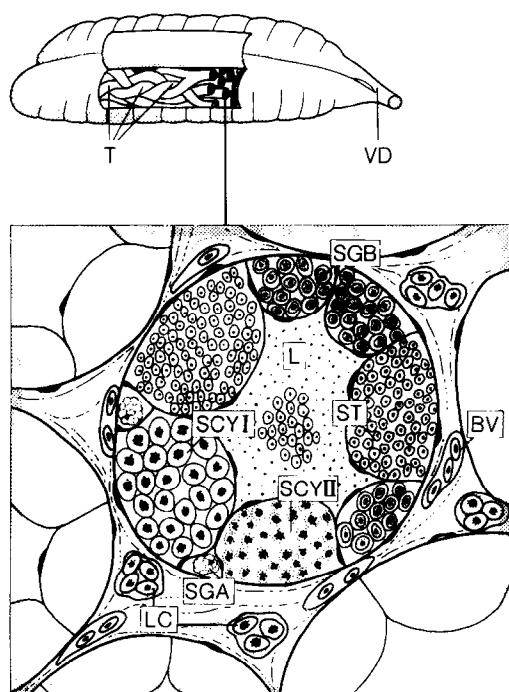
Fig. 2. Schematic representation of the testes of *Clarias gariepinus*. In detail: a transverse section through a seminiferous tubule (T). SGA = spermatogonium A; SGB = cysts with spermatogonia B; SCYI = cyst with primary spermatocytes; SCYII = cyst with secondary spermatocytes; ST = cyst with spermatids; L = lumen of a seminiferous tubule filled with sperm cells and spermatids; LC = Leydig cells; VD = vas deferens; BV = blood vessel.

tral lumen. The lamellae contain oogonia and oocytes in follicles at various stages of development (Fig. 3). Their morphology has been described by Richter and Van Den Hurk (1982) and Van Den Hurk and Peute (1985). The annual ovarian cycle can be divided into the same three periods as the testicular cycle, i.e. the breeding period, the resting period and the period of full gametogenesis.

During the breeding period, from May until August, ovaries are in the post-

TABLE 1

3β-HSD activity in interstitial Leydig cells (LC) during the annual reproductive cycle

Period	Stage	LC
Resting	Postspawning	+/++
	Preparatory	+
Full gametogenesis	Prespawning	++
Breeding	Spawning	+++

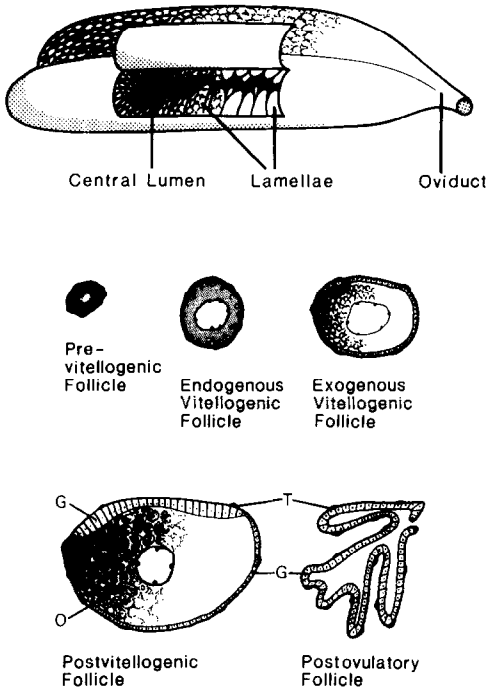+ = weak; + + = moderate; + + + = strong.

Fig. 3. Schematic representation of the ovary of *Clarias gariepinus* showing lamellae containing follicles. In detail: normal follicular development. G = granulosa; T = theca; O = primary oocyte.

vitellogenic or in the postovulation stage and show a strong 3β-HSD activity in the special theca cells and some interstitial cells, and the absence of 3β-HSD activity in the granulosa of the follicles (Table 2). Postovulatory follicles next to previtellogenic and vitellogenic follicles are present in postovulatory ovaries. In these the distribution and activity of the enzyme 3β-HSD corre-

TABLE 2

3β-HSD activity in interstitial cells (IC), special theca cells (STC) and granulosa of vitellogenic follicles (GVF) during the annual reproductive cycle

Period	Stage	IC/STC	GVF
Resting	Atresia	+ or + + +[a]	—
	Previtellogenesis	+	—
Full gametogenesis	Endogenous vitellogenesis	+	+
	Exogenous vitellogenesis	+ +/+ + +	+/+ +
Breeding	Postvitellogenesis	+ + +	—
	Postovulatory	+ + +	—

+ = weak; + + = moderate; + + + = strong; — = negative.
[a] + + + only in STC around some intact postvitellogenic follicles.

spond to those of the former stage. Fish in the postovulation stage were only sporadically found in the breeding period, indicating that ovulation and spawning may take place several times, and that after ovulation the ovary returns to the postvitellogenic stage. The continuing strong decrease in GSI, however, shows that after ovulation vitellogenesis is limited and does not lead to a restoration of the original number of postvitellogenic follicles.

The resting period, from August to March, comprises the stages of atresia and previtellogenesis. During this period the GSI is very low. Atresia was mainly encountered in August, September and October. It is characterized by a regression of many follicles. These atretic follicles do not show any 3β-HSD activity. In the remaining healthy follicles the 3β-HSD activity is also absent or restricted to some special theca cells. A similar situation is present in the interstitial cells of the ovary. At the stage of previtellogenesis most atretic follicles have disappeared, and numerous previtellogenic and some endogenous vitellogenic follicles are present in the ovaries.

The period of full gametogenesis begins in March and ends in May, and is characterized by an enormous increase in GSI (Fig. 1) due to a strong vitellogenic activity in the ovaries. It comprises two stages, i.e. endogenous and exogenous vitellogenesis. The former differs from the previtellogenic ovary by the presence of numerous endogenous vitellogenic follicles (400–500 μm in diameter), which show a weak 3β-HSD activity in their granulosa cells. The stage of exogenous vitellogenesis stands out by the presence of large (up to 1000 μm in diameter) exogenous vitellogenic follicles. These show a weak to moderate 3β-HSD reaction in their granulosa cells and a moderate to strong reaction in the special theca cells. Also in some interstitial cells a moderate to strong 3β-HSD activity can be found at this stage. Female catfish, caught near the warm springs of Lake Kinneret, showed vitellogenic ovaries as early as December and January.

Periodicity of pituitary gonadotropic development

Changes in the pituitary gonadotropin (GTH) content follow an annual cycle that, like the reproductive cycle, can be divided into the three periods, i.e. breeding period, the resting period and the period of full gametogenesis. In males, the pituitary GTH content reaches peak values at the beginning of the breeding period, in May, preceding the peak value in females by two months (Fig. 4). The pituitary GTH content in males decreases during the breeding period and continuous to do so till a minimum is reached shortly after the beginning of the resting period, in October. In females the decrease does not start before the end of the breeding period and leads to a minimum in pituitary GTH content in November. Before the end of the resting period, in February, and during the period of full gametogenesis an increased amount of GTH is stored in the pituitary (Fig. 4).
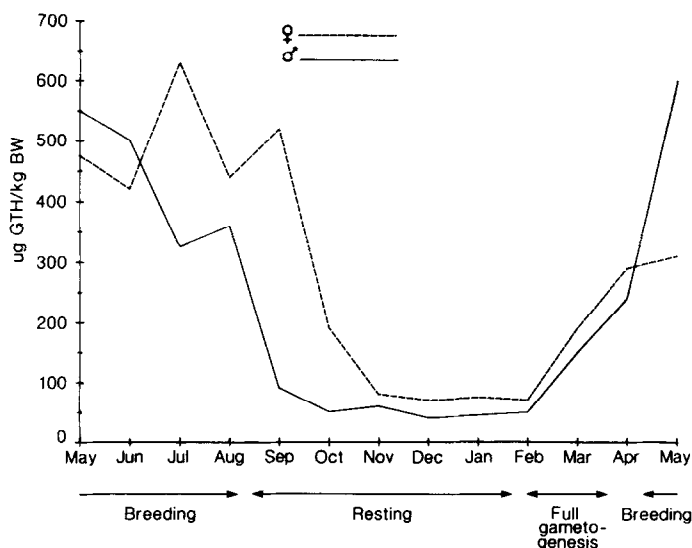
Fig. 4. Pituitary GTH content of male and female catfish during one annual cycle and expressed as μg/kg body weight.

The ultrastructure of the gonadotropic cells follows the seasonal variations in pituitary GTH content. These cells, for *Clarias gariepinus* first described and immunocytochemically identified by Peute et al. (1984), are located in the proximal pars distalis of the pituitary (cf. Van Oordt and Peute, 1983). The ultrastructure of the gonadotropes in male and female catfish is characterized by the presence of granular endoplasmic reticulum (GER) with dilated cisternae and by three types of gonadotropin-containing inclusions, i.e. granules, globules and large irregular masses (IMs) (Figs. 5 and 6).

During the breeding season, the gonadotropes are large and packed with GTH containing inclusions, mainly granules and globules. From the beginning of the resting period the GTH cells gradually decrease in size, and in both sexes are smallest in November. At the same time the ultrastructure of the gonadotropes changes gradually, particularly with respect to the number and size of the IMs (Fig. 7). That is to say, in males as early as July the IMs begin to augment in number and size and until October gradually occupy larger areas of the cytoplasm. In females the same phenomenon takes place between September and November. The periphery of the large and multiform IMs as a rule has a fibrillar structure (Fig. 8), the centre remains more or less amorphous. Parallel to the changes in the IMs, the granules and globules slowly decrease in number. They continue to do so when in November–December the IMs also disappear within a short time.

As early as December, half way through the resting period, the gonadotropes in both males and females begin to increase in size. They reach their maximal
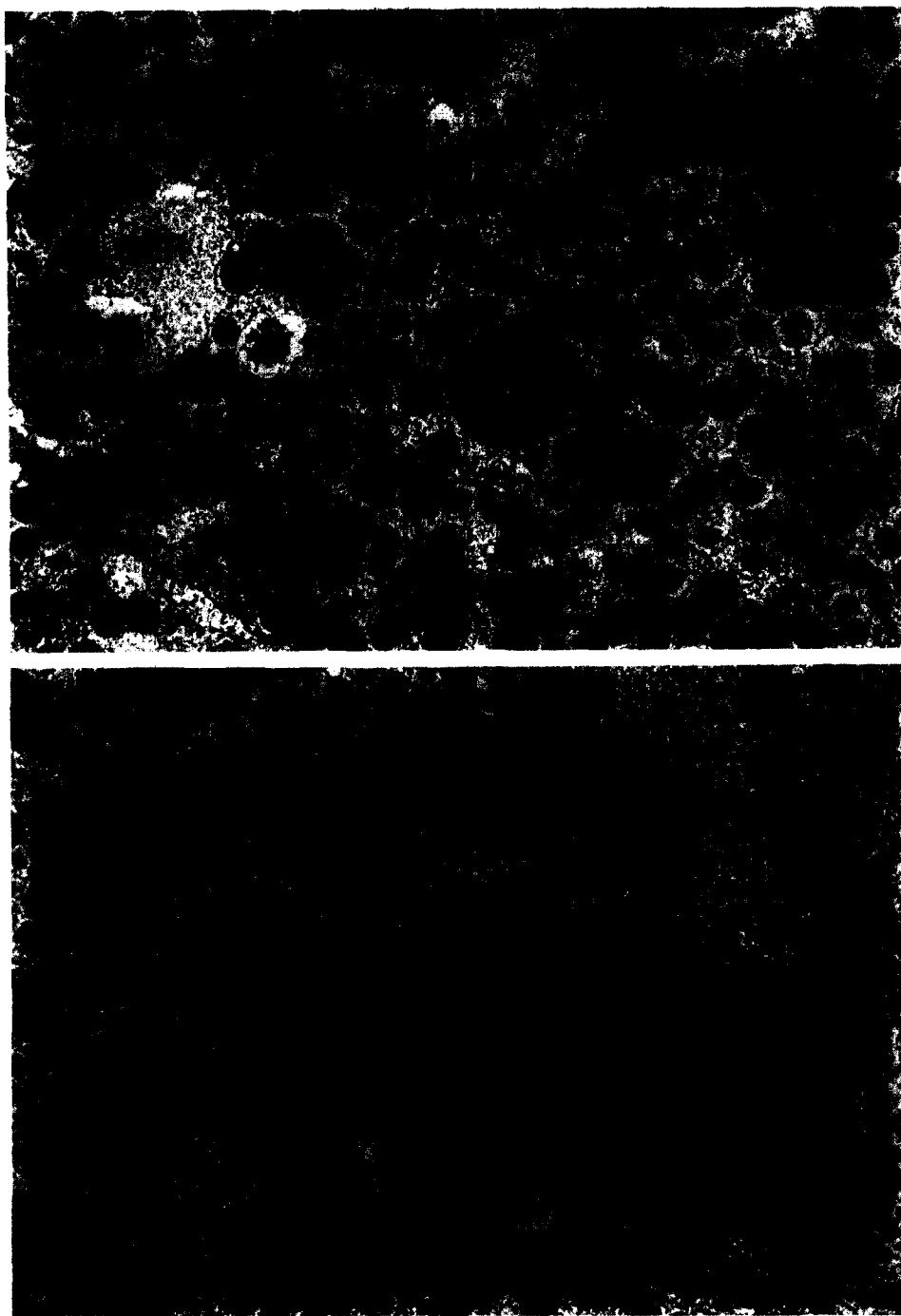
Figs. 5 and 6. Details of gonadotropic cells of catfish caught in May, showing secretory granules (Sg), globules (Gl), irregular masses (IM), cisternae of the granular endoplasmic reticulum (GER) and a neurosecretory fibre (Nf), 12 250×.
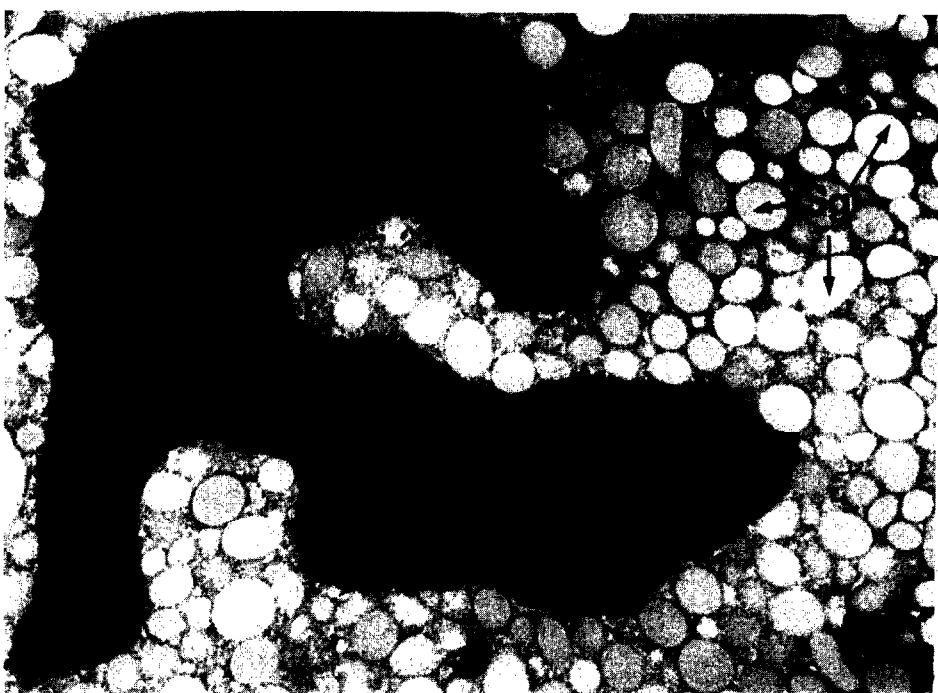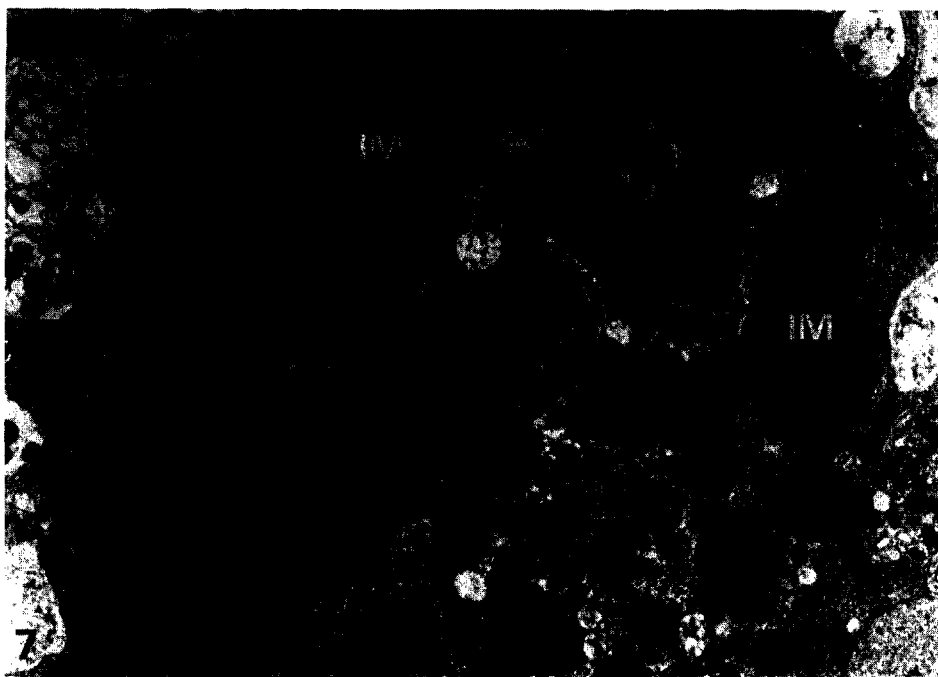
Fig. 7. Gonadotropic cell of catfish caught in November, with many irregular masses (IM), some of which have a nonhomogeneous structure, 12 250×.

Fig. 8. Detail of an irregular mass, with marked arylsulphatase-positive activity in central areas, the latter being surrounded by fibrous material; Sg secretory granules, 21 200×.

size at the transition from the resting period to the period of full gametogenesis. During the latter period also the ultrastructure of the gonadotropes changes. The area occupied by the GER and the Golgi complex increases, and that is followed by an increase in number of the GTH containing granules and globules.

DISCUSSION

The three periods of the reproductive cycle of the African catfish, *Clarias gariepinus*, in Northern Israel coincide with three stages in the ultrastructure and the hormone content of the gonadotropic cells in the pituitary. During the breeding period from May to August the peak in pituitary GTH content in males precedes the peak in females by 2 months. This difference corresponds to the difference in maximal steroidogenic activity in testes and ovaries respectively. It therefore seems that the strong biosynthetic capacity for steroid production, probably reflecting a strong secretion of gonadal steroids, is accompanied by an accumulation of GTH in the pituitary. Ultrastructurally this GTH accumulation is reflected by an abundance of secretory granules and globules. Comparable indications for a stimulation of GTH storage by gonadal steroids have been observed by De Leeuw et al. (1986) under experimental conditions, i.e., castration and steroid replacement leading to an increase in GTH release and storage respectively. It seems that in the African catfish during the breeding period, when gametogenesis in males and females has almost or entirely come to an end, the GTH release is not very strong (Resink et al., 1987a). Similar observations were reported for the Indian catfish, *Heteropneustes fossilis* (Sundararaj, 1959, 1960) and the Nile catfish, *Synodontis schall* (Rizkalla and Yoakim, 1975).

In male African catfish from the Hula Nature Reserve the plasma GTH concentration is low throughout the breeding period, except for a very short period around spawning (Resink et al., 1987a). It is this GTH surge which is most likely required for spermiation and for oocyte maturation and ovulation (Donaldson and Hunter, 1983). Female African catfish, raised and constantly kept under favourable laboratory conditions, never show such a GTH surge and consequently never show spontaneous oocyte maturation and ovulation, even though their ovaries are filled with postvitellogenic oocytes and their pituitaries contain large and granulated gonadotropes (Peute et al., 1984) and large amounts of GTH throughout the year (De Leeuw et al., 1985a). On the other hand, ovulation can be induced experimentally in such animals by evoking an artificial GTH surge. Depending on the method employed for inducing a massive GTH release, ovulation will take place 12–14 h after injection of the hormone (Richter and Van Den Hurk, 1982; De Leeuw et al., 1985b; Goos et al., 1987; Richter et al., 1987a).

When in nature, at the end of the breeding period, the resting period begins, the size and granulation of the gonadotropic cells and the GTH content of the

pituitary enter a period of gradual regression which lasts about 4–6 months. During these months the IMs in the gonadotropes initially increase in size and number, but have disappeared by December. Peute et al. (1986) have argued that these structures are involved in the intracellular degradation of GTH, the more so since they not only contain GTH, but also lysosomal enzymes, such as arylsulphatase (Fig. 8) and acid phosphatase (Peute et al., 1987). During the entire resting period low GTH concentrations were measured in the blood (Resink et al., 1987b). In all probability this reduced gonadotropic activity of the pituitary has led to the resting conditions of the gonads, reflected by the absence of gametogenesis and a weak 3β-HSD activity. It is not clear which factors initiate the regression of the gonadotropes. In males the first signs of regression were seen even during the breeding period, in July, when the water temperature was 25–28°C, and the daily photoperiod more than 12 h. Feeding conditions, however, were unsatisfactory in the Hula Nature Reserve during mid summer (Viveen, unpublished results). Under laboratory conditions, including a water temperature of 28°C and ample foor supply, African catfish show a continuous reproductive cycle with numerous ripe sperm cells and post-vitellogenic oocytes in testes and ovaries respectively (Richter and Van Den Hurk, 1982), but even under these favourable circumstances ovulation and spawning can be more readily induced during the first than during the second half of the year (Resink, unpublished results). These data together may point to an inherent rhythm in GTH secretion, strongly influenced by the external environment.

As in *Clarias batrachus* (Lehri, 1967, 1968) and in *Heteropneustes fossilis* (Sundararaj and Vasal, 1976), spermatogenesis and vitellogenesis in feral *Clarias gariepinus* are resumed in late winter and early spring, when the water temperatures are relatively low, but increasing daily. During that time of year Viveen (unpublished results) observed sunbathing of catfish in very shallow and warm (28°C) water of the Hula Nature Reserve at midday. This may indicate a positive effect of the ambient temperature on gonadal recrudescence. As in other fish (for review see Idler and Ng, 1983) gametogenesis in the African catfish depends on pituitary GTH. Accordingly, during the second half of the resting period the gonadotropic cells show various signs of a gradual recovery, such as an increase in GER, in Golgi complex and in numer of secretory granules and globules. The GTH concentration in the blood remains low during the period of full gametogenesis (Resink et al., 1987b), but obviously suffices for spermatogenesis, vitellogenesis and a moderate activity of the steroid producing cells in the testes and ovaries.

In conclusion, the African catfish in Northern Israel under natural circumstances exhibits a discontinuous reproductive cycle, regulated by annual changes in the activity of the gonadotropic cells in the pituitary. The critical points in these annual changes are a prespawning GTH surge and a postspawning regression of the gonadotropes. Under husbandry conditions these

two phenomena are suppressed, and a continuous production, storage and release of GTH prevail, leading to a continuous production and storage of sperm cells and postvitellogenic oocytes, and synthesis of gonadal steroids. This offers the possibility of obtaining viable eggs and healthy larvae throughout the year (Richter et al., 1987b).

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