Seasonal fluctuations in gonadotropin levels in the plasma and gonads of male and female tilapia, *Oreochromis mossambicus*

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Abstract

Syferkuil Dam is situated 8 km NW of the University of the North and comprises a series of eight interconnected rectangular dams, having cement sides and mud bottoms. Throughout the experimental period, male and female adult specimens of the mouthbrooding tilapia, *Oreochromis mossambicus* were collected for further analysis. Aspects of the reproductive physiology of *O. mossambicus* that were investigated included the role of gonadotropin hormones in reproduction. There are two distinct gonadotropins in *O. mossambicus*, luteinizing-like hormone (LH-like), and follicle stimulating-like hormone (FSH-like). Both of these hormones are secreted in response to increased water temperature and both are involved in enhancing spawning. The gonadotropins also provide the impetus for steroid hormone secretion to occur. Human chorionic gonadotropin ("HCG") plays a role in the final maturation of the oocytes within the female ovary. The results imply a close interaction between environmental cues and endocrine control of reproduction. Endocrine control cannot be sustained without the appropriate environmental cues required to stimulate reproduction.

Introduction

Seasonal cycles of gonadal activity have been described for many teleost species. The association of changes in gonad condition with plasma levels of gonadal steroids and the gonadotropins has proven to be a valuable tool in the development of an understanding of endocrine control of reproduction in teleosts (Cornish, 1993).

Although it has been ascertained in cyprinids that final oocyte maturation and ovulation are induced by a preovulatory gonadotropin surge, little is known about the plasma and gonadal changes in gonadotropin and steroid hormone levels during the reproductive cycle in *O. mossambicus*.

The role of hormones in the regulation of reproductive behaviour in fish is a highly investigated area of study. Evidence for the hormonal regulation of reproductive behaviour is based upon:

- the treatment of fish with exogenous hormone preparations, with or without prior gonadectomy; and/or
- the correlation of the timing of reproductive behaviour with endocrine activity as assessed by histological and cytological means.

More recently these techniques have been combined with the use of neurohormones, dopamine antagonists and other pharmacological agents. In comparison with these more traditional procedures which relied upon histological and cytological data, radio-immunoassay (RIA) assessment of plasma and gonadal hormone levels provides a more precise analysis of the relationship between endocrine state and behaviour (Liley et al., 1987), as well as the stage of gonad development.

The paramount importance of the pituitary gland in the control of teleost reproduction has been extensively reviewed by Dodd (1960), Hoar (1969) and Lam et al. (1978). Idler and Ng (1983) state that until 1975, data from chemical fractionation studies and bioassays supported the concept that the teleost pituitary elaborated a single gonadotropin which controlled all phases of the reproductive cycle including vitellogenesis, oocyte maturation, ovulation, spermatogenesis, androgen production and spermiation. Since 1975, reports on the isolation of gonadotropins from more teleostean species have appeared, and the results have shed some light on the controversial issue of the number of gonadotropins in this important class of vertebrates.

Farmer and Papkoff (1977) and Hyder et al. (1979) have shown that there appear to be two distinct gonadotropins in tilapia; one that resembles luteinizing hormone (LH-like) and another that resembles follicle stimulating hormone (FSH-like) in terms of their biological activity and chromatographic behaviour. Tilapia gonadotropins seem to be involved in stimulating spermatogenesis and androgen secretion in males.

Considerable experimental data have been collected on the role of gonadotropins in tilapia (Gissis et al., 1986; 1991; Levavi-Zermonsky and Yaron, 1986; Planas et al., 1990; Yaron and Levavi-Sivan, 1990; Levavi-Sivan and Yaron, 1992). Gissis et al. (1991) have demonstrated the dual hypothalamic control of gonadotropin release in tilapia, particularly in response to circulating gonadotropic releasing hormone (GnRH) levels. Levavi-Sivan and Yaron (1992) have shown the involvement of cyclic adenosine monophosphate (AMP) in the transduction of the short-term effect of GnRH on gonadotropin release in tilapia. The cyclic AMP seems to operate in an interconnected manner with the system of calcium influx.

Zohar and Billard (1984) have examined annual plasma gonadotropin and sex steroid levels in relation to teleost gonad cycles. The plasma gonadotropin levels increase only gradually during the major part of gonadal development (vitellogenesis, spermatogenesis) but increase sharply toward the end of gametogenesis; that is at the time of oocyte maturation and ovulation and before spermiation.

Burlakov et al. (1985) have shown that by measuring the levels of gonadotropin in the plasma of tilapia (*Oreochromis*

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aureus), it is possible to determine the sequence of events prior to and during spawning.

The present study examined the levels of the gonadotropin hormones in both plasma and gonads on a seasonal basis and related the results to other parameters considered to be involved in the regulation of reproduction in both male and female *O. mossambicus*.

Materials and methods

Each Monday morning at 08:00, 10 adult male and 10 adult female *O. mossambicus* were collected at Syferkuil Dam, 8 km NW of the University of the North using a seine net. The collection period spanned a full calendar year. A 2.5 mℓ sample of blood was collected from each fish at the dam using the cardiac puncture method before the fish were transported back to the University of the North campus in oxygenated water containing 20 mg/ℓ neutralised MS222 according to the method of Smit (1980). Fish were transported to the laboratory in this way to overcome the effects of handling stress that may have been encountered during netting. On arrival at the laboratory, measurements of the fish mass (g) and gonad mass (mg) were taken and recorded. These data were used to calculate the gonadosomatic index (GSI) for each fish analysed throughout the sampling period according to the formula of Roff (1983).

Both the plasma and the gonadal homogenate supernatants were analysed for the presence of endogenous human chorionic gonadotropin ("HCG"), exogenous FSH-like and LH-like. The concentrations of these hormones were measured in both male and female samples by RIA using human kits obtained from FRANSA (Cat. Nos. FSH-like: FSH-likeK-PR; LH-like: LH-likeK-PR; "HCG": "HCG"K-PR). Estradio 17-β levels were determined in females using the appropriate FRANSA RIA kit (Cat No. SB-ESTR). All readings of radioactivity were taken using a Beckman Gamma 8500 Microprocessor Counter.

Statistical analyses were carried out using the SAS program. Due to this being a field study, which may not be controlled as in the laboratory, a large variation in the size of the experimental animals occurred and also a degree of stress may have been encountered, the significance of the variation is not as great as in controlled laboratory conditions.

Results

All data shown in Tables 1, 2 and 3 are the mean monthly ± standard deviation values recorded for the parameters indicated. All data shown in Figs. 1 to 8 are the mean values only for the parameters indicated, combined with steroid data recorded by Cornish (1993).

Table 1 shows male and female *O. mossambicus* plasma and gonadal supernatant FSH-like concentration (mIU/mℓ) values for the entire experimental period. Male plasma FSH-like showed two significant ($p < 0.001$) dips in concentration during September and November whereas gonad FSH-like dropped significantly a month later ie: during October and December. Female plasma FSH-like was undetectable from June to August, whereafter a slight decrease was observed until January. Gonad FSH-like was virtually undetectable from September to February. No great variation in FSH-like levels in male plasma and gonads were observed. However in females, aside from March, plasma FSH-like levels were almost always higher than gonad levels.

Table 2 shows male and female *O. mossambicus* plasma and gonadal supernatant LH-like concentration (mIU/mℓ) values for the experimental period. Male plasma LH-like levels peaked during October and from March to May. Lowest levels were recorded during November to January. Female plasma LH-like levels were high from August to October whereafter they declined significantly ($p < 0.001$) until November. In general, male and female plasma LH-like levels were always higher than gonad LH-like levels.

Table 3 shows the levels of "HCG" (mIU/mℓ) as measured in both male and female *O. mossambicus* plasma and gonadal supernatant. It may be noted that in the case of male plasma and female gonadal supernatant, the only measurable quantity of "HCG" is noted during May when a concentration of <1.00 mIU/mℓ is noted. For the remainder of the experimental period, no "HCG" could be detected in either the plasma or the gonadal supernatant. In the case of the male gonadal supernatant, Table 3 shows that during May and February concentrations of <1.00 mIU/mℓ are observed. During January, however, a high value of 24.48 ± 1.44 mIU/mℓ is noted. During the remainder of the experimental period, no measurable "HCG" could be recorded. In

TABLE 1
FSH-LIKE CONCENTRATION (mIU/mℓ) MEASURED IN PLASMA AND GONADS OF MALE AND FEMALE *O. MOSSAMBICUS* (SAMPLES TAKEN PER MONTH, n = 40)

	♂ Plasma (mIU/mℓ)		♂ Gonad (mIU/mℓ)		♀ Plasma (mIU/mℓ)		♀ Gonad (mIU/mℓ)	
	mean ± sd		mean ± sd		mean ± sd		mean ±sd	
May	23.20	1.01	<1.00		17.16	0.99	<1.00	
Jun	11.91	0.77	< 1.00		< 1.00		< 1.00	
Jul	10.82	0.59	19.13	1.02	<1.00		18.86	1.07
Aug	12.46	0.91	15.49	1.10	<1.00		3.28	0.37
Sep	5.02	0.54	12.84	0.79	15.72	0.88	<1.00	
Oct	25.62	1.17	5.08	0.44	9.96	0.63	<1.00	
Nov	5.44	0.38	11.22	0.93	8.74	0.69	<1.00	
Dec	10.16	0.87	<1.00		7.22	0.72	<1.00	
Jan	13.18	1.15	7.06	0.51	5.64	0.56	<1.00	
Feb	27.70	1.21	9.22	0.81	8.38	0.91	<1.00	
Mar	35.10	1.47	11.78	0.92	11.82	0.95	19.42	1.12
Apr	28.73	1.09	10.98	0.74	14.11	1.13	<1.00	

TABLE 2
LH-LIKE CONCENTRATION (mIU/mℓ) MEASURED IN PLASMA AND GONADS OF MALE AND FEMALE *O. MOSSAMBICUS* (SAMPLES TAKEN PER MONTH, n = 40)

	♂ Plasma (mIU/mℓ)		♂ Gonad (mIU/mℓ)		♀ Plasma (mIU/mℓ)		♀ Gonad (mIU/mℓ)	
	mean ± sd		mean ± sd		mean ± sd		mean ±sd	
May	44.46	2.24	12.38	0.89	31.42	2.01	<1.00	
Jun	23.73	1.92	14.11	0.94	< 1.00		<1.00	
Jul	14.80	0.89	25.82	1.01	13.76	0.91	<1.00	
Aug	17.18	1.67	13.68	0.89	39.88	3.28	<1.00	
Sept	25.18	1.84	7.58	0.74	89.66	5.77	18.86	1.03
Oct	52.24	3.01	4.28	0.33	28.02	2.26	18.42	0.91
Nov	5.38	0.67	10.82	0.68	5.04	0.75	5.32	0.66
Dec	4.46	0.48	2.28	0.27	5.48	0.65	<1.00	
Jan	5.48	0.61	7.96	0.75	8.56	1.32	<1.00	
Feb	25.02	1.06	11.58	0.97	19.82	1.18	<1.00	
Mar	47.74	2.98	12.22	0.88	17.26	1.91	<1.00	
Apr	46.99	2.43	12.07	0.91	19.38	1.45	<1.00	

TABLE 3
"HCG" CONCENTRATION (mIU/mℓ) MEASURED IN PLASMA AND GONADS OF MALE AND FEMALE *O. MOSSAMBICUS* (SAMPLES TAKEN PER MONTH, n = 40)

	♂ Plasma (mIU/mℓ)	♂ Gonad (mIU/mℓ)	♀ Plasma (mIU/mℓ)	♀ Gonad (mIU/mℓ)
	mean ± sd	mean ± sd	mean ± sd	mean ±sd
May	<1.00	<1.00	10.22 0.76	<1.00
Jun	non-detectable	non-detectable	non-detectable	non-detectable
Jul	non-detectable	non-detectable	10.44 0.78	non-detectable
Aug	non-detectable	non-detectable	<1.00	non-detectable
Sept	non-detectable	non-detectable	<1.00	non-detectable
Oct	non-detectable	non-detectable	<1.00	non-detectable
Nov	non-detectable	non-detectable	<1.00	non-detectable
Dec	non-detectable	non-detectable	41.50 1.98	non-detectable
Jan	non-detectable	24.48 1.44	12.18 0.88	non-detectable
Feb	non-detectable	<1.00	9.62 0.64	non-detectable
Mar	non-detectable	non-detectable	14.36 1.05	non-detectable
Apr	non-detectable	non-detectable	12.49 1.32	non-detectable

the female plasma however, there appears to be a measurable concentration of "HCG" throughout the experimental period with a high concentration of 41.50 ± 1.98 mIU/mℓ being observed in December. In the months of August through November, concentrations of <1.00 mIU/mℓ were measured.

Figure 1 represents the relationship between GSI (%) and the gonadotropins (FSH-like and LH-like) concentration (mIU/mℓ) in male *O. mossambicus* plasma and Fig. 2 represents these same parameters in the gonadal supernatant. Figure 1 shows that a good relationship exists between FSH-like and LH-like in male plasma (r = 0.72). Plasma LH-like levels were almost double those of FSH-like. Figure 2 shows a significantly weaker relationship in male gonadal supernatant (r = 0.59). In plasma, only one peak for both FSH-like and LH-like was observed immediately prior to the peak in GSI. However, in the gonads, two peaks were observed, the highest being in July with a second significantly lower peak for the two hormones in November.

Figure 3 represents the relationship between GSI (%) and the gonadotropins (FSH-like and LH-like) concentration (mIU/mℓ) in female *O. mossambicus* plasma and Fig. 4 represents these same parameters in the gonadal supernatant. Figure 3 shows that a relatively poor relationship exists between FSH-like and LH-like (r = 0.48) which peaks with maximum gonadosomatic index (GSI) in September. Gonadal supernatants (Fig. 4) on the other hand, suggested an extremely strong relationship between FSH-like and LH-like (r = 0.99) with an inverse relationship between both hormones and GSI. This may also be noted in Tables 1 and 2. In the gonadal supernatant, a seemingly much closer

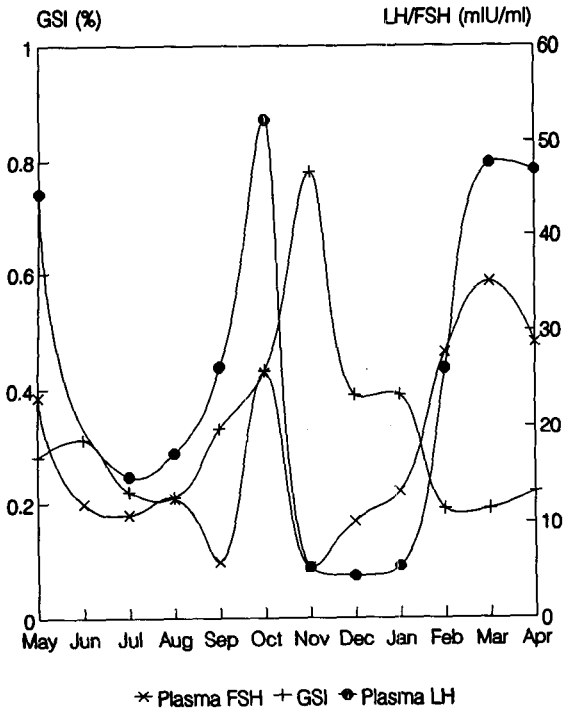

Figure 1
Plasma gonadotropin levels and GSI in male
O. mossambicus

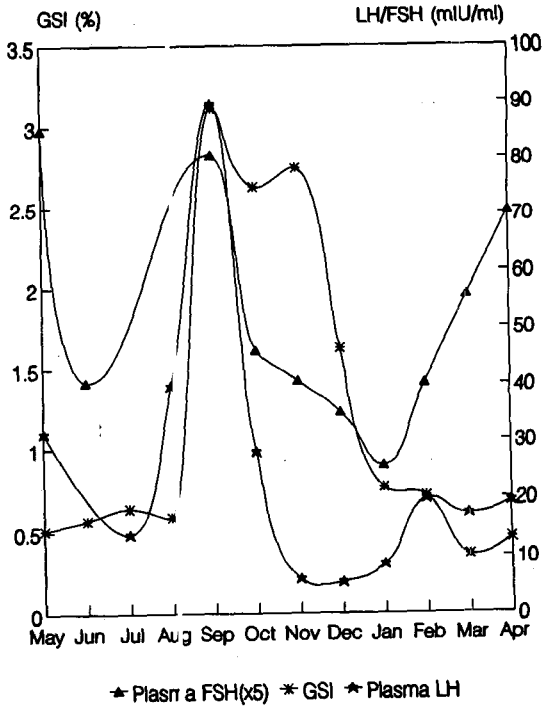

Figure 3
Plasma gonadotropin levels and GSI in female
O. mossambicus

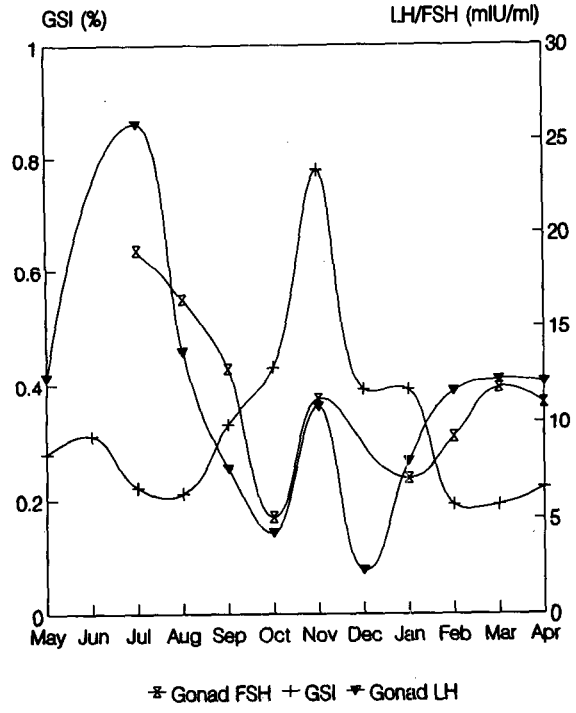

Figure 2
Gonadal gonadotropin levels and GSI in male
O. mossambicus

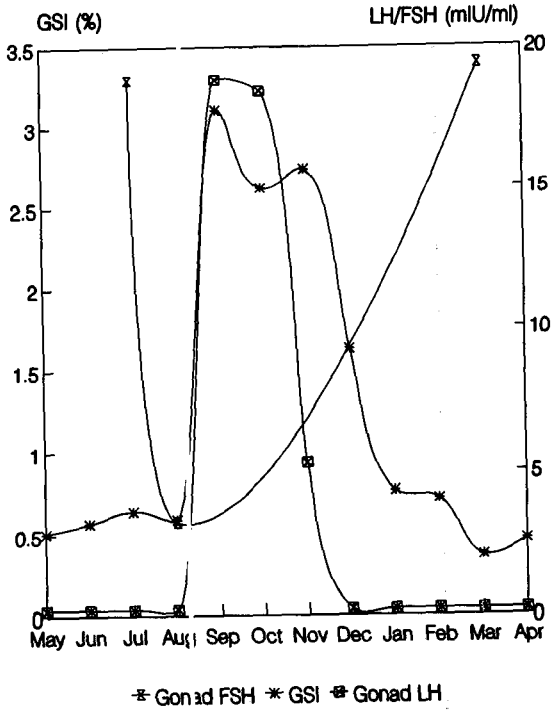

Figure 4
Gonadal gonadotropin levels and GSI in female
O. mossambicus

relationship exists between FSH-like and LH-like. Tables 1 and 2 show that for the majority of the experimental period a value of <1.00 mIU/mℓ was recorded for both FSH-like and LH-like and that it is only during the months of July and March that high values are observed.

Figure 5 represents the relationship between gonadotropin and steroid reproductive hormones in male *O. mossambicus* plasma and Fig. 6 represents these same parameters in the gonadal supernatant. In the plasma, testosterone appears to reach a maximum value during September and a second, slightly lower peak is observed during January. Both LH-like and FSH-like appear to have three peaks. In LH-like, they are of similar magnitude and are noted during May, October and March. In FSH-like the peaks are also of a similar magnitude, but are much lower than is the case in LH-like. The FSH-like peaks may be seen during August, October and January, prior to the LH-like peaks. Maximum peaks for both hormones are reached in October at maximum GSI. In the case of the gonadal supernatant (Fig. 6), testosterone reaches maximal values during August and November with the latter being highest and coinciding with maximal GSI. LH-like however, reaches a peak value during July as does FSH-like. A second smaller peak for these hormones was observed during November.

Figure 7 represents the relationship between the gonadotropins and steroid hormones in female *O. mossambicus* plasma and Fig. 8 represents these same parameters in the gonadal supernatant. In Fig. 7 LH-like may be seen to reach a maximum during September which coincides with maximal GSI. Thereafter a smaller peak is reached during January and February. Although FSH-like also reaches a high during September, the magnitude of its peak is much lower than that for LH-like. Smaller variations also occurred during November and December and February and March. Progesterone may be seen to reach a peak during September/October/November and February whereas estradiol 17-β, which is present in much lower concentrations than progesterone, is high during August, October and February (Cornish et al., 1996).

Figure 8 shows the gonadal supernatant, in which it may be noted that the levels of LH-like and FSH-like appear fairly similar during the experimental period when they could hardly be detected during August to November. In the case of progesterone and estradiol 17-β, the former is present in much greater concentrations than the latter. Estradiol 17-β increases during August, September and October and reaches a peak of 5.02 ± 0.12 ng/mℓ during September whereas the levels of progesterone seem to fluctuate throughout the year to reach four different peak levels during June, August, October and January. It is, however, at its maximal value of 117.40 ± 5.63 ng/mℓ during January.

Discussion

The concentration of a circulating hormone results from the rates of secretion into and the clearance from the plasma. Interpretation of plasma hormone fluctuations in fish appears to be linked with the pituitary and gonad secretion rates.

Males

Figures 1 and 2 show the relationship between GSI and the gonadotropin levels in both the plasma and gonads of male *O. mossambicus*. During this study, human FSH-like, "HCG" and LH-like kits were used to determine the different gonadotropin levels. The results suggest that two different gonadotropins are

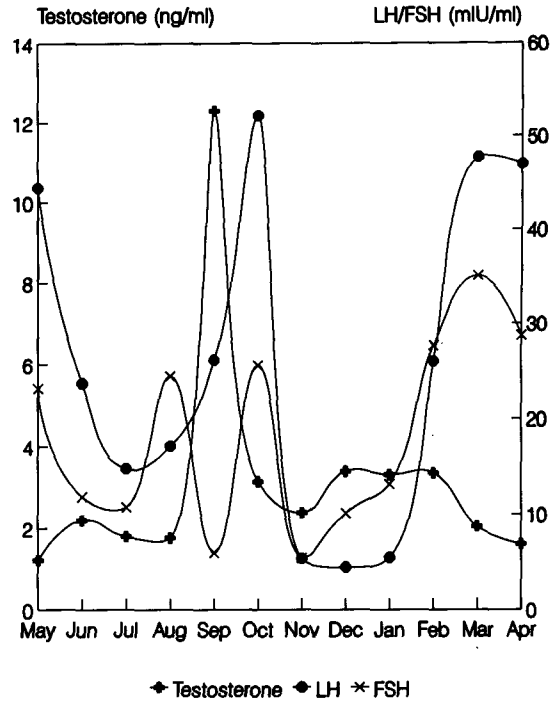

Figure 5
Plasma reproductive hormone levels in male
O. mossambicus

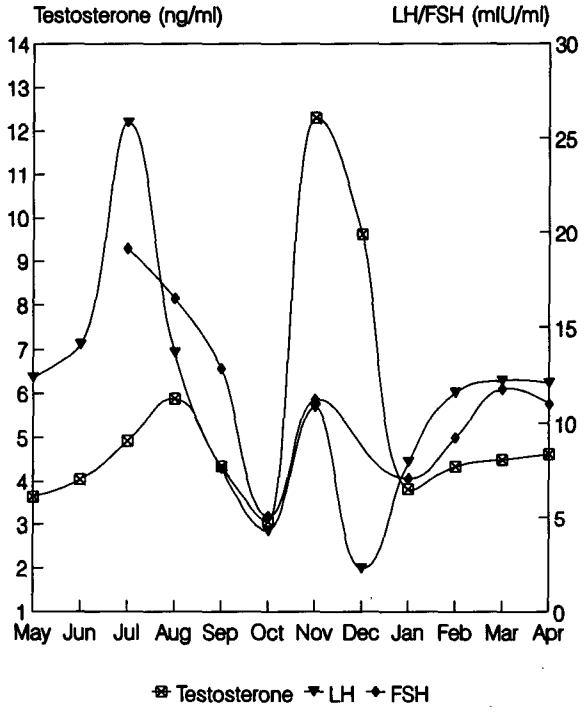

Figure 6
Gonadal reproductive hormone levels in male
O. mossambicus

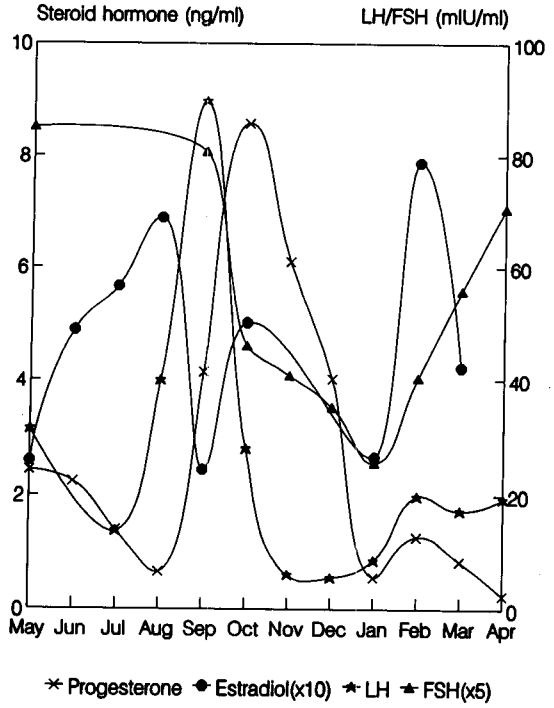

Figure 7
Plasma reproductive hormone levels in female
O. mossambicus

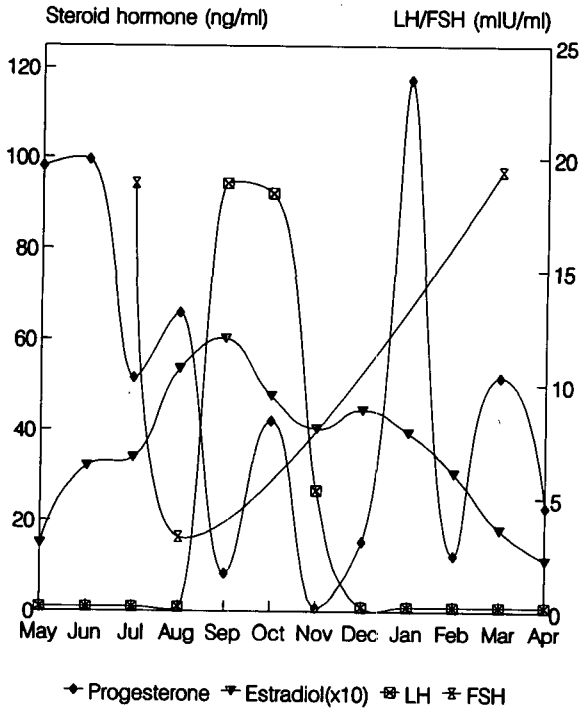

Figure 8
Gonadal reproductive hormone levels in female
O. mossambicus

released by the pituitary, one being FSH-like-like and the other LH-like-like. "HCG" has limited FSH-like and extended LH-like qualities (Schoonbee et al., 1978). No detectable "HCG" activities were recorded in male plasma during the entire period. However, some activity was recorded in male gonads during January. This coincided with the increase in male gonad FSH-like and LH-like observed during the same period which may be purely incidental. Van Oordt and Peute (1983) indicated that the gonadotropic cells in the pituitary have a key position in the brain-pituitary-gonadal axis. It seems that gonadotropin secretion depends on the increase in water temperature. This was also confirmed by Kobayashi et al. (1986) for goldfish. They showed that plasma gonadotropic hormone (GTH) levels increased with a rise in temperature which is also apparent in the present study on *O. mossambicus*. High levels of gonadotropins are associated with increasing GSI levels. The latter is preceded first by an FSH-like followed by an LH-like surge. A similar pattern was observed in male gonads which precede the plasma values by two months. This observation suggests that when plasma levels of the two gonadotropins are low, gonadal levels are high. Plasma levels of gonadotropins appear low when these are shifted to the gonads for maturation. When the gonads mature, plasma levels of gonadotropins increase on a short-term basis indicating positive feedback of gonadotropic pituitary secretion induced by steroids produced by the gonads. Thus when gonadal steroids increase, FSH-like secretion is increased. During early spermatogenesis or in the spawning testis, 11-ketotestosterone is actively responsible for a negative feedback action on the pituitary. The occurrence of "HCG" in female plasma from December onwards, suggests the secretion of an inhibitory gonadotropic factor which could not be detected in the female gonads. It is therefore suggested that in females, "HCG" in the plasma suggests that it is not necessary for this hormone to act on the gonads but rather to inhibit gonadotropic secretion by the hypothalamo-hypophyseal axis. The results suggest that multiple maturation peaks occur during the breeding period for males in various groups. The older males spawn first, followed by the younger group. Four spawning peaks for males are observed during the breeding season. Older males therefore experience lower gonadotropin increases whereas young males have a comparatively larger increase in gonadotropins. Although this may be indicative of pituitary sizes being similar in both small and large males, the larger gonadotropin release amongst young males could be due to a manifestation of male sexual characteristics whilst the adults are stable, regulated by a negative feedback action on the pituitary. GSI levels would then be suppressed in this way. It appears that the two gonadotropins measured in this study are important as stimulants for the production of testosterone in male *O. mossambicus*.

Besides the effects of high temperature on the gonad, changes in the daily pattern of gonadotropin secretion may also affect gonadal development. Reproductive behaviour and pheromones may influence the daily fluctuation of gonadotropin levels in Cichlids. Male gonadotropin levels are high during winter, suggesting that there is an initial commencement of gonadal development during this season. The peaks that are observed in Figs. 5 and 6 for both FSH-like and LH-like are due to the release of testosterone earlier in the breeding cycle. The lower testosterone levels are probably due to its transformed molecule 11-keto-testosterone being more abundant as it is the maturation-inducing androgen. The plasma peak in September confirms the fact that testosterone is more involved during testicular development and that it has a positive feedback role on the pituitary to secrete the higher LH-like hormone values recorded.

Females

Similar cycles for FSH-like and LH-like in two age groups were recorded during the breeding cycle. It consisted of an increase in FSH-like followed by a concomitant increase in LH-like. In general, female FSH-like and LH-like levels were lower than male values in both the plasma and gonads. "HCG" levels were also recorded in female plasma which were lower than the comparative LH-like levels. No "HCG" levels were detected in female gonads. This suggests that "HCG" corresponds to LH-like which confirms its activity as suggested by Schoonbee et al., (1978). The presence of "HCG" in the plasma only suggests an inhibitory role for gonad development in a manner similar to "HCG" secretion in humans. The occurrence of a double cycle corresponds with the possibility that mouthbrooders guard their eggs and fry for approximately six weeks. This behaviour will suppress sexual activity and the levels of sexual hormones involved. After juvenile independence the temperate environment would allow for another cycle to commence. The lower levels of FSH-like and LH-like in female plasma and gonads may be related to the larger size of the ovaries in females when compared to males at the same development stage. It is possible that gonadotropin stimulation of ovarian theca cells may have a more prolonged effect than is the case with Sertoli and Leydig cells in the testes.

Canario and Scott (1990) have shown in a study on the dab, *Limanda limanda* and the plaice, *Pleuronectes platessa* that oocytes are always more responsive to "HCG" than to steroids. In the present study, female *O. mossambicus* plasma levels of "HCG" were very high (41.50 ± 1.98 mIU/mℓ) in December and remained fairly constant through the months of January until March (±11mIU/mℓ). The high value recorded in December is difficult to explain, however, as already stated, "HCG" plays a role in the final maturation of oocytes in both the dab and the plaice (Canario and Scott, 1990). "HCG" levels observed during March, May and July could be involved with oocyte maturation. As previously indicated, "HCG" may act as an inhibitory hormone as a precursor to the resting phase of gonads. It may thus be a rate-limiting factor for the preparation of gonads for the next spawning cycle. A more comprehensive study would be required to verify this.

The foregoing information indicates that male FSH-like and LH-like levels peak slightly prior to the female values. This suggests that females secrete some hormone, or perhaps a pheromone or breakdown products of steroids excreted in the female urine, which are responsible for the males being ready for spawning prior to the females reaching a peak GSI. The synchrony of the gonadotropin release in both sexes most probably facilitates ovulation and spermiation to occur at the same time, thereby optimising the chances of successful fertilisation.

Gonadotropin secretion is stimulated by a rise in the temperature of the water in which the fish live. The gonadotropins appear to be an important cue for ovarian maturation in females and the stimulation of testosterone release and consequent sperm production in male *O. mossambicus*. Mating behaviour and synchronised gonadotropin hormone secretion may also play a role in optimising successful fertilisation by mediating the chain secretion of steroid hormones and prostaglandins.

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