A SEASONAL INVESTIGATION INTO THE REPRODUCTIVE PHYSIOLOGY OF THE TILAPIA, *OREOCHROMIS MOSSAMBICUS*, (TELEOSTEI, CICHLIDAE) IN THE NORTHERN TRANSVAAL.

Daryl A. Cornish

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by

Daryl Archibald Cornish

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Declaration

I declare that this thesis hereby submitted by me, Daryl Archibald Cornish, to the University of the North for the degree of Doctor of Philosophy in Physiology has not been submitted by me for a degree at another university and that this is my own original work.

Signed .....

Daryl A. Cornish

Dated 25/11/1993

Dedication

This Thesis is dedicated to my mother, Marjory Cornish and my father, Archibald ("Snow") Cornish. Without their sacrifices, encouragement and enthusiasm, I would not be what I am today. Thank you.

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Abstract

Syferkuil Dam is situated 8km northwest 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 environmental factors like rainfall, photoperiod, relative humidity, wind speed, environmental and dam water temperature and dam water pH in reproduction; various general observations pertaining to sexual dimorphism, body and gonad mass, body and gonad length and blood and gonad pH; a light and electron microscope study of the gonads; the chemical composition of the plasma and gonadal supernatant and the reproductive hormone profiles in the plasma and gonadal supernatant.

The environment plays an important role in at least initiating the commencement of the reproductive cycle in ***O.mossambicus***. An increase in photoperiod, rainfall and environmental and dam water temperature are the cues for gonadal maturity to occur. Male development seems to be dependant on female development in that it "lags" behind the female by two months. Male gonadosomatic index reaches a peak of 0.78 ± 0.12% during November as opposed to a maximum of 3.11 ± 0.72% being reached by the female during September. A definite relationship exists between mass and length for both the parent fish and the gonads in both males and females.

Blood and gonadal pH show an inverse relationship when breeding is prevalent. Male gonads are more alkaline (7.72 ± 0.29 during September) and females more acidic (6.94 ± 0.16 during September).

Oreochromis mossambicus exist as distinct males and females. Male and female gonads are housed in separate individuals as paired, elongated, dorsolateral bodies. Their function is to produce mature, viable spermatozoa in the male testes and ova in the female ovaries.

The chemical composition of the plasma and gonadal supernatant varied considerably over the experimental period. Sodium and calcium are involved in initiating and maintaining sperm motility during the spawning period (spring to early summer). Potassium is responsible for keeping the sperm immobile during the winter months when gonadal development is occurring.

Glucose, lipids, lactate and proteins are all involved in energy production or providing a protective function to the developing gonads. The presence of urea indicates protein metabolism is happening.

The hormonal investigation yielded some interesting results. There are two distinct gonadotropins in ***O.mossambicus***, luteinizing hormone (LH), and follicle stimulating hormone (FSH). 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. Testosterone stimulates the development to maturity of the male testes. Progesterone concentrations show two peaks during the breeding cycle, possibly as a result of larger and smaller sized experimental specimens being used. Estradiol 17-β also shows two peaks in concentration. The first peak is toward the end of the ovarian cycle, corresponding to a resumption in oocyte growth. The second peak corresponds to a rapid vitellogenic growth phase in the oocytes.

This study has shown that three distinct stages - breeding season, resting season and a gonadal recrudescent season may be distinguished in both male and female

O.mossambicus. During the female resting season there are low levels of steroid hormones in general. However, during ovarian recrudescence, there is an increase in trophic and steroid hormones. This relates to a period of endogenous vitellogenesis that occurs during winter. After winter, photoperiod begins to increase leading the breeding cycle into a state of exogenous vitellogenesis, wherein a second steroid (predominantly estradiol) surge is observed. In males, testosterone peaks prior to the gonadotropins because the former hormone is more important for sperm development whereas the gonadotropin luteinizing hormone is important in the hydration process.

The results imply a close interaction between environmental cues and endocrine control of reproduction. The endocrine control cannot continue without the appropriate environmental cues required to stimulate reproduction. For practical purposes, the most important environmental cue for the artificial propagation is an increase in water temperature. This may be used as a starting point for breeding in controlled laboratory conditions when attempting to manipulate artificial breeding by hormonal intervention.

CHAPTER 1

Introduction

The naturally occurring fish populations of the world are becoming increasingly endangered as a result of pollution, dams, bridges and various other environmental factors. In general, there is also great pressure on the fish stock numbers to be maintained as a consequence of commercial fishing and over - fishing. Furthermore, the rapidly expanding human population of the earth consumes increasing amounts of food derived from both agriculture and natural aquatic systems as well as fish farms. In addition, angling in this country is an extremely popular past time and thus compliments the necessity to maintain the water impoundments fully stocked with fish. This occurs all over the world, especially in the Asian countries. In South Africa, however, freshwater fish farming is still in the developmental stage and has many problems that need to be solved in order that a good quality protein may be produced for human consumption. Currently, no information is available on local economically viable intensive fish culture systems. Many attempts have been made to claim success in this regard, but the fact that the large business conglomerates have not attempted such business ventures, suggests that it is not economically viable.

Among the important issues in aquaculture is reproduction of aquatic animals under intensive husbandry conditions. In this regard, it is well known that many fish species, particularly indigenous fish species, can be grown and spawned in captivity, but fail to reproduce spontaneously under such conditions. It is therefore necessary to investigate their reproductive biology to develop techniques for artificial propagation. In recent years, several reports also became available on the genetic manipulation for the culture of aquatic animals. However, certain limitations prohibits this type of research in South Africa. On the other hand, most of the research undertaken in South Africa on freshwater fish are undertaken by universities and other institutes of basic research. It is therefore

essential that such fundamental and applied research groups coordinate their activities in efforts to establish a national fundamental basic and applied research effort in conjunction with a suitable training programme.

The reproductive physiology of freshwater fish is of great interest and importance due to the fact that several species have been successfully bred artificially in captivity in South Africa. Such techniques were introduced into South Africa by Schoonbee ***et al*** (1978), employing mostly exotic species. Several other attempts are known to exist for indigenous species, but the success rate is curtailed by insufficient knowledge on the basic understanding of reproduction principles. Such knowledge on the understanding and manipulation of reproductive physiology for indigenous species, is not well documented. It is therefore essential to gain knowledge and experience on the control of development, growth, differentiation and maturation of the reproductive system. This can be achieved by understanding the internal organ physiological, cellular and molecular systems regulating reproduction. Another important aspect involves understanding the environmental signals required for successful reproduction of different freshwater fish species. A sound fundamental knowledge on the issues mentioned, may contribute to developing a uniform artificial cost effective technique that can be applied to most fish species.

Most studies on teleost fish are carried out in standardized laboratory, artificial conditions. It is, however, essential to study fish in their natural environment where they are exposed to a larger variety of environmental factors. In nature, such factors are largely responsible for determining the timing of reproduction and thus the reproductive strategy of the species. Thus knowing the ecology of a species is an essential basis for the study of it's reproductive physiology as well as for adapting it to fish culture. Bruton (1979) has studied the environmental factors of ***Clarias gariepinus***. It is essential to evaluate the reproductive physiology and its endocrine regulation for this species for fish culture. It is therefore necessary to establish the cyclical events taking place

under natural conditions in the gonads and the pituitary that are directly responsible for stimulating gametogenesis and steroid production in the gonads. The basis for physiological and applied studies require histological and biochemical data about cyclical changes in organs and tissues involved in reproduction.

Many studies on the different ***Tilapia*** species have been undertaken in Africa. Amongst these, ***Oreochromis mossambicus*** is the best known in South Africa. It is well known that ***O.mossambicus*** is a temperate species and that it may be bred in captivity by increasing aquatic temperature. However, no background information on the endocrine control of reproductive cycle in an aquatic environment which undergoes dramatic seasonal changes has been collected. It was therefore necessary to undertake an extensive investigation on the influence of environmental factors on the endocrine control of reproduction in this species. This is essential to develop techniques to manipulate artificial propagation for further and future laboratory experimental purposes.

In teleosts, the regulation of reproductive processes includes a complex interaction between the brain, pituitary and gonads. Several authors indicated that such reproductive changes have a seasonal cyclic basis linked to environmental factors. Thus exogenous and endogenous impulses are integrated in the brain from where the secretion of gonadotropin by the pituitary gland is regulated. It therefore implies that environmental factors are responsible for stimulating the onset of the reproductive cycle in freshwater fish and that this period of reproduction is limited to a few months of the year (Bruton, 1979). It can therefore be assumed that the onset of such reproductive cycles for any particular species will show geographical differences.

The most general research approach to reproductive physiology focuses on the pituitary gland. The latter secretes a number of peptide hormones that control a wide spectrum of physiological processes in vertebrates. Over the past two

a failure of a GTH surge. Several successful attempts have been made to artificially induce maturation and ovulation of fish in captivity and although a multitude of investigations have completed to outline the regulation of reproduction, no serious attempts have been made to define the regulation of reproduction in ***Oreochromis mossambicus***.

It is generally known that in mammals, the release of follicle stimulating hormone (FSH) and luteinizing (LH) is controlled by releasing factors from the hypothalamus (Guyton, 1986). This appeared also to be the case in teleosts (Peter, 1983; Donaldson and Hunter, 1983) where this hormone was described as gonadotropin releasing hormone (GnRH). These are generally referred to as neuropeptides. Chang & Peter (1983) and Peter et al. (1978) also discovered the existence of a gonadotropin release inhibiting factor (GRIF) whose function is similar to dopamine. Applying a dopamine antagonist potentiates the GTH release (Dufour ***et al.,*** 1984). The latter acts on the gonads to stimulate their development which results in the secretion of steroids. This in turn, exerts a negative feedback on GTH release (Sundararaj and Goswami, 1968; Billard and Peter, 1977) to promote gonad development.

The reproductive physiology of freshwater fish is therefore of great interest and importance due to the fact that several fish species have been successfully bred artificially in captivity. ***Oreochromis mossambicus***, (see FIG 1.1) a freshwater fish found in rivers and dams in the Northern Transvaal, is no exception to the above statement, and consequently is currently under consideration as a candidate species for commercial aquaculture programs in this area. In order to undertake programme of this nature, it is essential to become acquainted with the environmental factors responsible for the onset of reproduction in this species. Many studies have been undertaken in this regard on Tilapia in South Africa, but no information is available on the physiological regulation of reproduction in the Highveld region of the Far Northern Transvaal. In general, Teleosts reveal a complexity of reproductive behaviours in terms of their gonadal structures. The

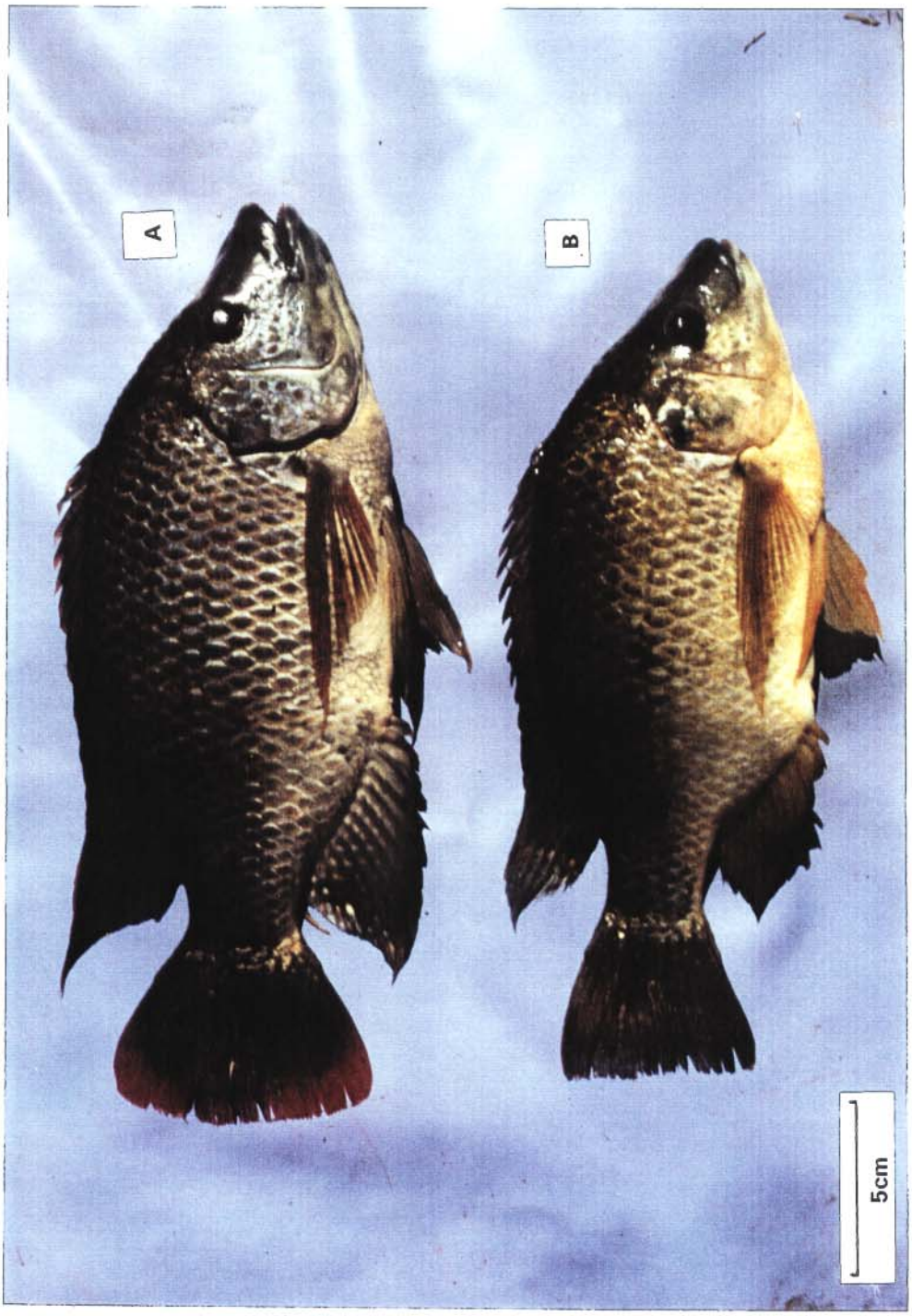


FIG 1.1: Photograph of male (A) and female adult ***Oreochromis mossambicus***.

basic physiological functions of the fish gonads are to produce fertilizable gametes, which are essential for the survival of a species.

The reproductive efficiency of the tilapia results directly from several biological or ethological characteristics which include the following:

* The making of a nest combined with a nest protecting behaviour (Lowe - McConnell, 1959; McBay, 1961; Bruton & Boltt, 1975; Ruwet ***et al.***, 1976; Philippart & Ruwet, 1982).

* A sequential oviposition immediately followed by the fertilization of each group of ova (Shaw & Aronson, 1954; Philippart & Ruwet, 1982; Mélard, 1986).

* The occurrence of parental care provided to the eggs immediately after they have been fertilized. In mouth-brooding species (***Oreochromis***), the eggs are brooded in the buccopharyngeal cavity; this behaviour often combined with a migration of the breeding fish to a planted and thus protected area.

* Reproduction may begin quite precociously for small sized fishes (Lowe - McConnell, 1958; 1983; Ruwet ***et al.***, 1976).

* Successive reproduction cycles enable a female to produce a new batch of fry every 4 to 6 weeks (Arrignon, 1969; Rothbard & Pruginin, 1975; Ruwet ***et al.***, 1976; Mélard & Philippart, 1981) except in environments exposed to significant seasonal variations (Moreau, 1979).

Controlled breeding programs not only ensure the continued existence of economically viable fish species, but may also assist in satisfying an ever increasing demand for protein for human consumption.

The importance and relevance of studies on certain parameters of fish blood

(and/or plasma) associated with reproduction, has recently become of significant interest to the fish biologist, particularly for fish production during aquaculture. A fundamental understanding of the regulation of reproduction in ***O. mossambicus*** will therefore greatly enhance the artificial propagation techniques for this particular species. Many efforts are currently used to facilitate artificial breeding of this species in captivity by control of the aquatic environment.

Although steroid hormone secretion within the fish gonad is controlled by the secretion of the gonadotropic hormones, this latter group of hormones may well influence gonadal development themselves (Upadhyay, 1977; Crim & Idler, 1978). Even though information regarding the hormonal control of maturation and ovulation in teleosts does exist, the effect of the time lapse between ovulation and spawning on fertilization and hatching rates is not yet well documented. Furthermore, the Gonadosomatic Index (GSI) may be used to indicate a seasonal breeding pattern. It is therefore necessary to determine the gonadotropic and steroid hormone concentrations for each and every gonadal developmental stage during the entire breeding cycle. This information will assist the researcher in terms of administering appropriate doses of hormone and at the appropriate point in the breeding cycle when attempting to artificially propagate ***O. mossambicus***. Knowledge of the breeding cycle and associated endocrine and biochemical changes for ***O. mossambicus*** may lead to greater success rates in artificial breeding programs. The present study involves an investigation into the reproductive cycle of ***O. mossambicus*** to establish a complete reproductive hormone profile which includes the histology and electron microscopic examination of gonadal development.

Further, an investigation into the physiological and biochemical status of the gonadal homogenates in relation to the same parameters in the serum will contribute toward the development of a commercial breeding program for ***O. mossambicus***.

In the light of the above information on freshwater fish production, several successful attempts have been made in South Africa to breed several freshwater fish species in captivity in South Africa. ***Clarias gariepinus*** (Prinsloo ***et al.***, 1989), ***Cyprinus carpio*** (Prinsloo and Schoonbee, 1984), ***Ctenopharyngodon idella*** and ***Hypothalmicthys molitrix*** (Schoonbee ***et al.***,1978), ***Schilbe S mystus depressirostris*** (Kruger and Polling, 1984) were successfully undertaken with pituitary extracts in South Africa. In spite of the above successful attempts, no information on the regulation of reproduction of ***Oreochromis mossambicus*** on the highveld in South Africa is available. This study was therefore undertaken to achieve this objective and was designed to include the following information:

1. The role of both aquatic and geographical environmental factors in the regulation of reproduction in males and female ***Oreochromis mossambicus.***

2. General macroscopic observations on physical measurements of gonadal development over the annual reproductive cycle on a monthly basis.

3. Histology and ultrastructure of gonadal development.

4. Hormonal profiles in ***Oreochromis mossambicus***.

5. Biochemical analysis of the plasma and gonadal supernatants.

6. General discussion on the regulation of reproduction in ***Oreochromis mossambicus.***

CHAPTER 2

Role of environmental factors

2.1 Introduction

In this study, on the reproductive biology of ***Oreochromis mossambicus***, the role of environment factors were considered to be of major importance. Many environmental and internal factors are thought to act as cues for the initiation of complex behavioural, physiological, biochemical and neuroendocrine changes controlling the pre- and postspawning processes of feeding, reconditioning, gonadal maturation and ovulation. Johnston ***et al.*** (1992) have shown that photoperiod may alter the normal cues such that gonadal maturation in Atlantic salmon (***Salmo salar***) occurs when daylength is short and water temperature is rising. Other studies have demonstrated that gametogenesis and spawning may be accelerated with compressed light cycles. Bromage (1987) has shown that time of first spawning may be advanced in female trout with photoperiod manipulation. Lam (1983) in Hoar, Randall and Donaldson (1983) states that photoperiod does not affect the timing of first sexual maturity in ***Sarotherodon*** (now ***Oreochromis***) ***mossambicus***. Puberty may occur in either continual darkness or continual light, thereby implying an endogenous rhythm.

Although photoperiod is considered to be an important regulator of oocyte development and spawning time, photoperiod does not act alone in controlling reproductive function. The extent to which water temperature acts synergistically with photoperiod has been poorly described. Billard and Breton (1977), Manning and Kime (1985) and Beacham and Murray (1988) have shown that it is likely that oocyte size and the time required for final egg maturation in Atlantic salmon may be governed by water temperature.

Beamish (1976) has stated that spawning of certain species may be inhibited in

acidic waters, thus indicating a possible important role of pH of the environment water.

In both tropical and subtropical species, peak spawning activity is often associated with rainfall (Schwassmann ,1971; 1978; 1980; de Vlaming, 1974; Lowe-McConnell, 1975; Billard and Breton, 1978; Gibson, 1978 and Liley 1980).

With the foregoing in mind, the role played by environmental factors such as rainfall, photoperiod, relative humidity, wind speed, environmental (atmospheric) temperature, dam water temperature and dam water pH were examined and related to a possible effect on the reproductive cycle of ***Oreochromis mossambicus***.

2.2 Literature Survey

The environment has long been considered to play an important role in the reproductive cycles of freshwater fish. Amongst those factors considered to be of importance are photoperiod, temperature, salinity, rainfall, lunar cycle and others. It is thought, that the external factors act on exteroceptors of the fish and through them on the hypothalamus - pituitary - gonad axis.

The influence of environmental factors has been reviewed within several fish species (Crim, 1982; Wootton, 1984; Zohar & Billard, 1984; Munro ***et al.***, 1990). Photoperiodic effects on reproduction have been reported for numerous cyprinids (Hontela & Stacey, 1990). A decreasing photoperiod generally inhibits spawning of both male and female fish. Poncin (1991) has shown in the barbel, ***Barbus barbus L.*** that this allows for two periods of reproduction; which contrasts with the situation known to occur in the salmonids (Bromage ***et al.***, 1984).

In tropical and subtropical species, peak spawning activity is often associated with rainfall, floods or the lunar cycle (De Vlaming, 1974; Lowe-McConnell, 1975; Schwassmann, 1971, 1978, 1980; Gibson, 1978; Billard & Breton, 1978; Liley, 1980).

It is not clear which of the terminal reproductive events (oocyte maturation or ovulation) is triggered or enhanced by rainfall, or whether spermiation and/or sperm release is involved. Further, it is not clear what specific factor or factors associated with rainfall may be involved in spawning stimulation. Bruton (1979) has suggested numerous related factors, like lowering of water temperature, dilution of electrolytes (eg: decrease in conductivity), increase in oxygen content and a change in pH. It is most likely that a consortium of factors is involved.

In subtropical or subtemperate regions (as is this study area - close to the Tropic of Capricorn), seasonal variations in photoperiod and temperature are relatively small. In those species that spawn in spring or early summer, gonadal recrudescence may often be stimulated as a result of increasing photoperiod, particularly when considered in combination with increasing temperatures (Lam, 1983). Fish are however, usually exposed to a gradual rather than an abrupt increase in photoperiod.

According to De Vlaming (1972), who reviewed the literature, photoperiod may play the major role in salmonids, while in cyprinids both photoperiod and temperature is of importance. Hilge (1989) indicates that De Vlaming stated that his results were inconclusive because they were mainly based on short term laboratory experiments and that the pre-experimental phase of the fish had not been taken sufficiently into consideration.

Photoperiod is also considered to be the most important environmental factor which modulates melatonin rhythms (Kezuka, ***et al.***, 1992). They state that melatonin is the time-keeping hormone in fish because of its cyclical appearance,

whereby melatonin levels in the pineal gland, blood and cerebrospinal fluid fluctuate in a rhythmic fashion. Bartke ***et al.*** (1982) have indicated that pinealectomy abolishes testicular regression in response to a short photoperiod and causes the regeneration of atrophic testes. These results have shown that melatonin and the pineal gland form an important intermediary in the action of photoperiod on the hypothalamic - pituitary - testicular axis.

The effects of temperature are also considered to be an important environmental factor regulating fish reproduction. It is thought that temperature may exert its effects by a direct action on gametogenesis (Lofts ***et al.***, 1968); an action on pituitary gonadotropin secretion (Breton & Billard, 1977; Peter, 1981); an action on metabolic clearance of hormones (Peter, 1981); an action on the responsiveness of the liver to estrogen in the production of vitellogenins (Yaron ***et al.***, 1980) or an action on the responsiveness of the gonad to hormonal stimulation (Jalabert ***et al.***, 1977; Bieniarz ***et al.***, 1978).

Rana (1990) has shown that low temperatures adversely effect the development of larvae whereas higher temperatures accelerate development in the tilapia, ***Oreochromis niloticus*** (L.).

Burns (1976) has shown that temperature is of utmost importance in gonadal development. He has shown that a critical temperature exists for testicular maturation which differs for ovarian development in the pumpkinseed, ***Lepomis gibbosus***. This is reflected in the gonadosomatic indices recorded for males and females respectively.

According to Okuzawa ***et al.*** (1989), an increase in water temperature induces vitellogenesis and spawning in cyprinid fishes. Their results for the cyprinid, ***Gnathopogon caerulescens*** indicate that the response to changes in photoperiod are most pronounced at higher water temperatures. At high temperatures, gonadal recrudescence is enhanced only at long photoperiods, whereas at low

temperatures it is enhanced regardless of photoperiod length.

pH is an important environmental factor to be considered due to the role it plays in terms of water pollution. Acidification of surface waters is one of the most serious problems of environmental pollution in North America (Fromm, 1980). Acid stress may impair vitellogenesis and even lead to spawning failure. These effects may be related to an upset in calcium metabolism and to a faulty deposition of yolk proteins in developing oocytes. High pH may also pose a problem to the reproductive activity of the fish living in such an environment. Due to the nature of the sampling site, similar problems could be encountered at Syferkuil Dam.

No literature could be found that relates or correlates either relative humidity or wind speed to freshwater fish reproduction. However, as both of these factors will play a role in both environmental and water temperature, it was deemed necessary to measure these parameters in this study and ascertain if either relative humidity or wind speed or both play a meaningful role in the reproductive cycle of the tilapia, ***Oreochromis mossambicus*** used in this study.

2.3 Materials and methods

Each Monday morning, 10 adult male and 10 adult female ***Oreochromis mossambicus*** specimens were collected at Syferkuil Dam, 8km north-west of the University of the North using a seine net. The period of collection lasted for a full calendar year. Before the experimental animals were transported back to the laboratory at the university campus for further analysis, a number of recordings pertaining to environmental conditions were made. These included both H_2O temperature (°C) and H_2O pH at 08h00 which was carried out using a portable immersible thermometer and pH meter respectively. For both of temperature and pH, the same three sites in the dam were used each Monday morning throughout the experimental period. The meters were obtained from Hanna Instruments.

The weather bureau provided the rainfall (mm), minimum & maximum daily air temperature (°C), relative humidity (%) and wind speed (ms^{-1}) for the collection site during the given sample period. The time of both the sun's rise and it's time of setting during the entire sampling period was also provided which allowed for the calculation of daylength (hrs) over the same period.

The environmental factors thus established were related to the GSI, standard fish length, parental fish mass, gonad mass and length.

2.4 Results

All data in TABLES 2.1; 2.2 and 2.3 are the mean monthly ± standard deviation values recorded for the parameters indicated. In the case of rainfall (TABLE 2.1) it is the total monthly rainfall that is indicated, and hence no standard deviation is indicated.

The following TABLE 2.1 shows the mean monthly rainfall (mm), daylength (hrs), relative humidity (%) and wind speed (ms^{-1}) at Syferkuil Dam.

	Rainfall (mm)	Daylength (hr)	Relative Humidity (%)	Wind Speed (ms^{-1})
	mean	mean ± sd	mean ± sd	mean ± sd
May	1	10.97 0.14	73.19 19.02	3.50 0.91
Jun	0	10.69 0.28	73.50 15.83	4.20 0.82
Jul	0	10.82 0.10	74.90 15.80	4.00 1.01
Aug	320	11.30 0.18	75.90 17.77	3.70 0.88
Sep	165	11.95 0.20	75.73 13.37	5.70 1.42
Oct	340	12.64 0.21	73.32 14.35	5.50 1.39
Nov	1096	13.25 0.15	71.30 13.59	5.50 1.81
Dec	1200	13.58 0.04	82.10 8.07	2.90 0.76
Jan	528	13.42 0.12	76.19 13.34	1.20 0.28
Feb	415	12.89 0.18	82.45 8.71	3.20 0.61
Mar	423	12.23 0.21	85.52 7.96	1.50 0.34
Apr	43	11.54 0.19	80.53 13.33	1.10 0.17

TABLE 2.1: Rainfall, daylight hours, relative humidity and wind speed at Syferkuil Dam, 8km northwest of the University of the North.

The following TABLE 2.2 shows the minimum, 08h00 and maximum air temperature at Syferkuil Dam.

	Min. Temp. (°C)	08h00 Temp. (°C)	Max. Temp. (°C)
	mean ± sd	mean ± sd	mean ± sd
May	9.69 2.73	12.30 2.80	25.12 3.57
Jun	4.32 2.17	6.76 2.97	19.53 3.53
Jul	3.77 1.60	6.10 2.39	20.02 2.50
Aug	7.22 2.47	9.70 2.19	20.69 4.34
Sep	11.78 2.40	14.91 2.34	23.45 4.30
Oct	12.75 2.89	16.87 3.67	24.92 4.09
Nov	16.98 2.04	20.40 2.99	27.83 3.57
Dec	18.19 1.35	20.83 1.97	27.72 2.24
Jan	17.40 1.84	20.61 2.10	29.15 2.65
Feb	17.30 1.76	19.74 1.57	27.01 2.40
Mar	16.76 1.99	19.10 1.53	26.87 2.75
Apr	13.22 3.35	15.74 2.92	25.15 3.08

TABLE 2.2: Minimum, maximum and 08h00 temperature at Syferkuil Dam, 8km northwest of the University of the North.

The following TABLE 2.3 shows H_2O temperature (°C) and pH at Syferkuil Dam.

	H_2O Temperature (°C)	H_2O pH
	mean ± sd	mean ± sd
May	15.20 0.20	7.52 0.14
Jun	13.50 0.60	7.52 0.11
Jul	14.30 0.90	7.50 0.09
Aug	14.30 0.70	7.27 0.12
Sep	20.30 0.90	7.50 0.16
Oct	24.50 1.10	7.42 0.07
Nov	25.70 1.30	7.41 0.13
Dec	25.10 3.90	7.30 0.11
Jan	24.80 1.20	7.23 0.08
Feb	24.60 1.10	7.26 0.21
Mar	20.70 0.60	7.31 0.17
Apr	18.40 0.30	7.38 0.13

TABLE 2.3: H_2O temperature and pH at Syferkuil Dam, 8km northwest of the University of the North (recordings per month, n = 12).

FIG 2.1 depicts the relationship between H_2O temperature (°C) and pH at Syferkuil Dam. It may be seen that dam water temperature reaches a minimum of 13.50 ± 0.60°C during June, which is winter in the northern Transvaal. Thereafter, as spring approaches in September, there is a sharp increase in temperature until a maximum of 25.70 ± 1.30°C is reached in November. Dam water temperature remains fairly high throughout summer and only falls again during April, which would represent the commencement of autumn. Dam water pH shows an inverse relationship to the temperature of the water except at the onset of spring when, immediately prior to the sharp increase in water

temperature, there is a marked decline in dam water pH to 7.27 ± 0.12. FIG 2.5 shows that this is also when air temperature begins to increase.

FIG 2.1: Water temperature and pH at Syferkuil Dam

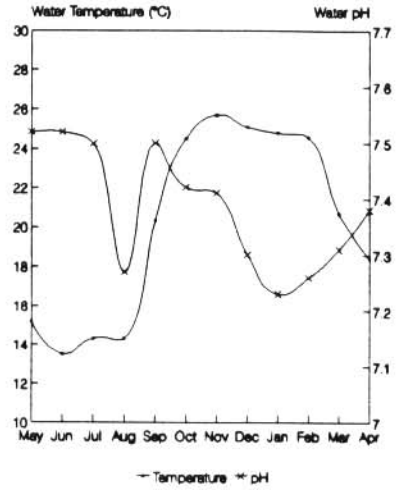


FIG 2.2: Water temperature at Syferkuil Dam and GSI in male and female *O.mossambicus*

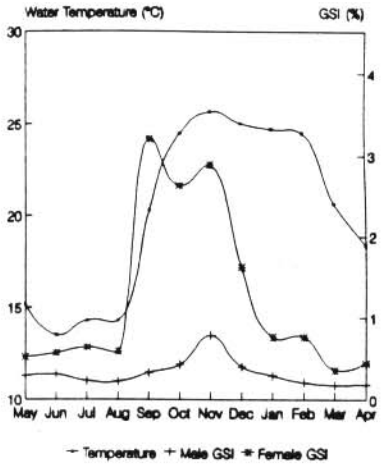


FIG 2.2 shows the relationship of Syferkuil dam water temperature (°C) and both male and female ***O. mossambicus*** gonadosomatic index (GSI) values. These GSI values are presented in Chapter 3, TABLE 3.2. It may be noted that at the same time as the water temperature begins to increase, so too does the female GSI increase markedly to reach it's maximum of 3.11 ± 0.72 during September. This is indicative of a "heavier" gonad which represents a higher degree of gonadal maturity. The female ***O. mossambicus*** GSI remains high until November which is when the male GSI reaches it's maximum of 0.78 ± 0.12, indicating that the male is now also reproductively mature. However, during August, males showed a non-significant increase in GSI.

FIG 2.3 shows the relationship of the mean monthly rainfall (mm) and the relative humidity as measured in the morning at Syferkuil Dam. The winter months showed no rain being recorded but during the hotter summer a maximum of

1200mm of rain fell during December. This appeared not to affect female GSI, but coincided with the initial increase in male GSI. The relative humidity was also considerably higher during the period July to September when the first rain fell and showed a marked increase with the higher rainfall measured during December. This occurred when male GSI showed its initial increase.

FIG 2.3: Rainfall and relative humidity at Syferkuil Dam

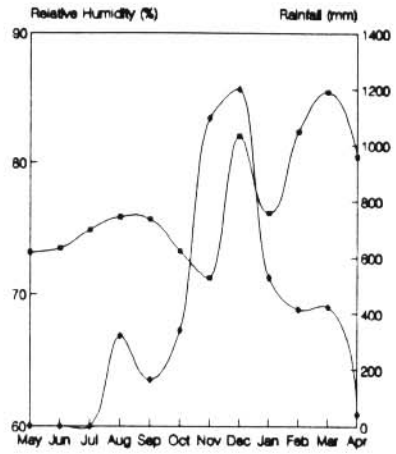


FIG 2.4: Rainfall at Syferkuil Dam and male & female GSI levels in *O.mossambicus*

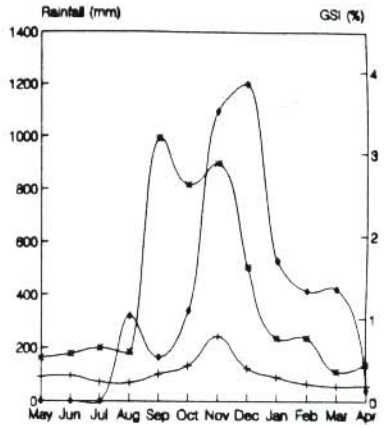


FIG 2.4 shows the relationship between male and female ***O. mossambicus*** GSI and the mean monthly rainfall at Syferkuil Dam. During the month of August an initial surge in rainfall of 320mm was recorded and this coincided with the increase in male GSI. Rainfall did not affect female GSI. However, during November when male GSI reaches it's maximum value, the rainfall for the month had increased considerably with 1096mm being recorded.

FIG 2.5 shows the minimum, 08h00 and maximum air temperature (°C) recorded at Syferkuil Dam. The data portrayed corresponds with that which may be seen in FIG 2.1 indicating the seasonal fluctuation in temperature at Syferkuil Dam. Air temperature started to increase during August.

FIG 2.5: Air temperature at Syferkuil Dam

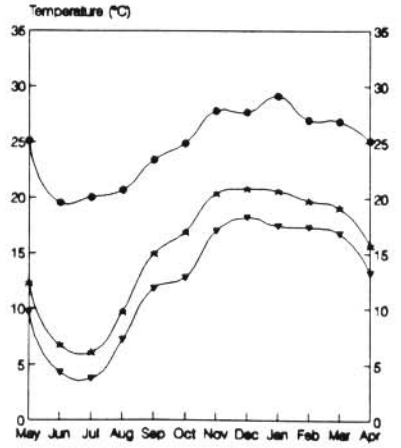
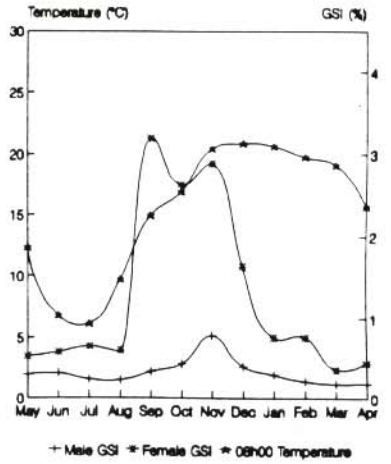


FIG 2.6: 08h00 air temperature at Syferkuil Dam and male & female GSI in *O.mossambicus*



In FIG 2.6 the relationship between male and female ***O. mossambicus*** GSI and the temperature as recorded at 08h00 at Syferkuil Dam are depicted. It may be noted that as may be seen in FIG 2.2 when the temperature shows an increase during early spring (August), the male and female GSI are at their lowest and thereafter increase to their maximum values.

FIG 2.7 indicates the photoperiod as recorded at Syferkuil Dam. It may be seen that daylight and darkness exhibit an inverse relationship with maximum daylight hours of 13.58 ± 0.04 hrs being measured during December. This is to be expected as the summer solstice is 21 December.

FIG 2.8 shows the relationship between male and female ***O. mossambicus*** GSI and the duration of daylight (hr) as observed at Syferkuil Dam. During the month of August, with the onset of spring, it may be seen that the length of daylight hours begins to increase substantially. This coincides with the change in female GSI. The significance of this change in photoperiod is observed in September when daylight an dark hours become equal and female GSI reaches its first peak.

However, the maximum daylength of 13.58 ± 0.04 hrs recorded during December appears to be more closely associated with the maximal male GSI of 0.78 ± 0.12 which is observed in November.

FIG 2.7: Light and dark hours at Syferkuil Dam

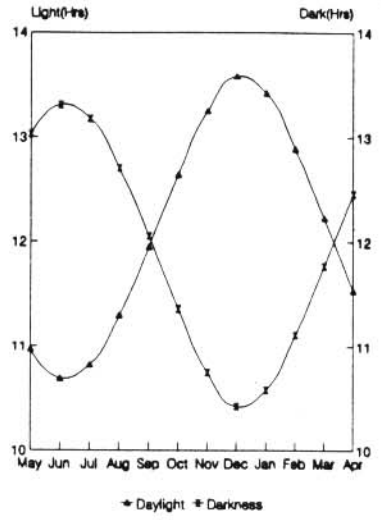


FIG 2.8: Daylight hours at Syferkuil Dam and male & female GSI in *O.mossambicus*

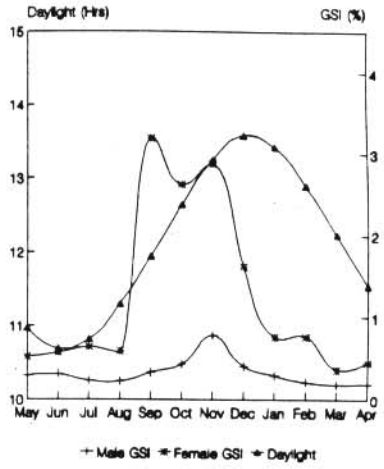


FIG 2.9 indicates the mean monthly wind speed (ms^{-1}) recorded at Syferkuil Dam during the experimental period. Wind speed reaches a maximum of 5.70 ± 1.42 ms^{-1} during September and a low of 1.10 ± 0.17 ms^{-1} during April.

FIG 2.10 represents the relationship between male and female ***O. mossambicus*** GSI and the mean monthly wind speed (ms^{-1}) as recorded at Syferkuil Dam. Wind speed increases during September which coincides with the female GSI peak value being observed. During November, the male GSI peak value of 0.78 ± 0.12 is reached and although wind speed is still relatively high (5.50 ± 1.81ms^{-1}) it immediately drops off quite markedly to a value of 1.20 ± 0.28 ms^{-1} by January.

FIG 2.9: Wind speed at Syferkuil Dam

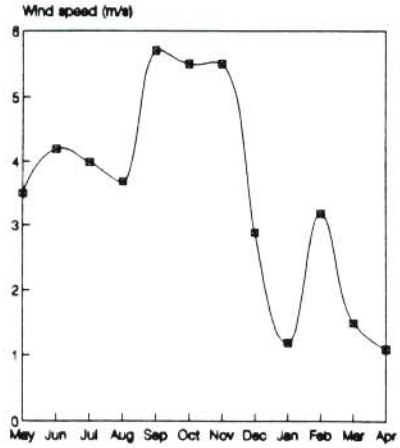


FIG 2.10: Wind speed at Syferkuil Dam and GSI in male and female *O.mossambicus*

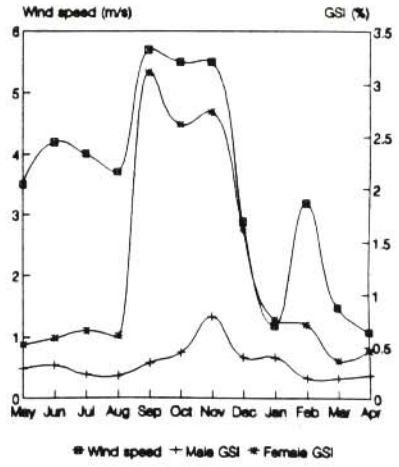


FIG 2.11: Water pH at Syferkuil Dam and GSI in male and female *O.mossambicus*

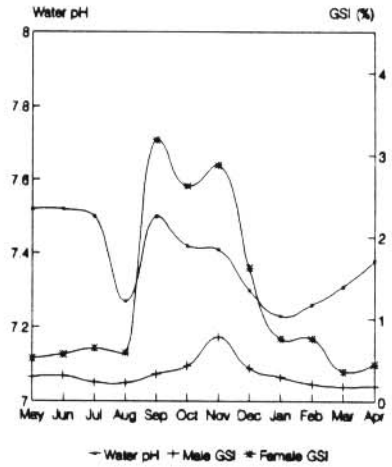


FIG 2.11 represents the relationship between water pH at Syferkuil Dam and the GSI in male and female ***O. mossambicus***. As water pH increases to a high value of 7.50 ± 0.16 during September, the female GSI reaches its peak of 3.11 ±

0.72. Once water pH starts to decline in November, the male GSI reaches its maximum value of 0.78 ± 0.12.

2.5 Discussion

2.5.1 Rainfall

The experimental area (far Northern Transvaal) has been gripped in the midst of a severe drought for some time, and yet the onset of the rainy season still appears to play a role in the reproductive cycle of ***Oreochromis mossambicus***. FIG 2.4 shows that the first rains of the new season fell in August which seems to have been the initial environmental cue for the female gonad to commence development toward maturity. Although the female reached and maintained a high level of gonadal maturity through the months of September, October and November; male development appears to be linked to the heavy rains recorded in November and December.

2.5.2 Photoperiod

It has been suggested that photoperiod is a more important regulator of reproduction than is temperature, with the latter acting only as a rate modifier (reviewed by Bye, 1987). FIG 2.8 indicates that, as is the case for rainfall, there appears to be a relationship between the onset of a longer daylength and the initiation of female gonadal development. This fact seems to be indicated by the increase in female gonadosomatic index during September (FIG 2.8). This coincided with the equalization of the same number of dark and light hours. Thus, the change reflected during August may be an important environmental cue to stimulate female GSI to reach a maximum in September. The maximal male gonadosomatic index is noted during November which is when daylength has virtually reached a peak for the summer months. However, an increase in male GSI also started during September.

2.5.3 Relative Humidity

FIG 2.3 depicts the mean monthly relative humidity as measured in the morning at Syferkuil Dam. It has already been suggested that the initial spring rains observed during August could be a possible stimulus for the female gonad to reach reproductive maturity. Although the relative humidity is not maximal during August, it does show a "mini peak" which then falls off during November, as the heavy summer rains fell. This is also the time when the male gonad reaches reproductive maturity and thus it is possible that this decline followed by an immediate and sharp increase in relative humidity could influence male gonadal development. It is difficult to explain the high relative humidity recorded during March, which is when little, if any, reproductive activity occurs.

2.5.4 Wind Speed

FIGS 2.9 and 2.10 would seem to re-enforce the ideas expressed regarding the role of the environment as a cue for first female and subsequently male gonadal development in ***O. mossambicus***. The high wind speed noted throughout the active reproductive period of ***O. mossambicus*** (September till December) would suggest that the dam water is more turbid during that period and thus there would be a greater availability of planktonic food material which could be utilized as an energy source. This could well be true for the sampling area (Syferkuil Dam) as it is a fairly shallow water impoundment (± 1.50m deep) which would suggest that the entire water body would be affected by any water movement.

2.5.5 Atmospheric temperature

The atmospheric temperature as recorded at Syferkuil Dam is depicted in FIGS 2.5 and 2.6. As all specimens were collected at 08h00 it was decided to relate only this temperature to the state of gonadal development. As has been noted for the other environmental parameters considered to be of importance to

gonadal development for this study, early spring (August and September) represents an increase in the mean daily temperature as recorded at Syferkuil Dam. This also coincides with the initial surge in gonadosomatic index for female ***O. mossambicus*** with male gonadal development lagging behind that of the female. It appears as if male development is stimulated by the attaining of maximal summer temperatures (November and December).

2.5.6 Dam Water Temperature

FIG 2.1 shows an inverse relationship between the dam H_2O temperature and the pH of the same dam H_2O. This inverse relationship is a well documented fact (Chemistry I). FIG 2.2 shows that the increase in dam H_2O during August appears to be the stimulus for female gonadal maturation to commence.

2.5.7 Dam Water pH

FIG 2.1 seems to indicate that the increase in H_2O pH at the same time as the H_2O temperature decreases plays an important role in terms of female gonadal development. In the case of the male, it would seem that the male requires a period of "conditioning" and it only reaches maximal maturity once the pH of the H_2O has declined and the H_2O temperature has reached it's summer maximum during November and December.

2.6 General

Most teleosts require appropriate aquatic conditions to ensure survival. Reproduction and its control are the only means of ensuring survival of any particular species. It is therefore essential to consider all the factors responsible for controlling reproduction in most indigenous freshwater fish species. At present fish broodstock management is more an art than a science, since very little control exists over the maturation of broodstock. The ideal situation is for

fish to undergo gonadal maturation spontaneously in accordance with its natural breeding season. This makes it desirable to control the gonadal development of broodstock in captivity to ensure their availability whenever required. Most environmental factors responsible for the regulation of reproduction are well known and may be used to manipulate broodstock control. From this observation it is evident therefore, that environmental factors contribute significantly to the onset of reproduction in freshwater fish. It therefore follows that some form of interaction between environmental factors and the sensory nervous system of fish occurs. The gland responsible for this interaction is the pineal gland (Jafri and Ensor, 1983). In order to identify and promote the artificial propagation of ***Oreochromis mossambicus,*** it is essential to identify the natural direct and indirect cues for the onset of reproduction in different species. In order to achieve this, it is necessary to determine the relationship between environmental cues responsible for the initiation as well as the triggering mechanism for regulating reproduction in ***O. mossambicus.***

It is well known that in nature, most indigenous freshwater fish species annual breeding season is limited to a few months. In this study, it was essential to determine the contribution of environmental factors to the onset of breeding in ***O. mossambicus*** in an isolated aquatic environment to differentiate between the indirect and direct factors responsible for triggering the reproductive cycle between males and females. In this regard, certain features of considerable biological interest that are associated with reproduction, should be considered. The results on environmental factors recorded in this study clearly suggests that significant differences for triggering the reproductive cycle occurred for male and female ***Oreochromis mossambicus***. It was therefore decided to discuss these under different sections to provide a clear outline for both males and females.

2.6.1 Indirect environmental factors

The above refers to those factors associated with geographical distribution and

must be clearly distinguished from direct aquatic environmental conditions. In this regard, it should be mentioned that most indigenous fish species are seasonal breeders (Bruton, 1979). **Tilapia** species, in general, are temperate zone fishes whose breeding is confined to a brief period during a definite season of the year (Fryer & Iles, 1972). The highveld area in the province of Transvaal (South Africa), is well known as having cold winter temperatures and hot summers. It is therefore expected that temperate fish, such as ***Oreochromis mossambicus***, may begin their breeding cycle as soon as climatic temperatures increase. All factors associated with this temperature change also have to be considered to determine the onset of the breeding season in conjunction with GSI for both males and females. From the results recorded in this study, the following factors made significant contributions.

Males

When comparing the extra-aquatic environmental factors with gonadal development in males, it appears that these indirect factors do not contribute significantly to the onset of the reproductive cycle in males. It appears that relative humidity has an indirect relationship with male GSI. The increase in air temperature from July onwards also suggests that it has a dampening effect on male gonad development. It is only when air temperature reaches its first plateau in September, that a change in male GSI is observed. Peak male GSI values are reached during November when air temperatures are highest. The results recorded for male GSI also increase at a much slower rate than those of females. Male GSI values may not be compared with female GSI values, since they are much lower than those of females. This is possibly related to the size and mass of male gonads which are generally smaller than those of females. Thus a small change in male GSI is more significant than those for females (See Chapter 3). The first significant increase in male GSI based on this observation, only occurs during September at the peak of female GSI. This suggests that male GSI changes are significantly related to direct aquatic environmental factors rather

than the indirect factors . This is clearly demonstrated when comparing daylight hours with male GSI values. It is only when daylight hours have reached a Summer value of 75% in October, that a significant increase in male GSI is observed. This indicates that male gonad development requires a different triggering mechanism than females. These observations explain why male gonad development is much slower than those of females and that it lags behind female values by a period of approximately two months when comparing peak GSI values for both sexes. It should be noted, however, that this observation is not size related in males.

Females

The geographic environmental cues involved in the onset of female reproduction appear to be linked to relative humidity, air temperature, photoperiod and wind speed. The results recorded suggest that they are certainly not directly responsible as a triggering mechanism but rather with the initiation of the reproductive cycle when compared to GSI values. The graphs presented in the section on Results indicate that the extra-aquatic environmental changes are responsible for subsequent changes in the aquatic environment. In this regard, relative humidity increases as a forerunner to the commencement of seasonal rainfall. This parameter also indicates an inverse relationship with air temperature. A similar relationship was also recorded for photoperiod. The latter suggests that increase in daylight hours plays a contributing role toward the onset of the female reproductive cycle. A positive relationship with wind speed was also recorded during the start of the rainy season. From the foregoing, it may be concluded that the indirect factors can be used as indicators for the forthcoming environmental changes after the winter period. It also appears that these factors are crucial to initiate the onset of the reproductive cycle in ***Oreochromis mossambicus***. It is for these reasons that a clear distinction be drawn between those environmental factors that are indirectly responsible for the initiation of the onset of the reproductive cycle in females and the direct aquatic changes that are

essential for triggering the immediate progress in female gonad development. It thus appears that the onset of the reproductive cycle in females require a "conditioning" process before triggering the onset of the reproduction cycle when compared with male ***Oreochromis mossambicus***. These observations also confirm the findings of Fryer & Iles (1972) and Balarin (1979) that most ***Tilapias***, including ***Oreochromis mossambicus***, favour temperate climates for artificial propagation. The distribution of ***Oreochromis mossambicus*** in the Highveld region suggests that the climate in general, supports the unconditional inhibition and natural control of their numbers as a result of wide temperature fluctuations. The cold winter temperatures therefore inhibit reproductive activities and also contribute to mortalities, especially of fry, in ***Oreochromis mossambicus*** in this region. Balarin (1979) indicated that excessive reproduction of Tilapia in temperate environments holds a significant disadvantage as a result of uncontrolled excessive reproduction. Thus for commercial purposes or natural control measures, the Highveld region in Transvaal is more suitable for the natural distribution and control of fish numbers.

2.6.2 Direct aquatic environmental factors

These factors refer to those that trigger reproduction activities within the natural aquatic environment. Gonad development in ***Oreochromis mossambicus*** appears to be more directly related to changes in water temperature, water pH, rainfall, photoperiod, and wind speed. Nevertheless, significant male and female gonad development changes were observed between both sexes of ***Oreochromis mossambicus***. Furthermore, such changes have a greater impact on female gonad development when compared to males.

Males

Male GSI values differ markedly from those recorded for females during the study period. In general, male GSI values started to increase during September and

increased significantly from the end of October to show a peak value in November whereafter a sharp decline was observed. This is in total contrast to female GSI values. These observations suggest that males either require different reproduction triggering mechanisms or that other factors, such as size, mask the initial changes in GSI. The reasons for this are not known, but may be related to different interpretations of environmental changes by the pineal gland.

From the above, it may be concluded that both direct and indirect factors contribute significantly to an increase in male GSI. It also appeared that the indirect environmental factors contributed to a greater extent to the increase in male GSI when compared with direct aquatic changes measured during this investigation. Close observations suggest that low relative humidity during November, high air temperatures, a drop in wind speeds and peak daylight hours coincided with peak male GSI. However, the initial change in male GSI during September appears to be linked to rainfall, change in air temperature and relative humidity. Exactly how these factors interrelate with each other is not known. It is, however, possibly associated with the algal blooms coinciding with low water pH. Algal blooms may be responsible for greater turbidity and less visuality which may also impact on the intensity of daylight hours. The number of dark hours and lunar cycles were not positively associated with male GSI values, but rather the change in photoperiod. Practical experience in angling for ***Tilapias,*** confirm these findings. When such blooms occur, the angling response of ***Tilapias*** is poor as a possible result of poor visibility in the water. Another contributing factor may be the extensive availability of food. This suggests that male reproduction requires special trigger mechanisms to promote gonad development. In contrast to females, the findings suggest that males have a greater significant association with indirect environmental factors as opposed to females where water quality parameters determine gonad development. However, this does not imply that water quality parameters do not contribute to male GSI values. Rainfall and relative humidity may be responsible for the onset of algal blooms. On the other hand , the continued rainfall may cancel algal bloom effects over a longer period,

with resultant greater penetration of daylight into the water. This may explain why male GSI is retarded when compared to female GSI. Thus, males require a greater intensity and perhaps also different environmental trigger actions than females. On the other hand, older males associated with increased size, may display totally different GSI characteristics when compared with younger and smaller males.

Females

Water temperature levels appeared to be the single most significant factor to act as a triggering mechanism for the onset of reproduction in females at the end of August and the beginning of Spring. GSI increases may have been preceded by the slight increase in water temperature observed during July. Significant relationships in female GSI were found with the two way increase in water temperature during this period. This may be associated with the age of the animals. It is possible that the oldest females that were in the minority, may have spawned first by the slight increase in water temperature. Nevertheless, this change in water temperature is positively linked to an increase in environmental temperature. This suggests that it takes time for water temperature to increase following the rise in air temperature. Thus it appears that the increase in female GSI requires a double temperature stimulus to trigger GSI. However, these findings differ significantly from those recorded by Nel (1978) who found that ***O. mossambicus*** permanently exposed to high aquatic temperatures as observed in Orlando Dam, south of Johannesburg (Transvaal), maintain a continuous breeding cycle throughout the year. Balarin (1979) also demonstrated this behaviour for most ***Tilapia*** species. During this investigation, it was also found that low aquatic temperatures inhibits the reproductive cycle in females. Such findings were also supported by Jalabert ***et al.***, (1977). This confirms that the most significant environmental cue for the onset of reproduction in this species, is linked to aquatic temperature.

Another significant contributor to the increase in female GSI, is linked to rainfall. It appears that the rainfall recorded during July / August preceded the change in water temperature. It is not known in what manner this contributed to increased GSI levels, but it may be postulated that the rainfall during this period diluted the water to reduce water conductivity. These in turn may act in conjunction with the increased water temperature, as a triggering mechanism to increase female GSI. Thus the increase in both water temperature and rainfall showed a significant relationship with the increase in female GSI. Furthermore, the sharp increase in female GSI correlated significantly with the first rainfall of the season. In addition, the two peaks in female GSI coincided with the two peak rainfall sessions during the breeding cycle.

The change in the afore mentioned water parameters also coincided with a sharp decline in water pH. This change cannot be explained in terms of GSI values, but may be associated with relative air humidity. The latter increase coincided with the drop in water pH. This change may be ascribed to an algal bloom in the dam at this particular time, since it is well-known that algae consume oxygen and produce large quantities of CO_2 (Schoonbee, 1988). This may explain the sudden drop in water pH that has an inverse relationship with CO_2. However, it is not known in what way this drop in water pH may act as a contributing triggering mechanism for the increase in female GSI. Most information related to this parameter, suggests that acid waters inhibit gonad development. The opposite was experienced during this investigation. It is possible that long term acid stress may have an inhibitory effect, but results recorded during this study suggest that short term exposure to low aquatic pH may actually stimulate the onset of female gonad development. This aspect, however, requires further investigation under controlled laboratory conditions.

Photoperiod also seemed to be connected to the sharp increase in female GSI. The only significant observations made, were that after the cold winter months, a slow change in photoperiod followed by a sharp percentage increase in daylight

hours occurred in September which continued right into December. Maximum female GSI coincided with equal number of daylight and dark hours. During December, a significant decline in female GSI was also observed. It is therefore suggested that the breeding cycle starts in August and ends during December. A plateau was reached at this time which continued into February. This suggests some "last minute" breeding activity, possibly associated with a second round of spawning. However, the above observations do not point to multiple spawning for ***Oreochromis mossambicus*** during the breeding season when considering the fact that this species is a mouth brooder. It does however indicate the possibility of different age groups spawning at different times during the breeding season, with possibly the oldest females spawning first, followed by the youngest females, thereby implicating a double cycle for two different age groups.

2.7 Summary

From the foregoing discussion it would appear that the role of the environment is important in terms of initiating and triggering gonadal development in ***Oreochromis mossambicus*** at Syferkuil Dam.

Female gonadal development is stimulated as a result of the spring changes and increase in rainfall, photoperiod, relative humidity, wind speed, environmental temperature and dam H_2O pH.

Female gonadal development is initiated as a result of the increase in dam H_2O temperature during the spring months.

Male gonadal development may well be initiated at the same time as that of the female. Howéver, there appears to be an approximate two month 'lag' period before reproductive maturity in the male is attained.

Comparison of all the environmental cues, suggest that extra-aquatic

environmental changes preceded the direct aquatic environmental changes to initiate the onset of reproduction in both sexes of ***Oreochromis mossambicus*** . This suggests that in general, rainfall, relative humidity, change in photoperiod, increase in air temperature and windspeed may be responsible for initiating changes in the aquatic environment to induce gonadal development. The triggering mechanism for sudden changes in gonadal development is linked to water pH, water temperature, windspeed and optimal photoperiod. Furthermore, the rate at which these changes occur are essential to initiate female gonadal development whereas in males, this did not appear to be significant. As a result of the significant weight changes in female gonads in relation to parental mass, female GSI is a more significant indicator of gonad development when compared to males. Thus a small change in male GSI is more indicative of sexual activity when compared to females.

It is recommended that further experimental work be conducted in the laboratory to determine the contribution of initial and sudden changes in both geographical and aquatic changes to gonad development. The reason for this suggestion is that the various environmental parameters that have been examined, could then more easily be manipulated. By controlling specific conditions, it would be possible to observe any direct effects that could be manifested in the reproductive development for individual and size related specimens of ***O.mossambicus***.

Alternatively, a second seasonal study could be undertaken and the same environmental conditions be observed. This could be of particular interest once the severe drought that is presently gripping the region, is broken.

It is conceivable that other environmental factors, such as oxygen content and/or turbidity of the dam water could play a significant role in the control of reproduction in ***O.mossambicus***. Thus, by conducting experiments either in the field or in the laboratory, whereby these parameters are naturally measured or are

manipulated, further clarity on the role of the environment on controlling or regulating the breeding cycle of ***O.mossambicus*** may be achieved.

Finally, the results of this investigation support most observations made for this species. However, supportive evidence of the pineal gland and the hypothalamo-pituitary axis is essential to describe the complete reproductive cycle in ***Oreochromis mossambicus***. The relative ease with which this species is bred in captivity, under controlled aquatic conditions, may necessitate investigations into their hormonal reproductive cycle under laboratory conditions.

CHAPTER 3

Reproduction in Oreochromis mossambicus

3.1 Introduction

Tilapia, probably Africa's best candidate species for aquaculture, is faced by the problems of small size due to frequent overpopulating of both culture environments and also impoundments in which they live. (Skelton, 1993)

With already more than 20 cultured species, Tilapias belong to a group of cichlid fish, which is particularly appreciated in the fish farming activity for its robustness, its wide distribution, its significant growth rate and its ease of reproduction.

The Tilapias are a major branch of African cichlid fishes that are generally deep-bodied, with a predominantly vegetarian diet that is reflected in their small teeth, fine pharyngeal teeth and extended intestines (Skelton, 1993).

Length-weight relationships have been examined by Taphorn & Lilyestrom (1983) in ***Curimatus magdalenae***, by Widodo (1986) in the guppy ***Lebistes reticulatus***, by Marshall & Echeverria (1989) in the monkeyface prickleback ***Cebidichthys violaceus*** and by Khumar & Siddiqui (1991) in the carp, ***Puntias sarana Ham***. In addition to the general observations made of the experimental fish, ***Oreochromis mossambicus***, it's distribution and sexual dimorphism provide an indication as to life cycle. Further to the length-weight relationships recorded, the gonadosomatic Index (GSI) has been used to establish or indicate the state of reproductive maturity in ***O. mossambicus*** (Nel, 1978).

Skelton, (1993) indicates that ***O. mossambicus*** breeds during summer, with

the female raising multiple broods every 3 - 4 weeks during the season. Males construct a saucer-shaped nest on the sandy bottoms of the impoundment; the female mouth broods the eggs, larvae and small fry. Although ***O. mossambicus*** grows rapidly and may mature in the space of a year, it is prone to stunting under adverse or crowded conditions.

By examining various aspects of the reproduction of ***Oreochromis mossambicus***, it is intended to provide a clearer basis for correlation with environmental factors, light and electron microscope observations, the chemical composition of both plasma and gonadal supernatant and also reproductive hormonal profiles. In this way, a clearer understanding of the breeding cycle of ***O. mossambicus*** may be realised.

3.2 Literature Survey

Tilapia are cichlid fish which prepare nests primarily for spawning. The fertilized eggs are incubated either in the mouth or in the prepared nests. Trewavas (1973) classified the tilapia into two distinct genera, namely ***Sarotherodon*** (now ***Oreochromis***) which are mouth-brooders and ***Tilapia*** which are substrate-brooders. In ***Oreochromis mossambicus*** the fertilized eggs are incubated in the mouth of the female.

As a general trend in fish, it is considered that factors effecting reproduction are likely to act through or on the gonads by modulating their development. For tilapias, this approach is facilitated by specific characteristics of the group. Baroiller & Jalabert (1989) have indicated the presence of precocious sexual dimorphism of the urogenital papilla (2-3 months after fertilization) which makes it possible to separate male from female fish.

Skelton (1993) has stated that in addition to the tilapiines constituting a major branch of African cichlid fishes; the natural distribution of the tilapiines is tropical

Africa and the Levant (near-Middle East), with certain species having been introduced to tropical and warm temperate areas around the world.

From Africa and the Levant, ***Oreochromis*** are fairly large, deep bodied, mouthbrooding cichlids, that are economically important to man. They are generally tolerant of wide temperature and salinity ranges, and the Mozambique tilapia (***O. mossambicus***) is able to live and breed in both fresh- and seawater. Within southern Africa, there are six indigenous and two introduced species of the genus ***Oreochromis***.

Munro & Singh (1987) report that reproductively - active males in many species of mouth-brooding cichlids establish territories from which they court and attempt to spawn with any ripe females in the vicinity.

The morphological aspects of fertilization in Tilapias has been studied by Bern & Avtalion (1990), who examined the relationship between the specific anatomy of tilapia (***Oreochromis***) gametes and their function.

Rojas (1988) , working on fecundity in ***Oreochromis aureus,*** has shown that the egg size apparently increases with a corresponding increase in the length of the parent fish.

Houzhen and Fujiang (1988), has shown that by studying the reproductive ecology of ***Tilapia nilotica***, the amount of egg mass involved in the sac is positively correlated with both the age and the length of the fish. It was also found that the quantity of dissolved oxygen contained in the water closely influences the condition of oestrous courtship and incubation in the process of mouthbreeding of the fish.

Studies on body mass, body length, gonad mass and gonad length are common in determining the reproductive stage of freshwater fish. From this data the well

documented Gonadosomatic Index (GSI) may and has been calculated for numerous freshwater fish species; thereby providing an indication of the state of reproductive maturity that the gonad has attained. The variation in GSI during the reproductive cycle of both males and females will also allow for the overall developmental stage of the fish. Considerable studies in this regard have been carried out by Fouché ***et al.*** (1985), Marshall & Echeverria (1989) Heins & Baker (1989), Shaikh & Jalali (1990).

The pH of both the blood and the gonad has been used as an indicator of reproductive maturity. Morisawa & Morisawa (1988) have reported such effects for rainbow trout and chum salmon. It is thought that spermatozoa acquire the potential for motility during their passage through the sperm duct. Although there is a critical pH value above or below which damage will occur to the spermatozoa, an increase in pH appears to be necessary to provide the spermatozoa with the ability to acquire motility.

3.3 Materials and methods

Each Monday, 10 adult male and 10 adult female ***Oreochromis mossambicus*** were collected at Syferkuil Dam, 8km north-west of the University of the North using a seine net. The period of collection lasted for a full calendar year. Before the experimental animals were transported back to the laboratory at the university campus for further analysis, a 2,5ml blood sample was collected at the dam using the cardiac puncture method. This blood sample was used in determining blood pH levels. The fish were then transported back to the University of the North campus in oxygenated water containing 20mg/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 were taken and recorded of the standard fish length (cm) (Day, 1969), fish mass (g), gonad mass (mg) and gonad length (cm). The

gonads were also classified according to the Kesteven (1960) macroscopic scale. The Gonadosomatic Index (GSI) was calculated for each fish analysed throughout the sampling period according to the following formula of Roff (1983):

$$GSI\ (\%) = \frac{\text{gonad mass (g)}}{\text{parent mass (g)}} \times 100$$

The gonads were then excised and homogenized using equal parts of distilled H_2O to the gonadal mass. After centrifuging each individual sample, the supernatant was then drawn off and stored frozen at -15°C together with their corresponding plasma samples.

The pH of the plasma and the gonadal homogenate supernatants were measured using a Copenhagen Radiometer Blood Gas Analyzer.

Statistical analyses were carried out using the SAS program at the University of the North main frame computer. 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. From a true statistical point of view, the term significant used in the text does not always comply with the classical statistical definition. The correlation coefficients (r) obtained could be used in a true statistical sense.

3.4 Results

All data presented in TABLES 3.1; 3.2; 3.3 and 3.4 are the mean monthly ± standard deviation values recorded for the parameters indicated.

The following **TABLE 3.1** shows the male and female ***O. mossambicus*** blood and gonadal supernatant pH values for the entire experimental period.

	♂ Blood	♂ Gonad	♀ Blood	♀ Gonad
	mean ± sd	mean ± sd	mean ± sd	mean ± sd
May	7.35 0.09	7.39 0.11	7.24 0.07	7.01 0.14
Jun	7.22 0.06	7.34 0.04	7.22 0.09	7.23 0.09
Jul	7.63 0.22	7.81 0.26	7.46 0.16	6.97 0.24
Aug	7.55 0.09	7.75 0.18	7.52 0.12	6.91 0.31
Sep	7.52 0.09	7.72 0.29	7.23 0.14	6.94 0.16
Oct	7.27 0.06	7.51 0.07	7.31 0.05	7.01 0.07
Nov	7.30 0.07	7.47 0.19	7.14 0.12	7.04 0.17
Dec	7.16 0.10	7.31 0.21	7.21 0.19	7.18 0.23
Jan	7.25 0.12	7.41 0.08	7.17 0.06	7.15 0.08
Feb	7.17 0.04	7.39 0.12	7.21 0.07	7.18 0.20
Mar	7.31 0.07	7.28 0.09	7.28 0.09	7.24 0.07
Apr	7.27 0.12	7.24 0.03	7.24 0.03	7.19 0.22

TABLE 3.1: Male and female ***Oreochromis mossambicus*** mean blood and mean gonadal supernatant pH levels (samples taken per month, n = 40).

The most significant observations recorded for male blood pH indicate significantly higher levels during the months of July till September. Thereafter it declines from September right through till February. Similar observations were made for gonad pH. In both instances it appears that pH reflects a pH surge from July to September. It suggests that this surge is essential for gonad maturity.

Females, on the other hand, also experience a similar type of blood pH shift over the same period as males. However, gonad pH showed a significant decline from July till September and is slightly higher from October to November. Thereafter gonad pH increases significantly up to April. This clearly indicates a

three phase gonadal pH cycle in females. The same observations were recorded for males.

The following TABLE 3.2 indicates the male and female ***O. mossambicus*** Gonadosomatic index (GSI) recorded during the period of investigation..

	Male GSI (%)	Female GSI (%)
	mean ± sd	mean ± sd
May	0.28 0.08	0.51 0.13
Jun	0.31 0.14	0.57 0.17
Jul	0.22 0.09	0.64 0.17
Aug	0.21 0.08	0.59 0.19
Sep	0.33 0.17	3.11 0.72
Oct	0.43 0.25	2.62 0.86
Nov	0.78 0.12	2.74 0.74
Dec	0.39 0.12	1.62 0.29
Jan	0.39 0.09	0.76 0.13
Feb	0.19 0.05	0.71 0.25
Mar	0.19 0.07	0.36 0.13
Apr	0.22 0.04	0.46 0.12

TABLE 3.2: Male and female ***O. mossambicus*** Gonadosomatic Index (GSI) (%) (samples taken per month, n = 40).

Male GSI values increased significantly ($p < 0.1$) from the months of September to January, reaching a peak during November. Male GSI's therefore suggested a three phase cycle, with the GSI peaking during November. TABLE 3.1 shows that the months of April, May and June may be regarded as a period of gonadal recrudescence. Thereafter, in July and August, the recrudescence is arrested until a period of maturation and ovulation related to size frequency distribution

is observed during September, October, November and December. February and March are considered to be the gonadal resting period.

In females, a similar three phase cycle was observed which preceded the male cycle. GSI remained significantly ($p < 0.01$) higher during September to December with an intermediate phase following during January and February. Thereafter female GSI declined significantly.

The following TABLE 3.3 shows parental and gonadal length (cm) and parental and gonadal mass (g) in male ***O. mossambicus*** recorded during the period of investigation.

	Parent Length (cm)	Gonad Length (cm)	Parent Mass (g)	Gonad Mass (g)
	mean ± sd	mean ± sd	mean ± sd	mean ± sd
May	28.21 1.40	7.18 0.87	387.18 30.58	1.10 0.03
Jun	28.16 1.67	7.16 0.91	394.05 75.15	1.22 0.06
Jul	28.49 0.92	7.24 0.55	378.65 94.11	0.88 0.03
Aug	27.89 1.54	6.96 0.91	370.87 66.61	0.79 0.04
Sept	26.14 2.16	6.25 1.22	313.66 97.26	1.07 0.07
Oct	23.65 3.13	6.46 0.96	238.68 79.09	1.06 0.07
Nov	23.05 2.34	6.03 1.06	229.52 70.92	1.80 0.04
Dec	25.24 2.09	6.67 1.00	304.16 97.99	1.17 0.04
Jan	25.02 1.86	6.28 1.06	298.39 99.39	1.17 0.07
Feb	24.72 1.59	6.52 1.49	293.84 76.75	0.54 0.04
Mar	20.78 1.39	4.87 1.40	172.33 66.45	0.33 0.06
Apr	25.09 2.42	6.15 1.21	271.95 90.54	0.60 0.06

TABLE 3.3: Male parent and gonadal length (cm) and mass (g) for ***O.mossambicus*** (samples taken per month, n = 40).

Parental length, mass and gonad length displayed a three phase cycle during the breeding period. However, gonad mass suggested four different phases. The most meaningfull increases were recorded during September / October, December / January and May / June. Notably, the highest parental masses in terms of breeding activity were also recorded over the same period.

The following TABLE 3.4 shows parental and gonadal length (cm) and parental and gonadal mass (g) in female ***O.mossambicus*** recorded during the study period.

	Parent Length (cm)	Gonad Length (cm)	Parent Mass (g)	Gonad Mass (g)
	mean ± sd	mean ± sd	mean ± sd	mean ± sd
May	25.28 1.42	5.71 0.38	262.37 58.67	1.34 0.06
Jun	25.03 1.49	4.99 0.41	248.70 53.33	1.42 0.05
Jul	25.06 1.30	5.00 0.58	261.39 50.42	1.67 0.06
Aug	25.00 1.02	4.67 0.62	259.89 35.19	1.52 0.06
Sept	24.29 2.68	5.63 0.71	256.58 71.03	7.52 0.36
Oct	24.12 2.35	5.46 0.78	252.78 75.91	6.62 0.37
Nov	24.46 2.24	5.10 0.54	240.40 67.48	6.68 0.29
Dec	23.99 2.27	4.96 0.73	240.77 66.99	3.45 0.37
Jan	24.63 1.93	4.79 0.69	242.19 74.69	1.61 0.10
Feb	25.09 1.48	4.84 0.93	263.26 70.71	1.87 0.17
Mar	22.78 1.80	4.23 0.67	227.19 77.18	0.70 0.15
Apr	26.28 2.00	4.71 0.89	282.59 91.19	1.32 0.13

TABLE 3.4: Female parent and gonadal length (cm) and mass (g) for ***O.mossambicus*** (samples taken per month, n = 40).

Females displayed a definite three phase cycle for the above-mentioned period. The most significant high gonad masses were, however, recorded during

September to December, in a declining fashion.

FIG 3.1 represents male and female GSI in ***O. mossambicus***. It may be noted that female GSI reaches a peak value of 3.11 ± 0.72 during September and remains relatively high until November, whereafter it declines rapidly. During this period, four different peaks were recorded, ie: September, October, November and December. the period from March to July showed a gradual increase in female GSI. In the case of the male, GSI is seen to be considerably lower throughout the experimental period and it only reaches it's maximum of 0.78 ± 0.12 during November. However, male GSI increased steadily from September and October and then declined sharply from February. Thereafter a period of gonad quiecense was observed.

FIG 3.1: Male and female GSI in *O.mossambicus*

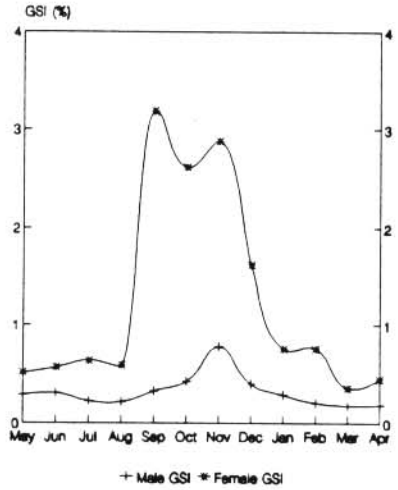


FIG 3.2: Blood and gonadal pH in male and female *O.mossambicus*

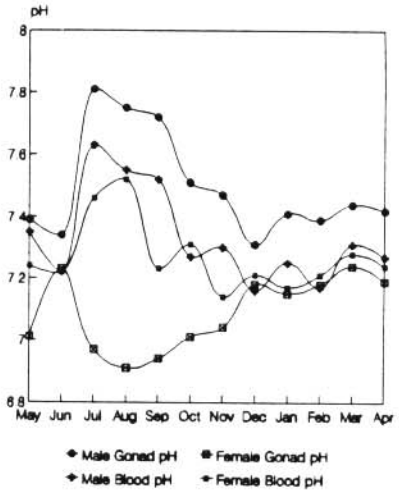


FIG 3.2 depicts the data presented in TABLE 3.1. Male gonadal pH reaches a peak of 7.81 ± 0.26 during July, which is considered to be mid-winter. Male blood pH follows a similar pattern to gonadal pH; however although blood pH also peaks in July it's maximum is only 7.63 ± 0.22. Female gonadal pH shows

an inverse relationship to male gonadal pH with the female pH reaching a low value of 6.91 ± 0.31 during August. However, female blood pH values tend to exhibit a similar trend to male ***O. mossambicus*** blood, with a high value for the female being 7.52 ± 0.12 and observed during August.

FIG 3.3 represents the relationship between GSI and pH in male ***O. mossambicus***. There appears to be an inverse relationship between the two pH parameters and male GSI, thereby clearly indicating the active phases of the male reproductive cycle. This may be clearly seen from July till September when pH in both the blood and gonadal supernatant is high and from June till August when pH is at it's lowest. Once male GSI reaches it's peak in November, both blood and gonadal pH have begun to decline.

FIG 3.3: Blood and gonadal pH and GSI in male *O.mossambicus*

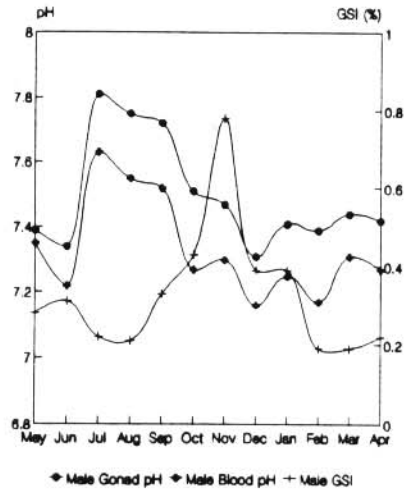


FIG 3.4: Blood and gonadal pH and GSI in female *O.mossambicus*

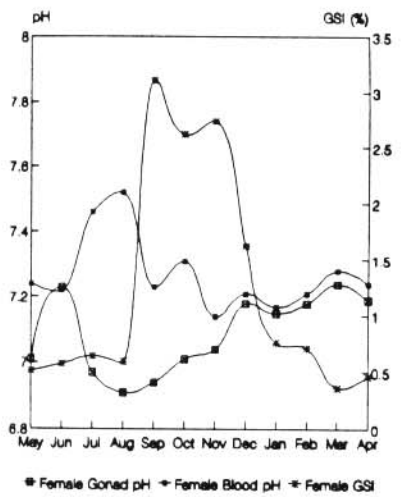


FIG 3.4 represents the relationship between GSI and pH in female ***O. mossambicus***. As has already been stated, it may be seen that in the case of female ***O. mossambicus*** there appears to be an inverse relationship between blood pH and gonadal pH, most notable during the months of June till November.

Female GSI reaches it's maximum of 3.11 ± 0.72 during September and remains high till November whereafter it declines markedly. An inverse relationship between blood and gonad pH also occurred. This ratio was actually smaller when compared with female gonad pH and GSI. These changes complied with all other physical measurements recorded for both males and females.

FIG 3.5 represents the relationship between parental length (cm) and mass (g) in male ***O. mossambicus***. A correlation coefficient of r = 0.99 was obtained indicating a very strong correlation between these two parameters. The parental fish mass and length are both maximal during winter and into early spring whereafter they both decline quite markedly. It is interesting to note that during November, when male GSI reaches a maximum, the mass of the parent fish is seen to be fairly low, 229.52 ± 70.92g.

FIG 3.5: Parental length and mass in male *O.mossambicus*

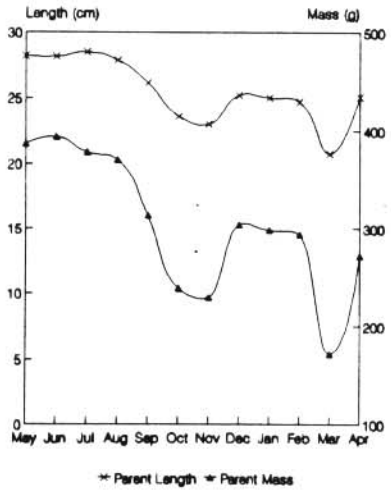


FIG 3.6: Parental length and mass in female *O.mossambicus*

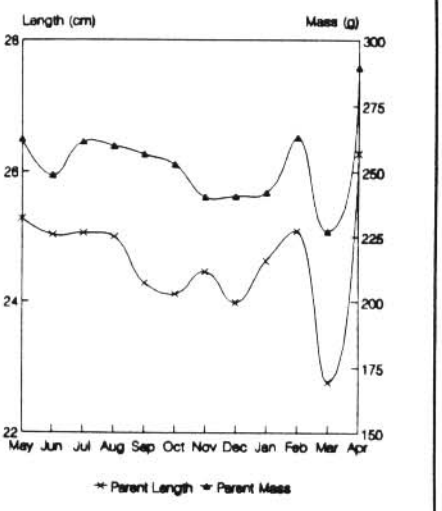


FIG 3.6 represents the relationship between parental length (cm) and gonadal mass (g) in female ***O.mossambicus***. There is a reasonably good correlation between these two parameters; a value of r = 0.54 was obtained. Gonadal mass

appears maximal during the summer months of December and January which may affect this relationship negatively from January onwards, when this parameter decreased greatly.

FIG 3.7 represents the relationship between parental length (cm) and mass (g) in male ***O.mossambicus***. A good correlation coefficient of r = 0.88 was obtained; although it seemed to be negative correlation between these two parameters.

FIG 3.7: Parental length & gonadal mass in male *O.mossambicus*

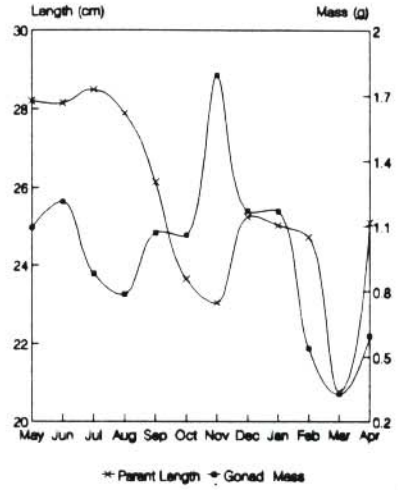


FIG 3.8: Parental length and gonad mass in female *O.mossambicus*

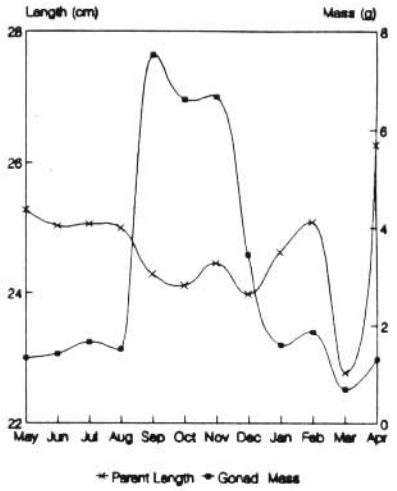


FIG 3.8 represents the relationship between parental length (cm) and gonadal mass (g) in female ***O.mossambicus***. Gonadal mass exhibits a positive relationship with parental length in the case of the female. The gonad reaches a maximum mass of 7.52 ± 0.36 g during September and remains high until November, whereafter it declines markedly. This pattern is also noted in female GSI during this period of the year (see FIG 3.1).

FIG 3.9 represents the relationship between the Syferkuil dam water pH and the

blood and gonadal pH in male ***Oreochromis mossambicus***. FIG 3.10 represents these same parameters in female ***O.mossambicus***. FIG 3.9 indicates that as water pH declines in July / August, so both the blood and gonadal pH shows an increase and thereafter follows a similar pattern for the remainder of the experimental period. FIG 3.10 shows that as water pH begins to decline, so the blood pH in females increase. However, at this same time, the gonadal pH declines markedly resulting in an "acidic female".

FIG 3.9: Water pH and blood & gonadal pH in male *O.mossambicus*

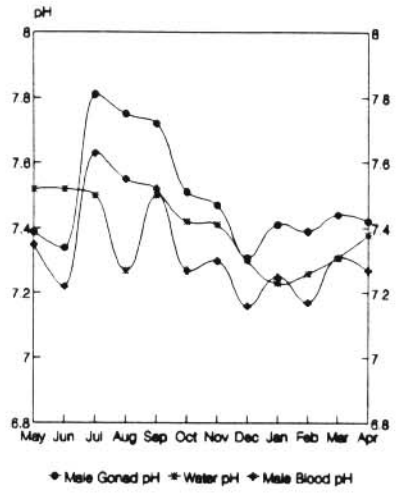
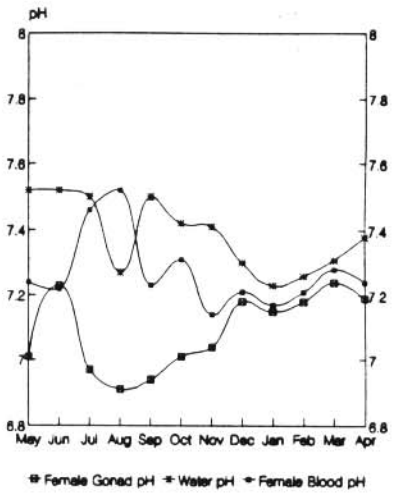


FIG 3.10: Water pH and blood & gonadal pH in female *O.mossambicus*



The relationship between parental length and gonad length is dispalayed in FIG 3.11 for males and FIG. 3.12 for females. In the case of males, the correlation coefficient of r = 0.59 shows an inverse relationship was indicated from the months of September onward to February. During this time male gonad lengths increased when compared to parental length. It also appeared as if the parental and gonadal lengths had a closer relationship when compared to females. The only significant increase in female gonad length was observed in September and it declined thereafter in a varying degree to February.

FIG 3.11: Parental length & gonadal length in male *O.mossambicus*

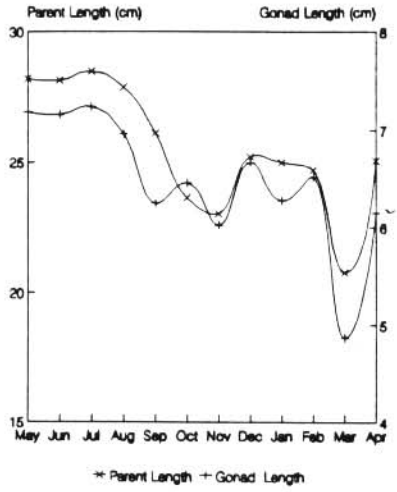
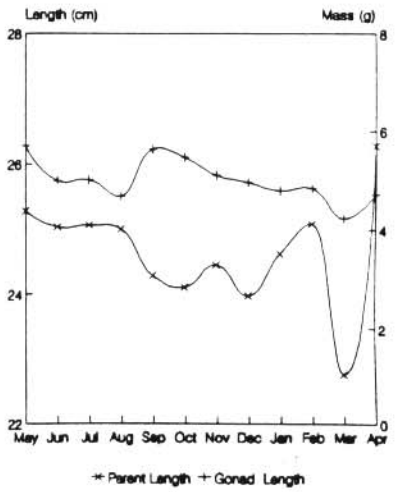


FIG 3.12: Parental length and gonad length in female *O.mossambicus*



3.5 Discussion

3.5.1 General observations

Syferkuil Dam is actually an interconnected series of eight dams with cement sides and mud bottoms. Although certain of the genera in these series of dams are substrate spawners (***Tilapia***), ***Oreochromis*** is a mouthbrooder. The fish collected throughout the experimental period ranged in size from a mean of 20.78 ± 1.39cm to a maximum mean of 28.49 ± 0.92cm in the male. The range of parental lengths recorded in the female was from a mean of 22.78 ± 1.80cm to a maximum mean of 26.28 ± 2.00cm. This may be ascribed to age differences for this species. Males displayed a wider size range than females. The adults were, as Skelton (1993) indicated, a silvery olive to deep blue-grey colour with the dorsal and caudal fins having red margins. The breeding males were noted to change colour, turning a deep greyish black with a white lower head and throat. This colour change seemed to commence in September and remain through October, November and early December; thus suggesting reproductive

activity within the male during this period.

Although Skelton (1993) has recorded a South African angling record of 3.265kg for ***O. mossambicus***, no fish of this size was captured in Syferkuil Dam during the experimental period. This was most probably due to the physical size of Syferkuil Dam and also the relatively high population density. Tilapias are known for prolific breeding in spite of insufficient available space. It therefore implies the presence of smaller specimens that are capable of reaching breeding maturity at an earlier age. In spite of predator fish, such as ***Clarias gariepinus***, in the pond, the numbers of ***O. mossambicus*** specimens were not actively controlled. This probably reflects the abundance of food in this impoundment. Similar observations were made by Balarin (1979).

3.5.2 Distribution and Sexual Dimorphism

The environment where the specimens were collected, may be considered as "confined", which Baroiller & Jalabert (1989) have indicated may rapidly lead to overcrowding and nanism. This semi-natural environment showed distinct overcrowding during certain times of the year - most notably in January and February which is immediately after the breeding season. The specimens collected for this study were found to be living freely in the dam, making sampling by net relatively easy. Thereafter their numbers declined markedly. This may be ascribed to predator action as well as climatic mortalities during winter. Skelton (1993) has stated that ***O. mossambicus*** occurs in all but fast-flowing waters and thrives in standing waters. Syferkuil Dam is a standing water body, which favoured the lifestyle of ***O.mossambicus***.

Oreochromis mossambicus occurs as distinct male and female fish. The gonads are paired structures, but were only clearly visible in mature or occasionally semi-mature specimens. For the major part of the year, the ovaries are a creamish colour, but become a deeper, yellowish colour during October and November

when they are full of ripe ova (eggs). The testes are long slender whitish structures, which in immature fish formed a thin tube, but increased in size with maturity and ripeness during September until November. In reproductively immature fish, the testes were often difficult to locate with the naked eye and equally difficult to remove for subsequent analyses to be conducted.

3.5.3 Body Mass, Body Length, Gonad Mass and Gonad Length Relationships

As may be observed in FIGS 3.5 and 3.6, there is a very definite relationship between the length and the mass of both the male and female ***O.mossambicus*** specimens used in this study. This is to be expected as both of these parameters are related to age, which plays a key role in determining both length and mass of the fish. It should be pointed out that during the period of this investigation, females with fertilized eggs or larvae in their mouths were not used for sampling. This probably explains the observation that the larger specimens breeding earlier than the smaller ones. At the end of August and during September the larger males and females were therefore more sexually active. As the breeding cycle continued, the smaller and younger specimens matured and joined the breeding cycle. It is also possible that the dominance of the larger males and females for breeding preference at the onset of the breeding cycle, inhibited the younger specimens from actively participating in the breeding cycle. Such results were confirmed by the fact that, in general, females were shorter than males. Further, females were also heavier than males in the same size group. Another contributing factor was that female gonadal mass during this period was almost double that of males. Thus, females show more significant characteristics than males. Any change in gonadal mass relative to size, will be more explicitly displayed in females.

FIG 3.7 shows that through the months of September till January, there is a great fluctuation in male gonadal mass as compared to the length of the fish from which the gonad was removed. It should be noted that at the onset of the

breeding cycle for females in September, male gonad mass showed an initial inverse relationship with fish length. Thereafter it changed to a more direct relationship for males being observed. This clearly suggests that the differences in male sizes are involved. This implies that the smaller male fish, experience a bigger increase in gonad mass relative to their size, when compared with the larger males. It may therefore explain the differences in the relationship between male gonad mass and fish length in the smaller specimens. Male GSI also reflects a three phase breeding cycle for males that also seems to be related to fish size. It may be due to the nesting activities of larger specimens which guard the nests after spawning to clear the way for the smaller specimens to continue their breeding activities. This is due to the fact that the testes are fully mature so as to be ready to release mature spermatozoa to fertilize the equally mature female ova being spawned during this period of the year. Once the testes are spent (February and March), there is a rapid decline in the gonadal mass and fish length. From February onwards, the males collected, were predominantly of a smaller size. However, it is clear that the mass increase in male gonads does not occur to the same significant extent as it does in females. The male to female GSI ratio outside the breeding cycle is 1 : 2, whereas during the breeding period it may vary from 1 : 4 to 1 : 10. This reflects differences in size of sperms and eggs; volume of gonads; mass of mature eggs before spawning as well as the increase in gonad mass associated with hydration in females. Hydration in males does not show the same significant changes that are noted in females. This contributes to a smaller increase in male GSI than female GSI. In females, a clear inverse relationship with fish length was observed. The changes in gonad mass also coincided with peak breeding times. This increase in gonad mass reflects an increase in egg size and related gonad activity to promote egg maturity. Female gonads also hydrate prior to spawning. All these factors contribute to extra mass of the female gonad during the breeding cycle. A similar female size distribution in spawning was observed for females. It appears as if a four phase female cycle occurred which suggests two spawning cycles for the larger and smaller females during the breeding season. It is, however, not certain whether the females have

a multiple spawning gonad in contrast to a multiple fractional spawning cycle. The findings in Chapter 4 suggest that the last mentioned possibility is more likely. The mass differences between male and female gonads, suggest that the semen volume and mass during this period is much lighter than the corresponding female gonad masses recorded during the same period. It also appears as if the relative gonad mass increase differs with fish size. Thus the smaller the male, the greater the increase in gonad mass that occurred.

FIG 3.8 exhibits the relationship of parental length and gonadal mass in female ***O. mossambicus***. There is, however, a far greater increase in ovarian mass during the months of September till December, due to the greater mass of the individual ova present in the ovary. In contrast to males, female gonad mass shows a positive correlation with size. These appear to be related to vitellogenesis and the general gonad metabolic activity as may be noted in TABLE 6.5.

A far more relevant parameter in determining reproductive maturity is that of Gonadosomatic Index (GSI). FIG 3.1 confirms the information gleaned from FIGS 3.5; 3.6; 3.7 and 3.8 regarding gonadal maturity. GSI is an expression of the total gonadal mass to the total body mass in terms of a percentage;- thus when GSI is maximal, the gonads are at their heaviest or at their most mature stage prior to spawning. This figure further confirms the ideas discussed in Chapter 2. The results that have been found and discussed in this chapter will also be referred to when examining the histological preparations made of the gonads over the experimental period. It is worth mentioning again that FIG 3.1 indicates that the female reaches reproductive maturity prior to the male, and therefore, in addition to the environmental cues discussed in Chapter 2, female maturity may well be a stimulus for the commencement of male gonadal development. In addition to this, it seems that the more mature fish breed first, followed by their smaller counterparts. Finally, the comparison of male and female GSI's in FIG 3.1 suggest that peak male GSI's reflects a "time lag" of two months for males which may indicate the possibility that spawning is induced only when the males

are ready for breeding. This, however, is a false conclusion, since male parental lengths and gonadal lengths, including the relative masses, suggest that larger specimens display a smaller change in GSI than smaller fish.

3.5.4 Blood pH

FIG 3.2 shows that the concomitant increase in blood pH in both male and female ***O. mossambicus*** blood correlates fairly well with the increase in dam water pH noted in FIGS 2.1; 3.9 and 3.10. It would appear that the pH of the dam water has some effect on both male and female blood pH values and that fish blood pH values are not well controlled. This may be expected as fish live in close association with their aquatic environment. It is also well known that fish blood has a poor buffering capacity (Smit, 1976). Most important, however, is that from June to the end of August, male and female blood pH values followed the pH of the dam water. The latter was associated with algal blooms at the end of the winter season as a result of increased relative humidity and the first rainfall after winter. On the other hand, dam water pH may serve as a triggering mechanism to initiate gonad activity. It is, thus, possible that these changes in pH of the dam water (as seen in FIGS 2.1; 3.9 and 3.10) may directly or indirectly affect the various physiological systems which may in turn initiate changes in reproductive function, particularly in the male.

The increased dam water pH (FIGS 2.1; 3.9 and 3.10) suggests that the findings of Fromm (1980), whereby the acidification (pH ≤ 6) of surface waters in North America led to vitellogenesis being impaired in the flagfish ***Jordanella floridae*** and Beamish (1976) who states that acid stress leads to spawning failure, possibly due to an upset calcium metabolism, are not a factor at Syferkuil Dam with females. The decline in water pH is such that it will not have an effect on inhibiting breeding activity, since water pH levels did not drop below a value of seven.

Comparison of male and female blood pH values indicate a close relationship until the end of August. Thereafter, an inverse relationship was observed until the end of February. Such changes were related to gonad pH which were different in males and females. It does, however, indicate that gonad metabolic activity in both males and females is related to the blood pH.

3.5.5 Gonadal pH

FIG 3.2 also shows a seemingly inverse relationship between male and female ***O.mossambicus*** gonadal pH. During the month of August, which is prior to the surge in GSI, the male gonad becomes more alkaline and the female gonad becomes more acidic. These results suggest that an "acidic female" and an "alkaline" male are required to initiate breeding, which would confirm the suggestion that fish have a poor buffering capacity.

There is more to be learned from FIGS 3.3 and 3.4, where the relationship between blood and gonadal pH and GSI are depicted for males and females respectively. There appears to be a definite inverse relationship between pH and GSI in both male and female ***O. mossambicus***. This inverse relationship between male gonadal pH and GSI, together with the fact that the female gonads reach maturity prior to the male suggests that once the female is in its breeding condition there could be certain other factors that increase the volume of the sperm, culminating in spawning. It appears that there is a common factor in the blood and gonads of male fish which is responsible for the change in the pH levels. When considering GSI and pH values for both males and females, they are of particular interest in the smaller specimens. It suggests that in smaller males, the nature of the gonad activity appears to be different from the larger males at the onset of the breeding cycle. These observations suggest a greater gonad activity. A similar observation was made for females with a contrasting difference in gonad activity which appears to be lower in the smaller females.

The poor buffering capacity of fish blood referred to earlier, suggests that during the breeding cycle, females showed a greater blood buffering capacity than males. This suggests that the nature of the metabolic activities in females are different when compared to males. These observations therefore indicate:

a) less controlled metabolic activity within the testes
b) the possibility that male steroids are more acidic in nature
c) greater mobilization of amino and fatty acids in females
d) greater anaerobic activity in female gonads during the breeding cycle

These findings do not imply that all fish are breeding at the same time (November), but rather that most are. This would concur with Skelton (1993), who indicated that ***O. mossambicus*** spawns on more than one occasion during the breeding season, ie: they are multiple or fractional spawners. This results in ripe eggs being released in batches and thus explains the fact that the GSI and gonadal masses remain high for a few months (September till December/January) and only show a gradual decline.

From FIGS 3.3 and 3.4 it appears that the changes in both blood and gonadal pH could be a further stimulus to at least the initiation of gonadal development in both male and female ***O. mossambicus***.

In general, it appears that major gonadal differences occur when comparing small and large males during the breeding period. It seems that gonad development in smaller males is more susceptible to acid stress in the water. This may affect the fertilizing ability of their sperm cells. This suggests poorly developed and less mature sperm cells, and sperm motility associated with a lesser production of larvae.

3.6 Summary

From the foregoing discussion, the following factors appear to be of importance in the reproductive cycle of ***O. mossambicus***;-

During each breeding cycle, more males and females participate actively. This compliments the suggestion that the bigger animals breed first, followed by the smaller specimens. Furthermore, the results obtained in this investigation, suggests a double cycle for each size group during the breeding period.

The water quality in the dam allows for uncontrolled breeding within the warm summer months. Although predator species are part of the fish population in this series of interconnected dams, their numbers are not sufficient to control ***O. mossambicus*** numbers. This results in smaller individuals that reach maturity at a smaller size.

At certain periods of the year, overcrowding in the relatively confined Syferkuil Dam became a reality, and may adversely affect the reproductive cycle of ***O.mossambicus***.

Reproductive development is associated with changes in blood and gonadal pH as well as an increase in GSI values which correspond with the state of gonadal maturity.

Blood and gonadal pH values are associated with changes in reproductive metabolism.

Male breeding behaviour appears to be stimulated by female reproductive maturity, and male reproductive maturity appears to be the "rate limiting" factor in the breeding process.

It is strongly recommended that a series of experiments be conducted under controlled laboratory conditions to investigate the differences as indicated in the discussion.

During the spawning period, it would be interesting to sacrifice a limited number of fish daily and to record the differences ascribed to blood and gonadal supernatant pH at regular intervals. This could provide a clearer indication and/or explanation regarding the suggestion that an "acidic" female and an "alkaline" male are required for optimal breeding to occur.

CHAPTER 4
Gonadal Development in Oreochromis mossambicus

4.1 Introduction

Most of the existing fish species belong to the bony fishes (Teleostei). Sexual maturation and ultimately spawning are thus important aspects in the life history of freshwater fishes. The majority of studies on reproductive activity have employed gonadosomatic indices (GSI) (Ralston 1981), macroscopic classification of gonad ripeness (Nzioka 1979; Harris 1986) or direct observations of spawning (Robertson 1983; Moyer 1984). Extremely few studies provide histological and/or electron microscopical analyses of gamete development. Considerable work has been carried out on gonadal development in Tilapias (Garcia & Phillip, 1986; Chao ***et al.***, 1987; Zhu, 1987; Fishelson, 1988; Yeheskel & Avtalion, 1988; Hwang & Sun, 1989; Nakamura & Nagahama, 1989). For this reason the general development of both male and female ***O. mossambicus*** are described in order that the traditional GSI information may be supplemented.

The study of spermatogenesis, which is defined as the production and development of male gametes is well documented for all vertebrates. Variations in mature teleost spermatozoa exist and thus this study was undertaken in order that such differences, if any would be noted either histologically or with the aid of an electron microscope.

According to Tricas & Hiramoto (1989), the relationship between ovarian weight and body size often changes with the stage of oocyte development. The production of viable eggs is obviously needed for species survival. A thorough knowledge of oocyte development is a prerequisite for a proper evaluation of reproductive condition. Selman & Wallace (1989) have provided a detailed review of teleost oocyte growth and development. It appears that oogonia may be found throughout the life of the female in most teleosts. These mitotically dividing cells

represent a stem-cell population that gives rise to oocytes and ultimately to eggs. In mature ("ripe") ovaries of many fish, oocytes of variable size and stage of development are randomly distributed.

The results obtained in this study should assist in identifying reproductive activity during both the active and non-active reproductive periods. The various seasonal developmental stages identified, together with GSI may serve as the basis from which further reproductive studies of this species should be carried out.

4.2 Literature Survey

The origin and history of the germ cell line in fish and the development of the gonads, with particular emphasis on the mode of sex differentiation, have been studied in several species. Besides morphological characterization of early germ cells and developing gonads using light microscopy (Wolf, 1931; Dildine, 1936; Johnston, 1951; Yoshikawa & Oguri, 1979; van den Hurk & Slof, 1981; Lebrun, ***et al.***, 1982; Bruslé, 1983; Parmentier & Timmermans, 1985; Selman & Wallace, 1989), electron microscopy has also been used to study the ultrastructure of early germ cells (Satoh, 1974; Bruslé & Bruslé, 1978a; Hogan, 1978; Hamaguchi, 1982; Selman & Wallace, 1989) and gonadal differentiation (Satoh, 1974; Bruslé & Bruslé, 1978b) in a number of teleosts.

The gonads of all vertebrates originate and develop as developing reproductive lines of peritoneal thickened folds (Franchi, 1962; Hoar, 1969) in the dorso-lateral region of the peritoneal space. In most Teleosts the primordial germ cells develop from the dorsal peritoneum and then migrate to the developing genital ridges (Katz ***et al.***, 1976). The genital ridge is a fairly compact blastema, sharply demarcated on the nephric side but virtually part of the epithelium on the coelomic side. A combination of amoeboid movement and passive transport, assisted by the inherent movements of the embryonic tissues, is responsible for this migration (Hardisty, 1967). According to Eckstein & Spira (1965) and Dadzie

(1969) hormonal control mechanisms are of considerable importance during this process.

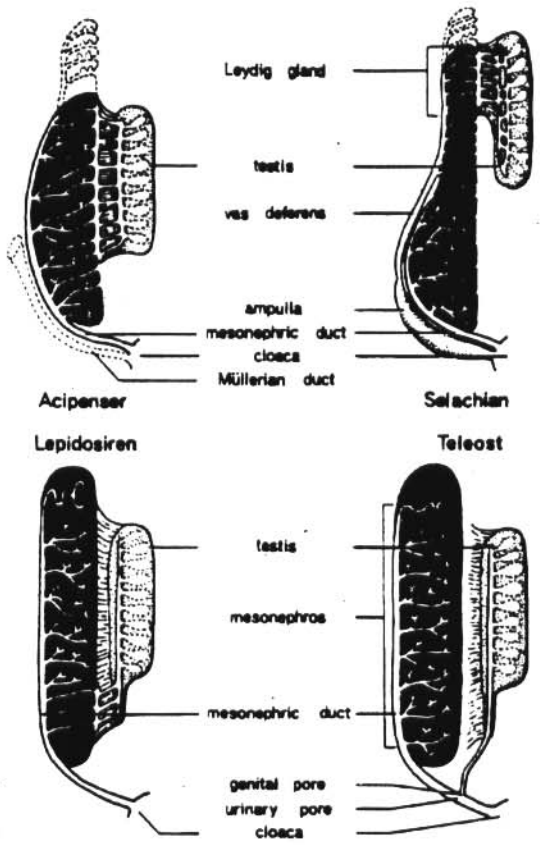
Hoar (1969) states that in the majority of vertebrates, each gonad develops from two closely associated peritoneal cell proliferations. The more laterally situated cortex originates as a lengthened ridge which is destined to develop into an ovary. The medulla, which originates from a more medial cell proliferation that also develops from the interrenal tissue, will develop into a testis. Usually the cortex or medulla develops more rapidly, whilst other cell development is inhibited. Thus the individual's sex is determined fairly early in it's embryonic development (Hoar, 1969). The differentiation of the sexes may possibly be influenced by the positioning of the germinal cells within the undifferentiated gonad. The majority of teleosts develop into a female when these germinal cells are situated in the cortex and into a male when said cells are present in the gonadal stroma (Witschi, 1957; Hoar, 1969).

The characteristic pattern of gonadal differentiation from two distinct peritoneal components are encountered by all elasmobranchs and tetrapods (Chieffi, 1949; 1952; 1967). In contrast to this, the gonads of cyclostomes and teleosts develop from a single component thereby resulting in the gonad developing entirely within the peritoneal cortex. There is therefore no constructive contribution by the interrenal components (Hoar, 1969). Paired gonads as they are encountered in vertebrates originate from bilaterally situated primordial components. In species where only a single gonad is present, the primordia appear to fuse early in the gonadal development (Franchi, 1962).

FIG 4.1 shows the origin of the different male gonoducts from the mesonephric system. In the Teleostei there is no connection between the mesonephros and the gonad at maturity and the vas deferens is quite separate from the ureter or mesonephric duct. It is generally assumed that the main gonoduct has been derived from the mesonephric duct during the phylogeny (Hoar, 1983).

Oreochromis mossambicus are considered to have testis of the tubular type.

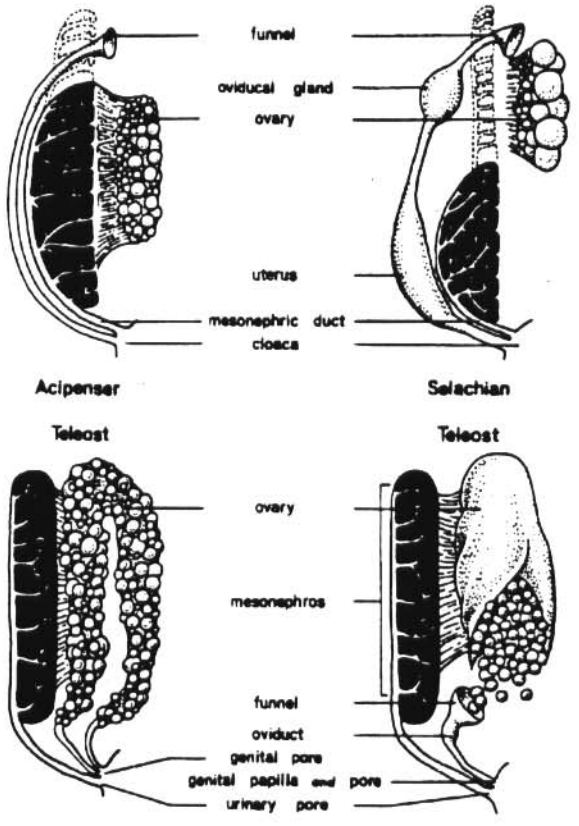
FIG 4.1 Representative types of urino-genital systems in male fish.



In virtually all vertebrates, the ova are discharged into the peritoneal cavity and find their way to the outside through oviducts (Müllerian ducts), which pass from the open anterior funnels to the cloaca. FIG 4.2 shows the different urogenital systems in female fishes. In the Teleostei, the oviducts are posterior continuations of the ovarian tunic. The ovary in some teleosts has a central ovarian cavity continuous with the oviduct while in others the oviducts are para-

ovarian (Hoar, 1983).

FIG 4.2 Representative types of urino-genital systems in female fish.



There has been a considerable amount of work carried out on the gonadal development of freshwater fish, and in particular the genus ***Oreochromis*** (Garcia & Phillip, 1986; Chao ***et al.***, 1987; Yunlin, 1987; Fishelson, 1988; Yeheskel & Avtalion, 1988: Hwang & Sun, 1989; Nakamura & Nagahama, 1989). In the case of Garcia & Phillip (1986), oocyte development in ***Oreochromis aureus*** by means of histological sections of the gonads were analysed by sampling at different seasons during the year. Chao ***et al.*** (1987), worked on different

species of tilapia (***O. aureus***, ***O. mossambicus***, ***O. niloticus***, ***Tilapia zilli***), examining the testes, which are of the tubular type, and the sperm.

Yunlin (1987) has observed gonadal development in young ***Tilapia*** (***Oreochromis***) ***niloticus*** from first hatching until the attainment of sexual maturity. As early as the third day after hatching, genital ridges may be noted. Thereafter, on the ninth day, large primordial germ cells may be seen in the dorsal intestinal mesentery. It is on the twelfth day that two different sexual glands may be distinguished; one that contains large sexual cells and the other that contains smaller sexual cells having large nuclei. On the fifteenth and eighteenth days respectively, primitive ovaries and testes may be observed; with distinct spermatogonia appearing twenty one days after hatching. Sexual differentiation for both sexes seems to occur between eight and twenty days after hatching.

Bern and Avtalion (1990) have examined various morphological aspects of fertilization in Tilapias using the electron microscope. This work involved an observation of the specific anatomy of tilapia gametes for both sexes.

Relatively little information concerning the physiology of developing gonads and differentiating germ cells is available.

4.3 Materials and methods

Histology of the gonads

Each Monday, samples were collected as described in Chapter 3. As the gonads were excised, they were fixed in Bouin's Fixative. The composition of Bouin's Fixative is as follows:

150 ml 80% Ethyl alcohol
60 ml 40% Formaldehyde
15 ml Glacial acetic acid

1 g Picric acid crystals

The same procedure was used throughout the experiment and the material was then subjected to standard histological procedure, using Shandon Histoplast, which is a mixture of purified paraffin wax and plastic polymers as the embedding medium. The intact gonads were serially sectioned at 6μm. Sections were stained with Harris' Haematoxylin and Eosin. Specimens were then observed and photographed using a Reichart-Jung Michestar 120.

Scanning electron microscopy (SEM) of the gonads

One half of all specimens collected were prepared for electron microscopic examination. Upon removal both male and female gonads were fixed in 2% glutaraldehyde in phosphate buffer at pH 7,4. They were subsequently post-fixed in 1% OsO_4 before being dried using the critical point method, and sputter coated with gold. Specimens were examined with an Hitachi 450 scanning electron microscope operating at 20kV.

Transmission electron microscopy (TEM) of the gonads

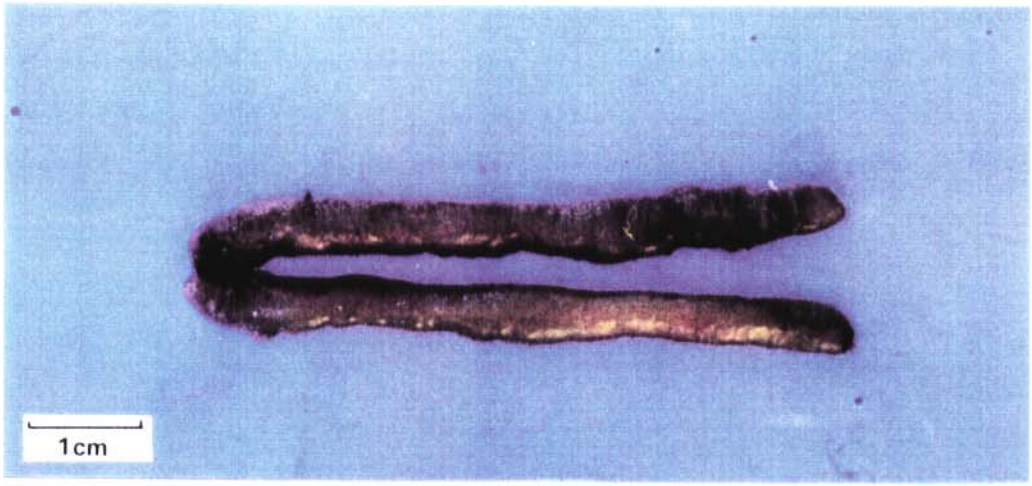
The specimens collected to be examined for ultrastructure using the TEM, were prepared using standard sample preparation (Weakley, 1981) and subsequently examined with a Phillips EM 301 transmission electron microscope operating at 80kV.

4.4 Results

The male

The testes (FIG 4.3) are soft elongated cream coloured bodies that are suspended from the dorsal wall of the visceral cavity. The testes are bilaterally symmetrical structures that are enclosed by a capsule and are subdivided into a number of relatively straight and slightly coiled canaliculi. Caudally they gradually

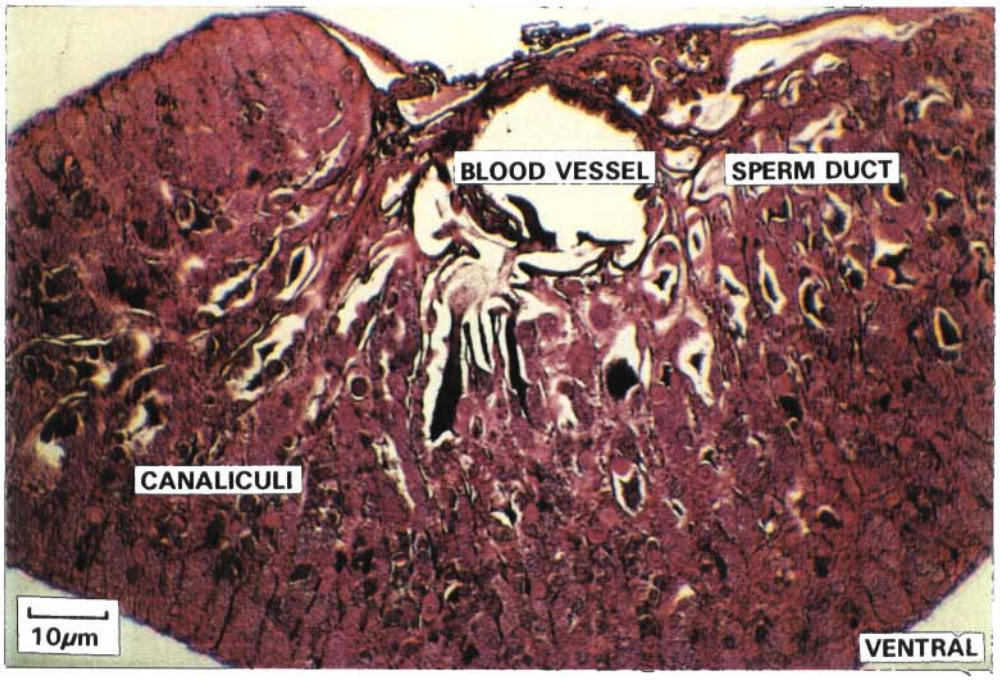
FIG 4.3: Photograph of male ***O.mossambicus*** testis removed from adult fish.



end up in hollow tubes that unite in the gonoduct. The size of the testis appears to be correlated with the size (and stage of reproductive maturity) of the fish and also on the amount of spawning that has occurred. The longest testes found were 7.24 ± 0.55cm, and the heaviest testes measured were 1.22 ± 0.06g.

The testis is also highly vascular, as indicated by the presence of a major ventral blood vessel (FIGS 4.4 and 4.9).

FIG 4.4: Light micrograph of cross section of ***O.mossambicus*** testis to show the dorsal blood vessel, sperm duct and canaliculi.

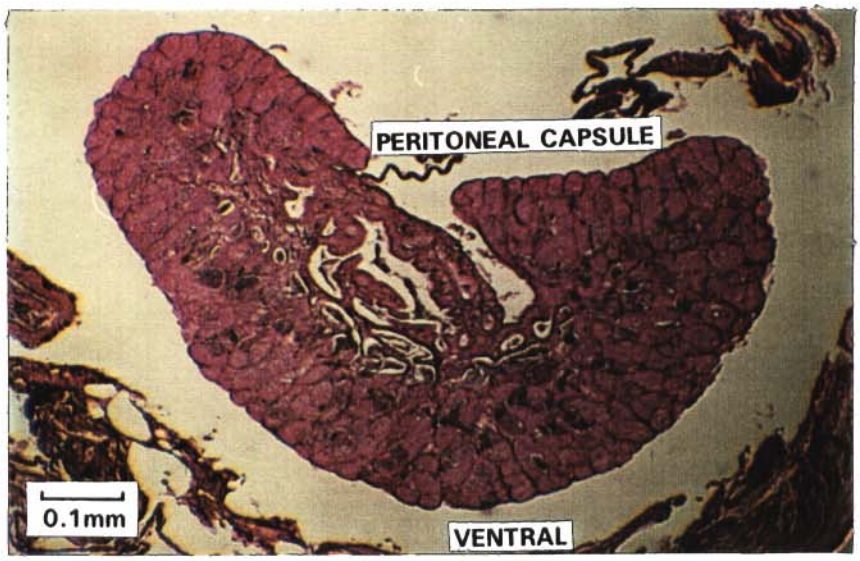


The greater part of the testis space may be seen to be occupied by tube-shaped lumina directed perpendicularly to the median, ventral and lateral testis walls (FIG 4.4). At one end it opens up into a large lumen running longitudinally through the dorsal part of the testis and gradually narrowing to form a gonoduct (FIGS 4.4 and 4.9). This gonoduct, which is the main sperm duct (vas deferens) arises from the posterior mesodorsal surface of the elongated testis and leads to the urogenital papilla. This vas deferens may be traced anteriorly in a connective tissue groove of the testis along with the spermatic blood vessels and nerves.

The walls of the testis and also of the tube-shaped lumina may be seen to

consist of a basal layer of connective tissue, collagenous fibres and smooth muscle fibres. Within the testis, there is a spermatogenic epithelium covering the walls. Externally, the testis is covered by a very thin peritoneal epithelium (FIG 4.5).

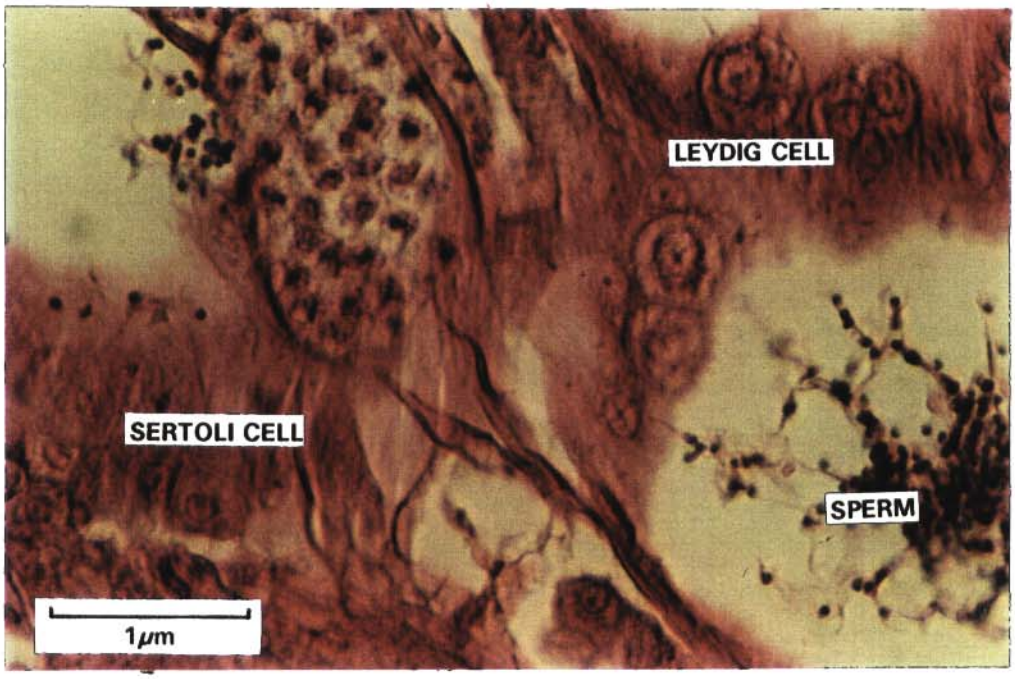
FIG 4.5: Light micrograph of cross section through ***O.mossambicus*** testis to show the thin peritoneal capsule enclosing the testis.



Located between the basal layers of the tubules and peripherally closed by the basal layer of the testis wall there are interstitial areas. Blood and lymph vessels, nerve fibres, mast cells, fibroblasts and various unidentified mesenchymal cells may be distinguished in these interstitial areas. Within the areas between the interstitial tissue are found large groups of Leydig cells (FIG 4.6). These cells are irregular polyhedral cells having large spherical nuclei and a prominent nucleolus. An initial study by Cornish ***et al.***, 1990 has shown that the Leydig Cells are the sites of 3β HSD synthesis and such steroid dehydrogenases leaves little doubt that of the role of the Leydig Cells is that of steroidogenesis. These findings are consistent with those described by Hoar and Randall (1983) for the ***Tilapias***. It

is possible that the 3β HSD could function as a steroid glucuronide or even a type of pheromone.

FIG 4.6: Light micrograph of sperm duct to show Sertoli, Leydig and sperm cells.



Within the testis of the adult male ***O. mossambicus*** there is a layer containing two types of cells that cover the lumina walls. These cells are the Sertoli cells that, as is generally accepted, have a trophic function and the spermatogenic cells that after proliferation and transformations, give rise to the spermatozoa (see FIGS 4.7 and 4.8).

The spermatogenic cells lie embedded in a loose reticular structure devoid of cell boundaries. When the sertolian nuclei are free from the spermatogenic elements they are vesicular in shape, whereas when they lie against the spermatogenic

elements they are often spindle-shaped (FIG 4.7).

FIG 4.7: Light micrograph of cross section of ***O.mossambicus*** sperm duct to show Sertoli and Leydig cells and developing spermatids.

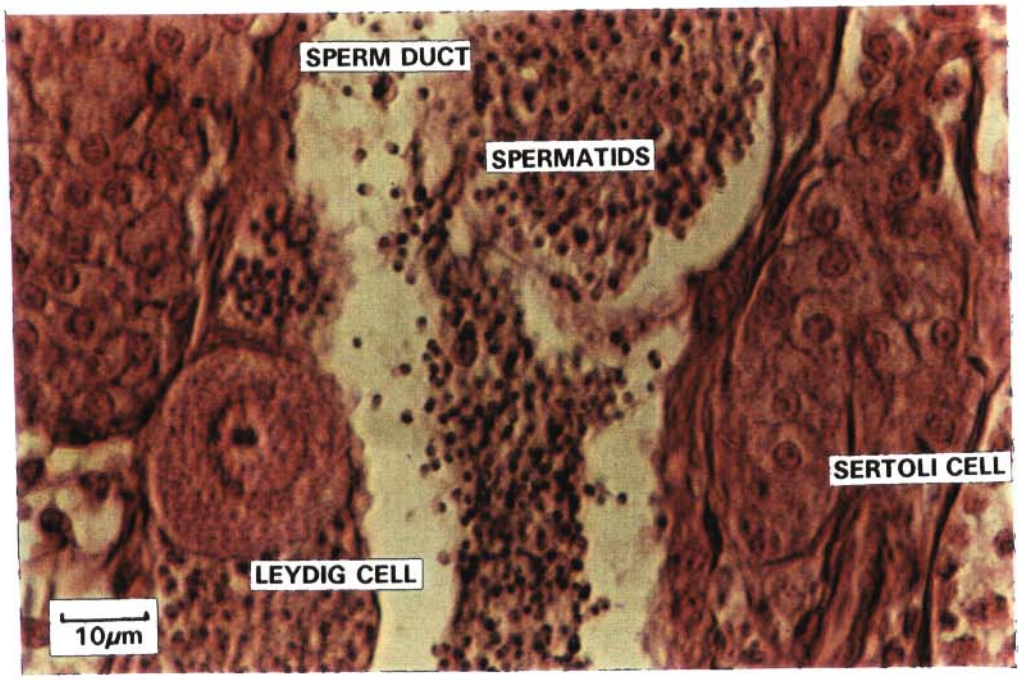


FIG 4.8 shows that the spermatogonia in male ***O. mossambicus*** lie close to the basal membrane of the seminiferous tubule lumen, with the more advanced stages being more centrally located. The lumina of the seminiferous tubules may be seen to be densely packed with spermatozoa, particularly during spring and early summer. The large cell seen in FIG 4.8 is most probably a Leydig Cell that is responsible for testosterone secretion.

FIG 4.8: Light micrograph of cross section of ***O.mossambicus*** sperm duct to show Sertoli, Leydig and sperm cells.

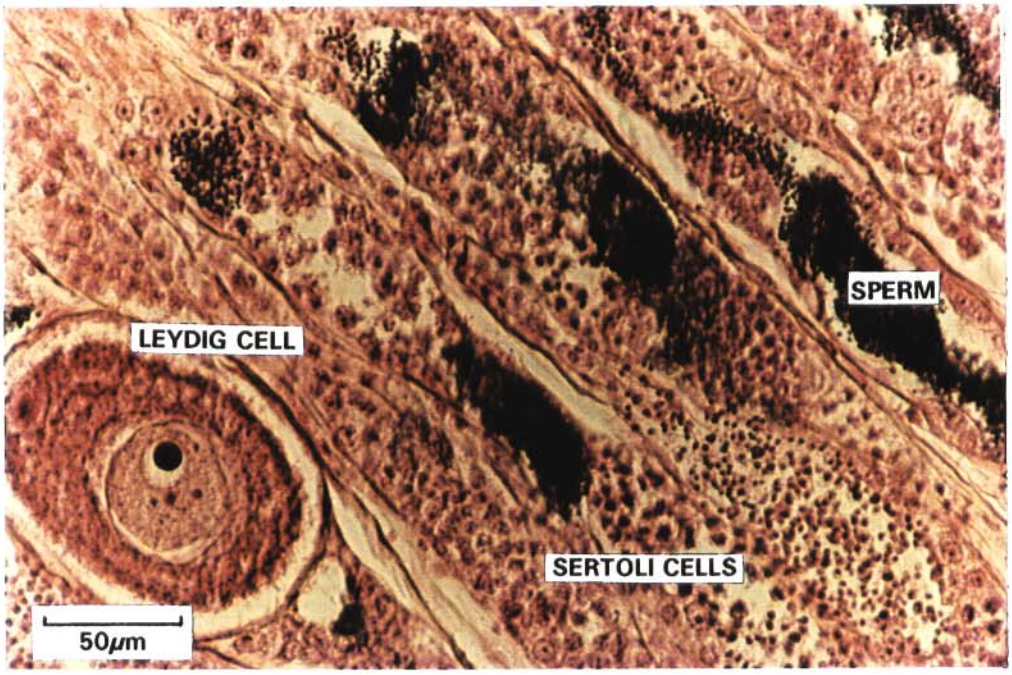


FIG 4.9 is an electron micrograph of the testis of male ***O. mossambicus***. The testis may be seen to be a bilaterally symmetrical structure enclosed by a capsule and being subdivided into a number of relatively straight and slightly coiled canaliculi (seminiferous tubules). FIG 4.9 shows that developing spermatids appear to have a more wrinkled head than do mature sperm (Fig 4.10). Further, mature sperm do not possess an acrosomal cap, but beneath the head is a "collar" (FIG 4.11). This "collar" region could be formed by an extrusion of the plasmalemma and an indentation of the nucleus as described by Stoss (1983). This region also contains mitochondria (Fig 4.12). The remainder of the sperm head contains the chromatin material (Fig 4.12). Mature sperm were found to have a total length of 18.40 ± 2.40μm, of which the tail (flagellum) constitutes the longest portion (11.50 ± 1.30μm). The dimensions of mature sperm heads

FIG 4.9: SEM of cross section of ***O.mossambicus*** testis to show the dorsal blood vessel (BV), sperm duct (SD) and canaliculi (C).

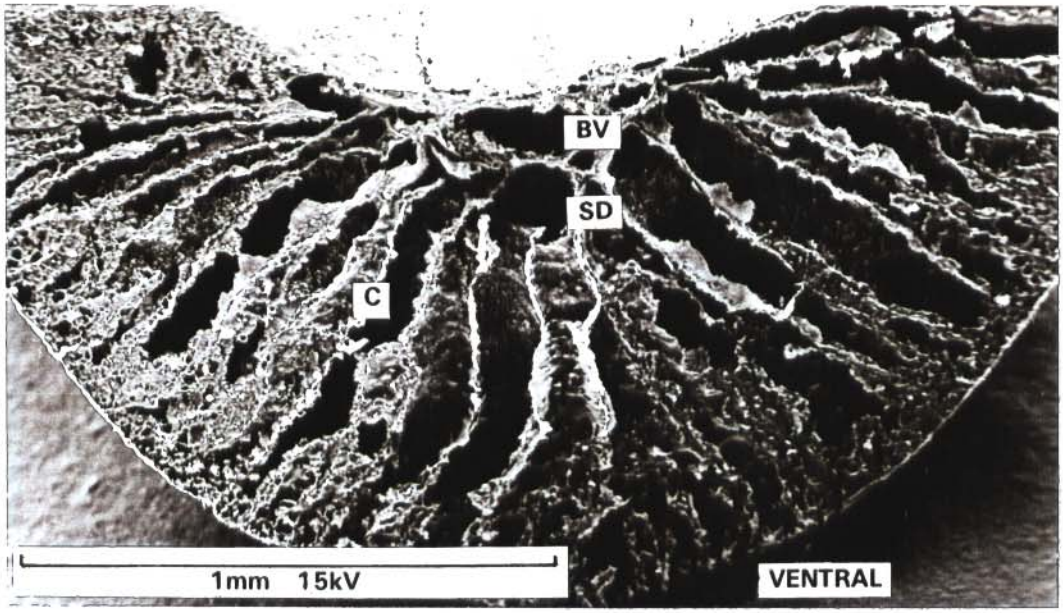


FIG 4.10: SEM of cross section of ***O.mossambicus*** sperm duct to show developing spermatids with wrinkled heads (H).

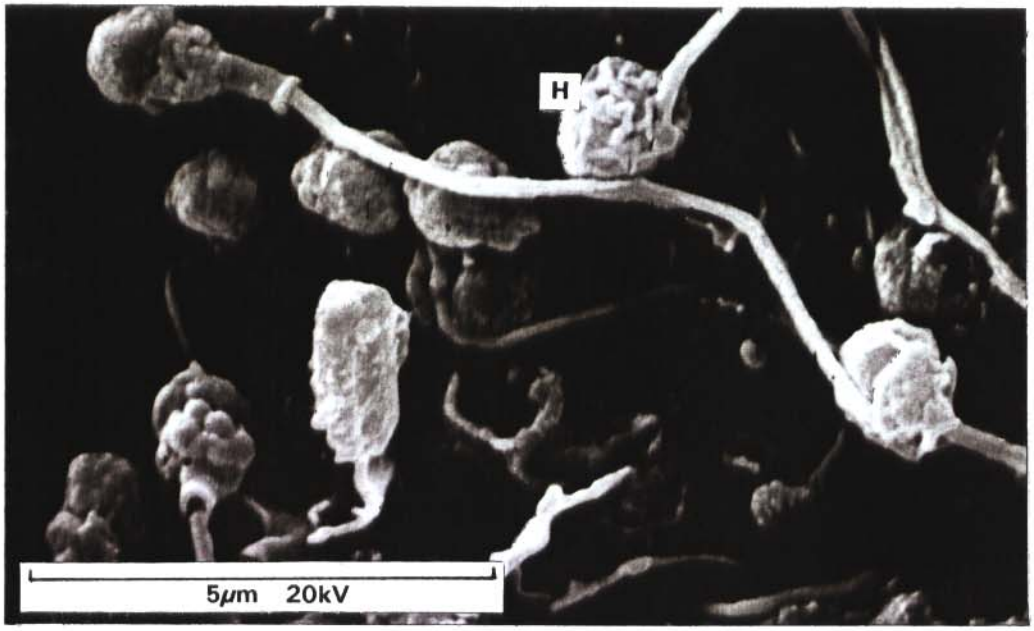


FIG 4.11: SEM of ***O.mossambicus*** sperm cells to show head (H), collar (S) and tail (T) regions.

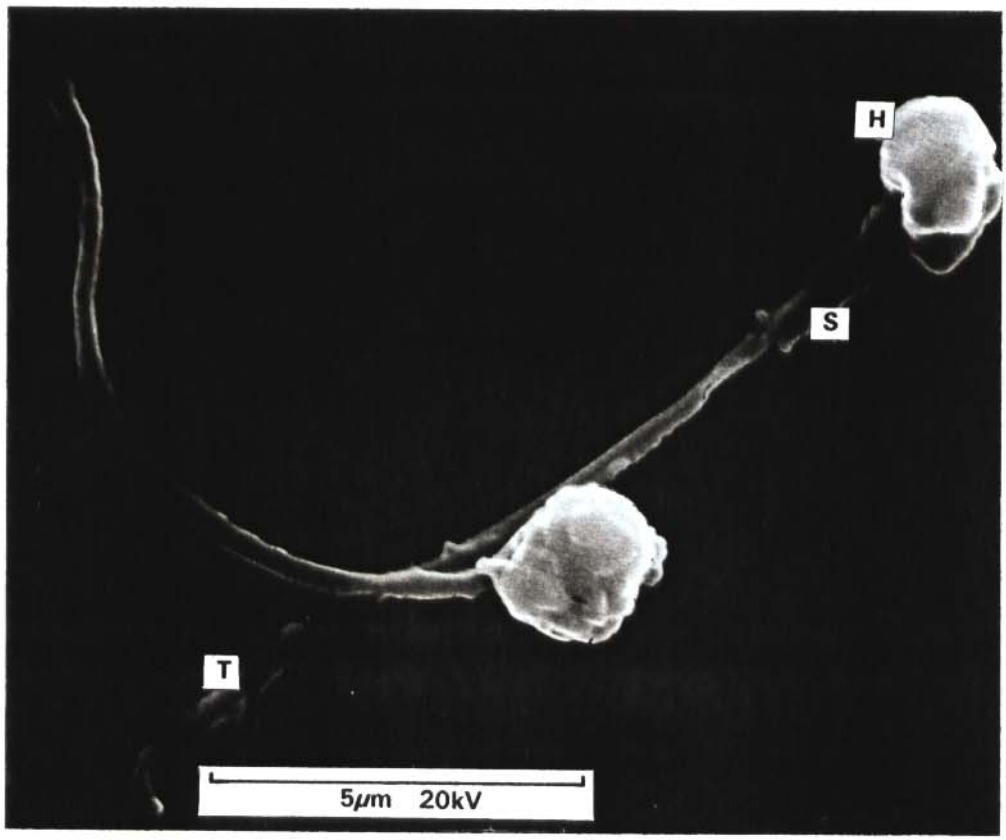


FIG 4.12: TEM of ***O.mossambicus*** sperm to show head (H), collar (S) and tail (T) regions.

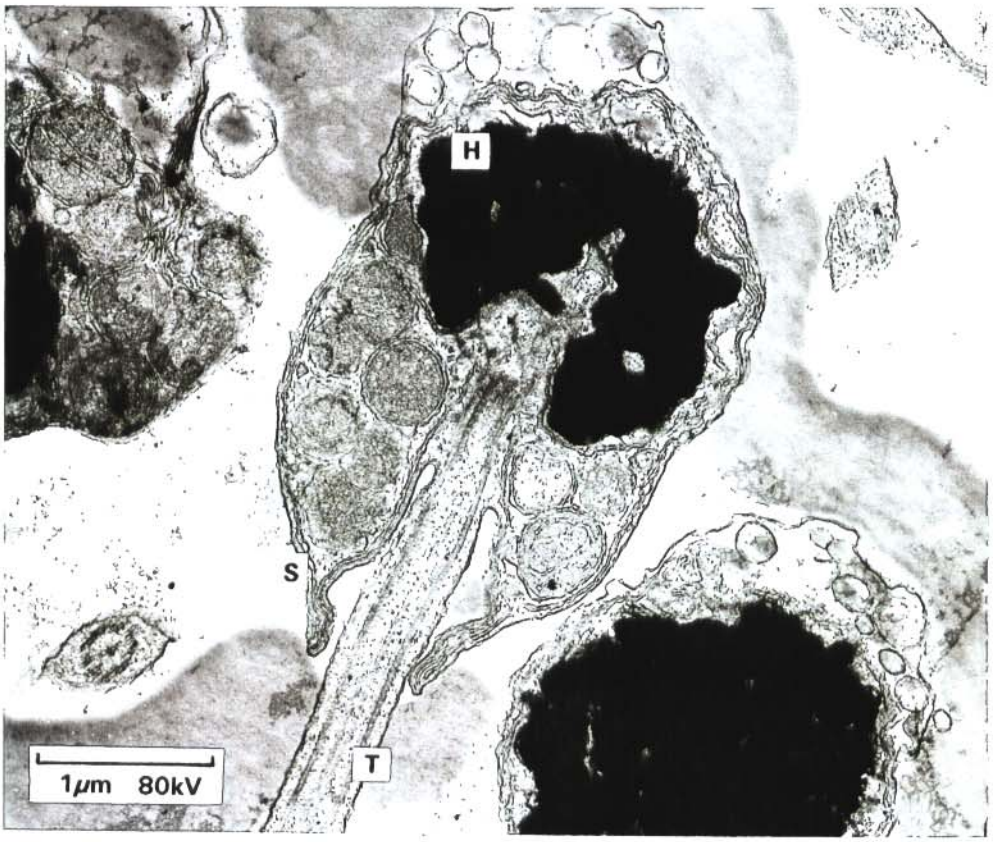
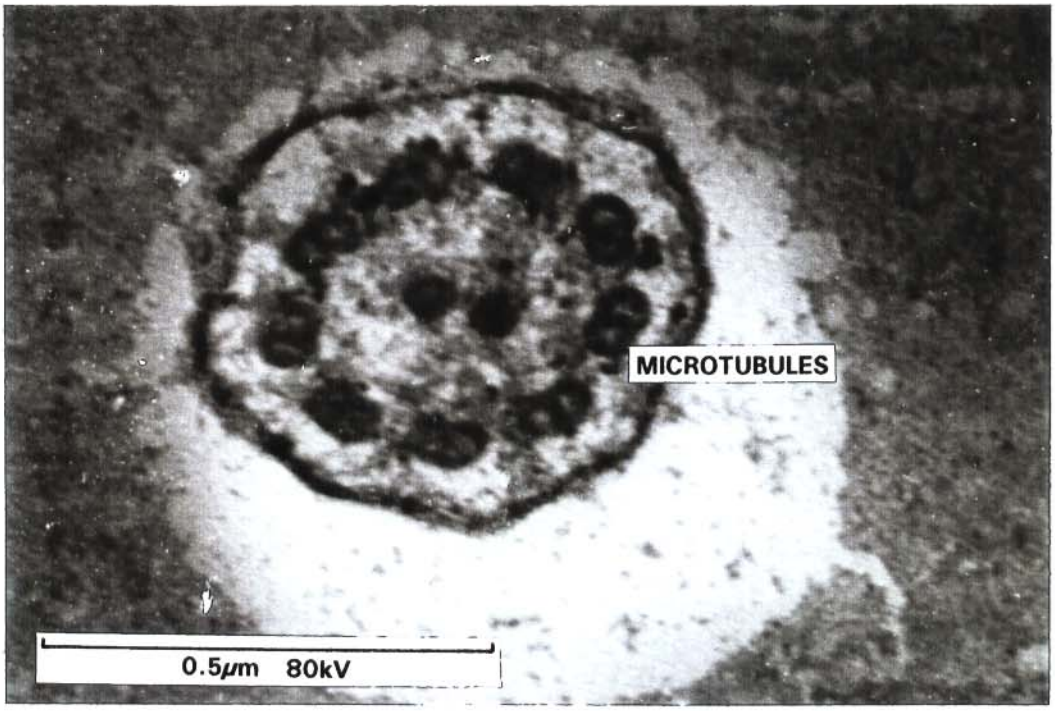


FIG 4.13: TEM of cross section of ***O.mossambicus*** sperm tail to show 9 + 2 microtubule arrangement.

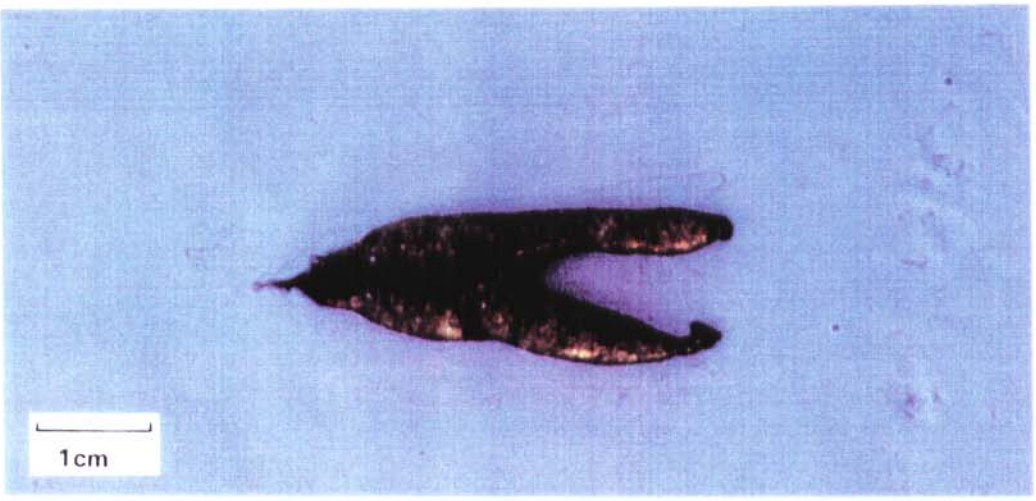


were observed to be 2.70 ± 0.70μm in length and 2.10 ± 0.80μm in width. The "collar" region measured 1.80 ± 0.50μm. FIG 4.13 shows that the flagellum contains the typical 9 + 2 arrangement of nine pairs of peripheral microtubules and one pair of central tubules.

The female

The ovaries of ***O. mossambicus*** (FIG 4.14) are paired, bilaterally symmetrical structures. The ovary wall consists of a single layered germinal epithelium which enspheres the tunica albuginea. The tunica albuginea is comprised of muscle and connective tissue. FIGS 4.15 and 4.16 show that the tunica albuginea projects at places into the ovary lumen and lamellae are formed in which oogonia and

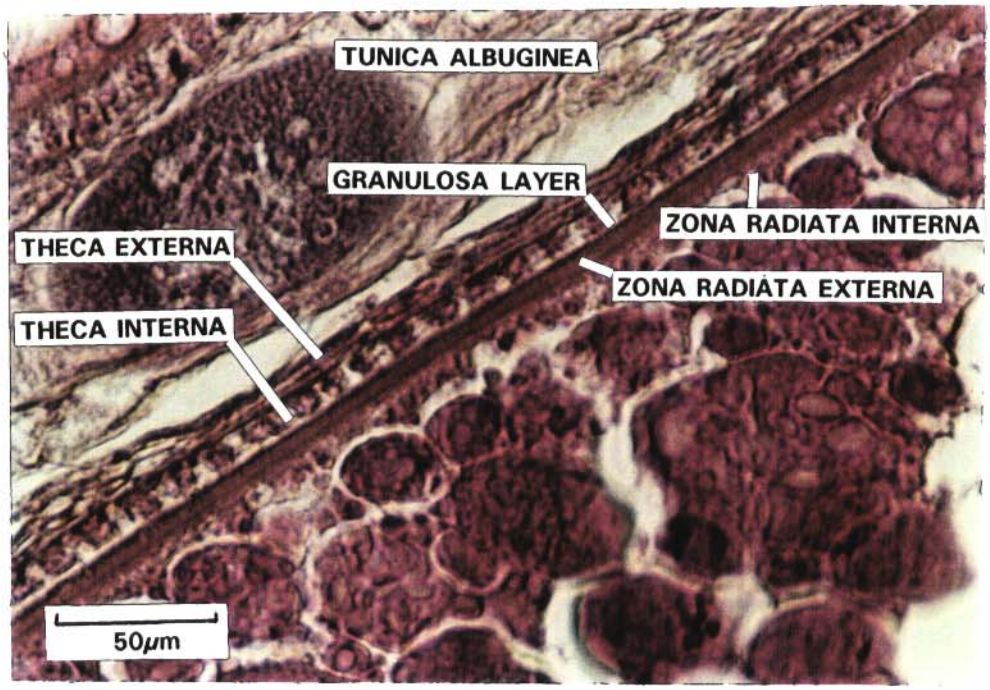
FIG 4.14: Photograph of female ***O.mossambicus*** ovary removed from adult fish.



follicle cells are present. The oogonia appear to be located in a type of connective tissue nest (see FIG 4.25). When the primary growth phase commences the primary oocytes, ensphered by prefollicle cells, migrate out of the oogonial nests and deploy themselves within the stroma.

Primary follicle cells which have originated from prefollicle cells consist only of a single layer of cells, but during mitosis different follicle layers develop, as may be seen in FIGS 4.15 and 4.16. The first surrounding follicle layer, the granulosa layer, is very distinct while the outer layer (theca layer) differentiates into two layers, namely, the theca interna and theca externa. Special theca cells occur in the theca externa and possess large nuclei in a central position. Special theca cells are observed as single cells or groups of cells. As the oocyte matures, the

FIG 4.15: Light micrograph of cross section of ***O.mossambicus*** ovary to show tunica albuginea, theca interna and externa, granulosa layer and zona radiata interna and externa.



zona radiata develops between the oolemma and the granulosa layer. The zona radiata demonstrates a transversal banded appearance which implies that microvilli from the oolemma are overlapping and associate by means of desmosomes with the prolongations from the granulosa layer (FIG 4.15) A dark zone, the zona radiata externa, and a light zone, the zona radiata interna, may be distinguished.

FIG 4.16: Light micrograph of ***O.mossambicus*** ovary to show tunica albuginea, granulosa layer and zona radiata interna and externa.

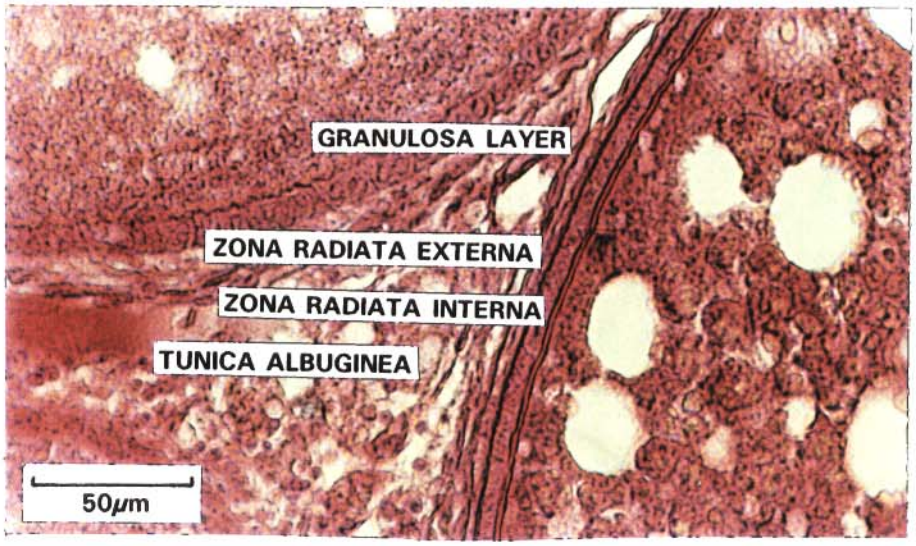
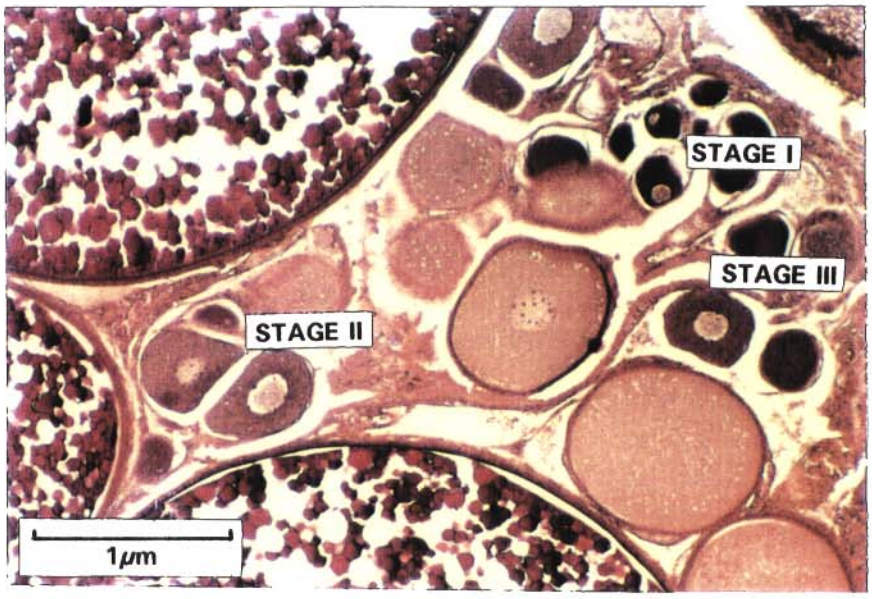


FIG 4.17: Light micrograph of ***O.mossambicus*** ovary to show Stage I, II and III oocytes.



The stages that a growing oocyte in ***O. mossambicus*** passes through before being transformed into an ovum may be classified as follows.

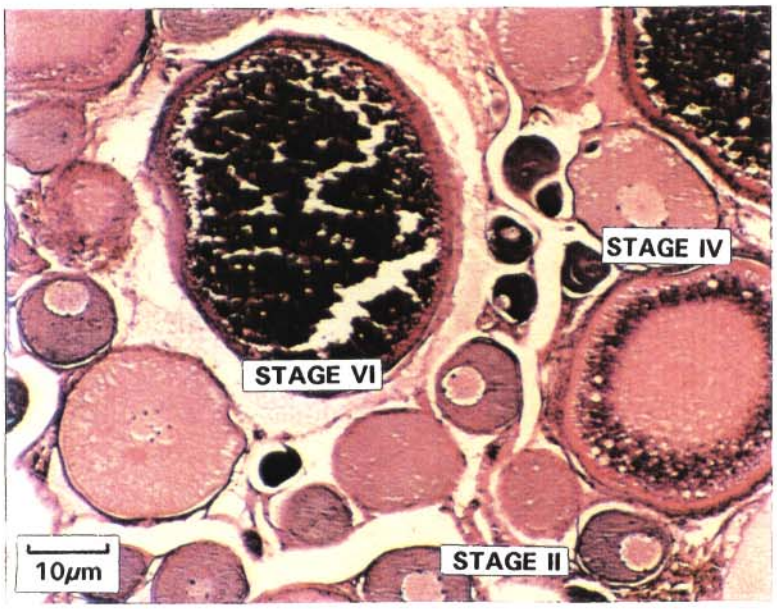
Stage I: chromatin-nucleolar stage (FIG 4.17)

It may be noted that the oocyte possesses a large nucleus that is centrally located. There is relatively little ooplasm present. The nucleus contains an eccentric primary nucleolus, as well as smaller nucleoli, with a homologous distribution of chromatin. A thin follicle layer surrounds the oocyte.

Stage II: peri-nucleolar stage (FIG 4.18)

The ooplasm begins to show more distinct zoning. The nucleolus divides into a number of smaller nucleoli which become arranged on the periphery of the nuclear membrane. There is a flattened follicular layer that surrounds the oocyte at this stage.

FIG 4.18: Light micrograph of ***O.mossambicus*** ovary to show Stage II, IV and VI oocytes.



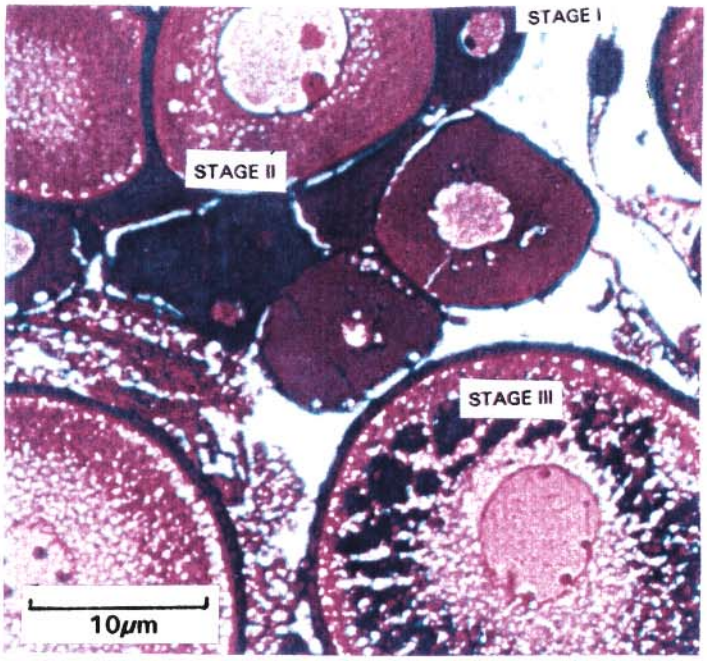
Stage III: yolk vesicle stage (FIG 4.19)

At the periphery of the oocytes, cortical alveoli containing yolk elements begin to develop. The nuclear membrane seems to have a more irregular outline with several nucleoli seen pushed into the granulations. A few nucleoli are beginning to extrude into the ooplasm.

Stage IV: vitellogenetic stage (FIG 4.20)

At this stage the deposition of yolk granules and cortical alveoli development appear. There are more nucleoli that extrude from the nucleus. It appears as if the yolk granules and cortical alveoli are replacing the nucleus and nucleoli. The entire oocyte is filled with yolk granules and cortical alveoli. A distinct theca layer, zona radiata and granulosa layer are present surrounding the oocyte.

FIG 4.19: Light micrograph of ***O.mossambicus*** ovary to show Stage I, II and III oocytes.



Stage V: maturation stage (FIG 4.21)

No nucleus may be seen and yolk granule deposition increases to an extent whereby the cortical alveoli and ooplasm are supplanted to the periphery of the oocyte.

Stage VI: ripe egg stage (FIG 4.22)

The large quantity of yolk granules results in the cortical alveoli and ooplasm becoming only a thin region at the oocyte periphery. A thin zona radiata and follicle layer may be seen at this stage.

Atresia (FIG 4.23)

Atretic oocytes may be seen during primary oocyte development (late summer and autumn).

FIG 4.20: Light micrograph of ***O.mossambicus*** ovary to show Stage IV oocyte with cortical alveoli development and two distinct nucleoli in the nucleus.

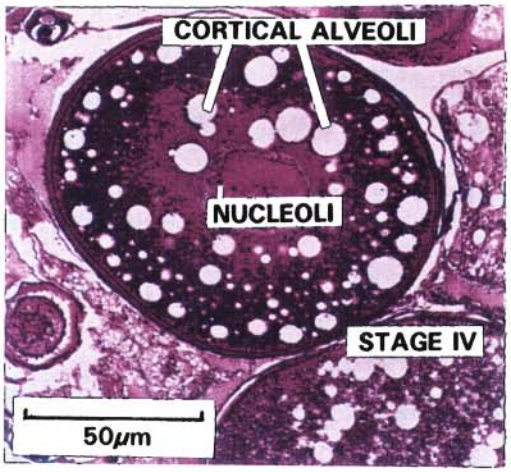


FIG 4.21: Light micrograph of ***O.mossambicus*** ovary to show Stage V oocyte with peripheral cortical alveoli.

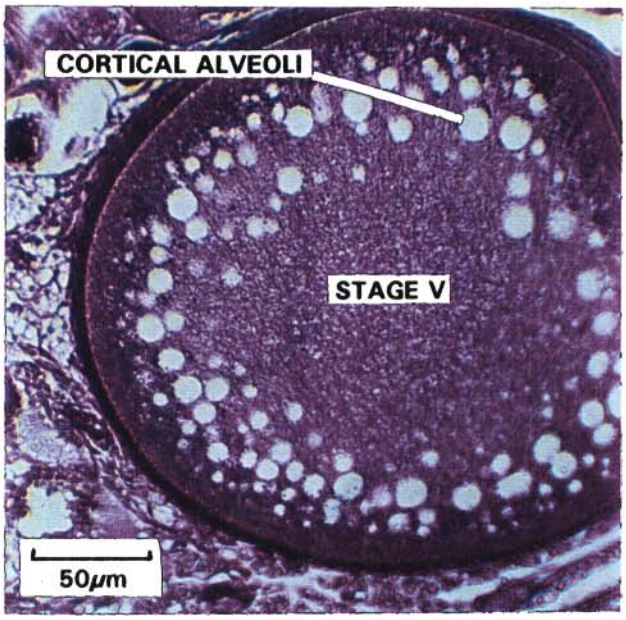


FIG 4.22: Light micrograph of ***O.mossambicus*** ovary to show a Stage VI oocyte.

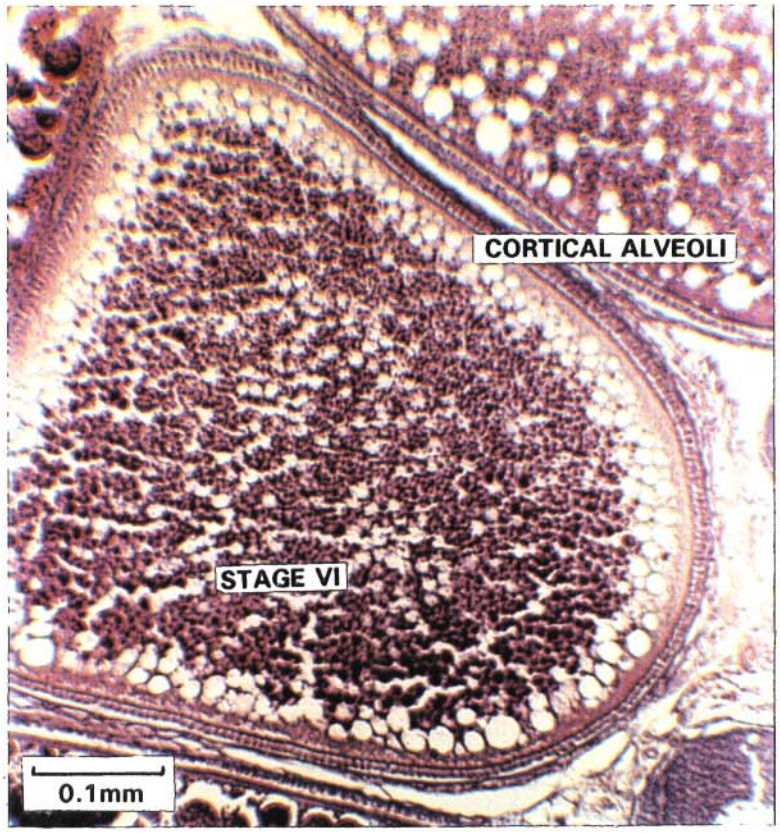
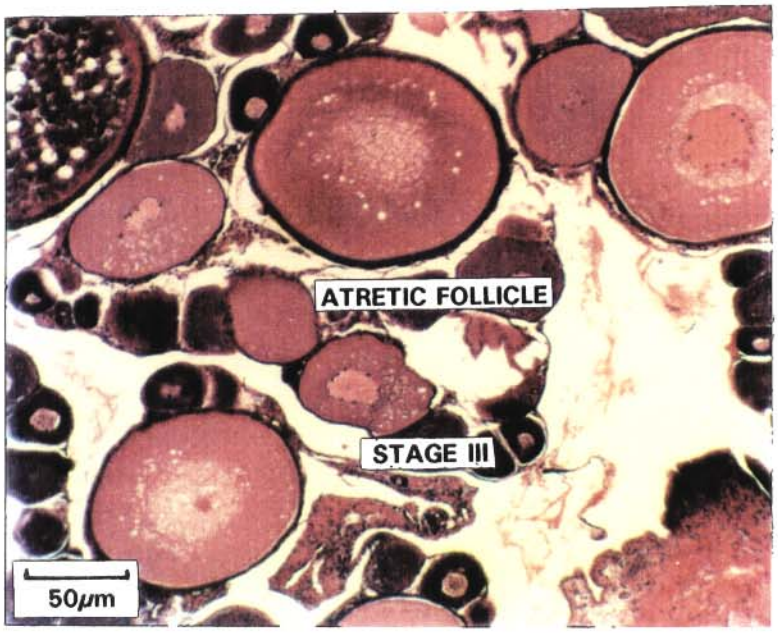


FIG 4.23: Light micrograph of ***O.mossambicus*** ovary to show an atretic follicle.



FIGS 4.24 and 4.25 represent scanning electron micrographs of the ovary of female ***O. mossambicus***. FIG 4.24 illustrates an ovary in cross section, showing a tough outer coat which encloses the loose stromal tissue and a number of developing follicles. A ventrally situated artery (A) may also be seen. To the left of this figure, an empty fractured follicle (F) may be noted. During midwinter, the female has a low gonad mass, thus, the follicles observed in FIG 4.24 are considered to be in an early stage of development. FIG 4.25 shows a cluster of such developing cells.

FIG 4.24: SEM of ***O.mossambicus*** ovary to show a ruptured follicle (F) and a dorsal artery (A).

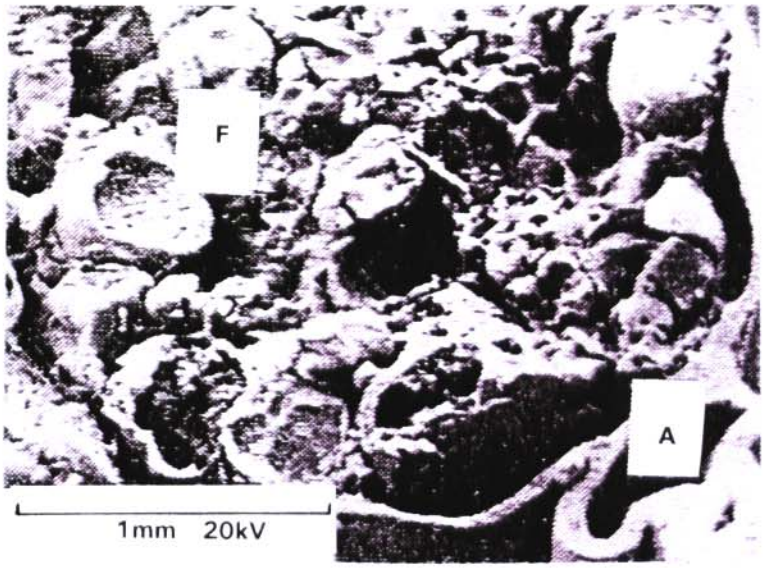
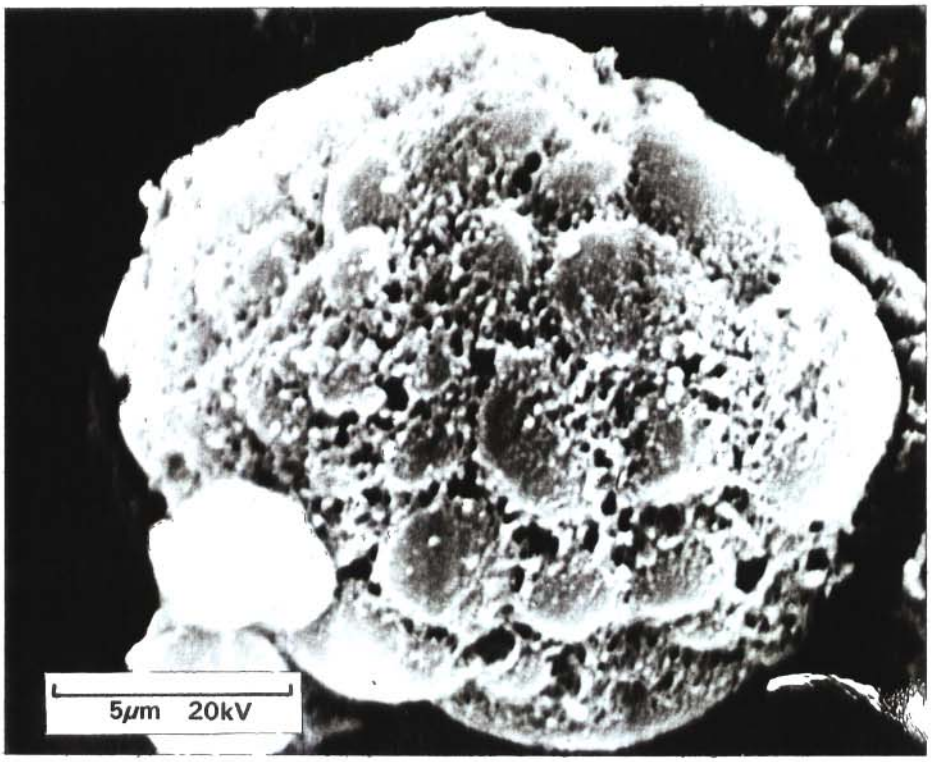


FIG 4.25: SEM of ***O.mossambicus*** ovary to show a "nest" of oogonia.



4.5 Discussion

The male

The histomorphology of the testis and ovary of ***O. mossambicus*** corresponds with descriptions for most other teleost species (Hoar, 1969; Nagahama ***et al.***, 1976; Wourms, 1976; Merchant-Larios, 1978; Riehl, 1978; Van Der Merwe ***et al.***, 1984; Selman ***et al.***, 1988).

The primordial germ cell development for ***Oreochromis mossambicus*** appears to correspond with the tubular testis type as described by Grier (1981) as opposed to the unlimited spermatogonial (acinar) testis that is present in some teleosts. Thus in ***O.mossambicus***, the process of spermatogenesis occurs within tubules

located within the testis. In this tubular testis, the resting germ cells are usually packed together at blind ends of the tubules near the periphery. However, during active spermatogenesis, nests of spermatogonia proliferate both from the ends of the tubules and from the germ cells along their walls. This then leads to the masses of sperm being located in cysts within the testis as is seen in ***O.mossambicus***.

Male ***Oreochromis mossambicus*** appear to exhibit a discontinuous cycle of germ cell development, with a fairly prolonged resting phase where spermatids are the predominant germ cells present. This corresponds with the results found for other Teleosts (Burke & Leatherland, 1984; Rosenblum ***et al.***,1987). Following the formation of spermatozoa within the seminiferous tubule lumens, a renewal of spermatogenesis was observed to occur throughout the spawning and post-spawning period; thereby resulting in a new generation of spermatids for the subsequent breeding cycle. The spermatogenesis may be triggered by both environmental and hormonal factors. It is also possible that there is an additional trigger provided in the form of pheromones being secreted by the female which are then detected by the male and initiate spermatogenesis.

A period of testicular quiescence is a general feature of seasonally breeding teleosts. In many instances, the quiescent testis is composed primarily of spermatogonia (Bhatti & Al-Daham, 1978; Shrestha & Khanna, 1978). In the lake chub, ***Couesius plumbeus***, (Ahsan, 1966) primary spermatocytes are formed prior to the quiescent phase, and in the brook stickleback, ***Eucalia inconstans***, (Ruby & MacMillan, 1970) and the three spined stickleback, ***Gasterosteus aculeatus***, (Borg, 1982) mature spermatozoa are the predominant germ cell stage during the quiescent period. In the brown bullhead catfish, ***Ictalurus nebulosus***, spermatogenesis is arrested at the spermatid stage in the quiescent testis. This appears to be the case in ***O. mossambicus*** with numerous spermatids being observed during the winter months (June and July), when the testis is quiescent. That there is such a diversity in the pattern of testicular development in teleosts

should not be surprising in view of the wide diversity in reproductive patterns exhibited by this group.

The roles played by the Sertoli and Leydig Cells have already been discussed. However, an initial investigation by Cornish ***et al.***, (1990) has shown results for 3β hydroxysteroid dehydrogenase (3β HSD) that compare favourably with those of Rosenblum ***et al.***, (1987) whereby the 3β HSD is confined to the Leydig Cells within the testes. This could also suggest the possibility of some aromatization of testosterone occurring.

In some teleosts, large structures often referred to as "seminal vesicles" have been reported (Hoar ***et al.***, 1983). These structures, which do not store sperm and thus do not seem to have the same function as do their counterparts in higher vertebrates do not appear to be present in ***O.mossambicus***.

The canaliculi (seminiferous tubules) observed using the electron microscope are most probably for the storage and subsequent maturation of the spermatids. Once they have reached maturity, the sperm will be transported to the dorsal sperm duct from where they will be released. This process of maturation of the spermatids coincides with the peak GSI of 0.78 $\pm$ 0.12 (%) being achieved in November. The levels of testosterone (see Chapter 6) that were measured in the gonad also reached a peak of 12.30 $\pm$ 2.70 ng/ml during November.

The "collar" observed in the sperm of male ***O. mossambicus*** is probably analogous to the mitochondrial collar and membranous sheath observed in ***Oreochromis macrochir*** by Van Vuren & Soley (1986). These mitochondria that become arranged as the mitochondrial collar are present in the cell from the spermatogonium stage and migrate to the "collar" region during spermiogenesis. This would suggest that the role of these mitochondria is to provide energy for the propulsion of mature sperm. Fawcett (1960) has shown teleost sperm to possess a single flagellum to provide the necessary motility to fertilize ova

external to the body of the female. The observations made in this study, on ***O. mossambicus***, differ from those for ***O. macrochir*** (Van Vuren & Soley, 1986) in that the latter possess a mature sperm tail having wings, which is not seen in ***O. mossambicus***.

The general histological and histochemical observations made for the testes of ***Oreochromis mossambicus*** suggest that the testes serve as an organ for -

Testosterone and possible pheromone synthesis and secretion. It thus may be considered to be primarily an endocrine organ.

The production of fertilizable sperm cells.

The site of seminiferous fluid production.

The region for maintaining high or low levels of spermatozoa in the testes by spermatogenesis.

Spermatogenesis occurs in cysts surrounded by Sertoli cells. This process involves the proliferation of spermatogonia through repeated mitotic divisions and growth to form primary spermatocytes. These then undergo reduction division to form secondary spermatocytes; the division of which produces spermatids which will then metamorphose into motile and potentially functional gametes - spermatozoa or sperm. This process of spermatid metamorphosis is often referred to as "spermiogenesis". the conclusion of this four stage development of the testes is culminated in male spawning.

The female

Oreochromis mossambicus has cystovaria with the oviducts joining each other to open as a single duct to the exterior. The ovarian wall consists from the outer to the inside of a tunica albuginea, germ cell epithelium, ovarian stroma and an inner layer of germinal epithelium. It also contains connective tissue, blood vessels and other inclusions that are typical of vertebrate ovarian stroma. Also, the different stages of oocyte development are present during the entire breeding

season, except during the mouthbrooding stage when stage 1 - 3 oocytes dominate. The histomorphological changes observed in the ovary, agreed in general with the macroscopic classifications of Kesteven (1960) and Nikolsky (1963). In general, a double ovulatory cycle was observed within the breeding season. Thereafter, climatic conditions changed after the mouthbrooding period that probably inhibited further maturation of the ovaries. This is followed by a resting period. At the end of July, a period of ovarian recrudescence stared to indicate the onset of a new breeding cycle.

In general, the female gonad of ***Oreochromis mossambicus*** showed a similar pattern to the maturity classification of Kesteven (1960) in terms of the stages through which the ovary passed. However, in the present study there was an abundance of stage I to stage III oocytes present in the ovary at almost any point in the reproductive cycle. This would therefore suggest the possibility of this fish being a multiple or fractional spawner.

The histomorphology of the ovary of ***Oreochromis mossambicus*** corresponds with the descriptions for other teleosts (Hoar, 1969; Nagahama ***et al.***, 1976; Wourms, 1976; Merchant-Larios, 1978; Riehl, 1978; Selman ***et al.***, 1988; van der Merwe ***et al.***, 1988). It is in the size of the oocytes and their nuclei where differences at the different developmental stages may be detected. With the aid of seasonal variation in oocyte stages and GSI, the reproductive cycle could be divided into different recrudescent phases. Stage V oocytes are characterized by the presence of large quantities of yolk elements. It is probable that it is the presence of these oocytes that are responsible for the increasing size and consequent increase in oocyte mass. Thus it appears to be the stage V oocytes that influence the GSI. Stage IV oocytes (vitellogenic stage) also consist of yolk elements but it was noticeable that when GSI reaches a peak, a smaller number of these stage IV oocytes may be detected. Thus stage IV oocytes do not seem to influence the gonadal mass.

The stage I and II oocytes do not consist of any yolk elements and therefore do not contribute toward the gonadal mass. There were only a few stage I and II oocytes observed throughout the study period. It is the stage III oocytes that appeared to be present throughout the study in relatively high numbers. Minimum GSI values indicate fish where spawning has already occurred with an increase in GSI in the next breeding season indicate a commencement of the next breeding cycle. GSI increases markedly during August which coincides with an increase in the number of stage V oocytes observed. The maximum number of stage V oocytes may be seen during September / October which is indicative of the final stages of maturation occurring.

In general, six oocyte development stages were identified which agreed with those identified by Fouché (1982) for ***Cyprinus carpio*** and van der Merwe (1984) for ***Labeo capensis***.

There appears to be oogonial nests that develop by mitosis, present in the ovary of ***O.mossambicus***. These nests develop by oogenesis whereby oogonia develop into mature oocytes. During final maturation, hydration of ooplasm occurs which is then responsible for the increase in size and mass of oocytes and consequently the ovary.

Stage I and stage II oocytes do not consist of any yolk elements, and therefore do not contribute toward the gonad mass as such. This is confirmed by the relatively low number of stage I and stage II oocytes being present in the gonads when the GSI reached a peak in September (spring). GSI reached a minimum during March, which would indicate that spawning is now complete. The gradual increase in GSI noted during the subsequent months would be indicative of the commencement of the next breeding cycle. Yaron ***et al.***, (1980) found that the GSI reached a minimum during artificial summer conditions and Spieler ***et al.***, (1977) also stated that GSI was at a minimum during longer photoperiods. These findings correspond with those of Van der Merwe ***et al.***, (1988) where GSI in

Labeo capensis reached a minimum during January (summer).

The sharp increase in ovarian mass is ascribed to vitellogenesis and hydration of oocytes. The latter may be responsible for an 80% increase in ovarian mass, since the number of oocytes do not increase during this period. As the new breeding cycle commences, so there is an increase in the number of stage III oocytes within the ovary. This indicates that there is an increase in primary oocyte development during April and May. This is confirmed by the increase in GSI observed for female ***O. mossambicus*** during these months. Also, this could be indicative of a double ovulatory cycle.

The gradual decrease in stage III oocytes and subsequent increase in stage IV oocytes (vitellogenic stage) indicates that active yolk production is occurring and there is a further increase in GSI. Stage IV oocytes also appear to have considerable yolk elements, however, the increase and decrease in GSI does not appear to correspond with the numbers of stage IV oocytes seen and therefore it is possible that stage IV oocytes do not effect the gonadal mass.

In maturing oocytes, the follicle membrane differentiates to form theca cells which increase in numbers to form a granulosa having both a glandular and a nutritive function (Hertig & Barton, 1972). The theca then forms two layers - the zona pellucida, which separates the ovum from the granulosa. This layer is in turn separated from theca interna by the membrana propria. In general, endocrine mechanisms are responsible for this oocyte development.

Oocyte maturation occurred during early spring when the number of stage V oocytes together with GSI showed a marked increase. The number of stage I, II, III and IV oocytes present now decreased. Stage V oocytes are characterized by the presence of large quantities of yolk elements, which are the main reason for the increasing size and consequent increase in mass of the oocyte (Craik, 1979). The number of stage V oocytes may therefore influence the GSI. The fluctuations

which may be seen throughout the breeding cycle could possibly be ascribed to the fluctuations in the numbers of stage V oocytes that are seen throughout the experimental period.

The results of this investigation suggests that a cyclic development of oocytes occurs during the breeding season. Synchronized spawning for both sexes were observed which was age dependant. This was evidenced by the varying periods of GSI measurements during the breeding period. In this regard, seasonal aquatic changes contributed largely to this process. It is also evident that this species display a double ovulatory cycle, limited by climatic conditions. Balarin (1979) indicated that this species will continue with breeding if the prevalent aquatic environmental conditions allow it to proceed. The climatic changes in the Highveld area, however, have a dampening effect on this process, primarily as a result of temperature changes. This inhibits the uncontrolled breeding of this species in nature. Although they are found as a majority in those impoundments where they occur naturally, environmental conditions and the presence of predator species, limit their numbers. Spawning appears to have occurred predominantly during October till December, after which GSI decreased. The increase in GSI observed during November together with the high standard deviation values calculated for September, October and November may be ascribed to the presence of pre-spawned gonads and spawned gonads. Thus during the breeding season it is evident that the potential for breeding always remains as a result of the fact that stage 1 - 3 oocytes are always present, but that mouth brooding inhibits the natural development process.

Whilst GSI values (see also Chapter 3) provide a good indication as to the level of gonadal development, they are not the only parameters to be used.

4.6 Summary

From the foregoing discussion, the gonadal development in both male and female ***O. mossambicus*** is of utmost importance in the understanding of both it's reproductive physiology and breeding cycle. It should be noted that to complete the picture; environmental factors, chemical composition of the plasma and gonads as well as the hormonal profiles need also to be considered.

Male gametes (sperm) are produced in the two bilaterally arranged testes; where the size of the testes appears to be correlated with the size and stage of reproductive maturity of the fish as well as the amount of spawning that has occurred.

The seminiferous tubules appear to be the site of spermatogonium maturation and storage. There is a dense packing of spermatozoa during spring and early summer.

Mature sperm do not possess an acrosomal cap, but rather a distinct head region that contains the chromatin material; a "collar" or neck region containing mitochondria to be used as an energy supply for mature sperm propulsion and a flagellum that is for sperm motility.

The female gonad has a number of developmental stages that may be identified throughout the breeding cycle. These phases may be classified as follows; the primary oocyte developing phase (late summer / early autumn); yolk production phase (autumn); maturation phase (winter); maturation phase and the final maturation and spawning phase (spring / early summer).

It is recommended that new and more advanced techniques for both embedding and sectioning material be employed for a study of both the pituitary and the gonads using the transmission electron microscope; in particular the female

ovary.

3β HSD activity, pheromones, vitellogenesis, electrophoresis of enzymes, proteins, fats, amino acids, immunochemical characterization of enzymes, the role of vitellogenin as a possible hormone carrier or vitamin uptake or other biochemicals involved in oocyte or sperm growth and or development all need to be examined.

An examination of the pituitary gland would allow for the identification of specific cells responsible for gonadotropin secretion. This would lead the researcher into a histochemical examination as employed by van der Merwe ***et al.*** (1984) of the gonads and thereby provide microscopic evidence regarding the secretion of hormones during the reproductive cycle of ***O. mossambicus***.

A further recommendation, that could provide interesting results would be to arrest the process of fertilization at the point of the sperm entering the ovum. This sample may then be examined using both a scanning and a transmission electron microscope.

CHAPTER 5

Hormonal Profiles in Oreochromis mossambicus

5.1 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. Correlations between seasonal changes in plasma levels of gonadal steroids and gonad condition have been well documented in a number of freshwater fish species (Crim & Idler 1978; Lambert ***et al.,***1978; Fostier & Jalabert 1978; Scott ***et al.,*** 1980a,b; Lamba ***et al.,*** 1983; van der Merwe ***et al.,*** 1987).

Histochemical research has shown that steroid hormones, which are important for reproduction, are formed in the adrenal glands and gonads of many freshwater fish species. Steroid production in the ovary has been observed in the granulosa cells and/or theca cells of developing and mature oocytes. Post ovulatory and interstitial cells are also sources of sex steroids in the ovary. The occurrence of steroid production in different cells of the ovary may be related to different phases of oocyte development. Adrenal steroids are secreted prior to gonad steroids to promote vitellogenin production for oocyte development. This was confirmed by many investigators who studied seasonal changes in the plasma levels of steroid hormones or gonadotropins (Crim *et al* 1973; Crim *et al* 1975; Fostier *et al* 1983; Kagawa *et al* 1983; Kobayashi *et al* 1986; Santos *et al* 1986; Rosenblum *et al* 1987; Pankhurst & Conroy 1987).

Although it has been ascertained in cyprinids that final oocyte maturation and ovulation are induced by a preovulatory gonadotropin surge, little information on

the plasma and gonadal changes in gonadotropin and steroid hormone levels during the reproductive cycle in ***O. mossambicus*** is known.

The present study examined the levels of the gonadotropin and steroid 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***.

5.2 Literature Survey

The role of hormones in the regulation of reproductive behaviour in fish is probably the most investigated aspect of the present study. Evidence for the hormonal regulation of reproductive behaviour is based upon

a) the treatment of fish with exogenous hormone preparations, with or without prior gonadectomy, and/or
b) 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 neurohormone antagonists and other pharmacological agents. In comparison with these more traditional procedures which relied upon histological and cytological data, radioimmunoassay assessment of plasma and gonadal hormone levels provides a more precise analysis of the relationship between endocrine state and behaviour (Liley ***et al.***, 1987).

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 Bun (1983) states 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 appears to be two distinct gonadotropins in tilapia; one that resembles luteinizing hormone (LH) and another that resembles follicle stimulating hormone (FSH) in terms of their biological activity and chromatographic behaviour. Tilapia gonadotropins seems to be involved in stimulating spermatogenesis and testosterone secretion in males.

Considerable experimental data has been collected on the role of gonadotropins in tilapia (Gissis ***et al.***, 1986; Levavi-Zermonsky & Yaron, 1986; Planas ***et al.***, 1990; Yaron & Levavi-Sivan, 1990; Gissis ***et al.***, 1991; Levavi-Sivan & Yaron, 1992). Gissis ***et al.*** (1991) have demonstrated the dual hypothalamic control of gonadotropin release in tilapia, particularly in response to circulating GnRH levels. Levavi-Sivan and Yaron (1992) have shown the involvement of cyclic 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 only increase (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 aureus***), it is possible to determine the sequence of events prior to and during spawning.

The literature devoted to fish steroids is extremely vast. Fostier ***et al.*** in Hoar and Randall (1983) have reviewed the literature extensively. In the present study, it was decided to consider only the physiological role of gonadal steroids in reproduction and that the steroids that would be examined were testosterone, estradiol - 17β and progesterone. Sex steroids function at various levels. They are important in the genesis of the gonad both in differentiation and maintenance of somatic tissues (mainly gonadal ducts) and in gametogenesis. When gametes are ready for fertilization, steroids act to bring the sexes together, stimulating the development of morphological characteristics and modulating sexual behaviour.

During vitellogenesis an increase in plasma estrogen levels, mainly estradiol 17-β that correlates with the growth of vitellogenic oocytes has been observed in many species. In the tilapia ***Sarotherodon*** (now ***Oreochromis***) ***aureus***, the initiation of spawning by increasing water temperature is followed by a rise in testosterone levels (Katz & Eckstein, 1974).

Rothbard ***et al.*** (1987) have examined the changes in steroid concentrations that occur during sexual ontogenesis in tilapia. Their results indicate that although both testosterone and estradiol increase during sexual ontogenesis, it appears to be the former that is responsible for the process of sex differentiation. Smith and Haley (1988), working on female ***Oreochromis mossambicus***, have reported that the estradiol profile in tilapia is similar to that reported by MacGregor ***et al.*** (1981), whereby there is no decline in estradiol levels prior to oocyte maturation. Testosterone appears high in these mouthbrooders in the latter half of the brooding period. Testosterone levels fall upon the cessation of mouthbrooding behaviour. It is not yet established whether testosterone is directly involved in mouthbrooding behaviour or not. In contrast to the testosterone levels, Smith and Haley (1988) found that progesterone levels in mouthbrooders only increase

once mouthbrooding had ended. This rise in progesterone could be due to either a decrease in conversion to other steroids or an overall increase in hormone production.

The research on sex steroids in fish raises two categories of question. One is concerned with the validity of analytical methodologies and the other with the nature of the experimental procedure.

No meaningful literature could be obtained relating to studies conducted involving human chorionic gonadotropin (HCG).

5.3 Materials and methods

Samples were collected as described in Chapter 3.

Both the plasma and the gonadal homogenate supernatants were analysed for the presence of the gonadotropic hormones, follicle stimulating hormone (FSH), luteinizing hormone (LH) and human chorionic gonadotropic hormone (HCG). The concentrations of these hormones were measured in both male and female samples by radioimmunoassay (RIA) using human kits obtained from FRANSA **(Cat. Nos. FSH: FSHK-PR; LH: LHK-PR; HCG: HCGK-PR)**.

The concentration of the steroid hormones testosterone in the male and estradiol 17-β & progesterone in the female were determined using appropriate FRANSA RIA kits. **(Cat Nos. Testosterone: CM-TESTO; Estradiol 17β: SB-ESTR; Progesterone: CM-PROG)**. Both plasma and gonadal homogenate supernatants were analysed. All FRANSA RIA test kits made use of ^{125}I-labelled hormones, which are intended for use with human samples.

All readings of radioactivity were taken using a Beckman Gamma 8500 Microprocessor Counter.

Statistical analyses were also carried out and interpreted as described in Chapter 3.

5.4 Results

All data shown in TABLES 5.1; 5.2; 5.3; 5.4; 5.5 and 5.6 are the mean monthly ± standard deviation values recorded for the parameters indicated.

The following TABLE 5.1 shows male and female ***O.mossambicus*** plasma and gonadal supernatant follicle stimulating hormone (FSH) concentration (mIU/ml) values for the entire experimental period.

	♂ 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
Sap	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 5.1: FSH concentration (mIU/ml) measured in plasma and gonads of male and female ***O.mossambicus*** (samples taken per month, n = 40).

Male plasma FSH showed two significant ($p < 0.001$) dips in concentration during September and November whereas gonad FSH dropped significantly a month later **ie:** during October and December. Female plasma FSH was undetectable from May to August, whereafter a slight rise was observed until January. Gonad FSH was virtually undetectable from August to February. No great variation in FSH levels in male plasma and gonads were observed. However, in females, plasma FSH levels were always higher than gonad levels.

The following TABLE 5.2 shows male and female ***O.mossambicus*** plasma and gonadal supernatant luteinizing hormone (LH) concentration (mIU/ml) values for the entire experimental period.

	♂ 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 5.2: LH concentration (mIU/ml) measured in plasma and gonads of male and female ***O.mossambicus*** (samples taken per month, n = 40).

Male plasma LH levels peaked during October and from March to May. Lowest levels were recorded during November to January. Male gonad LH levels remained low during September to January. Female plasma LH levels were high from August to October whereafter it declined significantly ($p < 0.001$) until November. Female gonad LH levels were virtually non-detectable during September to February. In general, male and female plasma LH levels were always higher than gonad LH levels.

TABLE 5.3 shows male and female ***O.mossambicus*** plasma and gonadal supernatant human chorionic gonadotropin (HCG) concentration (mIU/ml) values for the entire experimental period.

	♂ 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				
Jul			10.44 0.78	
Aug			<1.00	
Sept			<1.00	
Oct			<1.00	
Nov			<1.00	
Dec			41.50 1.98	
Jan		24.48 1.44	12.18 0.88	
Feb		<1.00	9.62 0.64	
Mar			14.36 1.05	
Apr			12.49 1.32	

TABLE 5.3: HCG concentration (mIU/ml) measured in plasma and gonads of male and female ***O.mossambicus*** (samples taken per month, n = 40).

Male HCG levels were virtually undetectable, except in January in the gonads. However, in female gonads, HCG was never detected, but was present in the plasma from December to July at much higher levels than FSH. .

The following TABLE 5.4 shows male ***O.mossambicus*** plasma and gonadal supernatant testosterone concentration (ng/ml) values for the entire experimental period.

	Plasma (ng/ml)	Gonad (ng/ml)
	mean ± sd	mean ± sd
May	1.22 ± 0.26	3.64 ± 0.88
Jun	2.20 ± 0.56	4.04 ± 0.68
Jul	1.80 ± 0.78	4.92 ± 1.02
Aug	1.76 ± 0.28	5.88 ± 0.96
Sept	12.30 ± 2.27	4.34 ± 0.62
Oct	3.14 ± 1.01	3.06 ± 0.69
Nov	2.38 ± 1.55	12.30 ± 2.70
Dec	3.38 ± 0.89	9.62 ± 1.21
Jan	3.30 ± 0.59	3.82 ± 0.49
Feb	3.34 ± 0.63	4.34 ± 0.64
Mar	2.04 ± 0.83	4.50 ± 0.63
Apr	1.60 ± 0.29	4.62 ± 0.55

TABLE 5.4: Testosterone concentration (ng/ml) measured in plasma and gonads of male ***O.mossambicus*** (samples taken per month, n = 40).

Male plasma testosterone levels peaked during September and dropped to a lower level from October till February. Thereafter it declined further. Gonad testosterone levels were significantly ($p < 0.001$) higher only during November and December whereafter these levels remained almost constant. Gonad

testosterone levels were also higher than their corresponding plasma levels.

The following TABLE 5.5 shows female ***O.mossambicus*** plasma and gonadal supernatant estradiol 17-β concentration (ng/ml) values for the entire experimental period.

	Plasma (ng/ml)	Gonad (ng/ml)
	mean ± sd	mean ± sd
May	0.26 ± 0.04	1.49 ± 0.07
Jun	0.48 ± 0.02	3.19 ± 0.09
Jul	0.56 ± 0.04	3.38 ± 0.09
Aug	0.68 ± 0.04	5.34 ± 0.06
Sept	0.24 ± 0.02	5.02 ± 0.12
Oct	0.50 ± 0.01	4.75 ± 0.09
Nov		4.03 ± 0.09
Dec		4.44 ± 0.11
Jan	0.26 ± 0.03	3.94 ± 0.23
Feb	0.78 ± 0.03	3.04 ± 0.10
Mar	0.42 ± 0.05	1.81 ± 0.04
Apr	<0.01	1.13 ± 0.05

TABLE 5.5: Estradiol 17-ß concentration (ng/ml) measured in plasma and gonads of female ***O.mossambicus*** (samples taken per month, n = 40).

Female plasma estradiol levels gradually increased from May to July, reaching a peak during August. Thereafter it declined to reach virtually undetectable levels in December. Gonad estradiol levels gradually increased from March to reach a peak in September whereafter a gradual decline was observed until February. Gonad levels remained almost ten times higher than plasma levels.

The following TABLE 5.6 shows female ***O.mossambicus*** plasma and gonadal supernatant progesterone concentration (ng/ml) values for the entire experimental period.

	Plasma (ng/ml)	Gonad (ng/ml)
	mean ± sd	mean ± sd
May	2.44 ± 0.66	98.08 ± 10.66
Jun	2.24 ± 0.98	99.50 ± 11.85
Jul	1.40 ± 0.04	51.44 ± 4.46
Aug	0.66 ± 0.17	65.86 ± 5.83
Sept	4.14 ± 1.39	8.34 ± 0.68
Oct	8.58 ± 2.89	42.00 ± 8.75
Nov	5.10 ± 1.64	0.84 ± 0.36
Dec	4.02 ± 1.80	15.46 ± 1.89
Jan	0.56 ± 0.19	117.40 ± 5.63
Feb	1.28 ± 0.25	12.34 ± 2.74
Mar	0.84 ± 0.01	51.66 ± 4.70
Apr	0.24 ± 0.07	23.10 ± 2.81

TABLE 5.6: Progesterone concentration (ng/ml) measured in plasma and gonads of female ***O.mossambicus*** (samples taken per month, n = 40).

Female plasma progesterone levels were low from January to August whereafter they increased significantly to December. In general, gonad values were high from March to August and thereafter showed 4 significant ($p < 0.001$) dips in concentration during September, November, December and February.

FIG 5.1 represents the relationship between GSI (%) and the gonadotropins (FSH and LH) concentration (mIU/ml) in male ***O.mossambicus*** plasma and FIG 5.2 represents these same parameters in the gonadal supernatant. FIG 5.1 shows

FIG 5.1: Plasma gonadotropin levels and GSI in male *O.mossambicus*

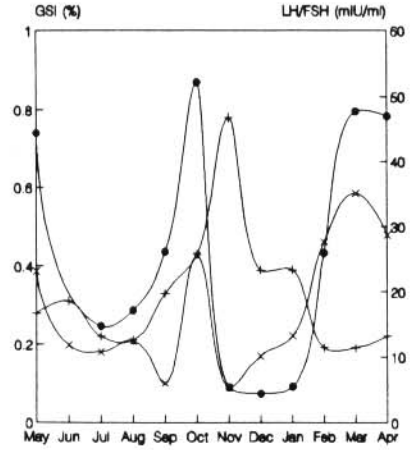
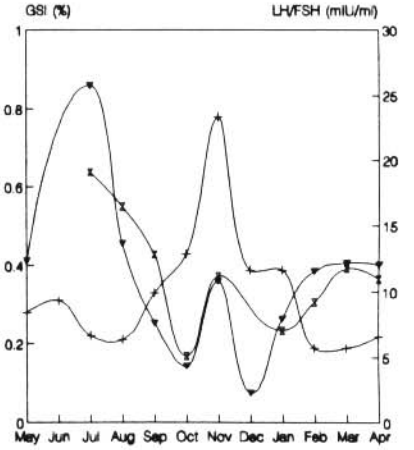


FIG 5.2: Gonadal gonadotropin levels and GSI in male *O.mossambicus*



that a good relationship exists between FSH and LH in male plasma (r = 0.72) Plasma LH levels were almost double those of FSH. FIG 5.2 shows a significant weaker relationship in male gonadal supernatant (r = 0.59). In plasma, only one peak for both FSH and LH 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.

FIG 5.3 represents the relationship between GSI (%) and the gonadotropins (FSH and LH) concentration (mIU/ml) in female ***O.mossambicus*** plasma and FIG 5.4 represents these same parameters in the gonadal supernatant. FIG 5.3 shows that a relatively poor relationship exists between FSH and LH (r = 0.48) which peak with maximum GSI in September. Gonadal supernatants (FIG 5.4) on the other hand, suggested an extremely strong relationship between FSH and LH (r = 0.99) with an inverse relationship between both hormones and GSI. This may also be noted in TABLES 5.1; 5.2 and 3.2. In the gonadal supernatant, where a seemingly much closer relationship exists between FSH and LH, TABLES 5.1 and 5.2 show that for the majority of the experimental period a value of <1.00

mIU/ml was recorded for both FSH and LH and that it is only during the months of July and March that high values are observed.

FIG 5.3: Plasma gonadotropin levels and GSI in female *O.mossambicus*

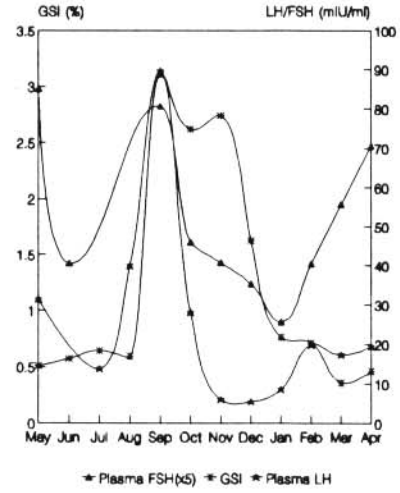


FIG 5.4: Gonadal gonadotropin levels and GSI in female *O.mossambicus*

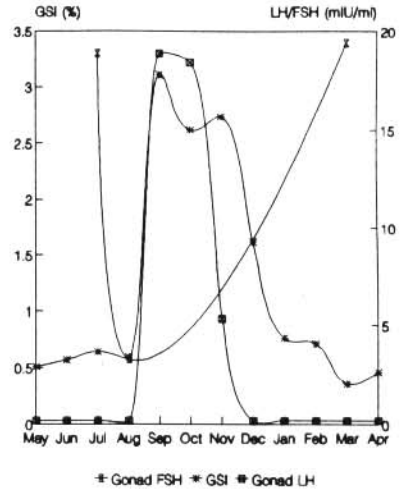


TABLE 5.3 shows the levels of human chorionic gonadotrophin (HCG) (mIU/ml) as measured in both male and female ***O.mossambicus*** plasma and gonadal supernatant. It may be noted that in the case of male plasma and female gonadal supernatant, the only measurable quantity of HCG is noted during May when 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 5.3 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 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.

FIG 5.5 represents the relationship between GSI (%) and the concentration of the steroid hormone testosterone (ng/ml) in male ***O.mossambicus*** plasma and gonadal supernatant. It may be seen that the concentration of plasma testosterone reaches a peak of 12.30 ± 2.27 ng/ml during September, which is two months prior to the maximum of GSI and gonadal supernatant testosterone levels in November. A correlation coefficient of r = 0.70 exists between gonadal supernatant testosterone level and GSI in male ***O.mossambicus***.

FIG 5.5: Steroid hormone levels and GSI in male *O.mossambicus*

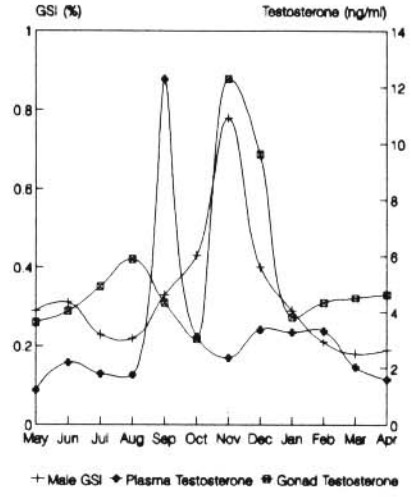


FIG 5.6: Plasma and gonadal steroid hormone levels and GSI in female *O.mossambicus*

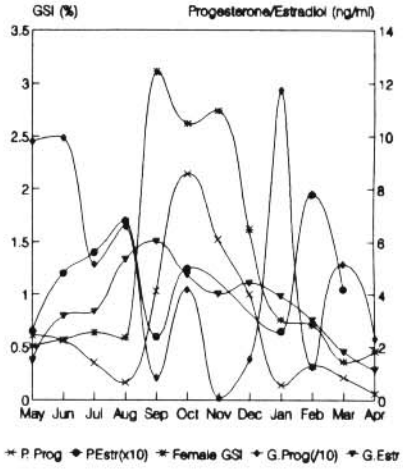


FIG 5.6 represents the relationship between GSI (%) and the concentration of the steroid hormones estradiol 17-β and progesterone (ng/ml) in female ***O.mossambicus*** plasma and gonadal supernatant. In FIG 5.6, the levels of progesterone in the gonadal supernatant have been divided by a factor of 10 in order to fit in with the axes used. It may be noted that female GSI reaches a peak during September. Plasma estradiol 17-β levels are high (0.68 ± 0.04 ng/ml) during August, whereas plasma progesterone reaches it's peak of 8.58 ± 2.89 during October. A very good relationship exists between plasma progesterone concentration and GSI (r = 0.85) in female ***O.mossambicus***. In the

gonadal supernatant, a good relationship exists between estradiol 17-β and GSI ($r = 0.67$) with both reaching maximum values during September. Gonadal progesterone concentration appears to exhibit an almost inverse relationship wit female GSI, although there is a small progesterone peak (42.00 ± 8.75 ng/ml) measured during October. The highest level of progesterone (117.40 ± 5.63 ng/ml) is seen in January when GSI has fallen considerably from the high noted during September till November.

FIG 5.7: Plasma reproductive hormone levels in male *O.mossambicus*

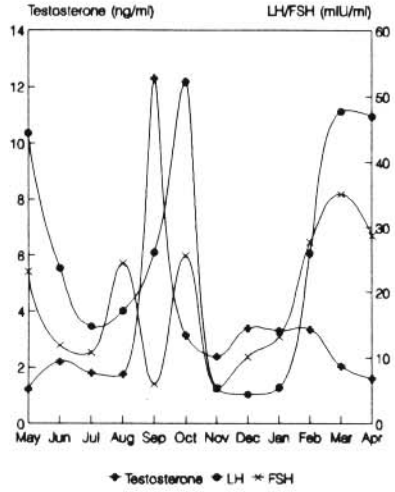


FIG 5.8: Gonadal reproductive hormone levels in male *O.mossambicus*

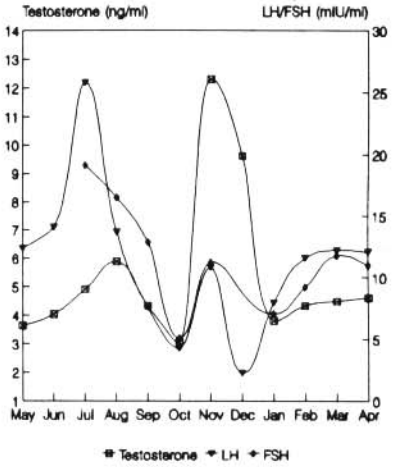


FIG 5.7 represents the relationship between gonadotrophin and steroid reproductive hormones in male ***O.mossambicus*** plasma and FIG 5.8 represents these same parameters in the gonadal supernatant. In the plasma, testosterone appears to reach a maximum value during September and a second, slightly lower peak is observed during January. Both LH and FSH appear to have three peaks. In LH, they are of similar magnitude and are noted during May, October and March. In FSH the peaks are also of a similar magnitude, but are much lower than is the case in LH. The FSH peaks may be seen during August, October and January, prior to the LH peaks. Maximum peaks for both hormones are reached

in October at maximum GSI. In the case of the gonadal supernatant (FIG 5.8), testosterone reaches maximal values during August and November with the latter being highest and which coincides with maximal GSI (see FIG 5.5). LH however, reaches a peak value during July and FSH does likewise. A second smaller peak for these hormones were observed during November.

FIG 5.9 represents the relationship between the gonadotropins and steroid hormones in female ***O.mossambicus*** plasma and FIG 5.10 represents these same parameters in the gonadal supernatant. In FIG 5.9 LH may be seen to reach a maximum during September which coincides with maximal GSI (see FIG 5.6). Thereafter a smaller peak is reached during January / February. Although FSH also reaches a high during September, the magnitude of it's peak is much lower than that for LH. Smaller variations also occurred during November / December and February / 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 (see TABLE 5.5).

FIG 5.9: Plasma reproductive hormone levels in female *O.mossambicus*

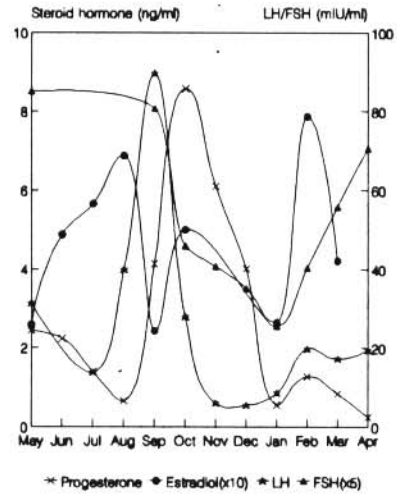


FIG 5.10: Gonadal reproductive hormone levels in female *O.mossambicus*

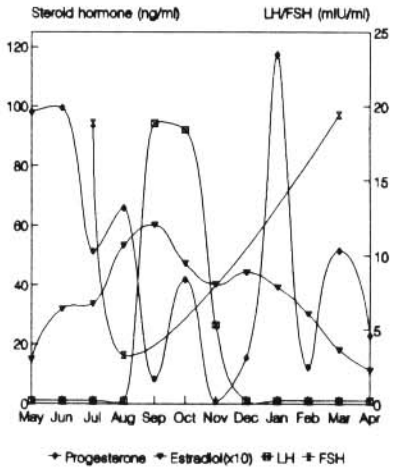


FIG 5.10 shows the gonadal supernatant, in which it may be noted that the levels of LH and FSH appear fairly similar during the experimental period when they could hardly be detected during August to November. In the case of progesterone and estradiol 17-β, the former is present in much greater concentrations 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 it's maximal value of 117.40 ± 5.63 ng/ml during January.

FIG 5.11: Syferkuil water temperature & plasma steroid hormone levels in male and female *O.mossambicus*

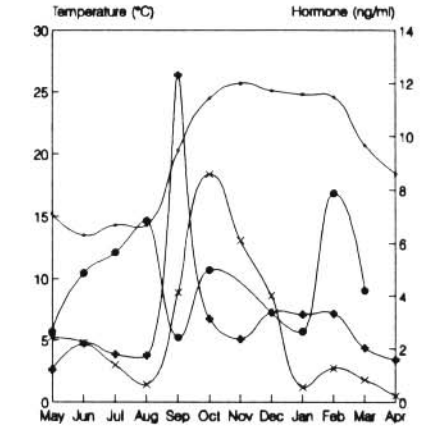


FIG 5.12: Syferkuil water temperature and plasma steroid hormone levels and GSI in *O.mossambicus*

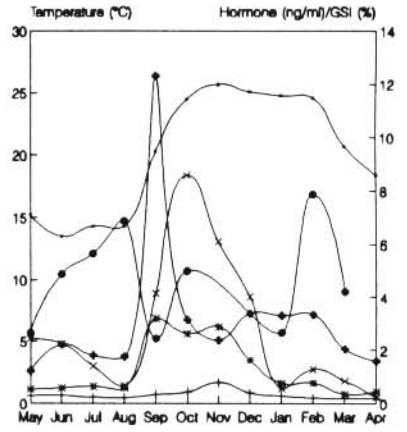


FIG 5.11 represents the relationship between dam water temperature (°C) and the concentration of steroid hormones (ng/ml) in male and female ***O.mossambicus*** plasma. In FIG 5.11 it may be noted that the temperature of the dam water increases sharply during September and remains high until February. Male testosterone also shows a peak during September and December to February. Female progesterone peaks in June, September to December and February reaching a maximum during October. In the case of female estradiol 17-

β, the highest concentrations are seen during August, October and February. Male plasma testosterone levels generally peak when female estrogen levels are low.

FIG 5.12 represents the relationship between Syferkuil dam water temperature (°C), GSI (%) and male testosterone and female steroid hormone concentrations (ng/ml) in ***O.mossambicus*** plasma, including male and female GSI. High male GSI values are seen to extend from September to January with a peak during November, which is two months after the maximum testosterone peak, but at a time when dam water temperature is still high. In the female, estradiol 17-β reaches a high concentration during August, October and February. It is during this period that female GSI levels reach a maximum. Progesterone also reaches maximum levels during this period. It therefore suggests that some female hormone, possibly estrogen breakdown products in the urine, may act as a pheromone to trigger testosterone production in males.

Fig 5.13: Plasma lipid and progesterone levels in female *O.mossambicus*

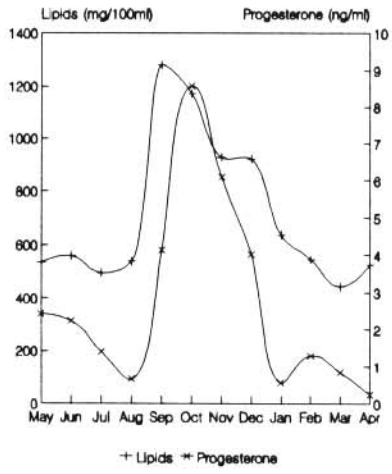


Fig 5.14: Gonadal lipid and progesterone levels in female *O.mossambicus*

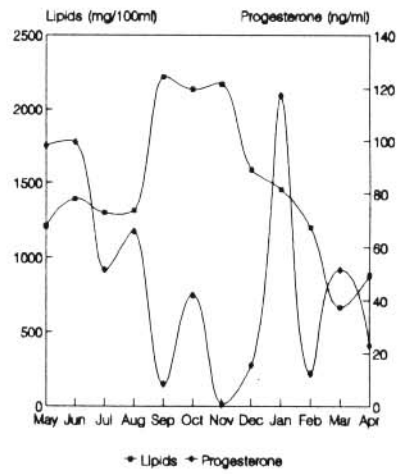


FIG 5.13 represents the relationship between lipid (mg/100ml) and progesterone (ng/ml) concentration in female ***O.mossambicus*** plasma and FIG 5.14 represents these same parameters in the female ***O.mossambicus*** gonadal supernatant. FIG 5.15 represents a compilation of FIGS 5.13 and 5.14. FIG 5.13 shows an inverse relationship with lipids and estradiol 17 β in plasma during August / September when peak values are reached. This was followed by an increase and subsequent decline in progesterone levels a month later. In the gonads, however, the same positive relationship between lipids and estradiol 17 β (FIG 5.14) was observed at much higher levels. FIG 5.14 appears to show an inverse relationship between lipid and progesterone concentration in the gonadal supernatant of female ***O. mossambicus***. In both FIGS 5.13 and 5.14, plasma and gonadal lipid levels reached a peak during the same month of September.

Fig 5.15: Plasma and gonadal lipid and progesterone levels in female *O.mossambicus*

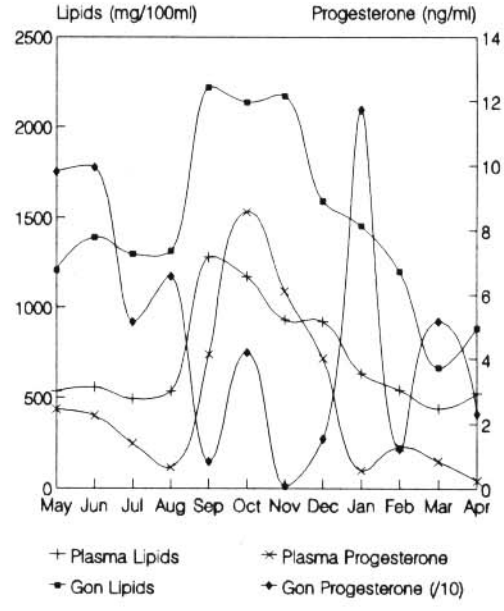


FIG 5.16 represents the relationship between lipid (mg/100ml) and estradiol 17-β (ng/ml) concentration in female ***O.mossambicus*** plasma and FIG 5.17 represents these same parameters in the female gonadal supernatant. FIG 5.18 represents a compilation of FIGS 5.16 and 5.17. FIG 5.16 shows that both plasma lipid and estradiol 17-β concentration shows a similar trend, with both reaching their peak concentration during September. FIG 5.17 shows that, unlike the lipid and progesterone gonadal supernatant concentrations (FIG 5.14), lipid and estradiol 17-β concentrations exhibit a similar trend. During September when lipid concentrations are at their maximum, the estradiol 17-β concentrations also reach a high level. FIG 5.18 shows that in the case of lipid and estradiol 17-β concentration in female ***O.mossambicus*** the trend appears very similar and a good relationship seems to exist between these parameters.

Fig 5.16: Plasma lipid and estradiol levels in female *O.mossambicus*

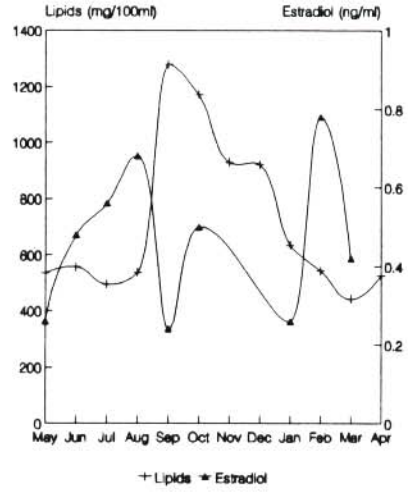


Fig 5.17: Gonadal lipid and estradiol levels in female *O.mossambicus*

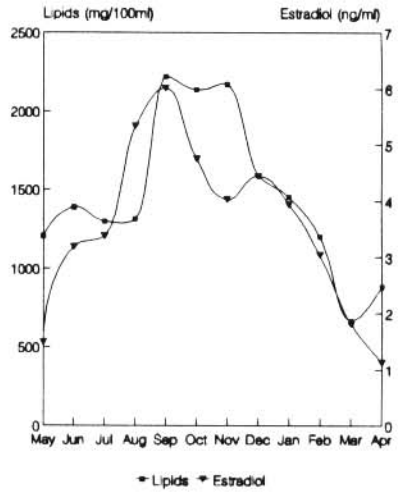


FIG 5.18: Plasma and gonadal lipid and estradiol levels in female *O.mossambicus*

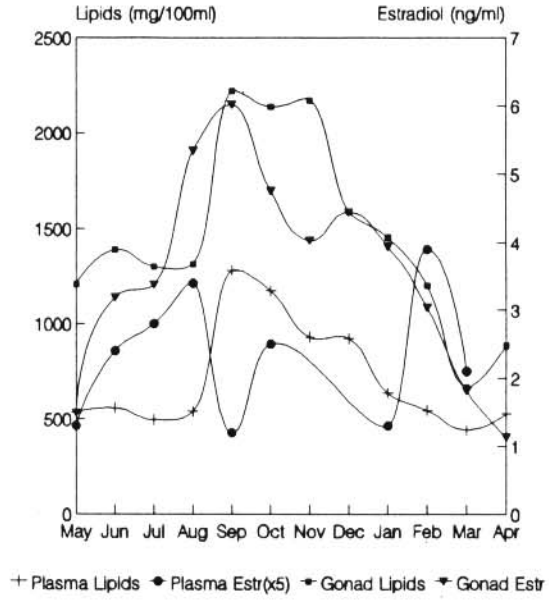


FIG 5.19 represents the relationship between lipid (mg/100ml) and testosterone (ng/ml) concentration in male ***O.mossambicus*** plasma and FIG 5.20 represents these same parameters in the male gonadal supernatant. FIG 5.21 is a compilation of FIGS 5.19 and 5.20. FIG 5.19 shows that a good relationship exists between plasma lipid and testosterone ($r = 0.67$) concentrations, with both parameters reaching a high concentration during September. In FIG 5.20 the relationship appears to be one month "out of phase". The gonadal testosterone level peaks during November with the gonadal supernatant lipid peak coinciding with maximum GSI (see FIG 5.5). FIG 5.21 shows that plasma testosterone, although initially lower than gonadal supernatant levels, reaches it's maximum prior to the gonadal supernatant testosterone peak. There is an initial surge of testosterone in the gonadal supernatant during August. Thus the two gonadal testosterone surges have opposing effects on plasma testosterone levels.

Fig 5.19: Plasma lipid and testosterone levels in male *O.mossambicus*

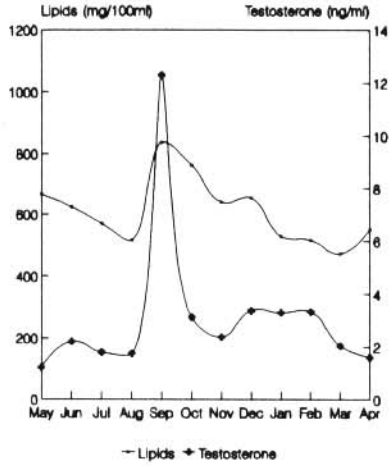


Fig 5.20: Gonadal lipid and testosterone levels in male *O.mossambicus*

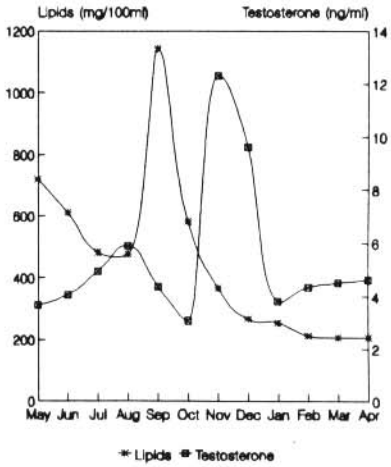
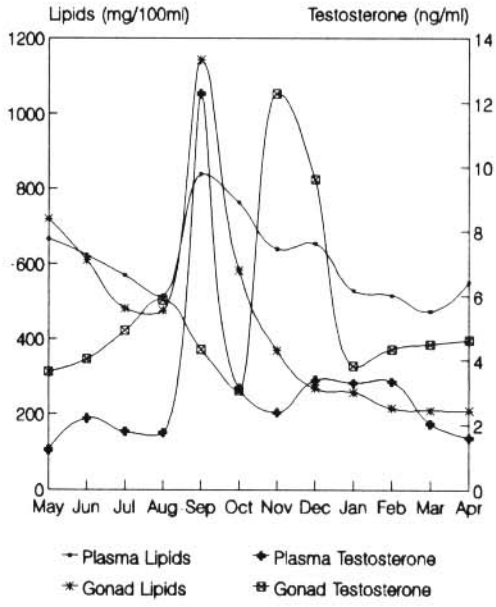


Fig 5.21: Plasma and gonadal lipid and testosterone levels in male *O.mossambicus*



5.5 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.

5.5.1 Gonadotropins

Males

FIGS 5.1 and 5.2 show the relationship between GSI and the gonadotropin levels in both the plasma and gonads of male ***Oreochromis mossambicus.***. During this study, human FSH, HCG and LH kits were used to determine the different gonadotropin levels. The results suggest that two different gonadotropins are released by the pituitary, one being FSH-like and the other LH-like. HCG has limited FSH and extended LH 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 and LH observed during the same period which may be purely incidental. It therefore appears as if only the FSH fraction of HCG could be detected during this time. It may therefore be safely assumed that male ***O. mossambicus*** reproduction cycles are controlled by two gonadotropins. The presence of HCG in male gonads suggest the secretion of an inhibitory trophic hormone by the gonads, since it was not detected in the blood. It may be similar to the some form of gonadotropin inhibitory hormone secreted by the hypothalamo- pituitary axis that are sifted rapidly from the blood to the gonads. These observations agree with similar findings in other teleost species. 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 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 GSI. The latter is preceded first by an FSH followed by an LH 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 therefore 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 to allow for inhibition of pituitary secretion by steroids by the gonads. Thus when gonadal steroids increase, FSH secretion is inhibited. This explains the inverse relationship between plasma and gonad gonadotropin levels. However, the occurrence of HCG in female plasma from December onwards, suggest 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 their is no necessity to this hormone to act on the gonads but rather to inhibit gonadotropic secretion by the hypothalamo-hypophyseal axis. The results also suggest that a double reproduction cycle occurs during the breeding period for males in two different age groups. The older males spawn first, followed by the younger group. Thus four cycles for males are observed during the breeding season. Furthermore, it appears as if an inverse relationship with mass and size occurs. Bigger males therefore experience lower gonadotropin increases whereas young males have a comparatively larger increase in gonadotropins. This cannot be explained, but may be indicative of pituitary sizes being similar in both small and large male specimens. 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 regression.

Male gonadotropin levels are high during winter, suggesting that there is an initial commencement of gonadal development occurring during this season. The peaks that are observed in FIGS 5.7 and 5.8 for both FSH and LH are due to the release of testosterone earlier in the breeding cycle. High progesterone levels therefore inhibit testosterone production.

Females

Two female gonadotropins were also noted for ***O. mossambicus***. A similar double cycle for both FSH and LH for two age groups were recorded during the breeding cycle. It consisted of an increase in FSH followed by a similar increase in LH. In general, female FSH and LH levels were lower than male values in both the plasma and gonads. Furthermore, HCG levels were also recorded in female plasma which were lower than the comparative LH levels. No HCG levels were detected in female gonads. This suggests that HCG corresponds to LH 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 carrying the eggs and larvae in their mouths for approximately six weeks. The temperate environment would therefore allow for another cycle in each age group to commence. In general, the lower levels of FSH and LH in female plasma and gonads may be related to the bigger size of the ovaries in females when compared to males at the same development stage.

5.5.2 Human Chorionic Gonadotropin (HCG)

This hormone could not be measured in any meaningful concentrations in male ***O.mossambicus*** plasma or female gonads (see Table 5.3). In the case of the male gonad, the high concentration observed in January (summer) could indicate a resting phase for the testes and the clearance of remaining gametes at the

conclusion of the breeding cycle. It could also be involved in the preparation of the testis for the next breeding cycle.

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 ***Oreochromis 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 & Scott, 1990). It is therefore possible that the HCG levels observed during March, May and July could be involved with oocyte maturation. As previously indicated, HCG may act as a 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.

Canario and Scott (1990) have also suggested that HCG may play a role in ovulation. This is unlikely to be the case in the bream, ***O.mossambicus*** as the high level of HCG was measured during December when ovulation was complete. There is however, still mouthbrooding occurring at that time, and so perhaps HCG is important in this process. Female plasma levels may be lower than those of males because of the greater shift of these toward the female gonads. In addition, smaller females displayed lower levels of FSH and LH than comparative sized males. The relationship with female gonadotropins appeared to be directly weight and size related.

The foregoing information suggests that male FSH and LH 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 milt preparation to occur at the same time, thereby optimizing the chances of successful fertilization.

5.5.3 Testosterone

This parameter was not measured in females. FIG 5.5 shows that there is a surge in the level of testosterone in the plasma prior to the surge observed in the gonad. FIG 5.11 shows that this increase in testosterone in the plasma could be associated with the increase in the dam water temperature which occurs at the same time (September - spring). Chapter 2 indicates that there is also an increase in daylength during this period, and photoperiod has been shown to be an environmental cue to a preovulatory surge in hormonal secretion in cyprinids (Aida, 1988). This occurrence may create a false interpretation of the results which will be outlined below. The foregoing results also suggest that an inverse relationship exists between male plasma and gonad testosterone levels. Two factors may be involved. First, a time lag may occur between the testosterone production in the testes and inter-renal tissue and their shift to the plasma before it may be measured at quantifiable levels. Second, the different peaks observed in the plasma, may be related to body size, ie: the bigger the specimen, the lower the testosterone levels. This corresponds with the size of the males collected during the breeding period. Gonad testosterone concentration shows a biphasic cycle for two age groups in males which may be size related. It appears as if the bigger males spawn first, followed by the smaller males. In addition this double testosterone cycle for each group, corresponds with ovarian development in females. The large testosterone concentrations correspond with female GSI development. Furthermore, the large testosterone surge in males during September, may result from a build up of testosterone in male plasma as a result of male testes being able to produce testosterone at a specific rate only. Thus,

when female GSI reaches a peak and spawning occurs, an immediate decline in male plasma testosterone occurs. This observation is supported by the relatively low production of testosterone recorded in the testes during the same period. The biphasic cycle in males seems to be related to the biphasic cycle observed in females. Although it appears that the male cycle "lags" about two months behind the female in terms of peak GSI's recorded for both males and females, hormone levels measured suggest the opposite. The synchronization of male and female reproduction cycle is therefore closely related to the mouthbrooding period of the females.

The increase in September, which was preceded by a much smaller peak during June suggests that the testosterone levels indicate the onset of spermatogenesis within the testis. The correlation of testosterone and GSI levels indicate that this androgen is associated with testicular development. FIG 5.7 shows that just prior to testosterone increasing, there is an increase in both gonadotropins measured, which may act as a stimulant, resulting in testosterone secretion. Testosterone in juvenile tilapia is known to be responsible for sex differentiation (Rothbard ***et al.***, 1987). Both plasma and gonadal testosterone levels show a bimodal increase. These results would appear to correlate with those for the brown bullhead (Burke ***et al.***, 1984), the black goby (Bonnin, 1979) and the blue cod (Pankhurst & Conroy, 1987) who all exhibit this bimodal profile of gonadal steroids. The significance of the bimodality could be that recrudescence occurs rapidly (less than one month). The relatively high levels of testosterone measured in the gonad during November (FIG 5.5) is most probably associated with the release of mature spermatozoa. Once the gonadal lipids reach a high during September (FIG 5.20), they could then lead to an increase in the testosterone levels and subsequent spawning.

In male teleosts, testosterone is typically elevated during spermatogenesis, and then falls at the onset of spermiation (Fostier ***et al.***, 1983). The lower levels of testosterone observed in this study, could reflect the synthesis of unmeasured

metabolites.

It appears that a time lapse exists between testosterone production and the male breeding cycle, which is associated with female gonadal maturity. Thus temperature appears to be the main cue causing testosterone to peak which leads to the gonads and subsequently, their gametes reaching reproductive maturity.

5.5.4 Estradiol 17-β

This parameter was measured in females only. Estradiol 17-β is secreted by both the female gonads and inter-renal tissues. In general, estradiol is responsible for stimulating vitellogenesis and is also secreted by female gonads during the pre-spawning period. Evaluation of the results in Table 5.5 and FIG's 5.11 and 5.12 reflects the importance of this hormone. Table 5.5 suggests that gonadal estrogen levels are generally higher than plasma levels. Plasma estradiol levels suggest no major changes, except during November / December. The latter observation suggests that most females were in the immediate postspawning period prior to gonadal recrudescence. From May to August a gradual increase in plasma levels was observed. This mild increase suggests a bimodal increase from both the gonads and the inter-renal tissues. It does, however, not explain why a major shift from the gonads to the blood occurs. This may be due to a decline in steroidogenic postovulatory follicles being present. It also suggests that this period corresponds with the major mouthbrooding phase of female ***O. mossambicus***. Furthermore, plasma estradiol levels confirm an increase in the immediate pre-spawning activity when compared with female GSI values. Gonad estradiol levels reflect a continuous maturing of females to prepare for the following spawning cycle. Estradiol is known to be secreted by the cells of the ovarian follicles that promote the development and maintenance of the female sexual characteristics. In humans it is the hormone (together with other hormones) that is responsible for controlling the female sexual cycle. Thus the

number of follicles that are ovulating would determine or at least contribute to the quantity of estradiol that is present in the gonads. Estradiol has been reported to stimulate vitellogenesis in Teleosts (Campbell & Idler, 1976; de Vlaming *et al.*, 1980; Smith & Haley, 1988). They have reported an increase in plasma estradiol levels once spawning commences, and remains high throughout the period of oocyte growth. These observations suggest that during this phase of undetectable estradiol levels, no vitellogenesis is required during the mouthbrooding period and that some females experience gonadal recrudescence. Another possibility to be considered, is that the mid-cycle decline in estradiol levels could be due to a rapid utilization of the hormone in stimulating vitellogenesis.

The estradiol peak observed in February (FIG 5.9) in female ***O.mossambicus***, would correspond to the results of Smith and Haley (1988), who observed that the estradiol levels increase markedly toward the end of the ovarian cycle, after mouthbrooding has ceased and oocytes resume their growth. As already mentioned, the second estradiol peak corresponds to a rapid vitellogenic growth phase in the oocytes. The initial estradiol peak may result in the oocytes being maintained through a "protective" effect similar to that suggested by Sundararaj and Goswami (1968). This protection could be to prevent the oocytes from becoming atretic.

The occurrence of a second estradiol peak (FIG 5.9) that is preceded by a rise in gonadotropin levels concurs with the results of Yaron and Levavi-Zermonsky (1986) for the common carp. This type of relationship may be expected between increased gonadotropin levels and a gonadotropin-dependent steroid synthesis.

Rosenblum ***et al.***, (1987) have shown a good correlation between circulating estradiol-17β and calcium levels in female teleosts. In the present study, increases in plasma estradiol in female ***O.mossambicus*** paralleled increases in both GSI and calcium levels (see Chapter 6), thereby confirming a role for

estradiol in vitellogenesis.

Pankhurst and Conroy (1987) have shown in the blue cod, ***Parapercis colias***, that the absence of high or detectable levels of estradiol may be due to only a proportion of follicles having estrogenic capacity at that time. The occurrence of stage 3 follicles throughout the entire period, suggest that estrogenic activity will always be located in female gonads. This could also be true for the present study on ***Oreochromis mossambicus***. At maturity, the relative proportion of estrogen synthesizing follicles may be larger, and there may also be a stimulatory effect on steroidogenesis associated with preovulatory increases in gonadotropin.

Another factor to be considered, is the effects of a preovulatory increase in estradiol that may be excreted ***via*** the urine. It is suggested that the estrogenic excretory products may act as a type of pheromone to prepare and attract males for the female ovulatory phase.

Although not the subject of this study, the role of estradiol has been stated to be important in sex inversion or reversal in Teleosts (Rothbard ***et al.***, 1987; Kime ***et al.***, 1991). Further investigation on ***O.mossambicus*** is required in this regard, in order that this suggestion may be verified or rejected.

In general, the results recorded for ***O. mossambicus***, correspond with those for most teleost fish and vertebrates.

5.5.5 Progesterone

Table 5.5 suggests that plasma progesterone levels are generally lower than gonad levels. It also reflects an inverse relationship between blood and gonad values. During the period of September till February, the gonads show a relatively sharp decline in progesterone levels with some mild fluctuation corresponding with the bimodal breeding cycle. Such fluctuations also follow the estradiol levels

recorded in the plasma and gonads. An inverse relationship was also noted between plasma and estradiol levels. Thus, when plasma progesterone levels are high, estradiol levels are low. These observations therefore give an indication of the stages of follicle development and gonadal recrudescence. Such levels also correspond with the bimodal cycle for the two age groups as suggested earlier.

Smith and Haley (1988) have shown that the progesterone levels in mouthbrooders do not increase until mouthbrooding behaviour has ended. Thus the sharp increase in gonad progesterone levels during the period of March to August, reflects the possibility of environmental factors inhibiting gonad development. It also confirms a resting period for this species before commencing the next spawning cycle at the end of August. This rise in progesterone could also be attributed to either a decrease in conversion to other steroids or to an overall increase in steroid hormone production. The present study on ***O.mossambicus*** indicates that the concentration of progesterone shows a marked increase during January which is after mouthbrooding has been completed (see FIGS 5.6; 5.10; 5.14 and 5.15). A progesterone peak some time after spawning has been shown by Smith and Haley (1987) to mark the time when steroidogenic - appearing postovulatory follicles begin to degenerate in mouthbrooders. The presence of two peaks for progesterone in the present study could be explained by the fact that fish of different sizes were sampled and that the younger fish complete their breeding prior to the older fish. The second peak is noted toward the end of the ovarian cycle. It is unknown whether progesterone is involved in final maturation in the tilapia.

Progesterone also seems to increase in concentration as a result of the increase in water temperature that is noted during September (spring).

FIGS 5.9 and 5.10 show that the levels of the gonadotropins are fairly high when the progesterone levels and GSI (FIG 5.6) have declined. As Kobayashi ***et al.***, (1986) have shown in goldfish, the levels of the gonadotropins are influenced by

water temperature and further that high levels of gonadotropin in summer could be due to a reduction of an inhibitory effect of gonadal steroids on gonadotropin secretion. It could be possible that the high levels of gonadotropins during the postspawning period are involved in the clearance of any remaining fertilizing gametes prior to the next breeding cycle commencing.

5.6 Summary

The foregoing discussion on the hormones involved in the breeding cycle of ***Oreochromis mossambicus*** show that reproduction is regulated by different hormonal influences in this species.

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 in male ***O.mossambicus***. Gonadotropin hormone secretion may also play a role in optimizing successful fertilization by playing a pivotal role in the secretion of the steroid hormones.

Testosterone, the secretion of which also appears to be influenced by the water temperature increase, provides an indication of testicular development, in particular spermatogenesis, prior to female spawning.

Progesterone appears to be of great importance in a mouthbrooder such as ***O.mossambicus***. The increase in progesterone subsequent to the completion of mouthbrooding behaviour may be due to a decrease in the conversion of progesterone to other steroids or to an overall increase in hormone production.

Estradiol 17-β appears to be involved in stimulating vitellogenesis to occur. The levels of estradiol that were recorded may reflect the proportion of estrogen synthesizing follicles that are present.

Human chorionic gonadotropin (HCG) or its equivalent, may play a role in the final maturation of oocytes in female ***O.mossambicus*** or an inhibitory role to ensure a resting phase prior to the next spawning cycle.

Future investigations on this species should concentrate on the following:

1. Histology of the hypothalamo - pituitary axis in both males and females to determine the nature of the gonadotropin secreting cells.
2. It would be of great interest to measure the levels of gonadotropins within the pituitary in order to establish a pituitary - gonadal axis of hormonal activity.
3. Development of suitable RIA techniques to verify and determine the existence of two pituitary gonadotropins as identified in other teleosts. It ought to be remembered that the FRANSA kits used in this study are designed for use with human samples. This would ensure that more accurate quantitative results could be obtained.
4. Examination of the hypophysis to determine the role of gonadotropin releasing hormones.
5. The role of other pituitary hormones in supporting gonadal maturity and the survival of larvae.
6. The fact that two gonadotropins were found, suggest the possibility of two different vitellogenins involved in the reproductive cycle.
7. Immunocytochemistry of the pituitaries and gonads in male and females to determine the origin of the steroid hormones.
8. The role of dopamine antagonists in the control of the reproduction cycle.
9. The role of pheromones in reproduction.
10. The role of pheromones in fertilization.
11. Application of suitable techniques for the artificial propagation of these species.

By conducting controlled laboratory experiments, hormones could be administered to the ***O.mossambicus*** adults used, and the subsequent effects, if any, could then be determined.

CHAPTER 6

Chemical Composition of Plasma & Gonadal Supernatant in Oreochromis mossambicus

6.1 Introduction

The general characteristics of the spermatozoa and ova of various fish species have been well documented (Clemens & Blake Grant, 1965; Cruea, 1969; Ginzburg, 1972; Guest ***et al.***, 1976; Jaspers ***et al.***, 1976; Scott & Baynes, 1980; Van der Horst, 1980; Baynes ***et al.***, 1981; Kazakov, 1981). However, aside from Kruger ***et al*** (1984), not that much literature is available on work conducted on ***Oreochromis mossambicus***. A possible reason is that this species breeds freely in captivity under controlled environmental conditions. It is also not an endangered species.

The chemical examination of the plasma and gonads have generally been an aid in the evaluation of the reproductive ability in mammals (Cruea, 1969). In fish, few chemical criteria have been established to judge their reproductive ability. Chemical criteria that are of importance are the presence or absence of inorganic and organic components and the osmolality and pH of both the plasma and the gonads in association with the reproductive cycle (Ginzburg, 1972; Scott & Baynes, 1980).

Scott & Baynes (1980) and Marshall (1986) have shown that salmonid spermatozoa quiescence in seminal plasma is associated with a high ratio of K^+ to Na^+ concentrations. There appear to be two ion transport processes that help maintain low Na^+ and high K^+ levels. They have further demonstrated a relationship between gonadotropin secretion and K^+ and Na^+ levels in salmonids.

Flik ***et al.***, (1989) have shown that in tilapia, a positive sodium balance is

maintained, especially in acid water.

Calcium has been shown to play an important role in fertilization (Hiramoto ***et al.***, 1989). The release of prolactin from the pituitary of the tilapia ***Oreochromis mossambicus*** has also been shown to be calcium dependent (Grau ***et al.***, 1986). The release of gonadotropin has also been shown to be not only dependant on gonadotropin releasing hormone (GnRH) secretion, but also to be calcium dependant (Mikolajczyk ***et al.***, 1990a,b). Working on the common carp (cyprinidae), these authors found that Ca^{++} acts as a second messenger in the action of GnRH on GTH release. Thus, it is not only GnRH that stimulates GTH secretion, but also basal gonadotropin secretion is calcium dependant.

Mukhopadhyay ***et al.***, (1986) have stated that mature eggs of fish contain a very large amount of protein rich yolk. Although it is known that the synthesis of protein and other cellular components in a species depends on its genome, it is conceivable that any seasonal variation in total protein may provide an indication of the reproductive development of the fish. Glucose, lactate and lipids, although more likely to be energy sources, may also provide an indication as to the stage of development of the gonads in ***O.mossambicus***.

The fact that Syferkuil Dam forms part of the effluent of a sewage system would suggest that urea levels could be important. Grubinko ***et al.*** (1987) have shown that urea levels are related not only to the environmental levels but also to the breakdown of arginine by arginase.

The object of this investigation was to measure the concentrations of sodium, potassium, calcium, glucose, total lipids, lactate, total protein and urea in both plasma and gonads of male and female ***O.mossambicus***. Cognizance has been taken of several problems and shortcomings that still exist, especially concerning the availability, handling, evaluation and analysis of fish plasma and gonadal samples.

6.2 Literature Survey

The chemical composition of both plasma and the gonads of freshwater fish may provide an indication of homeostasis and health status during the stage of reproductive development. The various parameters that have been examined and for which information could be obtained include sodium and potassium (Morisawa ***et al.***, 1983; Kruger ***et al.***, 1984; Morisawa & Morisawa, 1988; Flik ***et al.***, 1989; Marshall ***et al.***, 1989; Yamauchi, 1991), calcium (Lee & Hu, 1983; Grau ***et al.***, 1986; Urasa & Bonga, 1987; Hiramoto ***et al.***, 1989; Levavi-Sivan & Yaron, 1989; van Asselt ***et al.***, 1989; Mikolajczyk ***et al.***, 1990a,b; Munkittrick, 1991; van der Kraak, 1991), lipids (Rao & Rao, 1984; Besnard ***et al.***, 1989; Garcia-Garrido ***et al.***, 1990), proteins (Mukhopadhyay ***et al***, 1986; Mukhopadhyay ***et al***, 1987) and urea (Grubinko ***et al.***, 1987).

Electrolytes such as sodium could possibly fulfil a role in maintaining the osmolality of seminal fluid and thereby ensure the viability of sperm ***in vivo***, before the release and activation during the spawning activity. When present in high concentrations, potassium is thought to exert an inhibiting effect on the sperm and seem to keep spermatozoa immobile within the testis (Kruger ***et al.***, 1984). This seems to contrast with the effects of calcium, which when present in certain concentrations seem to activate the spermatozoa. Kruger ***et al.*** (1984) suggest that a possible correlation between potassium and calcium concentrations and the motility of sperm may exist.

Yamauchi ***et al.*** (1991) has reported a possible involvement of plasma sodium concentrations with growth hormone levels, with the hormone controlling ionic regulation.

Marshall ***et al.*** (1989b) working on the brook trout ***Salvelinus fontinalis*** state that low sodium and high potassium levels are important in maintaining the quiescence of spermatozoa in the sperm duct lumen. As a decrease in potassium

and/or an increase in sodium occurs extracellularly, sperm motility will be initiated. Sperm duct epithelium actively secretes K^+, apparently by an electrically silent mechanism and actively absorbs Na^+ electrogenically. In combination, these two ion transport processes help maintain low Na^+ and high K^+ in the seminal plasma (Marshall ***et al.***, 1989b). This seems to contrast to other results, including personal observations, whereby low Na^+ levels initiate sperm motility and possibly even lead to ejaculation.

Morisawa ***et al.*** (1983) have suggested that sperm of freshwater Cyprinidae become motile at spawning due to a reduction in the osmolality resulting from the dilutary effect of the aquatic environment. Sodium concentrations in the gonad were found to be lower than in the plasma, whereas the opposite was true for potassium. High potassium levels and low sodium levels seem to help in maintaining sperm motility.

Morisawa and Morisawa (1988), working on salmonid fishes, suggest that it is rather the increase in seminal bicarbonate concentration and pH that occurs as spermatozoa pass from the testis to the sperm duct that provides the sperm with their motility.

Considerable work has been carried out on the effects of calcium on fish reproduction. Mikolajczyk ***et al.*** (1990a,b) have examined the effects of calcium ions as a mediator in gonadotropin releasing hormone (GnRH) action on gonadotropin release in the common carp, ***Cyprinus carpio*** **L.** Their results have shown that gonadotropin secretion is calcium dependant. They have shown that an increase in calcium concentration is an essential step in GnRH action. The results of Levavi-Sivan & Yaron (1989) confirm that in tilapia, gonadotropin secretion is dependant on calcium. They have shown that the dependence of gonadotropin secretion on extracellular Ca^{++} in tilapia is in agreement with that observed in mammals where sustained LH release, in response to GnRH, is inhibited in a calcium - free medium. The immediate phase of LH release from rat

gonadotrophs is associated with intracellular calcium ion stores being utilized.

Hiramoto ***et al.*** (1989) have shown that a possible role for calcium is that of fertilization of eggs. Their results suggest that an increase in intracellular Ca^{++} concentration is the direct cause of the breakdown of cortical alveoli which then enhances fertilization. Gilkey ***et al.***, (1978), using eggs of the medaka, ***Oryzias latipes***, have shown that the cytoplasmic Ca^{++} release which starts at the site of sperm entry and is then propagated over the entire cortex occurs at fertilization. Van der Kraak (1991) suggests that in goldfish ovarian follicles, calcium may contribute toward the full expression of gonadotropin effects on steroid hormone production. Calcium seems to play an important role in GTH - induced steroidogenesis in preovulatory ovarian follicles within the goldfish. The precise manner in which GTH regulates intracellular calcium levels in goldfish ovarian follicles is unknown. This calcium, in contributing toward the steroidogenic actions of GTH may be mediated by an interaction of calcium with calmodulin.

Lee and Hu (1983) have reported the importance of calcium in many biological activities. They also indicate the importance of calcium in controlling the permeability of the cell membrane to other ions and to water, and is responsible for the integrity of cell-cell junctions. Their results for the grey mullet, ***Mugil cephalus*** **L**, suggest that calcium is indispensable to the embryonic development of the eggs.

Munkittrick (1991) indicates that in salmonids, serum calcium levels are used as a crude, indirect indicator for the reproductive developmental stage. The major yolk protein in many species is the large lipophosphoprotein, vitellogenin, which is produced in the liver and transported to the ovaries in the plasma (Mount ***et al.***, 1988). Because vitellogenin binds calcium ions, the concentration of bound calcium will rise. Thus total calcium levels may also be suggestive of increased transport of yolk proteins and of advancing reproductive development.

Very little information could be obtained pertaining to studies on Glucose, lipids, proteins or lactate for freshwater fish. Loir ***et al.*** (1990) have shown that protein is present in seminal fluid to a much lesser extent than it is in the plasma. The importance of the study of proteins for biochemical systematics is usually used to show that both qualitative and quantitative differences in fishes may be related to genetic variants. In this study it would be more likely that the quantitative differences in total protein levels could be related to a developmental stage of the gonad.

Rao and Rao (1984) have measured the levels of total lipids, phospholipids, free fatty acids and total cholesterol in ***Oreochromis mossambicus***. Their results suggest that lipids are utilized to mitigate any stressful condition and as reproduction is a high stress condition, considerable lipids ought to be present during the spawning period of this fish. When levels of lipid are reduced it could be as a result of increased glyconeogenesis being induced, particularly in the liver and muscle. Besnard ***et al.*** (1989) report that as a result of a seasonal study, neutral lipids may be indicative of sexual maturity.

6.3 Materials and methods

Samples were collected as described in Chapter 3.

Both the plasma and gonadal homogenate supernatants were analysed for sodium and potassium. The gonadal homogenates were diluted by a factor of two, whereas the plasma samples were analysed without dilution. All determinations were carried out with a Corning 450 Flame Photometer.

The calcium concentration in the plasma and the gonadal homogenate supernatants was established via the Colorimetric Method involving o-Cresolphthalein complexone, without deproteinization, using a Boehringer Mannheim test kit **(Cat. No. 204 382).**

Glucose concentrations were determined in both plasma and gonadal homogenate supernatants by the GOD-Perid Method, using Boehringer Mannheim test kit **(Cat. No. 124 036)**.

Total lipids, total proteins and lactate concentrations were also determined with the aid of Boehringer Mannheim test combinations **(Cat. Nos. 124 303; 124 281 & 124 842)** using the Sulfophosphovanillin reaction, Biuret Method and UV-Method respectively.

The concentration of urea in both the plasma and gonadal homogenate supernatants was determined by Berthelot's Reaction, using a Boehringer Mannheim test kit **(Cat No. 124 788)**.

All readings as required when using Boehringer Mannheim test combinations were taken on a Beckman model DU65 Spectrophotometer.

Statistical analyses and interpretations were as described in Chapter 3.

6.4 Results

All data presented in TABLES 6.1; 6.2; 6.3; 6.4; 6.5; 6.6; 6.7 and 6.8 are mean monthly ± standard deviation values for the parameters indicated.

The following TABLE 6.1 shows the concentration of sodium (mg/100ml) in both the plasma and gonadal supernatant of male and female ***O.mossambicus***.

	♂ Plasma (mg/100ml)	♂ Gonad (mg/100ml)	♀ Plasma (mg/100ml)	♀ Gonad (mg/100ml)
	mean ± sd	mean ± sd	mean ± sd	mean ± sd
May	187.00 18.36	222.80 17.36	195.67 17.64	235.50 18.77
Jun	205.50 11.12	148.33 10.15	212.38 14.71	125.75 5.70
Jul	208.33 17.43	161.73 13.99	217.11 15.33	151.00 11.53
Aug	209.00 18.51	188.00 13.11	222.75 22.55	192.00 13.80
Sept	240.00 12.83	148.34 12.62	247.33 24.11	15.75 2.36
Oct	262.67 23.38	98.71 9.31	296.00 16.57	24.00 2.94
Nov	282.00 25.35	53.19 6.17	308.98 21.75	23.71 2.68
Dec	249.20 16.31	24.29 4.42	280.14 18.37	29.33 3.06
Jan	258.50 23.40	46.80 4.67	241.21 12.85	24.67 4.45
Feb	230.40 22.56	51.17 7.11	253.50 13.50	22.00 1.93
Mar	245.33 23.01	54.67 5.95	252.77 16.11	23.45 1.61
Apr	218.00 13.95	52.22 4.22	226.67 18.88	22.00 3.49

TABLE 6.1: Na^+ concentration (mg/100ml) measured in the plasma and gonads of male and female ***O.mossambicus*** (samples taken per month, n = 40).

The following TABLE 6.2 shows the concentration of potassium (mg/100ml) in both the plasma and gonadal supernatant of male and female ***O.mossambicus***.

	♂ Plasma (mg/100ml)	♂ Gonad (mg/100ml)	♀ Plasma (mg/100ml)	♀ Gonad (mg/100ml)
	mean ± sd	mean ± sd	mean ± sd	mean ± sd
May	4.84 0.62	34.45 7.34	4.85 0.80	36.87 6.71
Jun	6.35 0.70	33.61 6.10	6.28 1.01	34.88 1.76
Jul	4.69 0.94	29.53 4.81	6.01 0.87	38.80 2.94
Aug	5.54 1.22	24.73 2.83	6.18 1.86	37.84 4.33
Sept	7.27 0.92	27.91 3.16	8.05 1.41	27.35 3.69
Oct	8.62 1.80	30.07 2.99	7.25 1.95	29.13 2.20
Nov	8.24 1.80	31.18 4.52	8.16 1.32	34.90 3.69
Dec	8.40 1.78	30.65 3.13	8.62 1.02	35.37 1.50
Jan	8.07 1.52	32.10 2.78	10.92 2.44	34.12 4.63
Feb	9.32 0.56	38.11 4.14	12.57 2.64	32.73 4.08
Mar	6.07 0.62	34.67 3.01	11.36 1.78	32.88 3.89
Apr	7.51 1.44	27.27 2.90	7.39 1.39	28.42 4.20

TABLE 6.2: K^+ concentration (mg/100ml) measured in the plasma and gonads of male and female ***O.mossambicus*** (samples taken per month, n = 40).

The following TABLE 6.3 shows the concentration of calcium (mg/100ml) in both the plasma and gonadal supernatant of male and female ***O.mossambicus***.

	♂ Plasma (mg/100ml)	♂ Gonad (mg/100ml)	♀ Plasma (mg/100ml)	♀ Gonad (mg/100ml)
	mean ± sd	mean ± sd	mean ± sd	mean ± sd
May	10.04 0.84	2.80 0.70	9.47 1.67	2.53 0.92
Jun	10.82 1.25	2.50 0.93	10.11 1.59	2.50 0.85
Jul	9.03 1.78	2.85 0.61	9.51 1.80	2.73 0.52
Aug	12.45 0.80	3.01 1.04	13.34 1.27	3.18 1.12
Sept	14.26 1.91	1.04 0.48	17.84 1.57	2.56 0.46
Oct	16.18 0.51	1.42 0.59	19.05 1.67	2.63 0.95
Nov	12.66 1.31	1.89 0.40	14.38 1.26	3.52 0.83
Dec	11.98 1.58	1.70 0.30	14.28 0.62	2.92 0.71
Jan	10.31 1.30	1.49 0.62	14.07 1.76	2.65 0.76
Feb	13.06 1.80	1.35 0.28	14.23 1.42	2.49 1.10
Mar	9.07 1.87	0.77 0.23	8.42 1.36	1.85 0.68
Apr	14.06 1.23	0.69 0.16	15.19 0.99	1.67 0.41

TABLE 6.3: Ca^{++} concentration (mg/100ml) measured in plasma and gonads of male and female ***O.mossambicus*** (samples taken per month, n = 40).

The following TABLE 6.4 shows the glucose concentration (mg/100ml) in both plasma and gonadal supernatant of male and female ***O.mossambicus***.

	♂ Plasma (mg/100ml)	♂ Gonad (mg/100ml)	♀ Plasma (mg/100ml)	♀ Gonad (mg/100ml)
	mean ± sd	mean ± sd	mean ± sd	mean ± sd
May	5.60 1.32	2.82 0.41	4.76 1.17	10.02 2.09
Jun	5.15 1.01	2.35 0.38	2.61 0.99	9.35 2.11
Jul	11.07 2.33	2.30 0.47	2.43 0.83	9.30 2.41
Aug	10.33 1.98	2.73 0.51	1.09 0.14	12.32 2.68
Sept	12.25 1.79	1.98 0.33	3.07 0.73	9.57 1.91
Oct	12.57 2.24	6.77 1.10	6.54 1.08	10.74 1.84
Nov	7.75 1.14	3.40 0.81	7.49 1.31	8.34 1.21
Dec	3.20 0.91	1.15 0.27	8.10 1.42	5.71 1.07
Jan	4.15 0.77	2.45 0.41	5.14 0.88	1.12 0.44
Feb	4.30 0.81	1.07 0.19	3.48 0.61	2.12 0.62
Mar	4.61 1.01	1.50 0.21	3.89 0.69	3.07 0.81
Apr	4.80 1.17	2.56 0.49	3.61 0.51	4.07 0.96

TABLE 6.4: Glucose concentration (mg/100ml) measured in plasma and gonads of male and female ***O.mossambicus*** (samples taken per month, n = 40).

The following TABLE 6.5 shows the total lipid concentration (mg/100ml) in both plasma and gonadal supernatant of male and female ***O.mossambicus***.

The following TABLE 6.6 shows the total protein concentration (mg/100ml) in both plasma and gonadal supernatant of male and female ***O.mossambicus***.

	♂ Plasma (mg/100ml)	♂ Gonad (mg/100ml)	♀ Plasma (mg/100ml)	♀ Gonad (mg/100ml)
	mean ± sd	mean ± sd	mean ± sd	mean ± sd
May	666.24 11.40	719.40 66.01	535.18 13.30	1209.21 100.64
Jun	624.20 9.90	610.14 19.69	558.00 47.89	1389.93 46.48
Jul	570.06 16.26	482.02 14.27	494.98 15.74	1300.15 52.24
Aug	516.24 83.72	476.04 12.13	538.16 72.60	1315.75 29.85
Sept	838.25 21.52	1143.88 49.63	1280.63 50.20	2222.05 30.63
Oct	762.40 18.34	582.46 12.13	1172.67 24.90	2138.89 56.71
Nov	639.90 20.36	368.41 22.25	929.33 29.05	2173.14 40.43
Dec	653.88 92.32	268.42 47.07	921.44 23.86	1591.24 31.48
Jan	529.20 66.51	257.27 11.57	636.17 60.56	1454.56 49.79
Feb	516.81 14.20	215.60 22.06	544.33 11.71	1201.81 43.79
Mar	473.62 36.78	210.12 13.63	443.15 18.99	666.39 34.71
Apr	552.09 76.30	209.29 13.42	526.13 25.48	883.80 65.04

TABLE 6.5: Total lipid concentration (mg/100ml) measured in plasma and gonads of male and female ***O.mossambicus*** (samples taken per month, n = 40).

	♂ Plasma (mg/100ml)	♂ Gonad (mg/100ml)	♀ Plasma (mg/100ml)	♀ Gonad (mg/100ml)
	mean ± sd	mean ± sd	mean ± sd	mean ± sd
May	2642.90 85.42	2302.60 56.53	2451.00 68.85	3518.80 100.80
Jun	2384.50 60.46	1407.90 59.02	2337.88 30.85	1627.67 79.80
Jul	2685.33 45.81	1603.60 63.10	2532.43 43.34	2242.00 80.95
Aug	2245.80 31.65	2038.70 58.18	2376.90 77.40	3900.70 160.44
Sept	2555.50 81.09	2061.50 135.15	4272.63 123.50	6082.11 247.28
Oct	3593.38 49.54	2403.63 34.24	5999.25 281.15	4313.00 92.37
Nov	2828.29 44.13	1708.10 172.81	4005.20 53.63	5303.38 159.63
Dec	2984.90 81.36	1515.10 84.51	3678.40 70.47	4256.00 116.50
Jan	2327.50 33.26	1371.80 51.56	2261.00 45.75	3138.17 113.30
Feb	2158.40 44.70	1176.10 29.44	2147.00 23.40	3565.43 243.75
Mar	2085.25 44.96	1229.30 78.71	2002.60 46.28	3159.00 71.35
Apr	3013.40 71.24	1061.89 86.65	3423.17 48.24	2679.00 103.59

TABLE 6.6: Total protein concentration (mg/100ml) measured in plasma and gonads of male and female ***O.mossambicus*** (samples taken per month, n = 40).

The following TABLE 6.7 shows the lactate concentration (mg/100ml) in both plasma and gonadal supernatant of male and female ***O.mossambicus***.

	♂ Plasma (mg/100ml)	♂ Gonad (mg/100ml)	♀ Plasma (mg/100ml)	♀ Gonad (mg/100ml)
	mean ± sd	mean ± sd	mean ± sd	mean ± sd
May	55.02 1.17	14.20 0.40	10.14 0.88	91.47 8.76
Jun	48.83 1.76	8.69 0.98	9.68 0.97	94.88 14.24
Jul	65.64 1.65	5.93 1.15	4.96 0.78	92.22 8.39
Aug	39.79 1.56	5.79 0.78	15.58 1.37	97.21 3.90
Sept	26.06 1.36	1.38 0.20	13.51 1.17	76.91 4.67
Oct	19.27 1.98	3.03 0.98	9.52 0.78	19.31 3.31
Nov	8.96 0.88	11.13 0.68	9.45 0.49	55.78 2.83
Dec	4.83 0.78	7.65 0.88	46.89 6.83	6.69 1.46
Jan	40.41 2.15	5.38 0.39	41.93 2.88	5.17 0.88
Feb	16.55 0.39	1.38 0.79	63.92 6.34	8.69 0.78
Mar	6.34 0.38	4.52 0.53	29.83 0.25	16.29 0.76
Apr	34.72 0.93	4.63 0.12	6.41 0.30	17.75 1.04

TABLE 6.7: Lactate concentration (mg/100ml) measured in plasma and gonads of male and female ***O.mossambicus*** (samples taken per month, n = 40).

The following TABLE 6.8 shows the Urea concentration (mg/100ml) in both plasma and gonadal supernatant of male and female ***O.mossambicus***.

	♂ Plasma (mg/100ml)	♂ Gonad (mg/100ml)	♀ Plasma (mg/100ml)	♀ Gonad (mg/100ml)
	mean ± sd	mean ± sd	mean ± sd	mean ± sd
May	4.99 2.83	63.92 17.75	4.01 2.79	75.34 24.26
Jun	22.25 4.76	39.06 7.70	23.33 8.35	67.98 17.88
Jul	148.76 21.08	49.12 7.56	168.55 10.86	80.06 16.77
Aug	13.22 4.53	41.16 6.24	11.77 3.79	78.21 12.36
Sept	4.65 1.55	17.32 4.28	12.33 3.92	134.74 13.60
Oct	21.25 4.26	93.67 23.06	20.20 3.02	189.61 28.70
Nov	19.68 6.52	68.80 7.41	21.53 2.76	195.39 12.49
Dec	17.63 2.72	30.56 4.80	13.07 3.32	141.14 12.50
Jan	11.72 3.02	45.59 7.38	13.89 2.70	75.52 6.88
Feb	16.57 4.06	37.93 9.54	10.06 2.23	113.96 8.36
Mar	16.78 4.05	28.70 5.86	17.82 4.37	38.61 7.21
Apr	15.74 4.44	26.10 2.50	19.37 6.88	62.87 12.44

TABLE 6.8: Urea concentration (mg/100ml) measured in plasma and gonads of male and female ***O.mossambicus*** (samples taken per month, n = 40).

FIG 6.1 represents the sodium concentration (mg/100ml) in male and female ***O.mossambicus*** plasma and FIG 6.2 represents the same parameters in ***O.mossambicus*** gonadal supernatant. FIG 6.3 represents a compilation of FIGS 6.1 and 6.2. FIG 6.1 shows that female Na^+ levels are generally higher than male values over the same period. In the plasma there is a good correlation (r = 0.92) between males and females with maximal values of 282.00 ± 25.35 and 308.98 ± 21.75 mg/100ml respectively being observed in November. Female plasma

levels show a wide peak between September and December whereas males have a similar, but lower peak during the same period. Thereafter both male and female plasma sodium concentrations decline. FIG 6.2 shows a poorer correlation ($r = 0.36$) between male and female gonadal supernatant sodium concentrations. In general, both male and female gonad levels are lower than plasma values. Furthermore, female gonad levels are significantly lower when compared with male values. In the female gonad, sodium concentration falls off markedly during September and remains low until the end of April. This is the opposite to that seen in FIG 6.1 where plasma values are represented. FIG 6.3 indicates that from September till April there appears to be an inverse relationship between plasma and gonadal Na^+ concentrations.

FIG 6.1: Plasma sodium levels and GSI in male and female *O.mossambicus*

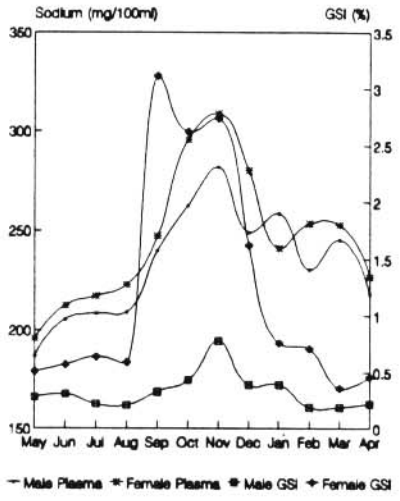
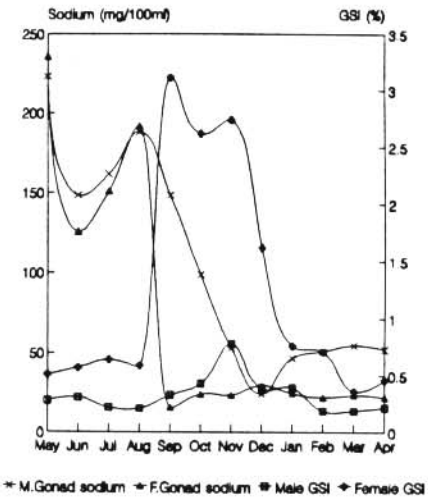


FIG 6.2: Gonadal sodium levels and GSI in male and female *O.mossambicus*



Male plasma sodium values (FIG 6.1) form a similar direct pattern to male GSI levels, although gonad levels are initially high and then gradually drop to a low in December with a slight increase thereafter. Female plasma sodium levels increase sharply from September and remains high until December whereafter a non-significant increase occurs. However, gonad levels (FIG 6.2) drop sharply in

September and remain low to April. Female gonad levels decrease sharply with increased GSI.

FIG 6.3: Plasma & gonadal sodium levels in male and female *O.mossambicus*

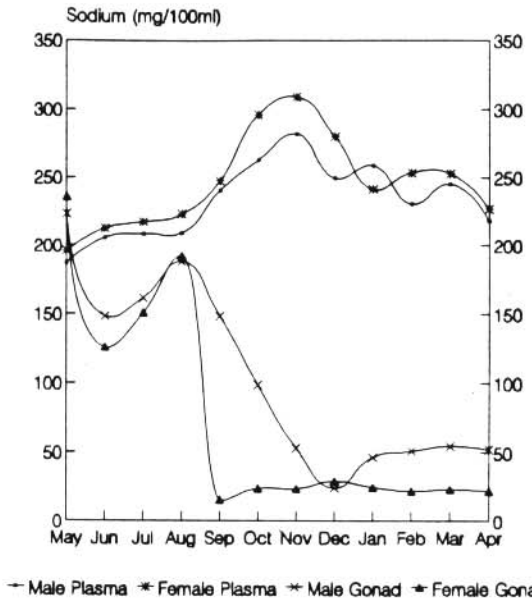


FIG 6.4 represents the potassium concentration (mg/100ml) in male and female ***O.mossambicus*** plasma and FIG 6.5 represents the same parameters in ***O.mossambicus*** gonadal supernatant. FIG 6.6 represents a compilation of FIGS 6.4 and 6.6. FIG 6.4 shows that in the plasma there is a fairly good correlation ($r = 0.62$) between males and females with maximal values of 9.32 ± 0.56 and 12.57 mg/100ml respectively being observed in February. Male potassium values show four peaks ie: June, October, December and February. It remains relatively high from September to February. Male plasma levels are generally lower than female values. Female values peak in June, September, November and February. These values generally remain high between September and March. FIG 6.5

FIG 6.4: Plasma potassium levels and GSI in male and female *O.mossambicus*

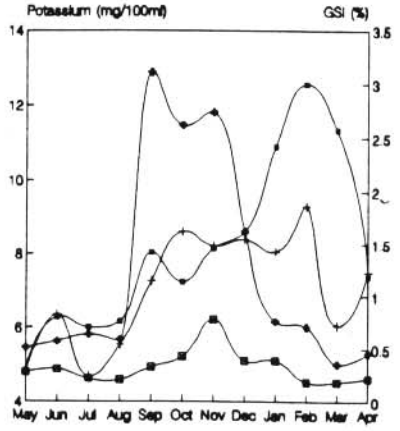


FIG 6.5: Gonadal potassium levels and GSI in male and female *O.mossambicus*

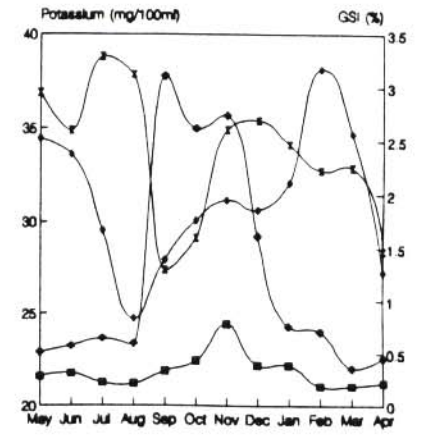
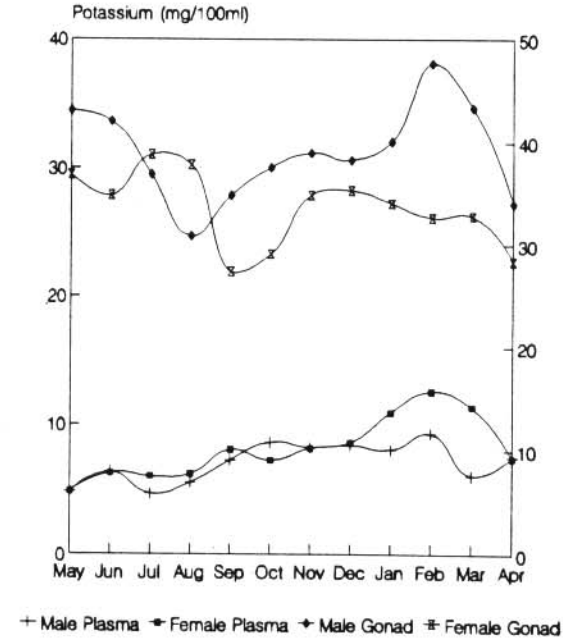


FIG 6.6: Plasma & gonadal potassium levels in male and female *O.mossambicus*



indicates that at certain times of the year there appears to be an inverse relationship between male and female gonadal supernatant potassium concentrations. Female levels peak during July till August, October till January and March. During July female gonadal supernatant potassium reaches a peak of 38.80 ± 2.94 mg/100ml and a month later in August male gonadal supernatant reaches a minimum of 24.73 ± 2.83mg/100ml. During December, when the potassium concentration in the female gonadal supernatant rises to supernatant rises to 36.37 ± 1.50 mg/100ml the male potassium concentration falls to 30.07 ± 2.99 mg/100ml. Male plasma values peak between May and July, September and December and January and March. FIG 6.6 shows that throughout the year, the concentration of potassium in both male and female ***O.mossambicus*** gonadal supernatant is significantly higher ($p < 0.001$) than has been measured in the plasma.

Male plasma potassium levels (FIG 6.4) show a sharp tetramodal increase from May right through to February and does not relate directly to male GSI increase, but follows a similar pattern. Male gonad potassium levels (FIG 6.5), however, shows a trimodal increase with the lowest levels recorded during August. Highest levels were recorded during January to March. Female plasma potassium levels followed female GSI and increased sharply after maximum GSI was reached. However, female gonad potassium (FIG 6.5) displayed an inverse relationship with GSI.

FIG 6.7 represents both the sodium (mg/100ml) and potassium (mg/100ml) concentrations in male and female ***O.mossambicus*** plasma and FIG 6.8 represents the same parameters in ***O.mossambicus*** gonadal supernatant. Male plasma sodium and potassium concentrations show a good correlation ($r = 0.72$), whereas in the females, the correlation coefficient obtained is $r = 0.40$. FIG 6.7 further indicates that Na^+ values in the plasma are generally higher than plasma K^+ concentrations throughout the year. In general, both plasma potassium and sodium levels increase from July and remain high throughout until

FIG 6.7: Plasma sodium & potassium levels in male and female *O.mossambicus*

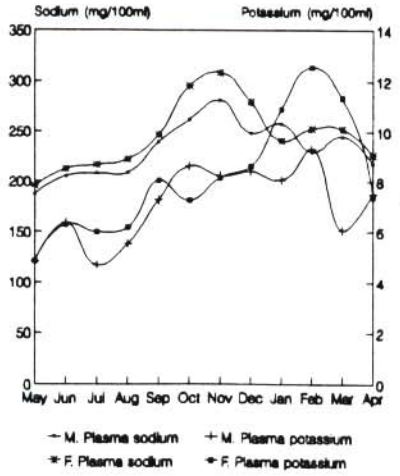


FIG 6.8: Gonadal sodium and potassium levels in male and female *O.mossambicus*

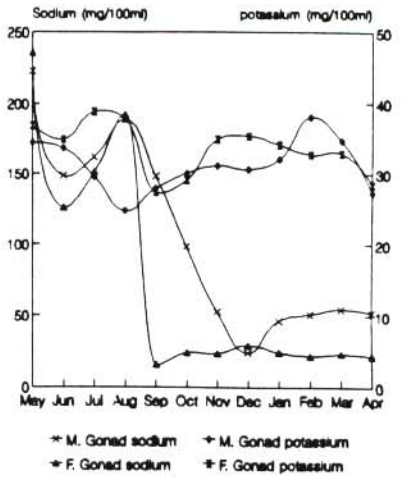
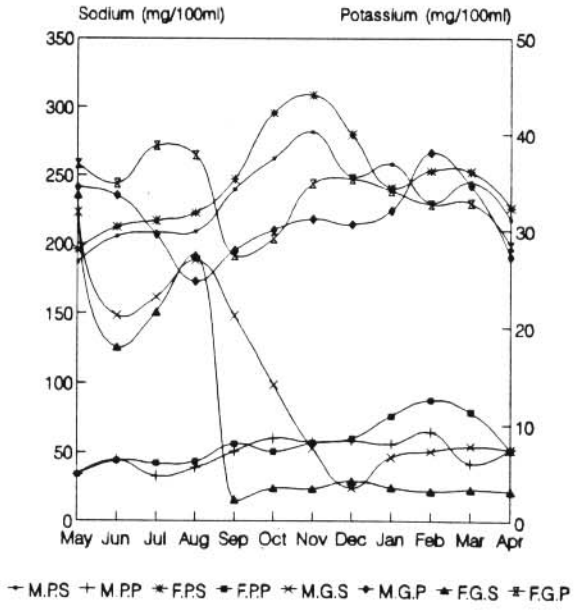


FIG 6.9: Plasma & Gonadal sodium and potassium levels in male and female *O.mossambicus*



February. FIG 6.8 shows that in the gonadal supernatant there also appears to be a relationship between male and female plasma Na^+ and between male and female K^+. FIG 6.8 also shows that in general, the level of potassium in the gonad is higher than the level of sodium; **ie:** there appears to be an inverse relationship from September until April. In FIG 6.9 it appears that there is a relationship between male and female gonadal supernatant K^+ and male and female plasma Na^+ levels. FIG 6.9 further indicates that there appears to be a similar relationship between male and female gonadal sodium and male and female plasma potassium levels.

FIG 6.10: Plasma calcium and GSI levels in male and female *O.mossambicus*

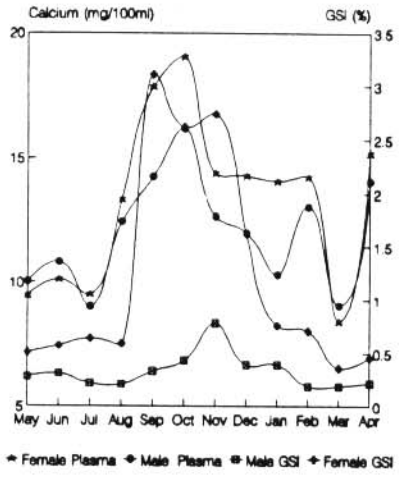


FIG 6.11: Gonadal calcium and GSI levels in male and female *O.mossambicus*

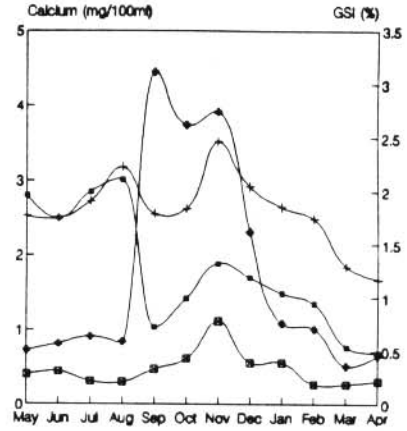


FIG 6.10 represents the calcium concentration (mg/100ml) in male and female ***O.mossambicus*** plasma and FIG 6.11 represents the same parameters in ***O.mossambicus*** gonadal supernatant. FIG 6.12 represents a compilation of FIGS 6.10 and 6.11. FIG 6.10 shows that in the plasma there is a very good correlation ($r = 0.92$) between males and females with maximal values of 16.18 $\pm$ 0.51 and 19.05 $\pm$ 1.67 mg/100ml respectively being observed in October. Male plasma values show peaks in June, August which extend to maximum

October, declines markedly in November to stabilize in December and increasing sharply in December. On the other hand, female plasma values show a similar trend, with plasma calcium levelling out between November and February whereafter a sharp decline is observed.

FIG 6.12: Plasma & gonadal calcium levels in male and female *O.mossambicus*

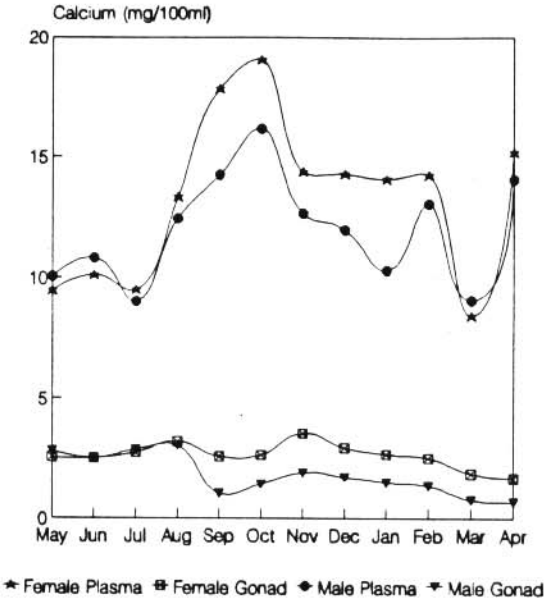


FIG 6.11 shows a correlation of r = 0.58 between male and female ***O.mossambicus*** gonadal supernatant calcium concentrations. Male gonad values increase steadily during July and August with a sharp decline during September. Another peak starts in October which continues into November whereafter a gradual decline continued to February. These male values clearly define a biphasic mode, starting with a sharp increase , followed by a longer phasic cycle. Females experience a similar biphasic cycle, but at much higher levels. FIG 6.12 indicates that the calcium concentration in both male and female ***O.mossambicus***

plasma is higher than in the gonadal supernatant throughout the year. Further, in the female, there appears to be an inverse relationship between plasma and gonadal supernatant calcium concentration. A similar relationship is also seen in male ***O.mossambicus.***

Both male and female plasma calcium levels (FIG 6.10) increased with GSI. However in males, this occurred one month prior to GSI whereas in females it followed the same pattern as GSI. Female gonad calcium levels (FIG 6.11), however, showed a similar but more extended relationship with GSI whereas in males , gonad Ca^{++} increased and decreased with GSI from September to April. Prior to September, an inverse relationship was observed.

FIG 6.13: Plasma glucose and GSI levels in male and female *O.mossambicus*

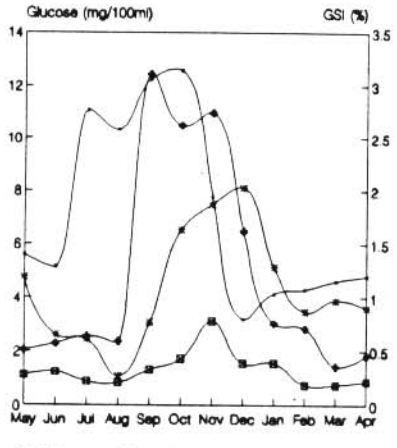


FIG 6.14: Gonadal glucose and GSI levels in male and female *O.mossambicus*

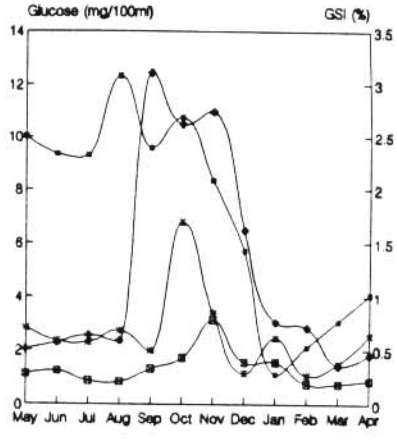
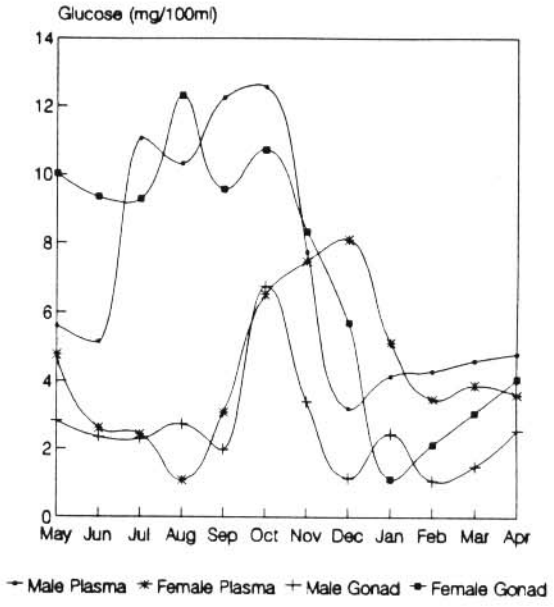


FIG 6.13 represents the glucose concentration (mg/100ml) in male and female ***O.mossambicus*** plasma and FIG 6.14 represents the same parameters in ***O.mossambicus*** gonadal supernatant. FIG 6.15 represents a compilation of FIGS 6.13 and 6.14. FIG 6.13 shows that in the plasma there appears to be an inverse relationship between males and females from June until December. Female

plasma glucose concentration reaches a minimum of 1.09 ± 0.14 mg/100ml during September whereafter it may be noted that male plasma glucose peaks at 12.57 ± 2.24 mg/100ml in October. Both males and females display a biphasic cycle.

FIG 6.15: Plasma and gonadal glucose levels in male and female *O.mossambicus*



In males, it extends from July to October whereafter a sharp decline occurs. Female plasma glucose levels show a steady decline from June to reach their lowest value in August. Thereafter it increases markedly until December whereafter a sharp decline occurs. In general, male glucose levels are higher than female values. In FIG 6.14, which represents male and female gonadal supernatant glucose levels, a direct relationship between the male and female is noted throughout the year. Female gonad levels are generally double those for males over the entire period. Females show a bimodal cycle which peaks during

August and November. Males displayed a three phase cycle; a low in August followed by a high in October and another low in January. FIG 6.15 appears to show a close relationship between male plasma and female gonadal supernatant glucose levels. A similar relationship is seen in the female plasma and male gonadal supernatant. Furthermore, in both males and females, there appears to be an inverse relationship between plasma and gonadal supernatant glucose concentrations.

Male plasma glucose levels (FIG 6.13) increased sharply from July and remained high until October. Thereafter it declined with male GSI. Plasma glucose levels showed such changes one month prior to male GSI. Such values were also higher than female values. Male gonad glucose levels, however, increased and changed with GSI (FIG 6.14). The lowest gonad glucose level was recorded during December whereafter gonad glucose levels increased again. In females, plasma glucose levels increased in September and remained high until December and declined with GSI whereafter it showed an increase, unrelated to the other parameters.

FIG 6.16: Plasma lipid levels in male and female *O.mossambicus*

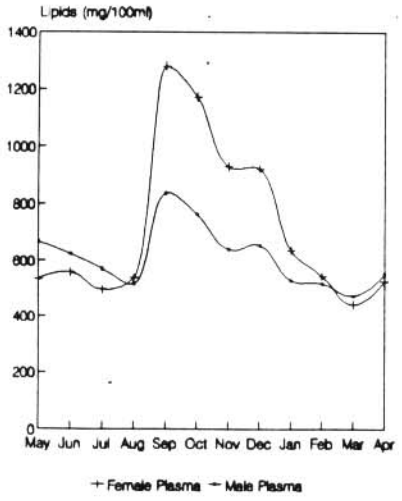


FIG 6.17: Gonadal lipid levels in male and female *O.mossambicus*

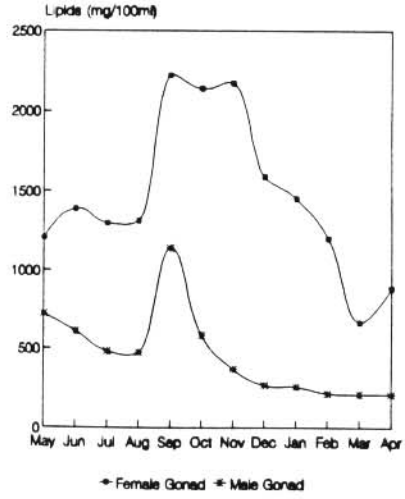
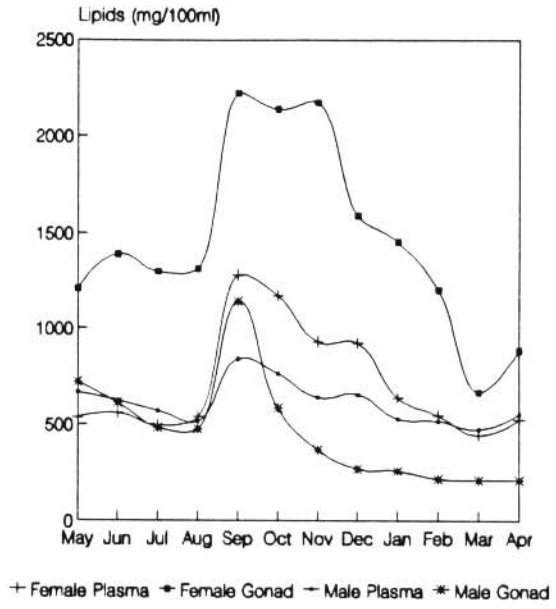


FIG 6.16 represents the lipid concentration (mg/100ml) in male and female ***O.mossambicus*** plasma and FIG 6.17 represents the same parameters in ***O.mossambicus*** gonadal supernatant. FIG 6.18 represents a compilation of FIGS 6.16 and 6.17. FIG 6.16 shows that in plasma there is a good correlation (r = 0.87) between males and females with maximal values of 838.25 ± 21.52 and 1280.63 ± 50.20 mg/100ml respectively being observed in September.

FIG 6.18: Plasma and gonadal lipid levels in male and female *O.mossambicus*



Thereafter, both male and female plasma lipid concentrations level out between November and December whereafter they decline. FIG 6.17 shows a slightly weaker correlation (r = 0.55) between male and female gonadal supernatant lipid concentrations. In this case, a single sharp increase is observed for both males and females during September. However, female levels remain high until November whereas male levels decline sharply in October to remain low

thereafter. As is noted in the plasma, gonadal supernatant lipid concentration reaches maximal values of 1143.88 ± 49.63 and 2222.05 ± 30.63 mg/100ml respectively in males and females during the month of September. However, it may be seen that in the female, gonadal supernatant lipid concentration remains relatively high till November, whereafter it declines markedly. FIG 6.18 shows that there is a close relationship between plasma and gonadal supernatant lipid concentrations in male (r = 0.80) and female (r = 0.90) ***O.mossambicus***. It is also apparent that lipid concentration in the female is higher than in the male throughout the experimental period. In general gonad levels for both males and females are higher than plasma levels, except in males from October to April where the situation is reversed.

FIG 6.19: Plasma protein levels in male & female *O.mossambicus*

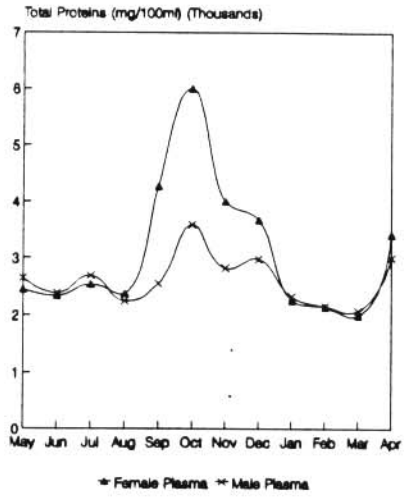


FIG 6.20: Gonadal protein levels in male & female *O.mossambicus*

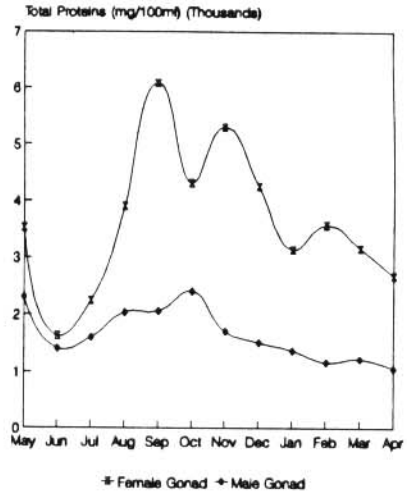


FIG 6.19 represents the total protein concentration (mg/100ml) in male and female ***O.mossambicus*** plasma and FIG 6.20 represents the same parameters in ***O.mossambicus*** gonadal supernatant. FIG 6.21 represents a compilation of FIGS 6.19 and 6.20. FIG 6.19 shows that in the plasma there is a good correlation (r = 0.87) between males and females with maximal values of 3593.38 ± 49.54

FIG 6.21: Plasma & gonadal protein levels in male & female *O.mossambicus*

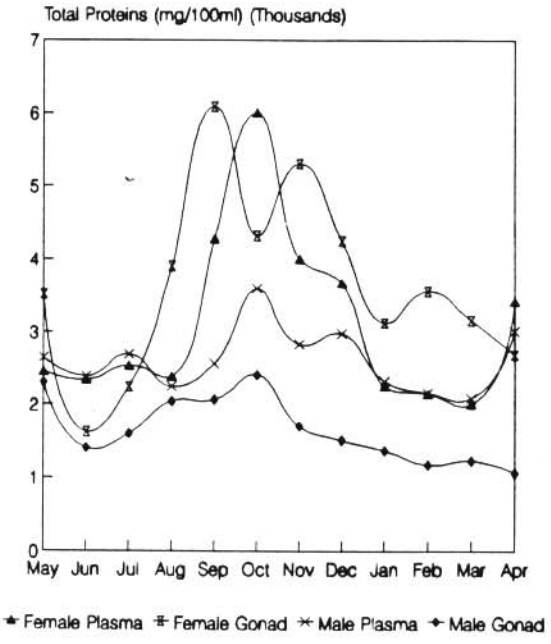


FIG 6.19 represents the total protein concentration (mg/100ml) in male and female ***O.mossambicus*** plasma and FIG 6.20 represents the same parameters in ***O.mossambicus*** gonadal supernatant. FIG 6.21 represents a compilation of FIGS 6.19 and 6.20. FIG 6.19 shows that in the plasma there is a good correlation ($r = 0.87$) between males and females with maximal values of 3593.38 ± 49.54 and 5999.25 ± 281.15 mg/100ml respectively being observed in October. In both cases, a biphasic cycle is observed in October and December. During this period, female plasma levels are higher in females. Apart from the period from September till December, the plasma protein concentrations in ***O.mossambicus*** appear to be much the same in both males and females. FIG 6.20 shows a much poorer correlation ($r = 0.49$) between male and female gonadal supernatant total protein concentration. Female levels are significantly higher than male values. Female values display three peaks during September, November and February.

During this period, all values recorded were higher than those recorded during June and July. The female gonadal supernatant total protein concentration reaches a maximum of 6082.11 ± 247.28 mg/100ml, whereas the male reaches it's peak of 2403.63 ± 34.24 mg/100ml one month later in October. On the other hand, male lipid levels displayed a biphasic cycle with peaks in August and October, whereafter these values decline gradually. Furthermore, female ***O.mossambicus*** gonadal supernatant total protein concentration appears to remain at a higher level than that observed in the male throughout the experimental period. In FIG 6.21 it may be noted that in the male, both plasma and gonadal supernatant total protein concentration reach a peak during October, although the plasma concentration is higher than that measured in the gonadal supernatant. This contrasts with the situation in the female, where both plasma and gonadal supernatant total protein concentration peak at a similar concentration, but the gonadal concentration peaks in September, whereas the plasma concentration reaches it's maximum one month later in October. Furthermore, the gonadal supernatant is generally higher than the corresponding plasma concentration for each month which is opposite to that recorded for males.

FIG 6.22 represents the lactate concentration (mg/100ml) in male and female ***O.mossambicus*** plasma and FIG 6.23 represents the same parameters in ***O.mossambicus*** gonadal supernatant. FIG 6.24 represents a compilation of FIGS 6.22 and 6.23. FIG 6.22 shows that in male plasma, there appears to be a peak in lactate concentration of 66.64 ± 1.65 mg/100ml during July and a second peak of a lower magnitude (40.41 ± 2.15 mg/100ml) during January. In female plasma, the lactate concentration increases markedly in December and remains relatively high until it reaches a maximum of 63.92 ± 6.34 mg/100ml in February whereafter it declines again. Female plasma lactate levels show a three phased increase in August, December / January and February whereafter a noticeable decline is observed. From May to September, male plasma levels are significantly higher ($p < 0.001$) than female values. From November to March,

FIG 6.22: Plasma lactate and GSI levels in male and female *O.mossambicus*

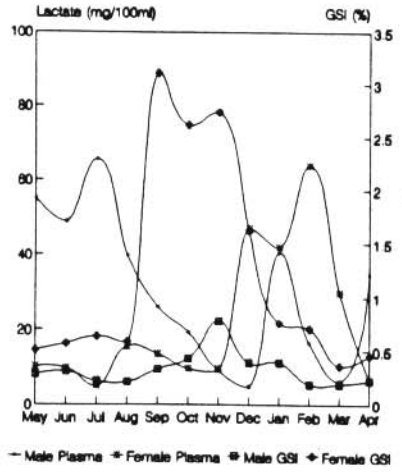


FIG 6.23: Gonadal lactate and GSI levels in male and female *O.mossambicus*

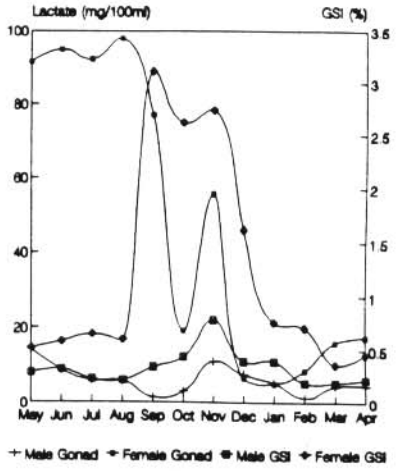
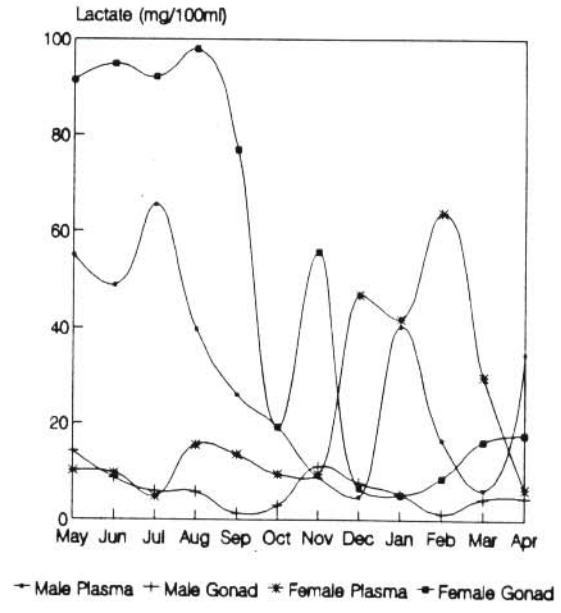


FIG 6.24: Plasma and gonadal lactate levels in male and female *O.mossambicus*



this position is reversed where female levels are significantly higher. FIG 6.23 appears to show a virtual year-round inverse relationship between male and female ***O.mossambicus*** gonadal supernatant lactate concentrations. The male lactate level attains a maximum of 11.13 ± 0.68 mg/100ml during November.

In general, levels display a four phase cycle during August, November, January and March. Females, on the other hand, have high lactate levels from May until September, whereafter the levels fall off markedly. Although a maximum of 97.21 ± 3.90 mg/100ml is reached in August, a second, smaller peak of 56.78 ± 2.83 mg/100ml may be noted during November, thus indicating a biphasic cycle. However, both males and females show two corresponding peaks during August and November. FIG 6.24 shows what appears to be a relationship between the female gonadal supernatant and male plasma lactate concentration and a second relationship between female plasma and male gonadal supernatant.

Male plasma lactate (FIG 6.22) levels increased sharply during July and gradually declined with increasing male GSI. During January, a sharp increase in male plasma lactate levels occurred again. Male gonad lactate levels (FIG 6.23) also varied with male GSI. Gonad levels, were however, considerably lower than plasma levels. Female plasma lactate levels (FIG 6.22) were relatively low and inversely related to GSI. After maximum female GSI was reached, plasma lactate levels increased significantly. Thereafter it declined rapidly in March. Plasma lactate levels were therefore high during low GSI in females and ***vice versa***. However, female gonad levels (FIG 6.23) were significantly higher ($p < 0.001$) during the period prior to maximum GSI and declined significantly ($p < 0.001$) during September and October whereafter it showed a sharp increase with GSI in November. This was followed by a rapid decline, fluctuating with female GSI.

FIG 6.25 represents the urea concentration (mg/100ml) in male and female ***O.mossambicus*** plasma and FIG 6.26 represents the same parameters in ***O.mossambicus*** gonadal supernatant. FIG 6.27 represents a compilation of FIGS

FIG 6.25: Plasma urea levels in male and female *O.mossambicus*

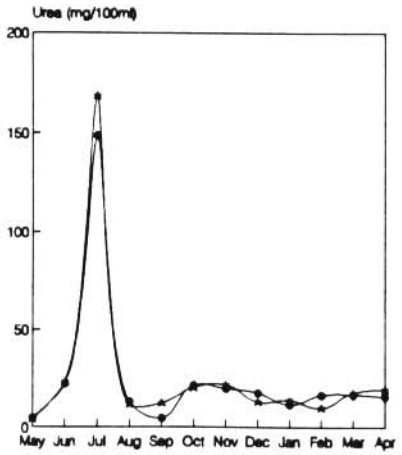


FIG 6.26: Gonadal urea levels in male and female *O.mossambicus*

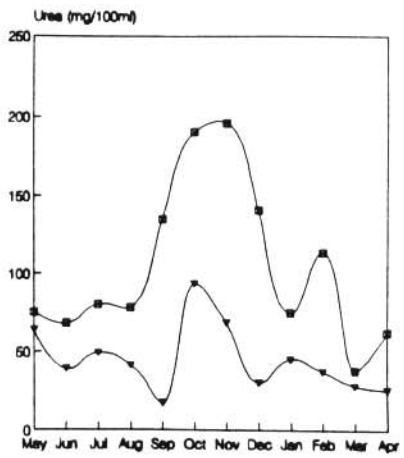
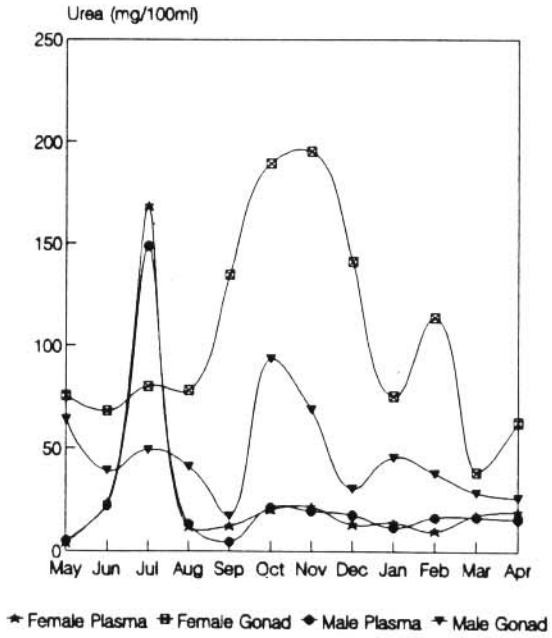


FIG 6.27: Plasma & gonadal urea levels in male and female *O.mossambicus*



6.25 and 6.26. FIG 6.25 shows that in the plasma there is an excellent correlation (r = 0.99) between males and females with maximal value of 148.76 ± 21.08 and 168.55 ±10.86 mg/100ml respectively being observed in July, which is during mid-winter in the northern Transvaal. Thereafter a sharp decline was observed with both male and female plasma urea levels remaining close throughout the experimental period. This was not observed in gonad values where an inverse relationship with plasma levels were recorded. FIG 6.26 shows a much weaker correlation (r = 0.54) between male and female gonadal supernatant urea concentration. In both males and females, peak urea levels are reached later in the year. Male gonadal supernatant urea concentration reaches a maximum of 93.67 ± 23.06 mg/100ml during October. Female gonadal supernatant urea levels show a marked increase in September, reaching a maximum of 196.39 ± 12.49 mg/100ml in November. In both cases, a triphasic urea cycle was observed. In females these occurred in July, October / November and February whereas in males it was observed during July, October and January. The last two peaks in males were observed one month earlier than in females. In FIG 6.27 it may be clearly observed that there is a "lag" period between plasma urea levels reaching their peak and the gonadal supernatant levels peaking at least two months later. It therefore appears that the high plasma levels in males and females during July may be environmentally or metabolically related.

FIG 6.28 represents the concentrations of urea; total protein and lipids (mg/100ml) in the plasma of male ***O.mossambicus*** and FIG 6.29 represents the same parameters in gonadal supernatant. FIG 6.30 represents the concentrations of urea; total protein and lipids (mg/100ml) in the plasma of female ***O.mossambicus*** and FIG 6.31 represents the same parameters in female gonadal supernatant. FIG 6.28 shows that in male plasma, urea concentration peaks in July, whereafter lipids peak in September and finally total proteins reach a maximum in October. A reasonably good correlation (r = 0.59) is noted between the concentrations of male ***O.mossambicus*** plasma lipids and total proteins.

FIG 6.28: Male plasma urea; protein and lipid levels in *O.mossambicus*

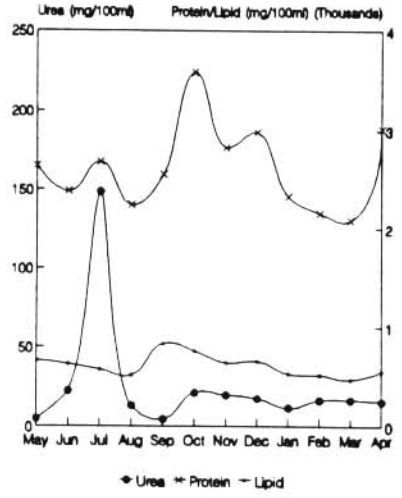
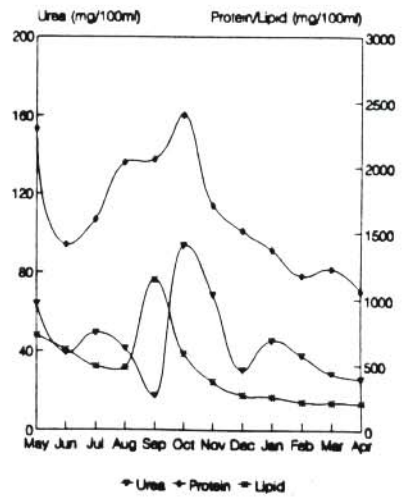


FIG 6.29: Male gonadal urea; protein and lipid levels in *O.mossambicus*



However, during this time, the high protein levels appeared to be the most significant. In male gonadal supernatant (shown in FIG 6.29) lipids reach a maximum in September, whereafter both urea and total protein concentrations peak in October. Good correlations between the concentrations of lipids and total proteins (r = 0.70) and urea and total proteins (r = 0.59) in male ***O.mossambicus*** gonadal supernatant may be noted in FIG 6.29.Peak urea levels correspond with peak protein levels in males.

FIG 6.30 shows that in female plasma, urea concentration peaks in July, whereafter lipids peak in September and finally total proteins reach a maximum in October. This is the same trend that is observed in male ***O.mossambicus*** and depicted in FIG 6.28. A good correlation (r = 0.87) exists between the concentrations of female plasma lipids and total proteins. In female gonadal supernatant (shown in FIG 6.31) lipids and total protein concentrations reach a peak in September, with total protein levels having a second, slightly lower peak during November, which coincides with the maximum urea concentration being attained at the low urea levels. The female gonadal supernatant shows good

correlations between the concentrations of urea and lipids (r = 0.87); urea and total protein (r = 0.71) and lipids and total protein (r = 0.72). The highest levels were recorded for urea and protein.

FIG 6.30: Female plasma urea; protein and lipid levels in *O.mossambicus*

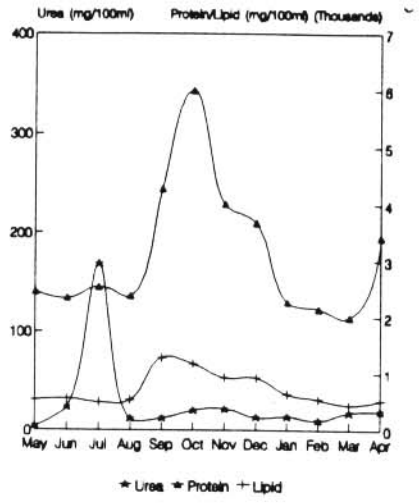


FIG 6.31: Female gonadal urea; protein and lipid levels in *O.mossambicus*

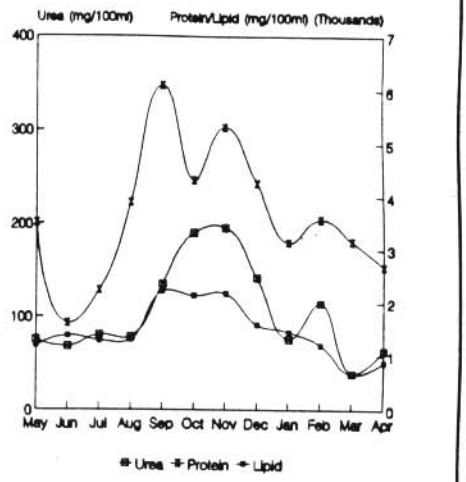


FIG 6.32 represents the relationship between plasma and gonadal supernatant urea concentration (mg/100ml) and GSI (%) in male ***O.mossambicus*** and FIG 6.33 represents these same parameters in the female. A better correlation between gonadal supernatant urea concentration and GSI may be noted in FIGS 6.32 and 6.33 than may be seen in the plasma. In the male, a value of r = 0.44 is obtained. Male gonad urea levels peak prior to GSI and also declines before GSI. Thus male peak urea levels always occur one month prior to maximum GSI in November. Plasma urea levels showed no relationship with GSI. In the female, this correlation value between GSI and gonad urea was r = 0.88. Gonadal supernatant urea concentration reaches a maximum two months after GSI peaks in September and declines with GSI. During February a small increase was again observed which coincided with GSI. Plasma urea levels are virtually the same in both males and females and did not vary with either GSI or gonad urea.

FIG 6.32: Plasma & gonadal urea levels and GSI in male *O.mossambicus*

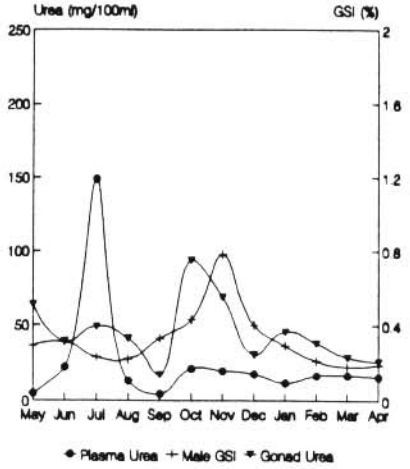


FIG 6.33: Plasma & gonadal urea levels and GSI in female *O.mossambicus*

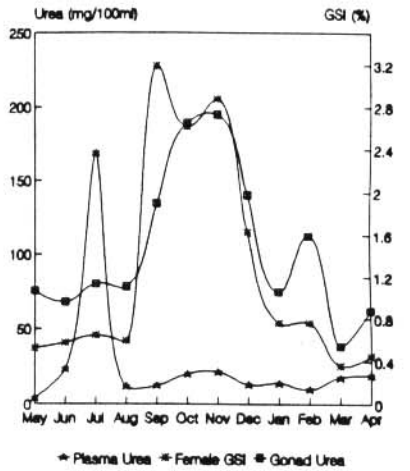


FIG 6.34: Plasma & gonadal protein levels and GSI in male *O.mossambicus*

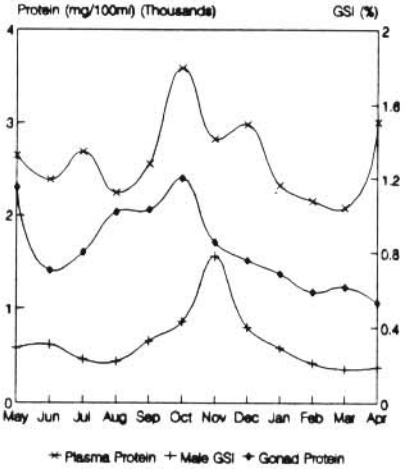


FIG 6.35: Plasma & gonadal protein levels and GSI in female *O.mossambicus*

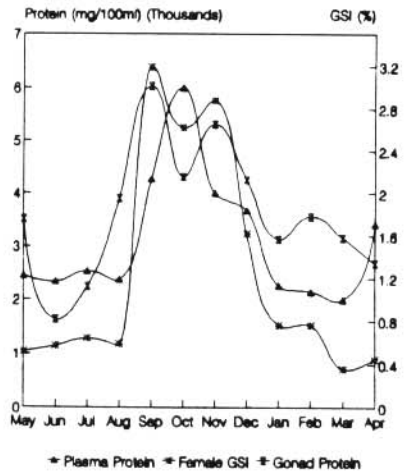


FIG 6.34 represents the relationship between plasma and gonadal supernatant total protein concentration (mg/100ml) and GSI (%) in male ***O.mossambicus*** and FIG 6.35 represents these same parameters in the female. In the female, good correlation coefficients are observed in the plasma (r = 0.83) and in gonadal supernatant (r = 0.84). In the male the corresponding correlation coefficients obtained are r = 0.42 in the plasma and r = 0.24 in the gonadal supernatant. In the male (FIG 6.34), both plasma and gonadal supernatant total protein reaches a maximum in October, which is one month prior to GSI peaking. In the female (FIG 6.35), the peak in GSI coincides with that observed in the gonadal supernatant, which is during September and follow GSI levels. Plasma total proteins follow a similar pattern with GSI, but one month later than GSI.

FIG 6.36: Plasma & gonadal lipid levels and GSI in male *O.mossambicus*

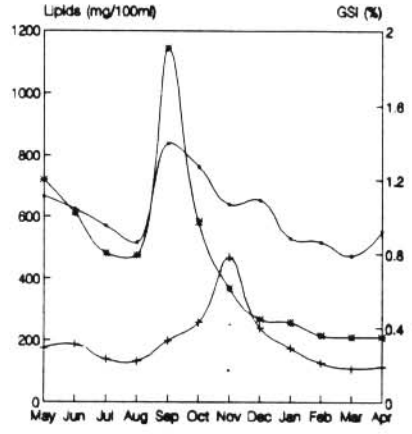


FIG 6.37: Plasma & gonadal lipid levels and GSI in female *O.mossambicus*

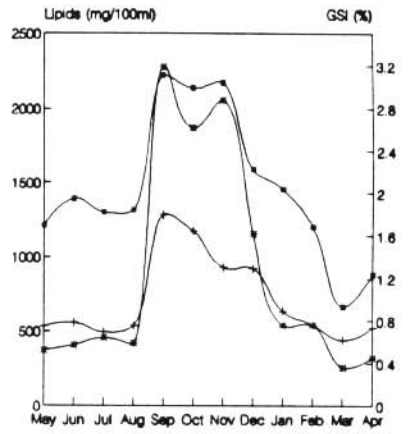


FIG 6.36 represents the relationship between plasma and gonadal supernatant lipid concentration (mg/100ml) and GSI (%) in male ***O.mossambicus*** and FIG 6.37 represents these same parameters in the female. FIG 6.36 indicates that a relationship does exist between plasma lipid concentration and GSI in male ***O.mossambicus***, with a correlation coefficient of r = 0.39 being noted always

one month prior to GSI. Furthermore, it may be seen that in the male, the lipid concentration in both the plasma and the gonadal supernatant reaches a peak during September, which is 2 months prior to the maximal GSI value being obtained in November. However, it does not follow the same pattern as GSI. In the female, (FIG 6.37), a far closer relationship is observed between GSI and both plasma (r = 0.96) and gonadal supernatant (r = 0.93) lipid concentration with plasma lipids at a much lower level. All three parameters reach their maximal value during September.

FIG 6.38: Plasma and gonadal urea levels and blood & gonadal pH in male *O.mossambicus*

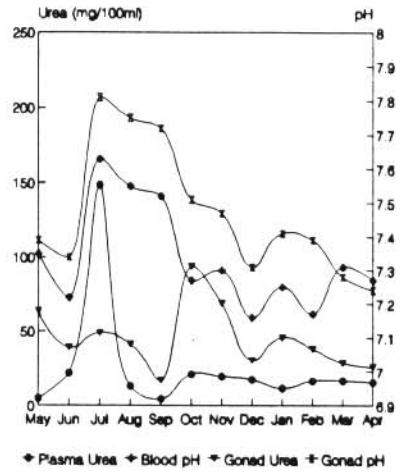


FIG 6.39: Plasma & gonadal urea levels and blood & gonadal pH in female *O.mossambicus*

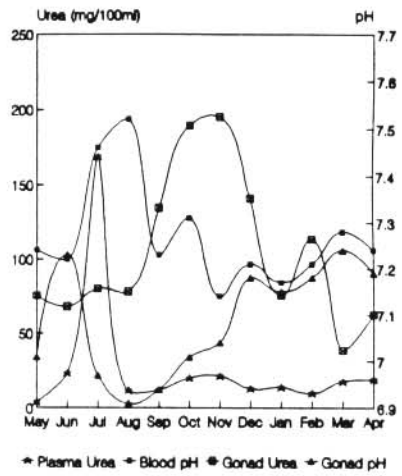


FIG 6.38 represents the relationship between plasma and gonadal supernatant urea concentrations (mg/100ml) and blood and gonadal supernatant pH in male ***O.mossambicus*** and FIG 6.39 represents these same parameters in the female. In the male, there appears to be a closer relationship between blood pH and plasma urea concentration than is observed in the gonadal supernatant. FIG 6.39, which represents the female, shows that the most significant relationship is observed in the gonadal supernatant where an inverse relationship between pH and urea concentration was noted during the months of May until November,

whereas in the blood/plasma there is an inverse relationship between August and February. It therefore seems that urea levels may contribute to a change in gonad pH values.

FIG 6.40 represents the relationship between plasma and gonadal supernatant total protein concentration (mg/100ml) and blood and gonadal supernatant pH in male ***O.mossambicus***. FIG 6.41 represents these same parameters in female ***O.mossambicus***. In FIG 6.40 it may be seen that there is a somewhat inverse relationship between plasma protein concentration and pH with a similar relationship being observed in gonadal supernatant. FIG 6.41, which depicts the situation in the female, also shows that there is an inverse relationship between total protein concentration and pH in both the blood/plasma and the gonadal supernatant, particularly during the period of June until December.

FIG 6.40: Plasma & gonadal protein levels and blood & gonadal pH in male *O.mossambicus*

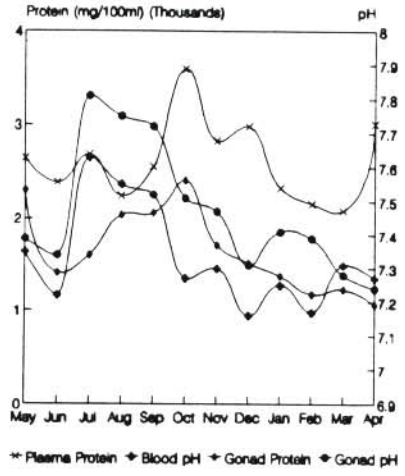


FIG 6.41: Plasma & gonadal protein levels and blood & gonadal pH in female *O.mossambicus*

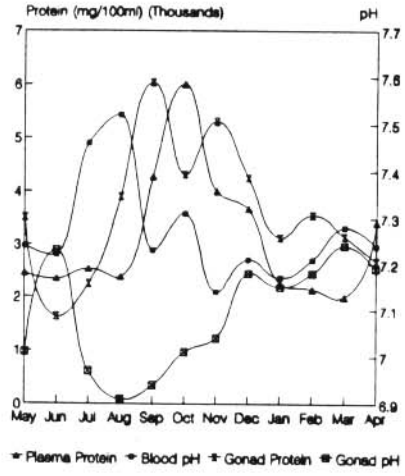


FIG 6.42 represents the relationship between plasma and gonadal supernatant lipid concentration (mg/100ml) and blood and gonadal supernatant pH in male ***O.mossambicus*** whereas FIG 6.43 represents these same parameters in the

female. As has been seen in FIGS 6.38; 6.39; 6.40 and 6.41, there appears to be an inverse relationship between plasma lipid concentration and blood pH in the male (FIG 6.42) and also in the female (FIG 6.43). A corresponding inverse relationship may be seen in male ***O.mossambicus*** gonadal supernatant lipid concentration and pH (FIG 6.42) and also in the female (FIG 6.43).

FIG 6.42: Plasma & gonadal lipid levels and blood & gonadal pH in male *O.mossambicus*

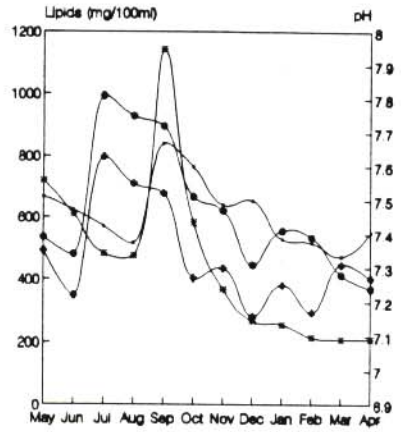


FIG 6.43: Plasma & gonadal lipid levels and blood & gonadal pH in female *O.mossambicus*

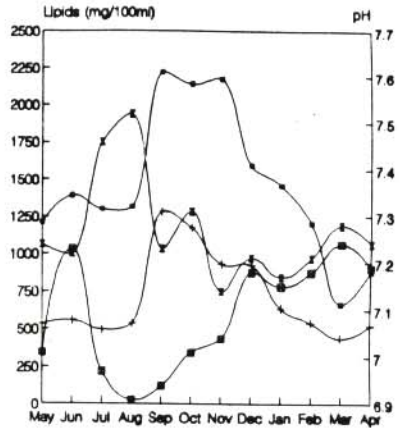


FIG 6.44 relates blood and gonad pH and plasma and gonad sodium levels. This indicates that an inverse relationship was recorded between blood pH and plasma sodium levels in males. Male gonad pH values showed a more direct relationship. As pH declined, gonad sodium levels also declined ie: as gonad acidity increased, sodium levels decreased. Thus an inverse relationship between plasma and gonad sodium levels was observed. In females (FIG 6.45) a similar pattern for plasma and gonads was observed with gonad sodium levels decreasing sharply with low gonad pH and remained low through to April.

FIG 6.46 relates blood and gonad pH and plasma and gonad potassium levels in males. Gonad pH and potassium were inversely related which was also the case

FIG 6.44: Plasma & gonadal sodium levels and blood & gonadal pH in male *O.mossambicus*

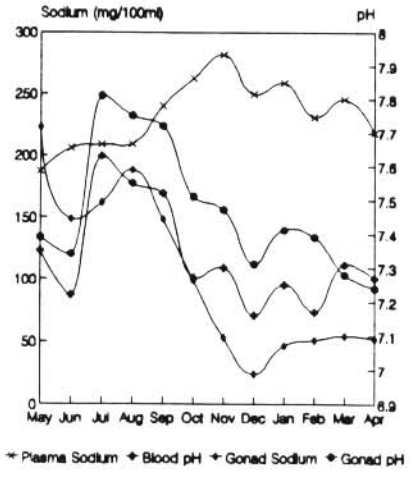


FIG 6.45: Plasma & gonadal sodium levels and blood & gonadal pH in female *O.mossambicus*

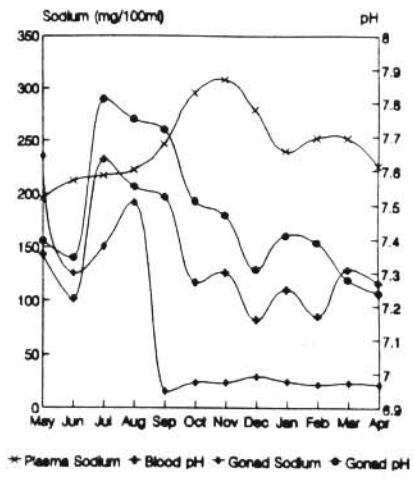


FIG 6.46: Plasma & gonadal potassium levels and blood & gonadal pH in male *O.mossambicus*

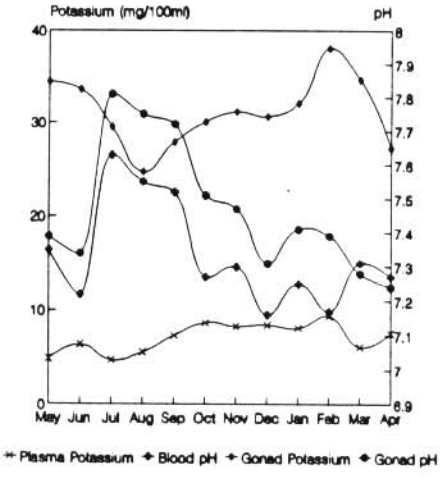
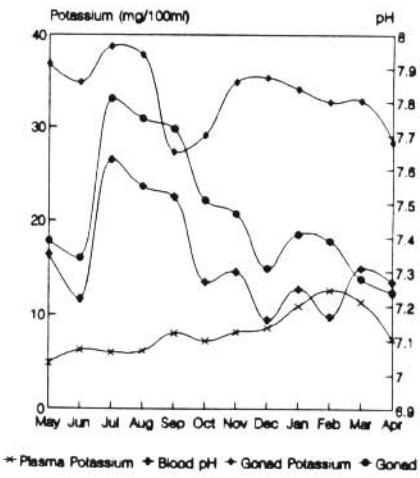


FIG 6.47: Plasma & gonadal potassium levels and blood & gonadal pH in female *O.mossambicus*



with the plasma parameters, but not to the same extent.

FIG 6.47 shows potassium levels in the plasma and gonads of females. Plasma potassium levels were relatively low throughout the experimental period and displayed an inverse relationship with blood pH. A similar, but a more significant relationship was observed in the gonads.

In males (FIG 6.48) an initial inverse relationship between blood pH and plasma and gonad calcium was observed which changed to a direct relationship in September as pH decreased with calcium. Gonad calcium was relatively low and fluctuated with pH. In females (FIG 6.49) plasma calcium and blood pH showed a fluctuating inverse relationship where the probability factor was not significant. Plasma calcium levels were, however, significantly higher than gonad values. No relationship between female gonad pH and calcium was observed, with calcium levels remaining low throughout.

FIG 6.48: Plasma & gonadal calcium levels and blood & gonadal pH in male *O.mossambicus*

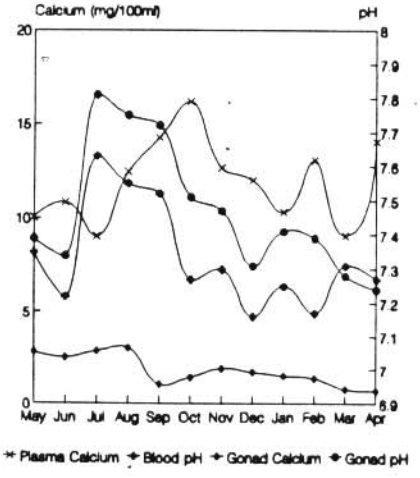
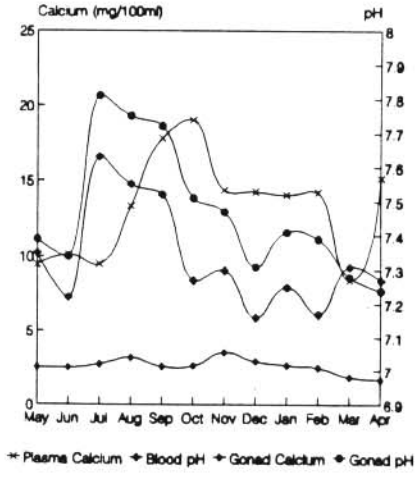


FIG 6.49: Plasma & gonadal calcium levels and blood & gonadal pH in female *O.mossambicus*



Male plasma glucose levels (FIG 6. 50) increased significantly ($p < 0.001$) during July and remained high until October whereafter it declined significantly ($p < 0.001$) until December whereafter it increased to gonadal resting period levels. Gonad glucose fluctuated throughout the experimental period with the most prominent increase occurring during October. Gonad pH did not relate significantly with glucose.

FIG 6.50: Plasma & gonadal glucose levels and blood & gonadal pH in male *O.mossambicus*

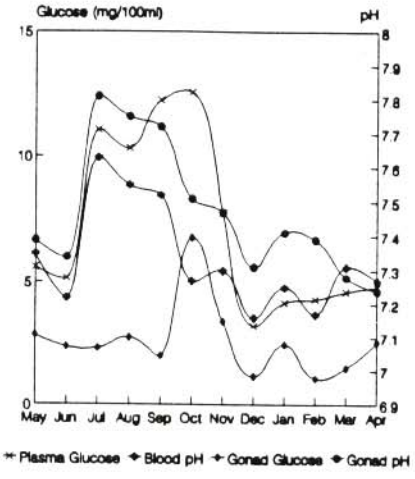


FIG 6.51: Plasma & gonadal glucose levels and blood & gonadal pH in female *O.mossambicus*

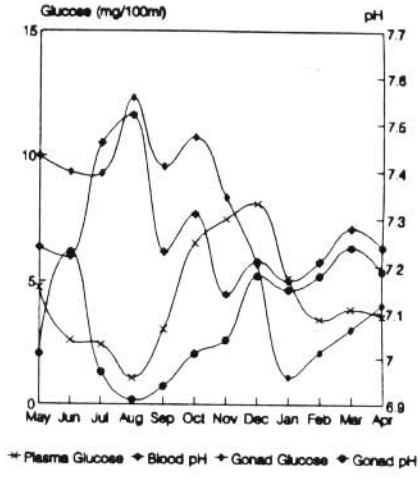


FIG 6.51 represents blood and gonad pH and plasma and gonad glucose levels in females. The most important relationship was that between plasma and gonad glucose levels which appeared to be inversely related. No significant relationship with pH was observed.

Plasma and gonad lactate levels in males did not show any significance with pH. However, from May to August plasma gonad levels were high and reached a peak again during January. Gonad levels remained low with higher levels only being recorded during November to January.

FIG 6.52: Plasma & gonadal lactate levels and blood & gonadal pH in male *O.mossambicus*

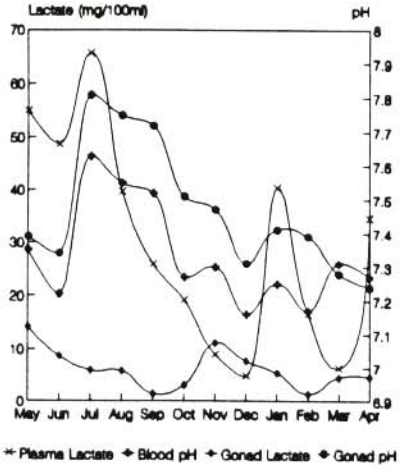
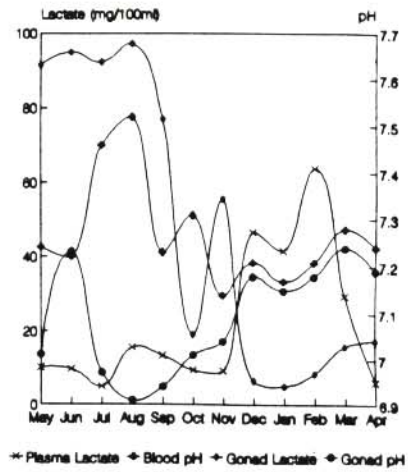


FIG 6.53: Plasma & gonadal lactate levels and blood & gonadal pH in female *O.mossambicus*



6.5 Discussion

6.5.1 Sodium and Potassium

Plasma Na^+ concentrations have been reported for numerous Teleosts over the past 30 years (see reviews of Holmes & Donaldson, 1969; Evans, 1979).

In general, the plasma contents of the blood are associated with homeostasis between the intracellular and extracellular fluids. Any changes in any blood parameters thus reflect changes in the functions of different organs or systems. It is for this purpose that the various blood parameters may be used as diagnostic tools in the evaluation of the functional activities of the body as well as determining the health status of fish (Blaxhall, 1972).

FIGS 6.1; 6.2 and 6.3 indicate the relationship that exists between the plasma and gonadal Na^+ concentrations recorded in ***Oreochromis mossambicus.*** In FIG 6.1 it appears that the levels of sodium in the plasma reach a peak that coincides

with the maximal GSI value recorded in November (males) and September till November (females) (see Chapter 3). Thereafter these values return to standard values recorded during February until June in both sexes. It is obvious from these figures that the changes in plasma sodium are associated with the reproductive cycle in both males and females. This increase in plasma sodium values may result from a haemoconcentration or osmoregulatory changes during the reproductive cycle. Since the reproductive cycle is under the control of gonadotropic hormone(s), it is reasonable to assume that such changes are associated with a neuroendocrine control of reproduction. In this regard, the inter-renal cells, which are homologous to the adrenal cortex in humans, may contribute to this response. These cells secrete cortisol, which was shown to play an important role in osmoregulation by Bern and Madsen (1992). In this study, it was also noted that blood pH levels decrease as GSI increases. Furthermore, according to Flik ***et al.***, (1988), prolactin also plays an important role in controlling sodium efflux across the branchial epithelium. This hormone reduces the branchial permeability to water and ions, including sodium in tilapia (Potts and Fleming, 1971). It has been demonstrated in several studies that the hormone prolactin, increases during gonad development (Bern and Madsen 1992). It is therefore suggested that the sodium / H^+ interchange in the gills may be responsible for this increase in plasma sodium levels in both males and females. Similar observations were recorded for Atlantic salmon kelt spawners held in freshwater (Johnston ***et al.***, 1987).

FIG 6.2 shows that the level of sodium in the gonads reaches a peak during August in both males and females, which is prior to gonadal maturity being reached. Several studies on reproduction of indigenous freshwater fish species, indicated that hydration of male and female gonads occur prior to spawning (Fouché, 1982; Van der Merwe, 1984; Fouché, 1985). In female ***O. mossambicus***, a sharp decline in sodium levels was observed with maximum GSI. This suggests that sodium levels in female gonads will decrease significantly prior to ovulation. No evidence is available for a shift in gonad sodium levels to the

blood. In males a similar observation was made, but the decline in sodium levels occurred gradually as GSI increased. Personal observations on sperm motility indicate that high sodium levels decrease sperm motility whereas low sodium levels have the opposite effect. Thus low sodium levels in male gonads may contribute to ejaculation. Kruger ***et al.***, (1984) have suggested that a correlation exists between the physical characteristics of the spermatozoa and the type of spawning. ***O.mossambicus*** carries out spawning activities after building nests in shallow slow-moving or standing water. The female usually releases several hundred eggs into the nest whereafter the male releases the semen in the vicinity of the eggs for fertilization to occur (Axelrod & Burgess, 1978).

It is possible that the increase in sodium levels may play a role in maintaining the osmolality of the seminal fluid and thereby ensure the viability and immotility of the sperm *in vivo*, before the release and activation during the spawning activity. Thus, the decrease in sodium concentration in the male appears to play a definite role in the initiation of sperm motility.

FIGS 6.4; 6.5 and 6.6 indicates the relationship that exists between the plasma and gonadal K^+ concentrations recorded in ***Oreochromis mossambicus***. In FIG 6.4, plasma potassium levels increase gradually during the pre-spawning periods when GSI in both males and females increase. However, in the post-spawning period a significant increase was observed in both males and females. This may be due to the high levels of pituitary and inter-renal hormones, such as prolactin and cortisol that are still present in the plasma. They may have an effect on osmoregulation as explained for sodium or they may be involved in shunting potassium to the gonads. Thus after spawning, the backlash of these high hormone levels may be responsible for the sharp increase in plasma potassium values in both sexes which may contribute to the decrease in blood pH values. Gonadal potassium levels (FIG 6.5)showed two peaks in both male and female ***O.mossambicus***. In all cases, gonad levels were higher than plasma levels. In FIG 6.6, it may be clearly noted that a definite relationship exists between the levels

of potassium in the plasma for males and females with a similar relationship existing for gonadal potassium levels. The high potassium levels recorded for the male would appear to agree with the ideas of Kruger ***et al.***, (1984) whereby they state that high concentrations of potassium in the seminal fluid might exert an inhibiting effect on sperm motility and seem to keep spermatozoa immobile within the testis. It therefore appears that in males a major shift of potassium to the gonads occur. Such levels remain high during the entire male reproductive cycle. The role of neuro-endocrine hormones in this regard still have to be evaluated. The same observation was made in female gonads. Prior to the increase in GSI, female gonad potassium levels increase sharply, followed by a significant decline with maximum GSI. This may be associated with hydration of the female gonads prior to spawning. Potassium was also shown to be directly involved in the release of hypothalamic hormones to increase gonadotropins in female rats. The high levels of potassium in the plasma after spawning suggest a feedback mechanism to the hypothalamus to increase gonadotropin secretion. This is of particular importance in the gonads of males and females where it may serve to increase the final gonadotropin surge for final maturation and ovulation or spermiation. Thereafter, potassium levels fluctuated with GSI, suggesting that different age groups are spawning at different stages during the breeding cycle. The role of potassium in maturing female oocytes needs further investigation.

6.5.2 Calcium

FIGS 6.10; 6.11 and 6.12 indicate that there is an increase in the level of calcium in the plasma of both sexes immediately prior to spawning. Corresponding gonad levels were proportionately lower. This contrasted with the findings of van der Merwe (1984) for ***Labeo capensis*** and Fouché (1985) for ***Clarias gariepinus*** where gonad levels were higher than plasma values. Calcium is regarded as a vitellogenic parameter and provides an indication of oocyte development through the mobilization of vitellogenin to the gonads, thus plasma calcium levels increase prior to spawning which is thought to be a function of

prolactin. The latter is regarded as a primary calcium retaining hormone (Bern and Madsen, 1992). These authors also suggest that evidence is available for estrogens to increase blood calcium levels. Nagahama (1990) suggested that estradiol 17-β is essential for vitellogenesis. Thus increased estrogen levels will be associated with the increased plasma calcium levels which were observed in this study. Furthermore, it was shown in rats that calcium is essential in stimulating the LH surge required for final maturation and ovulation (Sabatino ***et al.***, 1989). This finding was supported by Alila ***et al.***, (1989) who indicated that LH increased the calcium levels in the bovine theca derived luteal cells. Grau ***et al.***, (1986) confirmed that calcium is essential for prolactin release in ***O. mossambicus***. This coincides with the increased plasma calcium values recorded in both males and females prior to spawning. Cortisol has a similar effect. This relationship will be discussed with glucose. Nevertheless, male gonads showed an increase in gonad calcium levels during the early maturing phase from May to August whereafter it showed a marked decline in September. This may be related to gonad hydration. Although there is a decline in the calcium levels in the male gonad during the stage of female gonad maturity, male gonads displayed increasing levels during female ovulation. This corresponds with the work of Scott & Baynes (1980). They have also shown that a low concentration of calcium chloride (0.3 - 1.0 mmol/l) usually abolishes the effect of potassium concentrations (1 mmol/l) on sperm motility. It therefore appears that calcium and potassium jointly control sperm motility in males.

Further, Levavi-Sivan & Yaron (1989) have shown that in the presence of Ca^{2+}, a dramatic stimulation of gonadotropin (GTH) hormone occurs. This suggests that a relationship between the elevation of intracellular Ca^{2+} and GTH secretion exists in tilapia. This is consistent with the model of GnRH effect in the mammalian gonadotrophs where the increase in intracellular Ca^{2+} plays a key role in the relay of the effect (reviewed by Conn ***et al.***, 1987). FIGS 6.1; 6.2; 6.3 and 6.4 show that the gonadotropin levels increase during the same period of the reproductive cycle as the Ca^{2+} levels in male ***O.mossambicus***. This seemingly

dependence of gonadotropin secretion on extracellular Ca^{2+} in tilapia is in agreement with that observed in mammals (Hopkins & Walker, 1978; Stern & Conn, 1981).

Van Der Kraak (1991) has shown that calcium plays an important role in GTH-induced steroidogenesis in preovulatory ovarian follicles from the goldfish. This could also be the case in the female ***O.mossambicus*** used in the present study.

FIG 6.12 shows the increase in calcium levels in the plasma clearly and also the much lower and less marked increase in the gonads of ***O.mossambicus***. Hiramoto ***et al.*** (1989) have shown that upon fertilization of the eggs, there is a transient increase in Ca^{2+} levels in Teleosts. This could explain the results obtained in this study where a much lower concentration of calcium was measured in the gonads of female ***O.mossambicus*** than was measured in the plasma. Thus female gonad calcium levels reflect the maturity of oocytes in the ovary. The decline observed in association with GSI, probably reflects the effects of hydration.

Urasa and Bonga (1987) report that plasma calcium concentrations are a relative factor in controlling the corpuscles of Stannius activity (hormone release) in ***O.mossambicus***. The results of the present study would suggest that the possibility of a negative feedback relationship between the corpuscles of Stannius and plasma calcium exists.

6.5.3 Glucose, lipids, proteins and lactate

FIGS 6.13; 6.14 and 6.15 show the fluctuations in the glucose concentration measured in both male and female ***O.mossambicus***. In FIG 6.13 it may be noted that there is a marked increase in the concentration of glucose in male plasma during September and October and a much lesser increase in the gonad at the same time (FIG 6.14). This coincides with the increase in male GSI (see FIG 3.1) and thus this glucose is most probably important in terms of providing the energy

required to be used in courtship behaviour and subsequent release of sperm in order that fertilization may occur. Thus the role of glucose could be as an alternative or secondary source of energy, particularly for sperm motility. This increase in plasma glucose levels was also observed in ***Labeo capensis*** (van der Merwe, 1984) and ***Clarias gariepinus*** (Fouché, 1985). These authors indicated that this increase was associated with increased plasma cortisol levels in both males and females.

In female ***O.mossambicus***, FIG 6.13 shows a surge in glucose concentration in the plasma during the months subsequent to spawning and fertilization. As a mouth brooder, this is when the fish requires greater energy reserves, which could explain the increase in glucose during November until January. The female gonad (FIGS 6.14 & 6.15) shows a similar trend to the male plasma levels of glucose. Thus the female gonad, which is generally very immature during the months of June and July show a high glucose concentration, which may be used as an energy source for the ova which are beginning to develop. The inverse relationship between plasma and gonad glucose suggest a major shift from the plasma to the gonads to provide the nutritional and energy needs. Thus after spawning, a major increase in plasma glucose levels is observed during the mouthbrooding period. It therefore appears that a close interrelationship exists between the blood and gonads to meet the nutritional and energy requirements during the breeding and mouthbrooding periods of female specimens. These observations confirm the importance of the hypothalamo-hypophysial - inter-renal - gonad system during reproduction.

Seasonal variations occurred in total lipid concentration for both male and female ***O.mossambicus*** (FIGS 6.16; 6.17 & 6.18) in the plasma and gonads. Individual lipid types were not measured. Male gonad lipids were higher than plasma lipids during the early gonad developmental stages. Thereafter gonad lipids declined to lower levels when compared to plasma levels. This observation suggests a major shift to the gonads during this period in males. This gonadal lipid increase may

have a dual purpose. Lipids (in particular, phospholipids) are structural components of membranes (Beninger, 1984). The latter author indicated that phospholipids are the most actively degradable form of lipids that are sometimes considered as oocyte energetic reserves. Seasonal variations of phospholipid fatty acid contents are often related to changes in cell membrane properties. Thus the high concentration of lipids are related to gonad tissue development. Furthermore, it may also serve to provide supportive energy resources for spermatozoa. Similar observations were made for female gonads and plasma during the "reproductively active" months of September until December. This would provide for the synthesis of vitellogenin to support oocyte development as well as an important source of energy. The lower values of lipid measured during the winter months (May till July) suggest that there could be an increase in glyconeogenesis at this time. Lipids may also fulfil a protective function against any drastic temperature change that might occur when the mature spermatozoa are released.

The relationship seen in FIGS 6.42 and 6.43 between lipids and pH have been discussed in Chapter 3.

The specific role of protein in fish semen is unknown. It has been suggested that protein fulfils protective and nutritional roles (White & Macleod, 1963; Mann, 1964; Cruea, 1969). The present study has shown a much greater quantity of total plasma protein being measured in male ***O.mossambicus*** than the study of Kruger ***et al*** (1984). Due to the fact that protein is involved in the regulation of the colloidal osmotic pressure of body fluids, the protein that is present may indicate an osmotic role in the semen as well as a haemoconcentration effect in males. Female plasma and gonad protein levels were significantly higher. This observation coincides with maximum female GSI values. It relates to the production of vitellogenin by the liver and its transport to the ovaries to ensure oocyte development and maturation. These levels are not affected by gonad hydration but rather reflects oocyte growth and maturity. The nature of the

proteins determined in this study is not known. However, it may be of significant importance in the production of vitellogenin. The latter occurs naturally as a specific protein in mature males and females. Its synthesis by the liver is induced by estrogen (Ding ***et al.***, 1989). Oocyte growth seems to be a function of steroidogenesis as oocyte maturity increases prior to ovulation. Protein changes in the plasma and gonads therefore reflect changes in vitellogenin production and their transport to the ovaries for incorporation into the yolk of vitellogenic oocytes. The value of proteins is important in fishes as it is considered to be related to genetic variants (Mukhopadhyay ***et al.***, 1986).

The nature and distribution of vitellogenin in males and females and their relationship with specific proteins and lipids therefore explain the increases in proteins and lipids associated with gonad maturation. The non-phosphorous lipids may be made up of neutral lipids. It was shown in ***Tilapia nilotica*** that vitamins play an important role in transport of egg yolk proteins and the induction of steroidogenesis in the gonads.

The relationship seen in FIGS 6.40 and 6.41 between protein and pH have been discussed in Chapter 3.

FIGS 6.22; 6.23 and 6.24 show the seasonal variations in the lactate measured in the gonads and plasma of both male and female ***O.mossambicus***. Female plasma glucose and lactate levels showed an inverse relationship until November. These increased significantly during August, indicating a high anaerobic metabolic activity. This was supported by high female gonad lactate levels during the time prior to spawning. Gonadal glucose levels in females were also high during the same period. It therefore appears that anaerobic glycolysis is the major source of energy during this period. Mobilization of glucose is a function of cortisol by the inter-renal tissues which result in increased cortisol levels in the plasma and gonads prior to ovulation to meet the energy and nutritional demands during this period. Similar observations were recorded by Van der

Merwe (1984) and Fouchè (1985). On the basis of the limited data available for males, spermatozoa of externally fertilizing fish are capable of Krebs tricarboxylic acid cycle (TCA) metabolism (Gardiner, 1978). The presence of lactate could be an indication that it is being formed as a result of pyruvate kinase activity in the Embden-Meyerhof pathway in producing ATP to provide the necessary energy sources for sperm motility during fertilization. Furthermore, there appears to be an interdependence between lactate levels and pH in both the blood and gonads in this investigation.

6.5.4 Urea

FIGS 6.25; 6.26 and 6.27 indicate the seasonal variations in urea concentrations. Urea is considered in relationship to protein metabolism and total protein, particularly in animals having a seasonal sexual development. ***O.mossambicus*** has a defined breeding season (it is a summer spawner) and from FIGS 6.29 and 6.31 it may be seen that there is a clear relationship between urea and proteins in both male and female gonads. In both instances there is a surge in urea content measured that coincides with the increase in total protein measured during the "reproductively active" months of September till December. FIGS 6.28 and 6.30 shows the surge in urea noted in the plasma of both males and females prior to a protein surge (and gonadal maturity being reached). This could be due to the fact that the protein at this stage is being metabolized as reproductive maturity is taking place.

FIGS 6.32; 6.33; 6.34 and 6.35 support the suggestion of an apparent relationship between urea and protein that exists in both male and female ***O.mossambicus***. Protein metabolism indicates gonadal development and this may be noted in the similar curve for GSI. Thus the presence of urea indicates increased amino acid metabolism as a result of their mobilization from the tissues to promote the formation of egg yolk in mature oocytes as well as vitellogenin in male spermatozoa.

Gonad maturation in both males and females are associated with a decrease in the pH levels of the blood and gonads. Thus the mobilization of fatty acids and amino acids for the induction of vitellogenin by the liver, may contribute to the lowering of the pH values. This is also associated with the high lactate levels for anaerobic energy supply to the gonads. Such changes were more significant in females than males. The role of ascorbic acid in gonad steroidogenesis should also be considered to contribute to the gonad pH changes observed. It is also possible that such changes may be gonad size related, since male GSI's are significantly lower than female values. The increase in lactate levels in females may occur as a result of deficient blood supply to the larger gonads during the late maturation and ovulation phases. The low pH in male and female gonads may have a dual purpose. First, it may be related to closure of oocyte membranes and the formation of glucuronides responsible for fertilization to occur. Second, it appears that low pH causes a delay in ovulation to ensure that final maturation of the oocytes is completed. Ovulation in high pH freshwater may provide the stimulus for fertilization and larval development to occur.

6.6 Summary

From the foregoing discussion it would appear that the following aspects of the chemical composition of the plasma and gonads of ***Oreochromis mossambicus*** are of importance.

Sodium levels may play a role in maintaining the osmolality of the seminal fluid, with the initial increase in gonad sodium resulting in the inhibition of sperm motility. During spawning, a decline was observed which may serve to increase sperm motility. This is in contrast with potassium levels where a high concentration during gonad maturation exerts an inhibiting effect on sperm, keeping the spermatozoa immobile within the testis. In females, the high potassium levels in the gonads during maximum GSI, may coincide with the final LH surge required for ovulation to occur.

Calcium appears to assist sodium in controlling sperm motility and also to stimulate the release of the gonadotropin hormone. In female ***O.mossambicus***, calcium may also play an important role during the fertilization of the eggs. The latter appears to be related to prostaglandin synthesis and prolactin secretion.

Glucose could possibly be used as an alternative or secondary source of energy, particularly for sperm motility and for maturation of the ova.

Lipids, although important as structural components of cell membranes, may also provide energy to the developing gonads and to form part of egg yolk deposition. It may also fulfil a protective role against temperature fluctuations which released gametes may encounter.

Although considered to be largely protective, proteins may also be important in terms of the control of the colloidal osmotic pressure of body fluids, particularly the semen. In females, they are associated with vitellogenin production and incorporation into vitellogenic oocytes.

Seasonal lactate variations possibly reflect anaerobic glycolysis as a means of energy production in the form of ATP, possibly via pyruvate kinase activity in the Embden-Meyerhof pathway as a result of Krebs tricarboxylic acid cycle metabolism.

The presence of urea indicates protein metabolism is occurring, which would reflect gonadal development.

It is recommended that these experiments be conducted in a laboratory, where dietary intake may be monitored. The various parameters measured may also be manipulated in order that any possible effects on the reproductive cycle of ***O.mossambicus*** may be determined.

A pilot study (Cornish ***et al.***, 1990) has shown that sperm motility are of great interest and importance for fertilization of ovulated oocytes. Sperm was subjected to different concentrations of saline solutions and the time observed to either inhibit or initiate sperm motility.

In a future study of this nature, consideration should be given to the nature of the lipids associated with gonad development. Of significant importance will be the nature of the fatty acids, especially the polyunsaturated fatty acids, in neutral lipids. Such information will contribute to the nature of the prostaglandins involved in ovulation. In the same way, the role of cholesterol in oocyte maturation should also be determined as a precursor to steroidogenesis. This would contribute to a greater understanding of lipid utilization by spermatozoa and oocytes.

It may also be advisable to endeavour to measure the concentrations of triglycerides in both the plasma and the gonad and then relate such measurements to the parameters used in this study.

A suggestion as to gluconeogenesis occurring in ***O.mossambicus***, could be tested by analysing the amino acids that are present in both the plasma and the gonadal supernatant on a seasonal basis.

The role of ascorbic acid in gonad steroidogenesis should also be considered to contribute to the gonad pH changes observed.

Chapter 7

General Overview of reproduction in *Oreochromis mossambicus*

7.1 Introduction

Several hundred species from the family Cichlidae are known in the African lakes. Information on most of them are found in scientific publications. It was the purpose of this experimental undertaking to gather information on the reproductive physiology of ***Oreochromis mossambicus*** against the background of available knowledge on this particular aspect. This species is a teleost and as such, reproduction is expected to be similar in all teleosts but for adaptations to specific environments.

The population explosion in the developing "Third World" requires an ever increasing need for cheap sources of protein. In this way, more focus has been directed at fish farming as a possible alternative. The introduction of Tilapia culture in Africa, resulted in numerous reports on progress achieved in culturing this species. However, original hopes that Tilapia culture would make a significant contribution as a source of protein production, has faded with the significant disadvantage of excessive reproduction in culture ponds. This resulted in the production of small unmarketable specimens not acceptable to the general consumer. However, its relative ease of culture under tropical conditions, makes it suitable as a candidate species for commercial culture in South Africa.

Oreochromis mossambicus is classified as a mouthbrooder. In this regard it is essential to note that breeding in this species is associated with colour changes which occur slowly as the spawning period is approached. This is accompanied by elaborate breeding behaviours involving the establishment of territories, parental care and many other specializations. Since they are mouthbrooders, an essential feature is care of the eggs and larvae. Females collect the fertilized eggs

and brood them in their mouths. In this regard, they provide their own means of running water by carrying their eggs in the mouth through which a current of water passes. This suggests that females produce a limited number of eggs and the number brooded seldom exceed seven hundred. These afore mentioned characteristics, ensure that hybridization does not occur.

In general, the eggs of this species are very yolky and have a large water content. They are orange-yellow in colour and are essentially spherical in shape within the ovary, but after laying, they become ovoid. Egg and brood size has some biological significance which relates to the habitat in which they live. In crowded habitats, it is advantageous to produce a small number of large eggs to prevent predation pressure. Thus the larvae may be brooded to a large size before being released to face the hazards in a habitat, such as Syferkuil dam.

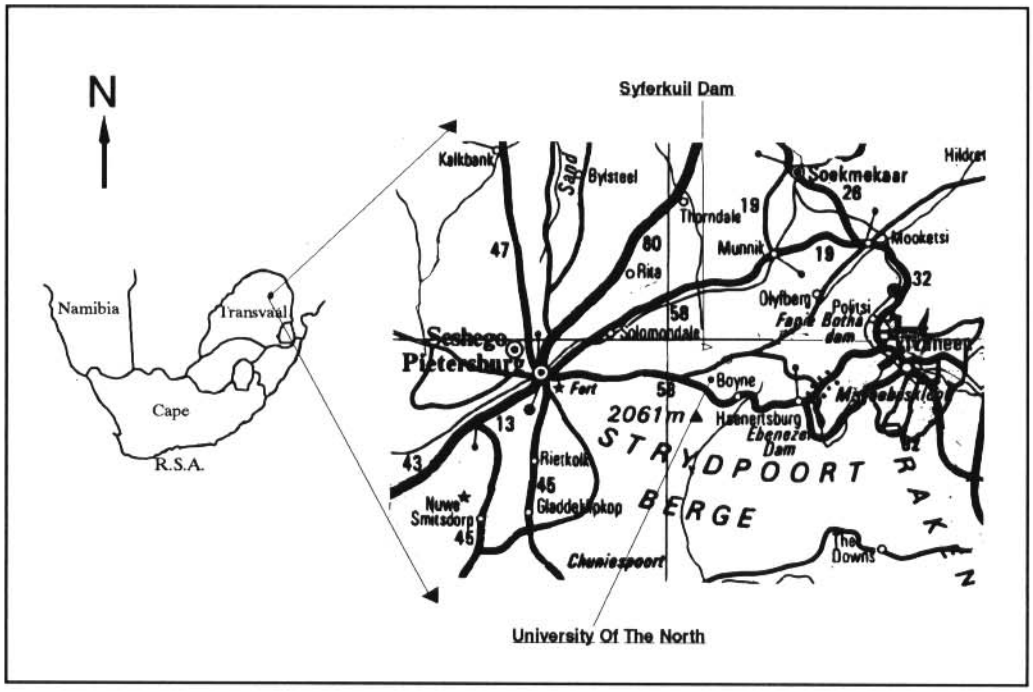
This study formed part of a series of investigations on the reproductive physiology of those species indigenous to the Far Northern Transvaal. It is aimed at determining some common denominators for different species to investigate the possibility of artificial propagation of freshwater fish species under similar conditions in an experimentally controlled simple laboratory setup that may be implemented for practical economic viability in future fish husbandry conditions. This study forms part of the initial investigation to establish suitable laboratory conditions to undertake further investigations to gain a fundamental knowledge on the regulation of reproduction in freshwater fish.

7.2 Site of collection

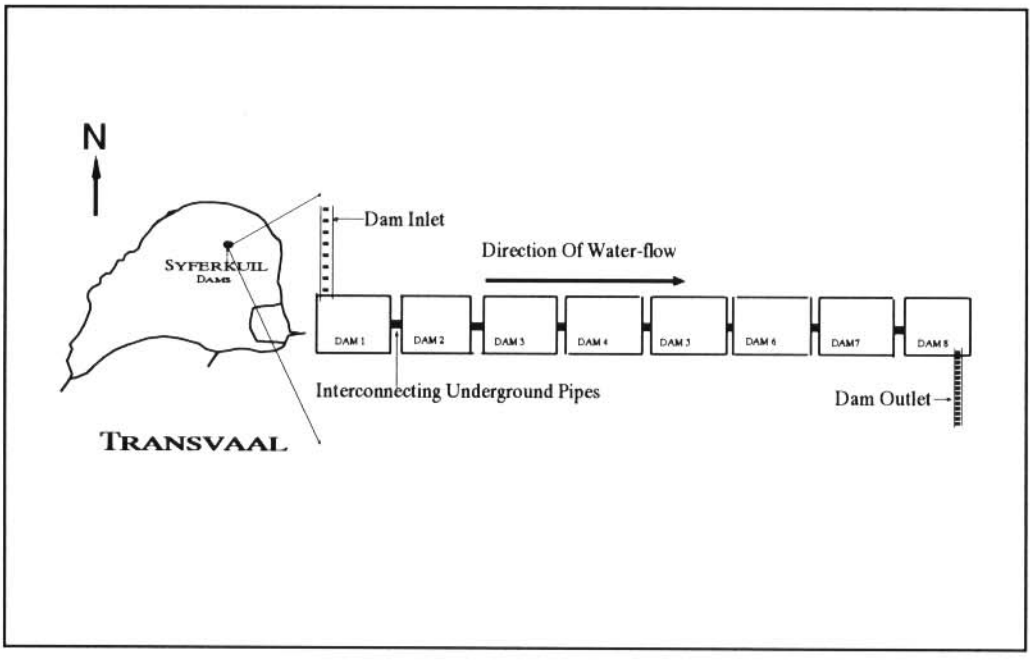
Syferkuil dam is situated 8 km northwest of the University of the North near the local township of Mankweng. It was primarily constructed for the purpose of using partly purified sewage effluent for irrigation purposes for the Syferkuil experimental farm as well as a water resource for irrigation of the University of

FIG 7.1: Geographical location of Syferkuil Dam (A) and a schematic representation of Syferkuil Dam (B).

A.



B.



the North campus grounds, in particular the sports fields. Its location suited the collection of specimens for this study to counter the costs involved in an experiment of this nature. The experimental farm and the sewage ponds were originally under control of the Department of Nature conservation (Ministry of Agriculture) of the Lebowa Government. During the late seventies, the Department of Nature Conservation started to investigate the possibility of polyculture systems in this area to accommodate a food supply to this rural community. No records for crop harvesting are available. Fig 7.1 shows the exact location of this series of dams. During this investigation it was noted that this series of dams is somewhat overcrowded and that a small number of predator fish was present in the pond. The size of most specimens were smaller when compared to those generally collected from rivers and large water impoundments. Of particular significance was the fact that during the breeding season, the larger specimens were easy to collect. The overcrowding also produced a large number of smaller specimens which become mature at a smaller size. During this investigation, care was taken not to sample specimens with eggs or larvae in their mouths.

7.3 Environmental conditions

The results recorded in this investigation points to a close relationship between environmental conditions and the aquatic environment of the ponds from which the eperimental fish were collected. Certain common features were identified that could activate the onset of the reproductive cycle. Although ***Oreochromis*** is well-known as a temperate species which could reproduce successfully in culture ponds, the results of this study suggest that the most important environmental cue to trigger reproduction was rainfall during the month of August. This resulted in a sharp increase in female GSI in September. Although many species have a pineal gland which is sensitive to photoperiod, etc., Lam (1983) indicated that the timing of sexual maturity in ***Oreochromis mossambicus*** may occur in continual darkness or continual light. This observation was confirmed in this

study. Although photoperiod and water temperature are important regulators in oocyte and sperm development in controlling reproductive function, water quality (rainfall) appeared to be the most significant environmental factor for the onset of reproduction in this species. All other parameters, such as temperature and photoperiod, acted synergistically to promote oocyte size and final egg maturation. In general, it was found that the age of the specimens determined the spread of reproductive activity. Each age group went through at least two successive reproductive cycles during the breeding period before climatic conditions intervened to inhibit reproductive progress. Other aquatic factors such as acidity, may inhibit reproduction. However, suitable rainfall activity overcame the negative criteria associated with reproduction. Furthermore, the cold winter aquatic conditions impeded repeated reproductive cycles throughout the year. This suggests that this factor may limit uncontrolled reproduction in large water bodies and rivers in the Highveld area of Transvaal. In conjunction with normal predator occurring species, their numbers are controlled naturally in such water bodies by environmental factors. Several new reports confirmed that this species show large mortalities during the cold winter season.

7.4 Gonad morphology

The Kesteven (1960) scale for reproductive maturity is generally used to describe the general macroscopic evaluation of gonad development in different fish species. It is generally used as a measure to describe the distribution of egg size during the reproductive cycle. This method was also applied in this study together with the histomorphological evaluation of gonad development in conjunction with certain cytochemical and endocrinological changes associated with gonad development. In South Africa, limited information on gamete development in Tilapian species is available. Most studies were conducted on the artificial propagation of freshwater fish species. Such methods were introduced from the Far East by Schoonbee ***et al*** (1978) as cited by Polling (1992). In the present investigation, greater emphasis was concentrated on determining male

and female sex steroid activity in the gonads, histological development and associated endocrine control of reproduction.

The successful reproduction of any species is dependent on the synchronization of spawning in both males and females as observed in this investigation. The successful breeding of this species in uncontrolled captivity in Syferkuil dam bares evidence to this assumption. Reproduction is therefore controlled largely by aquatic environmental factors such as rainfall and temperature.

7.4.1 Gonad and histomorphological parameters

Males

The breeding cycle of males displayed totally different characteristics when compared to females. Gonad length and mass varied with parental size and mass. In general, the period from February to April suggest that males undergo a short period of gonad quiescence. Large males could not be collected during this time. However, during the months of May to January, different sized males with large gonads were always available. These were most significant during May and June. The months of July and August revealed no significant decrease in gonad lengths, but gonad mass for similar sized males decreased significantly. It is suggested that during this period, the older males are in the process of preparing and establishing territorial grounds for spawning to occur. During the months of July and August, the mean gonad masses of males were significantly lower than those recorded in the preceding two months, although no significant differences in gonad lengths were recorded. However, from September onwards to January, male GSI's increased significantly with female GSI, suggesting that many more females are availably for nesting and spawning. During this period, male size and mass decreased significantly whereas GSI increased to reach a maximum during November. It therefore appears that during the peak breeding period, an inverse relationship between size and GSI occurred. This suggests that smaller and

younger males display greater sexual activity than larger males who are occupied with nesting and spawning activities. It therefore offers smaller males a greater opportunity to participate more actively in spawning activities with females when the larger males do not offer a threat to their activities.

The above observations do not provide an indication of their fertilization success during the breeding period. Histological and SEM investigations indicate that small males displayed different sperm characteristics than bigger males. A noticeable difference was the occurrence of a majority of immature spermatids in the gonads of smaller males. These are characterized by wrinkled sperm heads with smaller collars when compared with fully mature male sperm with smooth heads and longer collars. TEM micrographs of mature spermatozoa and spermatids, however, did not show any differences, apart from the wrinkled surface heads. It is possible that the greater spawning activities may have resulted in over production of secondary spermatids to compliment the spawning behaviour of gravid females. Thus it will be interesting to consider the fertilizing ability of such developing spermatids under controlled laboratory conditions. Studies of this nature should concentrate on glucuronide availability to conclude the ability of fertilization success of secondary spermatids.

Gonad morphology suggested a typical tubular testes with clear areas of steroid hormone production as indicated by 3β HSD activity as recorded for most fish species. Such activity is therefore not indicative of the fertilization ability of sperm cells.

Females

Oreochromis mossambicus has a typical syst ovarium (Hoar and Randall, 1983). The general histology of the gonads generally agree with the findings for most teleosts. The oocytes are embedded in the ovarium stroma, consisting of cell nests, connective tissue, smooth muscle and capillary vessels. It was also

evident that the blood cells do not come into physical contact with the spermatogonia and that a blood / gonad barrier exists. Blood from capillaries are drained into a main vessel of the gonads which was clearly observed (FIGS 4.4 and 4.7).

Oocyte development in ***Oreochromis mossambicus*** was characterized by six different development stages. Similar observations were made by Fouché (1982) for ***Cyprinus carpio***, van der Merwe (1984) for ***Labeo capensis*** and Fouché (1985) for ***Clarias gariepinus***. Most investigators used limited criteria to define the different oocyte developmental stages whereas in this investigation, detailed observations on the ooplasm and oocyte membranes, including yolk granules and globules have also been employed. Stage I and II oocytes are embedded in squamous epithelium with the ooplasm and nucleoplasm displaying significant staining characteristics. The differentiation of the *theca* made it possible to distinguish between stage III and IV oocytes. Stage IV oocytes were characterized by rapidly disappearing yolk vesicles. Stages V and VI oocytes also displayed their specific characteristics as outlined in Chapter 4. The size of the oocytes in the different stages of development showed significant differences as reported for other indigenous species. However, the ovaries of this species always contained oocytes in all different stages of development, even during winter. This suggests that even during the breeding cycle, all stage 6 oocytes do not ovulate, even after natural spawning. It is therefore difficult to distinguish between multiple spawning and fractional spawning. The occurrence of the different oocyte stages, especially pre-ovulatory oocytes, throughout the year under different aquatic conditions, suggests that ***Oreochromis mossambicus*** is a fractional multiple spawner. Atretic follicles undergo a period of resorption in a similar way as reported by van der Merwe (1984) and Fouché (1985).

The general increase in GSI is accounted for by two factors. These include vitellogenesis augmented by the liver whereby yolk is continuously deposited in the oocytes to reach maturity. A second contributory factor is hydration of the

ovaries prior to spawning. Hydration is caused by increased quantities of water accumulating in the gonads. The volume of water associated with hydration of the ovaries was not determined, but could be responsible for as much as 60% gain in gonad mass. It was obvious during this investigation, that all different stages of oocytes were present during any time in the breeding season, even after spawning occurred. Another important factor was that ***O. mossambicus*** spawned at least twice during the breeding season which extended from August to February. The available information indicates that ovulation and spawning occurred for at least two different age groups. It is suggested that the older specimens, according to size and mass, spawned first, followed by the younger specimens. Balarin (1979) ascribed this dual cycle to the fact that ***O. mossambicus*** is a mouthbrooder. After fertilized eggs were collected in the mouth, females nurse the eggs and larvae for a period of at least six weeks, sometimes longer, before releasing them into the aquatic environment. The major reason for this is to protect the larvae from predation by other species. Although this species is generally considered as a temperate fish, climatic constraints prevent them from a continuous breeding cycle in Syferkuil dam. During the mouthbrooding period, the gonads undergo a resting period during which stage III oocytes become plentiful. Fryer and Iles (1972) confirmed that these species are territorially bound for breeding to occur. It is thus highly unlikely that females will spawn more than twice within the breeding period within this specific environment, since spawning and mouthbrooding for two cycles may take up to four months. Late spawning activities were still observed during February, but thereafter, aquatic conditions inhibited further breeding of this species. This is in contrast to other indigenous species, such as ***Clarias gariepinus*** (Fouché, 1985) that are classified as single spawners. ***Oreochromis mossambicus*** is therefore not a seasonally bound breeding species and breeding proliferation is determined by aquatic conditions.

Female ovarian development during autumn and winter indicated the predominance of stage III ovaries. The occurrence of immature (Stage I) and

(Stage II) ovaries was significant at the beginning of each new breeding cycle as a result of new females forming part of the breeding stock. It must be emphasized that gonad development was only studied in potential spawners and that immature specimens were not considered as part of this investigation.The emphasis was on the regulation of the breeding cycle. It was also apparent that variations in the ecophysiological requirements, affected ovarian development and growth. This is concluded from the fact that varying gonad lengths and masses were observed during the breeding period. Gonad length did not vary significantly with parental length for the different sized groups. This was also evident when comparing parental and gonad mass for females. However, during Autumn gonad lengths were significantly shorter and increased slowly during winter. During the breeding cycle, gonad lengths increased significantly as well as gonad masses. These changes are ascribed to increased oocyte size. It was also apparent that autumn was a period of gonad quiescence, resulting from poorer aquatic conditions that are not conducive to breeding. In spite of this, gonad development proceeded during the cold winter months. This indicates that during winter, gonad recrudescence occurs to ensure gonadal maturation soon after spring was in the air. Thus at the end of August, stage IV oocytes dominate. The first rainfall that occurred, reacted as a triggering mechanism to promote stage V and VI oocyte development in the majority of females from September right through to November, with resultant spawning. Stage VI oocytes therefore occurred in stage V ovaries whereafter hydration occurs. The long extended period of increased GSI, does not imply that spawning is restricted to the period of December only. During the high GSI period recorded from September to November, females with eggs of larvae were always observed. This specific period is therefore an indication of the highest gonadal activity and spawning. In general, females were always smaller than males during the period of the investigation.

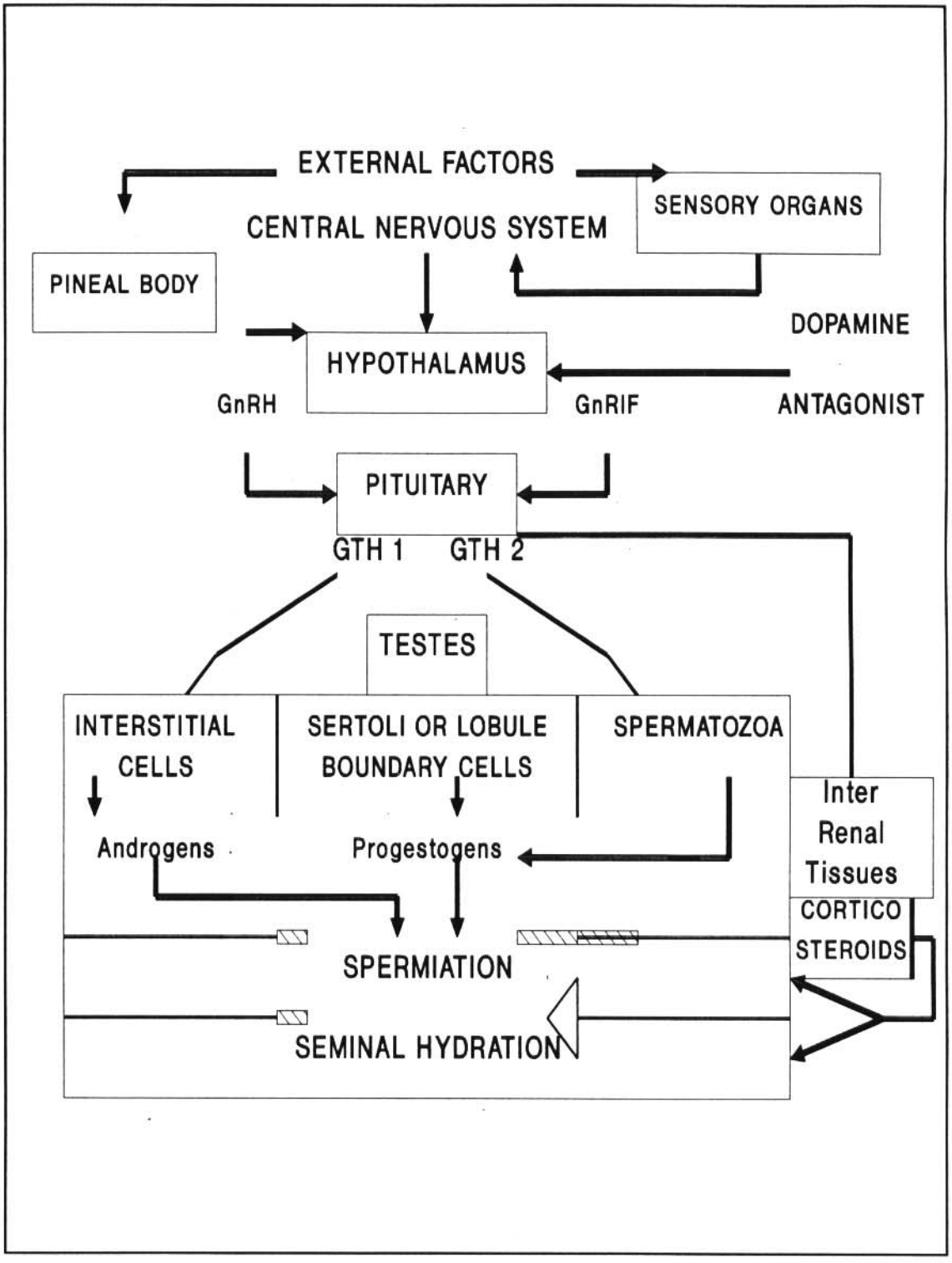
7.5 Endocrine regulation of reproduction

Teleost endocrinology forms a large segment of comparative vertebrate endocrinology. It is a field of research which draws enthusiasm from different laboratories over the entire world. In South Africa, limited investigations have been undertaken into this particular field, since the main application of fish research concentrated on artificial propagation of exogenous and indigenous species, and production potential of selected candidate species for culture and marketing purposes. However, the success of breeding programmes require a fundamental knowledge of the endocrine control of reproduction in both males and females. This will enable researchers to establish guidelines for artificial reproduction techniques for most candidate species. ***Oreochromis mossambicus*** is considered as a suitable candidate species for marketing purposes in South Africa. The aim of this investigation was to determine some aspects of the reproductive cycle which could be fruitfully applied for quality production of quality larvae in future research programmes.

FIG 7.2 provides a broad outline on the hormonal regulation of the male reproductive cycle as determined in this investigation.

Endocrine regulation of gonad development and the gonad cycle may be subdivided into different levels. Wegnez ***et al.***, (1977) indicated that endocrine mechanisms are genetically controlled with environmental factors providing the activating or inhibitory stimuli. FIG 7.2 suggests that the reproductive cycle is primarily controlled by external environmental factors which have been outlined previously in this chapter. Such environmental factors are detected by different sensory organs, including the pineal gland, which impact on the nervous system. Sensory organs of importance include taste, sight and smell as well as impulses detected by the lateral line organ. It appears that all these sensory systems are responsible for detecting water quality changes. Histological evidence suggests a secretory role for the pineal gland (Rüdberg, 1967). Jafri (1983) indicated that

FIG 7.2: Broad outline of hormonal regulation in male *Oreochromis mossambicus*.

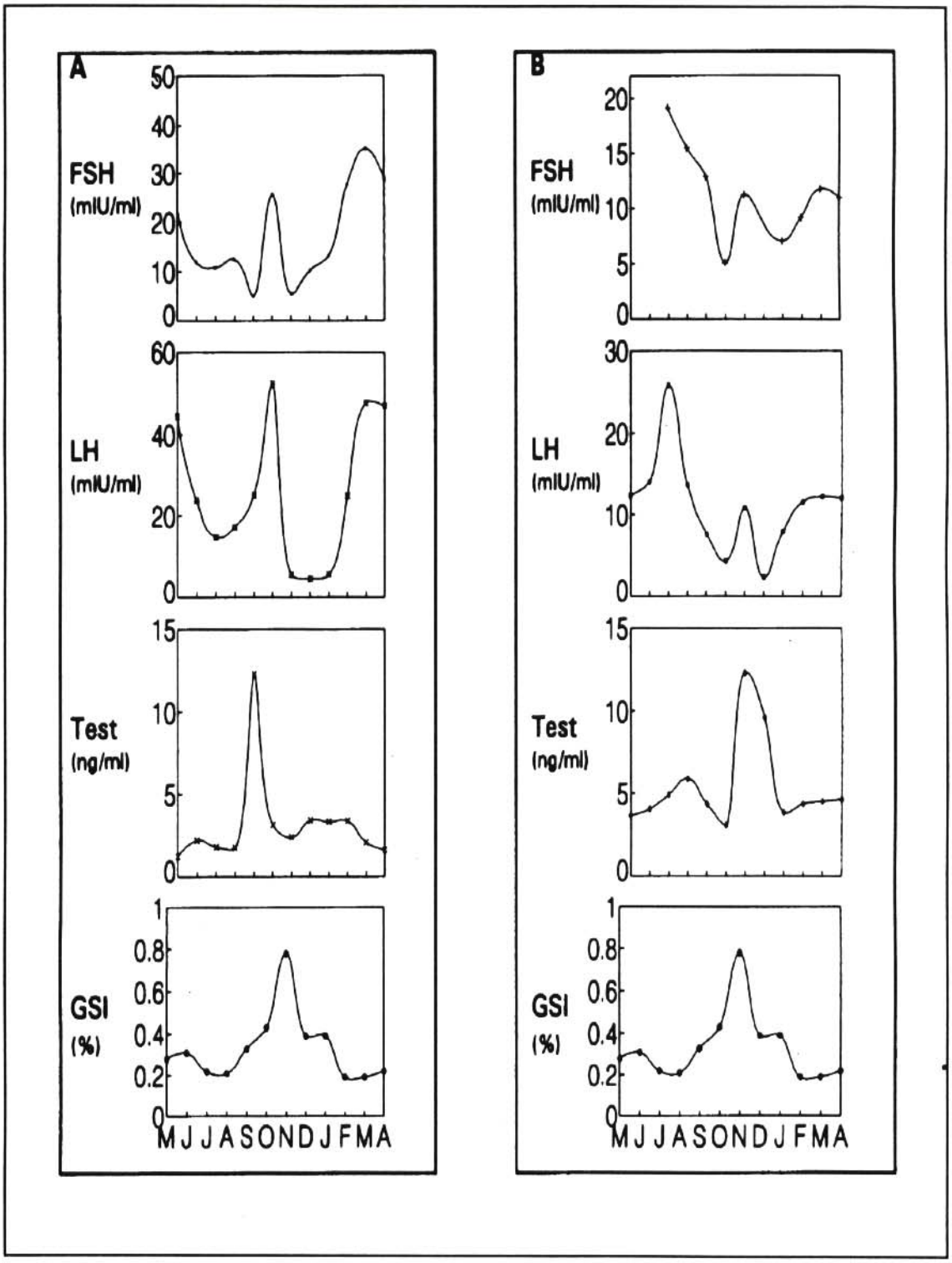


General Overview of Reproduction in *Oreochromis mossambicus*

the pineal gland of teleosts has an external semi-transparent area which suggests a light-sensitive role. Bern and Madsen (1992), on the other hand, indicated that the pineal gland also contains secretory cells. It secretes a hormone known as melatonin which affects pigment cells. This activity may be related to reproductive activity, since it is known that the function of melatonin is the transduction of information on day and calendar time and in the entrainment of endogenous rhythms. The pineal gland therefore forms the bridging gap with the hypothalamus. Thus the regulation of reproduction is controlled by environmental factors to trigger the onset of the cycle in a new breeding season.

The secretory activities of the pineal gland stimulates the hypothalamus to secrete different types of releasing factors which results in the pituitary gland secreting various trophic hormones (Abraham, 1983). The significance of this hypothalamo-hypophysial - interrenal - gonad axis is well-known in teleosts (Bern and Madsen, 1992). In this investigation, no attempts were made to monitor all the pituitary hormones. The emphasis was on pituitary gonadotrophic hormones directly involved in reproduction of which two were identified. Similar studies were undertaken by van der Merwe (1984) and Fouché (1985) for ***Labeo capensis*** and ***Clarias gariepinus*** respectively, employing the same methods applied in this study. For the purpose of this discussion, these hormones are referred to as GTH 1 and GTH 2 for the sole purpose of comparing it to international terminology. GTH 1 conforms to vertebrate follicle stimulating hormone (FSH) whereas luteinizing hormone (LH) is designated GTH 2. These observations therefore confirm the secretion of two different gonadotropins in ***Oreochromis mossambicus*** (see FIG 7.3). No attempt was made to identify the corresponding releasing hormones from the hypothalamus. According to FIG 7.3, after peak male GSI is reached in November, GTH 1 secretion reaches its lowest level. Thereafter it gradually increases in December followed by a sharp increase until the end of April. This is followed by a gradual decline from May to August with a final surge in October. This observation suggests that GTH 1 has a similar function to vertebrate FSH. It is therefore responsible for gonad development

FIG 7.3: Plasma and gonadal hormonal levels and GSI in male *Oreochromis mossambicus*. A: Plasma. B: Gonad.



GTH 1 and GTH 2. This suggests that both the GTH 1 and 2 surges were responsible for the final gonad surge of testosterone. The latter coincided with peak male GSI. However, it was not possible to measure other gonad steroids such as estrogens, progesterones and androgens. Also, the role of the inter-renal glands in this process of gonad maturation was not evaluated. General information on this aspect for teleosts is well-known (Bern and Madsen, 1992). However, analysis of plasma and gonad parameters associated with hormonal profiles, confirmed the involvement of the inter-renal tissues in male reproduction. This study therefore supports the general view that freshwater teleost reproduction is controlled by pituitary gonadotropins which play a regulatory role in teleost reproductive behaviour.

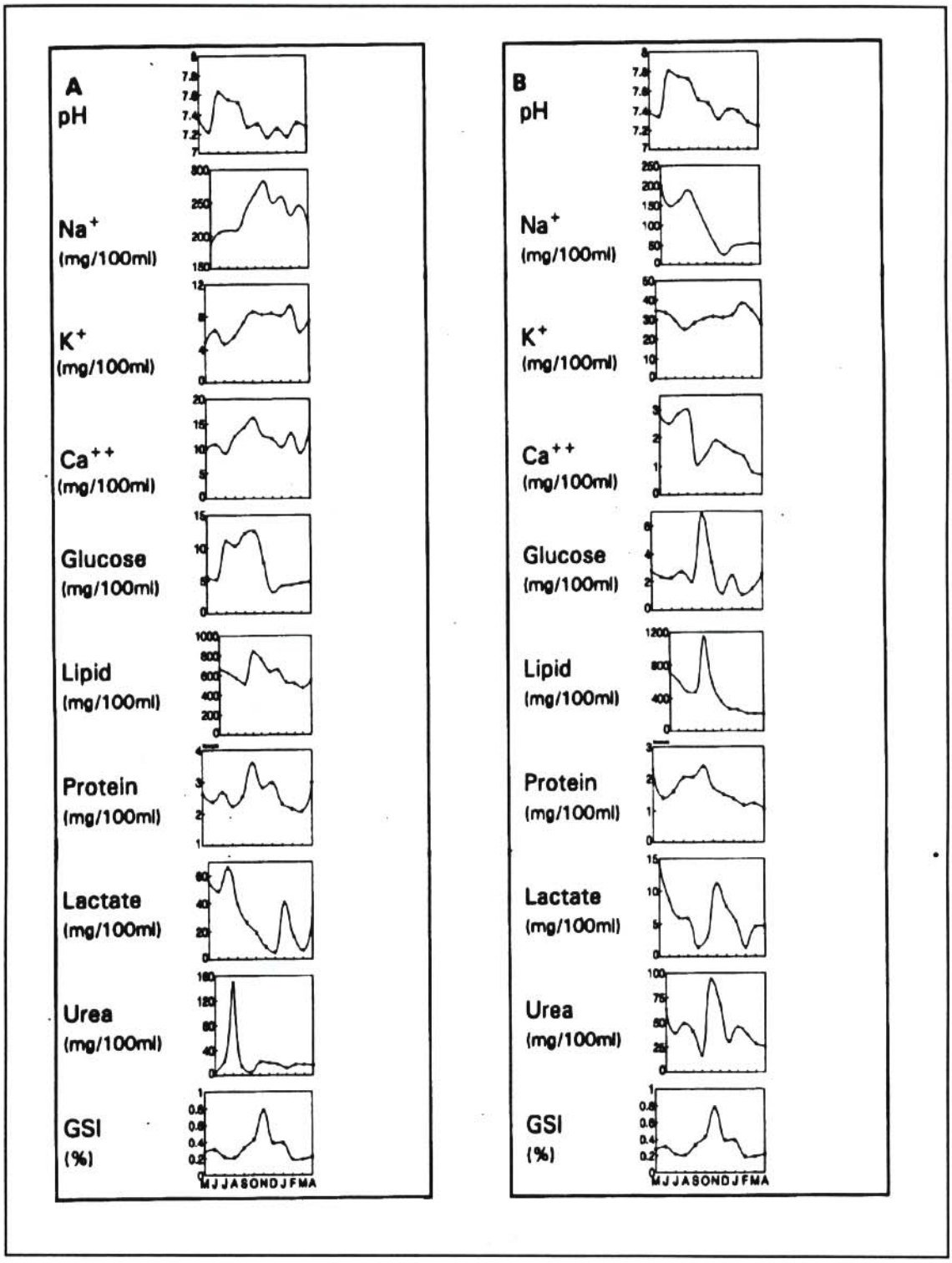
Blood and gonad parameter associated changes during reproduction

Analysis of male blood / plasma and gonad supernatant values during the study period, indicated two major groups of occurrences which either followed direct relationships between the plasma and gonad parameters or which showed inverse relationships. Direct relationships between the plasma and gonad parameters involved pH, K^+, glucose, lipids and proteins. Inverse relationships were obtained with Na^+, Ca^{++}, lactate and urea. Both groups of parameters provided an indication of the metabolic activities in the blood / plasma and gonads and the possible involvement of the inter-renal tissues in supporting gonad maturation. An overview of these activities are indicated in FIG. 7.4.

The most significant observation recorded in male blood was the gradual decline in pH from July to September. May and June showed lower blood pH values. The higher pH levels coincided with low plasma levels of GTH 1 and GTH 2. This corresponded with a non-significant increase in plasma testosterone and GSI values. However, gonad values for the hormones measured showed significant peaks during the corresponding periods, including gonad pH values. It can therefore be concluded that hormones in the plasma and gonads have no effects

on pH values during the early part of the breeding season. Such changes also conform to increased male activity, linked to age groups, during this period. If this assumption is correct, the role of the inter-renal tissues must also be considered. Inter-renal tissues are responsible for the secretion of different cortico - steroids (homologous to vertebrate adrenal cortex) and catecholamines from the chromaffin tissues (homologous to vertebrate adrenal medulla) as suggested by Bern and Madsen (1992). Although no hormones were analyzed in this gland, indirect measurement of their contributions may be made by measuring other blood / plasma and gonad parameters. Mineral metabolic activities indicate a gradual increase in plasma sodium associated with a high gonad level which shows a slight decrease during this period. Kruger ***et al.***, (1984) suggested that high gonad sodium levels inhibit sperm motility. The slight drop in gonad Na^+ values, suggest increased sperm activity. This is also confirmed by a drop in both plasma and gonad K^+ values. The latter authors also suggested that when sodium levels drop, high potassium levels inhibit sperm motility in the gonads of ***Oreochromis mossambicus***. Thus, the slight drop in gonad K^+ levels suggest increased sperm activity. However, the role of Ca^{++} in this process is not certain. In teleosts, since calcium levels are generally less homeostatic in teleosts. Bern and Madsen (1992) indicated that general consensus suggests the primary calcium regulating hormones include prolactin, steroids and cortisol. Furthermore, these authors also suggested that elevated extracellular Ca^{++} metabolism is essential for neuropeptide stimulation of gonadotropins and that it is essential for the fertilization process. Thus the plasma and gonadal Ca^{++} levels recorded in this study are in line with this hypothesis and supports the role of the inter-renal tissues in this regard. It should also be noted that the primary male gonad activity coincides with a drop in gonad sodium, increase in Ca^{++} and K^+ levels in conjunction with the two stage male gonad activity as suggested by Bern and Madsen (1992). Further evidence for the involvement of the inter-renal tissues is reflected in the measured glucose, lipid, protein and lactate levels. This implies a mobilization of carbohydrates, proteins and lipids to support gonad metabolism and energy requirements. It

FIG 7.4: Plasma and gonadal associated parameter levels and GSI in male *Oreochromis mossambicus*. A: Plasma. B: Gonad.



appears that cortisol is the most important hormone for this purpose. Hattingh ***et al.***, (1975) and Smit ***et al*** (1990) indicated a direct relationship between high plasma glucose and cortisol levels. It is therefore assumed that the high glucose, lipid and protein levels recorded, resulted from cortisol secretion from the interrenal tissues. Bern and Madsen (1992) also indicated cortisol receptors in the gonads, which may explain the high levels of these products during this period. It seems, however, that a general lag occurs with the transfer of these products to the gonads. Nevertheless, the significance of this observation suggests the necessity for gonad activity to be maintained. However, the significance of low blood pH may not be related to the plasma parameters measured. It may be due to the mobilization of amino acids and fatty acids for metabolic purposes. The increased levels of steroids may also be responsible for the decline in pH values. Lactate levels measured in the plasma, suggest that this parameter is not significant in the measurement of blood pH levels. Furthermore, it also appears as if male ***O. mossambicus*** blood has a low pH buffering capacity. Analysis of gonad pH levels suggest that two factors primarily contributed to a lowering of gonad pH. These include lactate and urea levels, thereby indicating high carbohydrate and protein metabolic activity in male gonads. In this regard, the gonad parameters displayed a more significant explanation for the decline in pH levels. An inverse relationship was shown with lactate and urea levels, thereby suggesting that these two factors are primarily involved in low gonad pH in males. It may also be associated with mobilization of fatty acids and amino acids. Another important feature to be considered, involves the possibility that the blood supply to the gonads is not sufficient to remove all the waste products. This implies that male gonads rely heavily on anaerobic glycolysis to meet the energy demands of spermatozoa.

In conclusion, the changes in male gonads involve a complex interaction of hormonal interaction in the hypothalamo-hypophysial - interrenal - gonad axis which is responsible for the supportive stages in male gonad development. Further studies on the involvement of other hormones in this axis, including

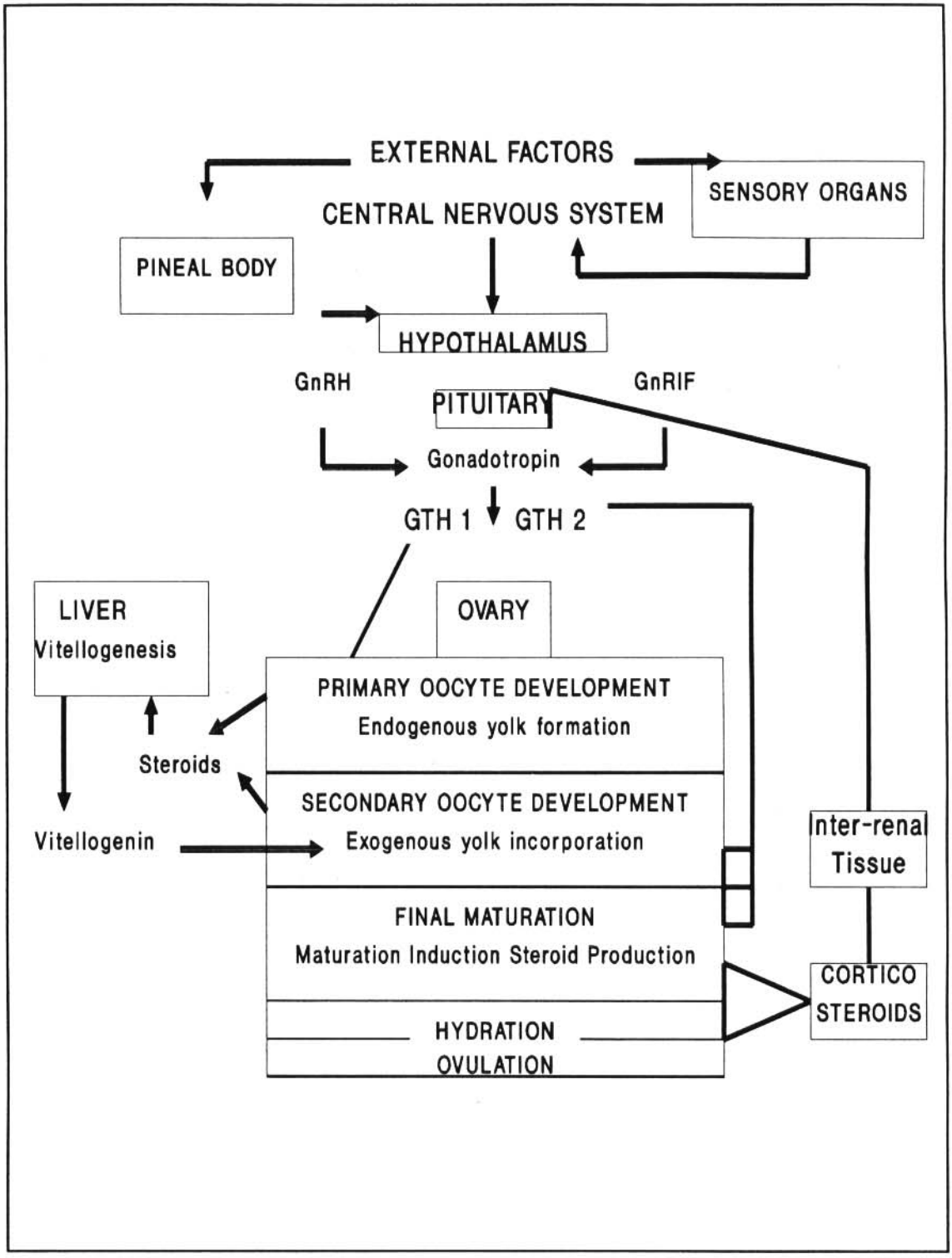
pheromones and related biochemical changes, will confirm the exact nature of changes associated with male reproduction. Although much information in general is available on male gonad maturation, the factors involved in the fertilization process needs to be further evaluated.

Female

FIG. 7.5 is formatted according to basic information provided by Bern and Madsen (1992). The outline presents the general endocrine regulation of female teleost reproduction. It is generally regulated by external as well as internal or endogenous factors. The external factors involved in regulation are similar to those described for the male cycle. In female teleosts, endogenous yolk formation and exogenous yolk absorption are controlled by pituitary gonadotropins. The latter stimulate interstitial tissue (stroma) and the oocyte layers of the secondary oocytes for the formation of sex steroids. These are responsible for the final maturation and ovulation of oocytes. The main vitellogenic steroid appears to be 17-β estradiol. The involvement of the inter-renal system through the hypothalamo-hypophysial - gonad axis, is also involved. These steroids are released into the circulatory system to promote vitellogenisis in the liver. The formation of excessive female steroids may have an inhibitory effect on the secretion of gonadotropins. The exact mechanism of action is not known, but it may operate ***via*** a negative feedback system on the hypothalamus. It is also possible that the sex steroids may act on the photoreceptors in the pineal gland to inhibit gonadotropin secretion.

Vitellogenin is taken up by the oocyte membrane and incorporated during the process of yolk formation. The corticosteroids from the inter-renal tissue is actively involved in oocyte maturation. Their secretion is stimulated by trophic factors from the hypophysis. The corticosteroids therefore also contribute to oocyte maturation, hydration and ovulation. Ovulation occurs through the intervention of prostaglandins.

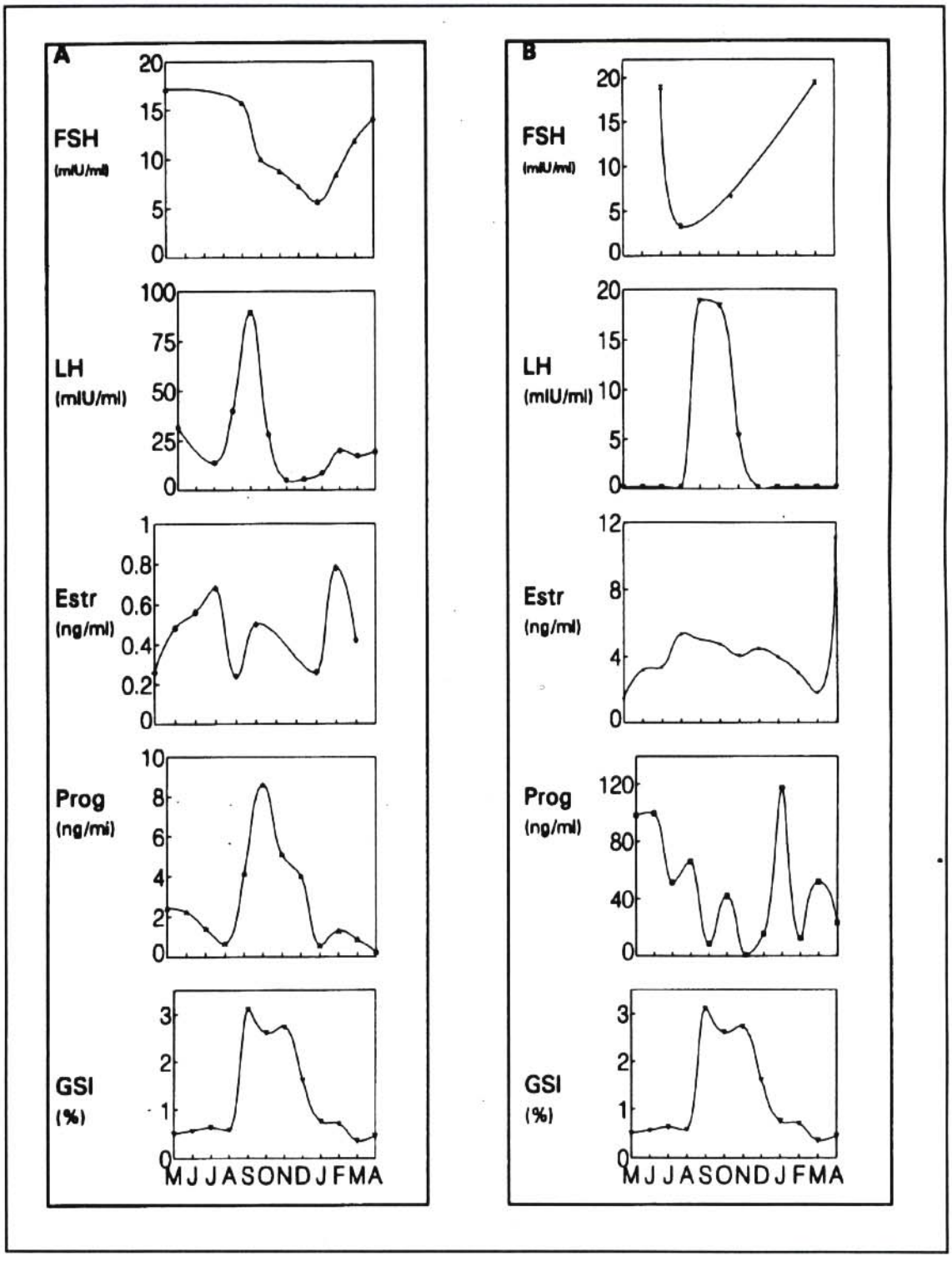
FIG 7.5: Broad outline of hormonal regulation in female *Oreochromis mossambicus*.



In general, the female reproductive cycle in female ***Oreochromis mossambicus*** is programmed in the same manner as males. Environmental factors stimulate receptors which are transported along nerve fibers to the central nervous system. The pineal gland is the most significant part that transducts these impulses to the hypothalamo-pituitary axis (Peter and Crim, 1979). This results in the activation of the neuroendorine system whereby the endogenous cycle for reproduction is initiated. Abraham (1983) indicated that the pineal gland influences the hypthalamo-hypophysial axis to secrete trophic hormones. The latter author also suggested that the pineal gland may act as an endocrine organ, since it contains glandular cells that may be responsible for the secretion of gonadotropins. This suggests therefore, that the pineal gland also stimulates the secretion of all pituitary hormones, including thyroid stimulating hormone and adrenocorticotropic hormone. Thus, the female cycle is not only controlled by the gonadotropins. In this study, the secretion of all the different trophic hormones in females were not monitored. However, Chang ***et al.***, (1983) clearly indicated the origin of these hormones. The purpose of this study was therefore aimed at verifying the secretion of gonadotropins only in ***O. mossambicus.*** Different opinions on the secretion of gonadotropins for female teleosts have been suggested. Kawauchi (1993) confirmed that such differences were cited by many researchers. In general, teleosts displayed two different gonadotropins that are similar to, but not the same, as vertebrate FSH and LH. It is for this reason that vertebrate radioimmunoassay kits for FSH and LH were used to verify the secretion of gonadotropins in ***O. mossambicus***. See FIG 7.6 on previous page.

Plasma GTH 1 and GTH 2 in females did not follow the same pattern as in males.GTH 1 (FSH) remained relativly high from May to September wherafter a sharp decline occurred during October. Therafter a gradual decline was observed until the end of January. The period from September to December thus indicates an inverse relationship between female GSI and GTH 1. It appears therefore that vertebrate FSH has a similar function to fish GTH 1. FSH in ***O. mossambicus*** therefore promotes the development of the female gonads after the resting

FIG 7.6: Plasma and gonadal hormonal levels and GSI in female *Oreochromis mossambicus*. A: Plasma. B: Gonad.



phase. Increased GTH 1 in the plasma was observed from January and continued to rise until peak GSI is reached in September. This observation suggests that the female gonads need a constant hormonal stimulus to ensure growth and development of the oocytes in females. In contrast, males showed a different response at much lower levels. It can therefore be safely concluded that female gonad function requires a more intense endocrine support to promote development and maturation of the oocytes. LH levels in female plasma also suggest a different secretion rate. It gradually increased during September to reach a maximum during October whereafter it declined significantly and remained low for the remainder of the breeding period. In males, plasma LH levels could be detected throughout the year. In contrast to the plasma values of FSH and LH, gonad levels of these two hormones displayed totally different characteristics. FSH declined sharply during August before peak female GSI is reached and gradually increased from November. Gonad LH, on the other hand, remained undetectably low during the entire year, except in September and October when maximum values are reached which coincided with peak female GSI. These occurrences suggest that FSH and LH in the gonads do not fluctuate to the same extent as in males. Another possibility to be considered for the lesser fluctuation observed in females, is the level of hydration when peak female GSI is reached. Hydration of the gonads may therefore mask the fluctuation in both FSH and LH levels. Gonad hydration involves an increase in the fluid content of the gonads as well as an increase in the size of the oocytes through the process of vitellogenesis (Babriker and Ibrahim, 1979). The degree of hydration is determined by estrogens (Wallace and Selman, 1978). In this regard, the role of the inter-renal tissues should also be considered. However, this was not determined in this investigation.

Evaluation of the plasma and gonad steroid levels in ***O. mossambicus*** suggest that both estrogens and progesterone are involved in this process of hydration. In the plasma, 17-β estradiol reaches peaks during August, October and February with a relatively slow decline from October to November. Gonad levels

remained relatively high from August to November, although these levels were generally lower than plasma values. Steroid production in the gonads was confirmed by 3 β HSD activity. As indicated previously, these effects may be masked by hydration of the gonads during this time. These observations confirm the findings of Wallace and Selman (1978) and is also indicative of the maturity stage of the oocytes and ovulation. Thus ovulation occurs predominantly from September to December as indicated by gonad progesterone levels. Plasma and gonad progesterone levels displayed similar fluctuations. Plasma progesterone levels remained high from September to December whereafter it declined and remained low throughout the remainder of the year. Gonad levels, however, showed peaks during June, August,October, January and March. Gonad peaks for progesterone are indicative of the resting phases in between ovulations by different female age groups. It therefore appears that the steroid levels are more indicative of oocyte development in the gonads, rather than the levels of FSH and LH.

A significant feature observed in this study, was the recording of human chorionic gonadotropin (HCG) levels in the plasma of females. This parameter was measured to determine the nature of the gonadotropins secreted. It was thought that one of the female gonadotropins may be similar to HCG, since it's basic structure suggests that it has limited FSH, but high LH activities (Schoonbee ***et al.***, 1978). Furthermore, in humans this is a placental hormone, secreted in conjunction with steroids, to prevent additional ovulations during the pregnancy period (Guyton, 1986). In females, this hormone was detected in the plasma only and not in the gonads. It was measured in the period from May to July, immediately prior to peak GSI in September and again in the period following ovulation. The contribution made by the presence of HCG is not very well understood, since different views can be attributed to its presence in the plasma only. Firstly, it's FSH and LH activities may be associated with the levels of FSH and LH in the post-ovulatory phase in females. However, when comparing this to both plasma FSH and LH levels, it shows no clear association, since an

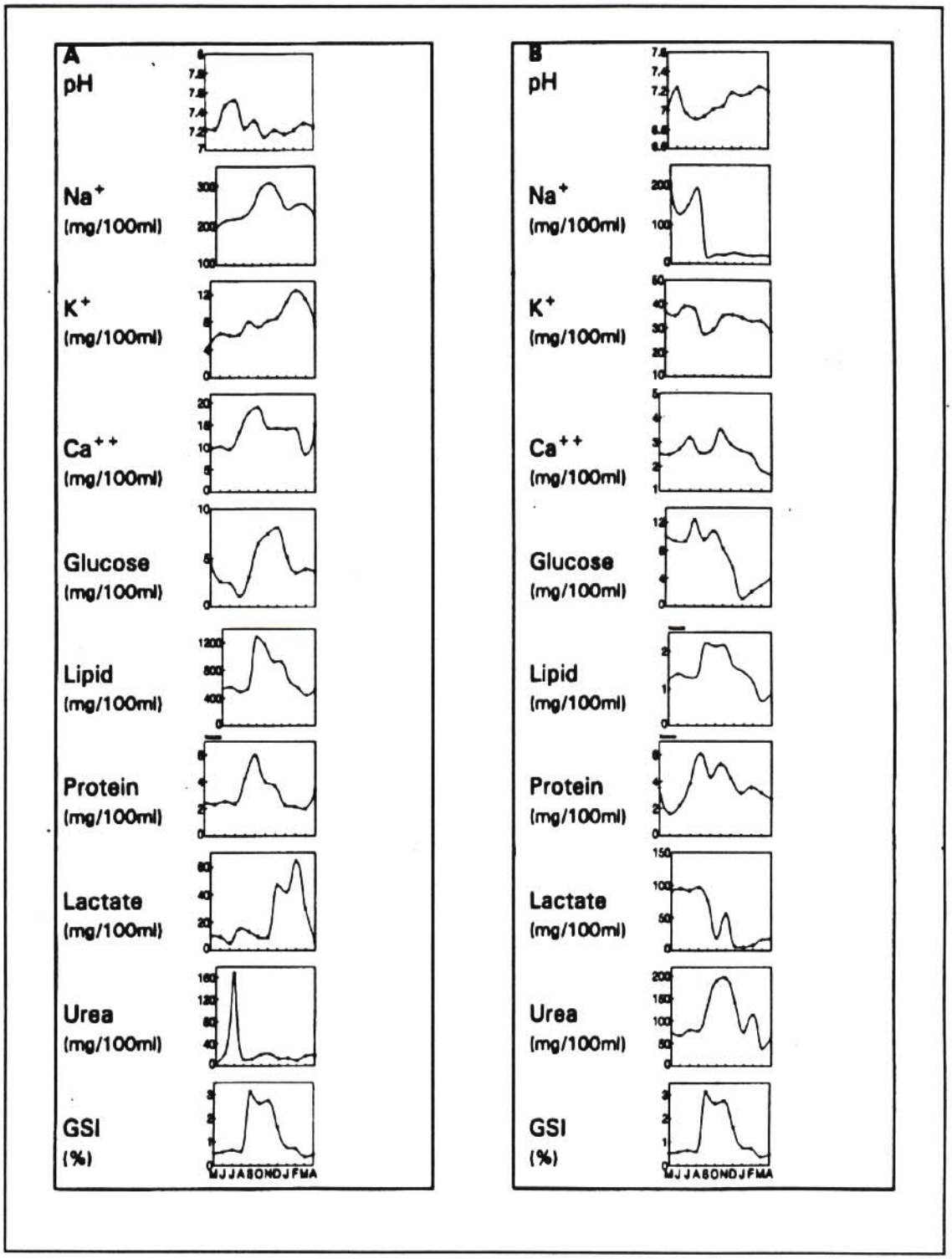
inverse relationship was observed. It therefore suggests that HCG has a totally different function. It therefore seems that this hormone may function in the same way as human HCG to prevent another cycle of oocyte maturation during the mouthbrooding phase of this species. It may also suggest the secretion of a gonadotropin inhibiting hormone to inhibit the secretion of gonadotropins, in spite of the feedback mechanisms involving steroid production in the gonads. HCG was determined in the plasma only and its absence in the gonads supports this possible role. It may therefore not be attributed to cross reactions for the various test kits used for determining the levels of gonadotropins, but rather provides for an inhibitory role for HCG. Another possibility is that cross reactions with other trophic hormones secreted by the pituitary may be involved. However, it does not make sense if considering TSH, MSH, ACTH or prolactin as alternatives, since these would have been present during peak GSI. Thus the only conclusion that may be reached, is that the presence of this hormone in the blood is as a result of some form of inhibitory activity to allow a resting phase for the gonads to allow sufficient time for gonadal recrudescense to develop.

These observations on the endocrine regulation of reproduction in female ***Oreochromis mossambicus*** were therefore found to be similar to vertebrate regulation of reproduction. The most significant difference was the fact that the onset of reproduction is controlled by environmental and aquatic conditions.

The measurement of chemical parameters in the blood / plasma of females contributed to a better understanding of the endocrine regulation of reproduction and supports the general principles used to evaluate the activities in the ovaries. All the parameters in the blood / plasma and gonads either show a direct or inverse realtionship with female GSI.

FIG 7.7 represents the blood / plasma and gonad chemical measurements for ***Oreochromis mossambicus***.

FIG 7.7: Plasma and gonadal associated parameter levels and GSI in female *Oreochromis mossambicus*. A: Plasma. B: Gonad.



Blood pH values fluctuate with female GSI. High blood values are recorded prior to peak female GSI whereafter a significant fluctuating decrease was observed. In most instances it showed inverse relationships with most plasma chemical parameters measured. It is therefore indicative of major blood changes occuring in reponse to changes occurring in the gonads and to meet the requirements for gonad development at the onset of the reproductive cycle. It is also indicative of a poor buffering capacity of female ***Orechromis mossambicus*** blood. In addition, it appears as if female FSH was not responsible for the decline in blood pH levels, but that it was associated with LH and steroid increases in the blood. Similar observations were made for female gonadal parameters. Gonad pH levels showed an inverse relationship with female GSI. Thus, as GSI increases, gonad pH levels dropped. It may therefore also be associated with metabolic changes in the gonads to provide the necessary nutrients for oocyte development and for vitellogenesis to continue. However, the low gonad pH levels are not clearly understood. It appears as if gonad FSH levels are not associated with this change, but that LH and steroid levels may be associted with the decline in gonad pH values. Thus the level of the gonadal hormones prior to the onset of reproduction in females, do not affect gonad pH values.The low gonad pH values suggest that it may delay ovulation until such time that oocytes are fully matured. It may also serve to reduce activity in the gonads to a point where other hormones from the inter-renal tissues or from steroids, such as prostaglandins, have reached a level to induce ovulation of the eggs. A similar proposal was made by Kügel ***et al.*** (1990) for ***Salmo trutta***. The role of corticosteroids and prostaglandins in oocyte maturation and ovulation should be determined in future studies of this nature.

The role of the reproductive hormones appear to be responsible for the many blood changes observed. The plasma elctrolytes, sodium, potassium and calcium increased during the period of maximum female GSI. Plasma Na^{+} levels remained high during the peak breeding season with K^{+} also increasing gradually. Ca^{++} showed a marked increase during peak GSI. The reason for such changes are

not known, but may be indicative of inter-renal corticoids to maintain homeostasis or haemoconcentration during peak gonadal development. The presence of high Ca^{++} levels are indicative of vitellogenesis in the liver. Consideration of gonad levels for these elctrolytes, suggest a totally diffenr picture. During peak GSI, gonad sodium levels drop significantly and remain low for the breeding season. This change may be indicative of sodium not being involved in gonad hydration to maintain osmolality during peak GSI. However, gonad levels of K^{+} and Ca^{++} suggest other roles for these two electrolytes. The relatively high K^{+} levels suggest that this electrolyte is responsible for gonad hydration ***via*** the corticoids of the inter-renal tissue, since only a slight drop was observed during hydration. Calcium, on the other hand, is indicative of vitellogenisis in the gonads as explained by Bern and Madsen (1992). The latter authors also suggested that increased calcium levels is a function of gonadal steroids as recorded in this investigation. Another role for Ca^{++} suggested by these authors, is that it regulates prolactin and cortisol secretion required for final maturation and ovulation. As indicated previously, cortisol promotes mobilization of carbohydates, proteins and lipids for energy purposes and incorporation into the oocyte yolk by vitellogenesis. This confirms the increase in glucose, proteins and lipids in the plasma and gonads during peak female GSI. Glucose values in the plasma are increased during this period, but it is also accompanied by an increase in lactate levels, particularly in the gonads. The latter may explain the decline in gonad pH values. The high glucose and lactate levels are also indicative of anaerobic glyclosis in the female gonads to provide in the energy demands. This situation also suggests the possibility of an inadequate blood supply to the gonads to accommodate exchange of nutrients and waste products.It is known that the female gonads have receptors for both cortisol and insulin. Future investigations of this nature should also involve the determination of these hormones in the gonads to reach clarity on the entire female reproductive cycle.It does, however, seem that a lag occurs between the transfer of these products to the gonads. Plasma levels also dropped and remained low after this transfer has occurred. Thus no increased blood circulatory levels of

these chemical parameters occurred after ovulation. This is in contrast to the males where a gradual decline was observed after peak GSI. It therefore appears that a closer relationship and more accurate regulation of gonadoropin and inter-renal secretion and plasma and gonad parameters exist in females when compared to males. The significance of these control mechanisms lies in the process of vitellogenesis and oocyte maturation and ovulation. The pituitary is responsible for the secretion of the regulatory gonadotropins which induce the formation of 17-β estradiol which plays an essential role during ovulation, accompanied by increasing levels of testosterone (not measured). Excess testosterone may serve as a precursor for some essential progesterones (Fouché, 1985). It is possible that ovulation may be a function of the sympathetic system which stimulates inter-renal medullary tissues to release catecholamines. Their function is contraction of the smooth muscle layer observed in the oocytes (Hall and Behrman, 1982) and formation of prostaglandins in the follicle layers to induce contraction in association with catecholamines (Jalabert, 1976). Such actions may be assisted by the cortico-steroids. It is therefore suggested that the regulatory hormones initiate the reproductive cycle through stimulation by external factors. As gonad development proceeds, the secretion of steroids by the gonads and inter-renal tissues are responsible by means of a negative feedback system to inhibit pituitary gonadotropic secretion and to promote the secretion of a gonadotropin inhibitor by the pituitary gland. Simulataneously, a positive feedback system may be involved to promote secretion of prolactin to assist in the final maturation of the oocytes. In addition, such changes may feed back to the inter-renal medullary tisuses (chromaffin system) to promote catecholamine secrection for the formation of prostaglandins to induce final ovulation. However, in ***Oreochromis mossambicus***, the role of thes hormones need to be evaluated in order to determine their interaction in gonad dvelopment. Thus the findings of this investigation confirm similar findings for other indigenous species in South Africa.

7.6 General

Male ***Oreochromis mossambicus*** is not seasonably bound to reproduction in the same manner as females. Although they are also temperate fish, it appears as if the gonad development in females is the primary stimulating factor responsible for initiating spermatogenesis. This was confirmed by 3 β HSD activity throughout the year in male testes, except during winter when less activity was observed. General gonad activities changed significantly during August in males. In general, stage V and VI male gonads were always visible , but were not prominent during June and July which were the coldest months of the year. Sperm development was monitored with SEM. These observations clearly distinguished between mature and immature sperm. Immature sperm were characterized by wrinkled heads and short collars as opposed to mature sperm which displayed smooth heads and longer collars. All other features characteristic of mature sperm were present. Furthermore, the testes appeared to be of the tubular type with seminiferous tubules filled with spermatocytes. The most significant difference observed in larger and smaller males, was the extent of GSI. Smaller males displayed higher GSI values when compared to larger males. This was portrayed in the lengths of the paired gonads which displayed no significant differences.Thus the extent of hydration in smaller males will be more significant as reflected in GSI.

Female ***Oreochromis mossambicus*** do not display a four season reproductive cycle as suggested by van der Merwe (1984) and Fouché (1985) for ***Labeo capensis*** and ***Clarias gariepinus*** respectively. The results clearly suggest that this species is a temperate one with repoduction occurring mainly during temperate conditions as confirmed by Balarin (1979). The endocrine mechanisms involved during gonad development conforms in broad outline with those known for other teleosts. Two stage gonadotropin secretions for gonad development have been identified that are similar to those described by Peute ***et al.***, (1986). It therefore confirms the two hormone hypothesis for many fish species.

The gonadotropins appear to be responsible for primary and secondary oocyte development through exogenous yolk uptake and endogenous yolk formation. These hormones also stimulate steroid production and vitellogenesis as confirmed by 3 β HSD activity. Unbound calcium binds with vitellogenin during yolk incorporation into the oocytes. Sex steroids produced by the oocytes appear to be primariy responsible for vitellogenesis and may contribute indirectly to oocyte maturation, hydration and ovulation. Their presence in gonad extracts after the initial ovulation phase may be linked to atresia of follicles. The role of mineralo- and glucocorticoids remain to be evaluated in future studies of this nature but may also be involved in oocyte development, hydration and ovulation as indicated by the mobilization of glucose, lipids and proteins. Oocyte development within the breeding season are characterized by primary oocyte development, followed by pre-vitellogenic oocytes, secondary oocytes, oocyte atresia, vitellogenin incorporation and yolk formation followed by oocyte hydration and ovulation. This sequence of events is followed by a resting phase and subsequent gonadal recrudescense.

Hormonal regulation of reproduction in males also corresponded to a two stage gonadotropin release by the pituitary gland. Thus FSH and LH was responsible for male gonad maturation. However, the nature of the interaction of these two hormones differed from the females. Male FSH did not dominate gonadotropin secretion, since LH levels were generally higher than FSH. Male gonad development was initiated in January and continued until May whereafter FSH levels declined. LH levels followed a similar pattern, but was generally higher than FSH levels. Both hormones also peaked at similar times in the plasma and gonads, thereby suggesting that both hormones are involved in gonad maturation and hydration. However, it appears that FSH is responsible for stimulation of steroid production by the Sertoli cells and that testosterone, together with LH is responsible for final maturation and hydration of spermatozoa. Thus, hormone levels in males are temperature dependent. A significant observation was the detection of HCG in the male gonad during January. This suggests that an

endogenous gonadotropin is formed in the testes to provide for a resting phase before testes development commences actively. It appears as if this factor is temperature dependant. Furthermore, it was not detected in the blood. This suggests that it is not secreted by the pituitary gland. the role of these hormones also follow male GSI levels, suggesting that during January male breeding activity is being reduced. The role of the inter-renal tissues were not evaluated, but the biochemical parameters recorded in the gonads, suggest the intervention of this system. These were evidenced in the mobilization of proteins, glucose and lipids as well as the associated changes in electrolytes. These changes support the possible involvement of mineralo- and glucocorticoids in gonad function. The gonad electrolytes suggest that sodium is responsible for the immotility of the sperm in the gonads which declines sharply after August. In contrast, gonad potassium remains high with a slight decline during the breeding season. This suggests that potassium is responsible for maintaining immobility of sperms in the gonads during the active breeding season. It may also explain the hydration of male gonads where potassium may be responsible for the increase in semen fluid.

CHAPTER 8

Résumé and Recommendations

8.1 Résumé

As a conclusion to this study, whereby a seasonal investigation into the reproductive physiology of the mouthbrooding tilapia, ***Oreochromis mossambicus*** was carried out at Syferkuil Dam, 8km northwest of the University of the North, a number of factors appear to contribute toward the initiation, regulation and maintenance of the breeding cycle. These factors are not listed in any order of priority, but include the following.

During the winter months (June and July) dam water parameters fall appreciably. This could well be the stimulus that prevents the initiation of female gonadal development. The aquatic environment, in particular, an increase in rainfall during September (spring), coupled with increasing photoperiod, environmental temperature, relative humidity, dam water pH and wind speed at the same time, appear to be the stimuli required for final gonadal maturity in the female.

Although male ***O.mossambicus*** gonadal development appears to be initiated at the same time as the female, there appears to be an approximate two month "lag" period prior to reproductive maturity in the male being attained. However, this was associated with the size of the males. Smaller males showed a more significant relationship between gonad and body mass.

This study has shown that reproductive development in ***O.mossambicus*** is associated with changes in blood and gonadal pH together with a concomitant increase in the gonadosomatic index (GSI) values calculated from the gonadal and parent fish masses. The role that is played by the blood and gonadal pH is

interesting in that it would appear that an "acidic female" and an "alkaline male" are required to optimize breeding. This then suggests that male breeding behaviour is stimulated by female reproductive maturity being reached. The male could therefore be considered to be the "rate limiting" factor in the entire breeding process and that urinary excretion of steroid metabolites may be the stimulatory factor to initiate nesting in this species.

The male gametes (sperm) are produced in the two bilaterally arranged testes, with the size of the testes being correlated to the size and stage of reproductive development. The spermatogonia mature and are subsequently stored in the seminiferous tubules, with a dense packing of spermatozoa being noted during spring and early summer. The mature sperm do not possess an acrosomal cap as do mammalian sperm. However, there is a distinct head region containing the chromatin, a "collar" or neck containing mitochondria for energy and a flagellum that provides the mature sperm with their motility.

The female gonad is also a paired structure in which a number of developmental stages may be identified. These stages include a primary oocyte developing phase (late summer / early autumn), a yolk production phase (autumn), an early maturation phase (winter) and a final maturation and spawning phase (spring / early summer).

A further aspect of the present study was to measure the concentrations of the reproductive hormones on a seasonal basis. The results obtained confirm the postulate that the hormones play a major role in initiating, regulating, controlling and maintaining the reproductive cycle in the tilapia, ***Oreochromis mossambicus***.

The gonadotropins', luteinizing hormone (LH) and follicle stimulating hormone (FSH), secretion is stimulated by a rise in the temperature of the water in which the fish live. These gonadotropins also appear to be an important cue for the release of the steroid hormone testosterone. The gonadotropins may optimize

successful fertilization of the released ova.

A number of chemical constituents that were measured in the plasma and gonads of ***O.mossambicus*** seem to play a meaningful role in the reproductive cycle of this fish.

Sodium levels appear to maintain the osmolarity of the seminal fluid, with an increase in concentration leading to the initiation of sperm motility. Potassium concentrations appear to contrast with those measured for sodium in that the former reach high levels during winter. Potassium, in such high concentrations, inhibit the spermatozoa's motility within the testis during this developmental period. As does sodium, calcium appears to play a role in regulating and initiating sperm motility. It does however, also seem to stimulate the secretion of the gonadotropic hormones luteinizing hormone (LH) and follicle stimulating hormone (FSH). Calcium further appears to be involved in the fertilization process of the spawned ova.

The glucose that was measured in both plasma and the gonads could be important as an alternative or secondary energy source, particularly for sperm motility and ovum maturation. Lipids probably also provide energy to the developing gonads. In addition, the lipids are important as structural components of cell membranes and may provide a protective role against temperature fluctuations encountered by the spawned gametes. Proteins are probably predominantly protective, but may also control colloidal osmotic pressure of the body fluids, particularly semen. The seasonal variations in lactate levels that were observed are indicative of adenosine triphosphate (ATP) production which is the primary energy source for all living systems. The urea measured would indicate that protein is being metabolized which is indicative of gonadal development and maturation.

Female ***Oreochromis mossambicus*** does not display a four season reproductive

cycle as suggested by van der Merwe (1984) and Fouché (1985) for ***Labeo capensis*** and ***Clarias gariepinus*** respectively. The results clearly suggest that this species is a temperate one with reproduction occurring mainly during temperate conditions as confirmed by Balarin (1979). The endocrine mechanisms involved during gonad development conforms in broad outline with those known for other teleosts. Two stage gonadotropin secretions for gonad development have been identified that are similar to those described by Peute ***et al.*** (1986). It therefore confirms the two hormone hypothesis for many fish species.

The gonadotropins appear to be responsible for primary and secondary oocyte development through exogenous yolk uptake and endogenous yolk formation. These hormones also stimulate steroid production and vitellogenesis as confirmed by 3 β HSD activity. Unbound calcium binds with vitellogenin during yolk incorporation into the oocytes. Sex steroids produced by the oocytes appear to be primarily responsible for vitellogenesis and may contribute indirectly to oocyte maturation, hydration and ovulation. Their presence in gonad extracts after the initial ovulation phase may be linked to atresia of follicles. The role of mineralo- and glucocorticoids remain to be evaluated in future studies of this nature but may also be involved in oocyte development, hydration and ovulation as indicated by the mobilization of glucose, lipids and proteins. Oocyte development within the breeding season are characterized by primary oocyte development, followed by pre-vitellogenic oocytes, secondary oocytes, oocyte atresia, vitellogenin incorporation and yolk formation followed by oocyte hydration and ovulation. This sequence of events is followed by a resting phase and subsequent gonadal recrudescence.

Male ***Oreochromis mossambicus*** showed a similar reproductive cycle to females, but with significant differences. In general, all males used for this study were sexually mature. The results suggest that males were generally of a bigger size than females. The testes are of a tubular type whereby mature spermatocytes are released into the seminiferous tubules. The occurrence of blood vessels were

positively identified by the fact that arteries appeared round, whereas veins had a more flattened appearance. Mature and immature spermatozoa were identified with SEM which indicated that immature sperms (primary and secondary spermatids) were characterized by wrinkles on the surface of heads and had a significantly shorter collar than mature spermatozoa. Semen hydration is primarily responsible for the increase in GSI. However, it appears that this hydration is more significant than in large mature males. Testes development is also not season dependent, although hydration is controlled by external environmental factors. It also appears that the urinary excretion of steroids in females, may act as a type of pheromone to stimulate gonad hydration and ejaculation. This was observed in the beginning of August when males were ready for breeding. Thus, testes development is already initiated during the colder months, since stage V and VI gonads were identified at the end of July and during August. During the entire breeding season, with the exception of December, these late stages of testes development were observed. The presence of 3 β HSD activity was noted throughout the year, but distinctly less during the coldest part of the year.

The results recorded in this study, support the role of environmental factors being responsible for an endogenous regulation of male reproductive activity. The presence of both FSH and LH activity also confirms a two stage control of spermatogenesis and testes development. It involves the sperm development directly or indirectly via the synthesis of steroids in the testes and possibly also by the inter-renal tissues. During the colder months of the year, the sperms are stored in the seminiferous tubules where they are maintained by the actions of steroids from the testes or inter-renal tissues. The measurement of electrolytes in the testes suggests an active role for corticosteroids. The results recorded suggest that FSH is responsible for spermatogenesis and testes development and that LH may be responsible for testes hydration together with testosterone. The latter appeared to be primarily responsible for gonad hydration. FSH may therefore be responsible for the induction of testosterone formation in the testes. It also involves LH. However, the role of other trophic hormones in the regulation

of the reproductive cycle still need to be confirmed in future investigations of this nature. Fluctuating levels of these hormones were observed during the breeding season. This may be an indication of the different ejaculatory phases when female gonadal development reaches a peak for the different age groups. Testosterone also seems to be dependant on water temperature as a stimulus to it being secreted. This hormone is important in terms of testicular development and maturation, in particular spermatogenesis.

Progesterone appears to increase in concentration prior to the completion of the mouthbrooding behaviour, and this could suggest that there is a decrease in the conversion of this hormone to other steroids, or it could indicate an overall increase in progesterone secretion. Estradiol 17-β seems to be important in the stimulation of vitellogenesis. The limited results received for human chorionic gonadotropin (HCG) and the equally limited literature to compare the results to, suggest that this hormone may play a role in the final maturation of oocytes in female ***O.mossambicus***.

8.2 Validity of the findings

Pituitary gonadotropins are not the only trophic hormones secreted by the pituitary gland. It is known that vertebrate gonadotropins are almost identical to fish gonadotropins with minor differences in amino acid sequence. Antibodies are developed specifically for the β subunits of vertebrate gonadotropins. Although minor differences may be evident, the possibility of cross reactions with other trophic hormones may have an influence on the values measured. It is therefore recommended that specific antibodies for fish gonadotropins be developed to determine their levels accurately. The same principle is applicable to the use of HCG RIA kits. It is conceivable that an inhibitory hormone could be secreted by the pituitary gland. The possibility also exists that an endogenous gonadotropin may be secreted by the gonads. This problem needs to be addressed. Thus the identity of the gonadotropins in ***Oreochromis mossambicus*** is unknown, although

the values recorded were considered as legal. However, the findings confirm a two hormone hypothesis for the regulation of reproduction in ***Oreochromis mossambicus***. However, reports by van der Merwe (1984) and Fouché (1985) suggest that all other hormones in fish are similar to vertebrate hormones, in particular the steroid hormones.

8.3 Recommendations

The present study has resulted in a number of questions being left unanswered and therefore a series of recommendations are presented here that will lead into future studies in order that a greater understanding of the reproductive cycle in ***Oreochromis mossambicus*** may be achieved.

In terms of the microscopic examination, a number of new techniques pertaining to both embedding and sectioning of the material have been established. This is of greatest importance in the study using the transmission electron microscope. An examination of the pituitary gland, in particular, a histochemical study would provide an indication as to the specific cells responsible for gonadotropin secretion. Such a histochemical study could also be conducted on the gonads in order to establish sites of synthesis and secretion of the steroid hormones. By arresting the fertilization process as the sperm enter the ova, an understanding of the fertilization process may be arrived at.

An initial investigation into sperm motility has already been started. A more detailed study, whereby sodium, potassium and calcium levels may be manipulated is envisaged. In addition to monitoring the dam water in which the ***O.mossambicus*** live for various heavy metals and pollutants, the plasma and gonadal supernatant levels of such pollutants and heavy metals could provide meaningful information as to a possible role in the reproductive cycle of this fish. It is possible, that this type of study will show up a number of negative aspects of the breeding pattern of ***O.mossambicus***.

If a sizeable enough sample could be collected, a hormonal profile for the gonadotropins within the pituitary gland would assist in finalizing the continuing debate as to whether there are one or more hormones constituting the gonadotropins in tilapia. An investigation into the gonadotropic releasing hormone (GnRH) levels could also be attempted. The present study made use of FRANSA kits to determine hormone levels in ***O.mossambicus***. These kits have been developed for use with human samples. Therefore, in order that more accurate, quantitative results for measured hormone concentrations be achieved, an assay specifically for the tilapia could be developed.

This study was restricted by a basic investigation into a few hormones only. It formed part of a series of investigations into the reproductive cycles of several indigenous freshwater fish species. Future investigations should concentrate on the role of other pituitary hormones, such as prolactin, TSH, MSH and ACTH in the reproductive cycle. The role of the inter-renal tissue should also be determined. Cyclic AMP is considered to be a second messenger in the mediation of a number of hormones actions on their target cells and tissues. The role of cAMP should be evaluated for freshwater fish reproduction, and in particular in ***Oreochromis mossambicus***. The evaluation of these trophic and steroid hormones would contribute significantly to a better understanding of oocyte maturation and ovulation and the nutrient and energy requirements during reproduction. The information gained in this way, will enable the evaluation of suitable methods to promote artificial propagation techniques under controlled laboratory conditions. Analysis of eggs and larvae for different hormones may change the strategy for manipulating reproduction in fish, since such information may be beneficially used to stimulate oocyte growth, maturation, ovulation and growth of larvae.

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Erratum

To PhD Thesis of Daryl A. Cornish

Pages 65 and 66
Yunlin, 1987 should read Zhu, 1987
Page 88
Van der Merwe *et al*, 1984 should read Van der Merwe, 1984
Page 90
Van Vuren & Soley, 1986 should read Van Vuren & Soley, 1984
Fawcett, 1960 should read Fawcett, 1970
Page 98
Fostier & Jalabert, 1978 should read Fostier & Jalabert, 1982
Page 240
Fishelson, L 1987 should read Fishelson, L 1988
Page 254
M^{c}Bay, LG 1963 should read M^{c}Bay, LG 1961

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Widodo (1986) and Khumar & Siddiqui (1991) were not found to be relevant and should be removed from the thesis.