# The effect of pH and selected chemical variables on the reproductive cycle of *Oreochromis mossambicus*

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## Abstract

Adult male and female *O. mossambicus* from Syferkuil Dam, situated 28 km NW of Pietersburg, were analysed for gonad mass and length, gonadosomatic indices, blood pH and gonad pH over a twelve-month experimental period. Male development seems to be dependant on female development in that it "lags" behind the female by two months. Male GSI reaches a peak of 0.78 ± 0.12% during November as opposed to the female maximum of 3.11 ± 0.72% during September. Blood and gonadal pH show an inverse relationship when breeding is prevalent. Male gonads are more alkaline (7.72 ± 0.29) and females more acidic (6.94 ± 0.16) during September. The chemical composition of the blood plasma and gonadal supernatant varied considerably over the experimental period. Glucose, lipids, lactate and proteins are all involved in energy production or provide a protective function to developing gonads. The presence of urea is indicative of protein metabolism. A breeding; resting and gonadal recrudescent season may be distinguished in both male and female *O. mossambicus*.

## Introduction

Many tilapias, including *O. mossambicus*, are good candidates for fish farming due to their robustness, wide distribution, significant growth rate and ease of reproduction. Tilapias are generally deep-bodied, with a predominantly vegetarian diet that is reflected in their small teeth, fine pharyngeal teeth and extended intestines (Skelton, 1993).

According to Skelton, (1993) the Mozambique tilapia (*O. mossambicus*) breeds during summer, with the female raising multiple broods every three to four 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 (Baroiller and Jalabert, 1989).

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. Tilapias are generally tolerant of wide temperature and salinity ranges, and *O. mossambicus* is able to live and breed in both fresh- and seawater.

Studies on body and gonad mass are common in determining the reproductive stage of freshwater fish and a gonadosomatic index (GSI) has been calculated to determine the reproductive maturity of gonads (Nel, 1978).

The pH of both the blood and the gonad is also used as an indicator of reproductive maturity (Morisawa and Morisawa, 1988). 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 (Morisawa and Morisawa, 1988).

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Length-mass relationships have been examined by Taphorn and Lilyestrom (1983) in *Curimatus magdalenae* and by Marshall and Echeverria (1989) in the monkeyface prickleback *Cebidichthys violaceus*.

The chemical composition of both the plasma and gonads of freshwater fish may provide an indication of homeostasis and health status during reproductive development. Lipids (Rao and Rao, 1984; Besnard et al., 1989; Garcia-Garrido et al., 1990), proteins (Mukhopadhyay et al., 1986; Mukhopadhyay et al., 1987) and urea (Grubinko and Yakovenko, 1987) have been examined.

The presence or absence of inorganic and organic components and the osmolality and pH of the plasma and the gonads in association with the reproductive cycle are considered to be important (Ginzburg, 1972; Scott and Baynes, 1980).

Mukhopadhyay et al. (1986) have stated that mature fish eggs 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*.

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 *O. mossambicus*. Their results suggest that lipids are utilized to mitigate any condition and as reproduction is a high stress condition, considerable lipids ought to be present during the spawning period of fish. When lipid levels are reduced it could be due to increased gluconeogenesis being induced, particularly in the liver and muscle.

**TABLE 1**
**MALE *O. MOSSAMBICUS* MEAN BLOOD AND GONAD PH, STANDARD LENGTH (cm), GONAD LENGTH (cm), FISH MASS (g), GONAD MASS (g) AND GONADOSOMATIC INDEX (%) (SAMPLES TAKEN PER MONTH, N = 40)**

| Month | Standard length (cm) | | Gonad length (cm) | | Fish mass (g) | | Gonad mass (g) | | Gonadosomatic index (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | mean ± sd | | 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 | 0.28 | 0.08 |
| Jun | 28.16 | 1.67 | 7.16 | 0.91 | 394.05 | 75.15 | 1.22 | 0.06 | 0.31 | 0.14 |
| Jul | 28.49 | 0.92 | 7.24 | 0.55 | 378.65 | 94.11 | 0.88 | 0.03 | 0.22 | 0.09 |
| Aug | 27.89 | 1.54 | 6.96 | 0.91 | 370.87 | 66.61 | 0.79 | 0.04 | 0.21 | 0.08 |
| Sep | 26.14 | 2.16 | 6.25 | 1.22 | 313.66 | 97.26 | 1.07 | 0.07 | 0.33 | 0.17 |
| Oct | 23.65 | 3.13 | 6.46 | 0.96 | 238.68 | 79.09 | 1.06 | 0.07 | 0.43 | 0.25 |
| Nov | 23.05 | 2.34 | 6.03 | 1.06 | 229.52 | 70.92 | 1.80 | 0.04 | 0.78 | 0.12 |
| Dec | 25.24 | 2.09 | 6.67 | 1.00 | 304.16 | 97.99 | 1.17 | 0.04 | 0.39 | 0.12 |
| Jan | 25.02 | 1.86 | 6.28 | 1.06 | 298.39 | 99.39 | 1.17 | 0.07 | 0.39 | 0.09 |
| Feb | 24.72 | 1.59 | 6.52 | 1.49 | 293.84 | 76.75 | 0.54 | 0.04 | 0.19 | 0.05 |
| Mar | 20.78 | 1.39 | 4.87 | 1.40 | 172.33 | 66.45 | 0 33 | 0.06 | 0.19 | 0.07 |
| Apr | 25.09 | 2.42 | 6.15 | 1.21 | 271.95 | 90.54 | 0 60 | 0.06 | 0.22 | 0.04 |

**TABLE 2**
**FEMALE *O. MOSSAMBICUS* MEAN STANDARD LENGTH(cm), GONAD LENGTH (cm), FISH MASS (g), GONAD MASS (g) AND GONADOSOMATIC INDEX (%) (SAMPLES TAKEN PER MONTH, N = 40)**

| Month | Standard length (cm) | | Gonad length (cm) | | Fish mass (g) | | Gonad mass (g) | | Gonadosomatic index (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | mean ± sd | | 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 | 0.51 | 0.13 |
| Jun | 25.03 | 1.49 | 4.99 | 0.41 | 248.70 | 53.33 | 1.42 | 0.05 | 0.57 | 0.17 |
| Jul | 25.06 | 1.30 | 5.00 | 0.58 | 261.39 | 50.42 | 1.67 | 0.06 | 0.64 | 0.17 |
| Aug | 25.00 | 1.02 | 4.67 | 0.62 | 259.89 | 35.19 | 1.52 | 0.06 | 0.59 | 0.19 |
| Sep | 24.29 | 2.68 | 5.63 | 0.71 | 256.58 | 71.03 | 7.52 | 0.36 | 3.11 | 0.72 |
| Oct | 24.12 | 2.35 | 5.46 | 0.78 | 252.78 | 75.91 | 6.62 | 0.37 | 2.62 | 0.86 |
| Nov | 24.26 | 2.24 | 5.10 | 0.54 | 240.40 | 67.48 | 6.68 | 0.29 | 2.74 | 0.74 |
| Dec | 23.99 | 2.27 | 4.96 | 0.73 | 240.77 | 66.99 | 3.45 | 0.37 | 1.62 | 0.29 |
| Jan | 24.63 | 1.93 | 4.79 | 0.69 | 242.19 | 74.69 | 1.61 | 0.10 | 0.76 | 0.13 |
| Feb | 25.09 | 1.48 | 4.84 | 0.93 | 263.26 | 70.71 | 1.87 | 0.17 | 0.71 | 0.25 |
| Mar | 22.78 | 1.80 | 4.23 | 0.67 | 227.19 | 77.18 | 0.70 | 0.15 | 0.36 | 0.13 |
| Apr | 26.28 | 2.00 | 4.71 | 0.89 | 282.59 | 91.19 | 1.32 | 0.13 | 0.46 | 0.12 |

Besnard et al. (1989) report that as a result of a seasonal study, neutral lipids may be indicative of sexual maturity.

The fact that Syferkuil Dam forms part of the effluent of a sewage system would suggest that urea levels could be important. Grubinko and Yakovenko (1987) have shown that urea levels in fish 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 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. An examination of these aspects of the reproduction of *O. mossambicus* is intended to provide a clearer basis for correlation with blood pH, gonadal pH and environmental water pH.

## Materials and methods

Every Monday morning, 10 adult male and 10 adult female *O. mossambicus* specimens were collected at Syferkuil Dam, 28 km NW of Pietersburg using a seine net. Syferkuil Dam is an interconnected series of 8 dams with cement sides and mud bottoms. The period of collection lasted for 12 months. A 2.5 mℓ blood sample was collected at the dam using a heparinised syringe and the cardiac puncture method to determine blood pH levels. The blood was then centrifuged to obtain a plasma sample. The fish were then transported back to the laboratory in oxygenated water

containing 20 mg/ℓ neutralised MS222 according to the method of Smit (1980). In the laboratory, standard fish length, gonad length, fish mass and gonad mass were recorded. The GSI was calculated for each fish analysed throughout the sampling period according to the formula of Roff (1983).

The gonads were then excised and homogenised using equal parts of distilled water to the gonadal mass. After centrifugation the supernatant was drawn off and the pH measured using a Copenhagen radiometer blood gas analyser.

Glucose concentrations were determined in both plasma and gonadal homogenate supernatants by the GOD-Perid method, using a 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 and 124 842) using the sulphophosphovanillin 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.

## Results

Tables 1 and 2 show that the male fish collected throughout the experimental period ranged in size from a mean of 20.78 ± 1.39 cm to a maximum mean of 28.49 ± 0.92 cm. The range of lengths recorded in the female was from a mean of 22.78 ± 1.80 cm to a maximum mean of 26.28 ± 2.00 cm. 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 from September to November, turning a deep greyish black with a white lower head and throat.

Figure 1 shows that male gonadal pH reached a peak of 7.81 ± 0.26 during July, which is mid-winter. Male blood pH followed a similar pattern to gonadal pH; however, although blood pH also peaked in July its maximum was 7.63 ± 0.22. Female gonadal pH showed an inverse relationship to male gonadal pH with female pH reaching a low value of 6.91 ± 0.31 during August. However, female blood pH values tended to exhibit a similar trend to male *O. mossambicus* blood, with a high value for the female being 7.52 ± 0.12 during August.

According to Fig. 2 there appears to be an inverse relationship between male blood and gonadal pH and 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 was high and from June till August when pH was at its lowest. Once male GSI reached its peak in November, both blood and gonadal pH had begun to decline. The most significant observations recorded for male blood pH indicated significantly higher levels during the months of July till September. Thereafter it declined from September right through till February. Similar observations were made for gonad pH. In both instances it appears to reflect a pH surge from July to September that could be essential for gonad maturity.

Male GSI (Fig. 2) was considerably lower than female GSI (Fig. 3) throughout the experimental period, only reaching a maximum of 0.78 ± 0.12 during November. Tables 1 and 2 show gonad mass in females to be considerably higher than in males, particularly just prior to spawning which also affects GSI. However, it increased steadily during September and October and then

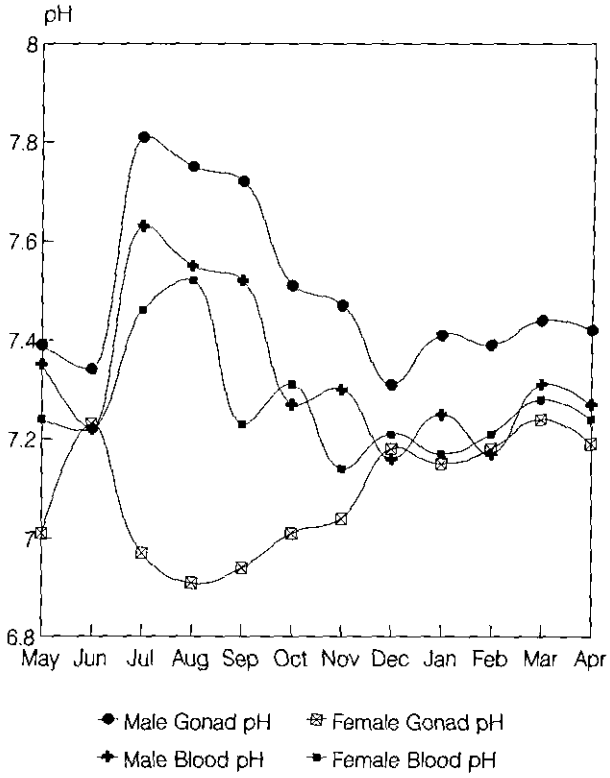


***Figure 1***
*Mean monthly blood and gonadal pH in male and female* O. mossambicus

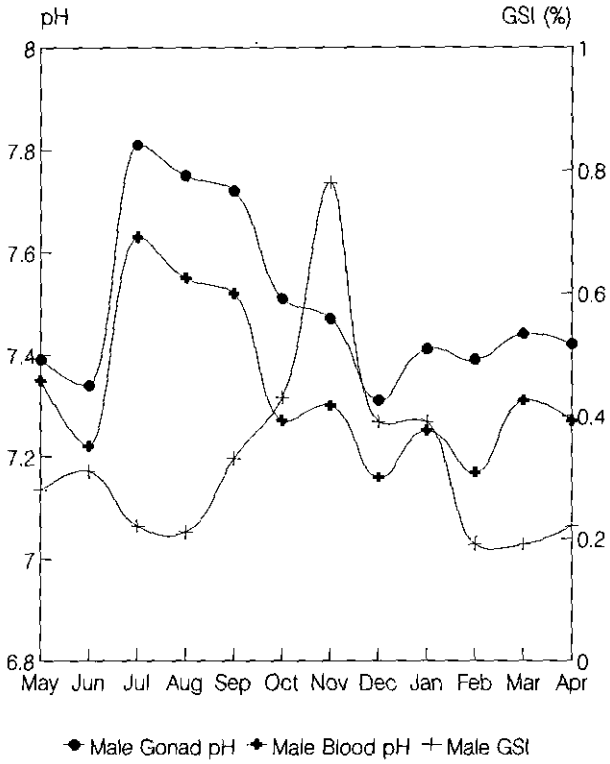


***Figure 2***
*Mean monthly blood and gonadal pH and GSI in male* O. mossambicus

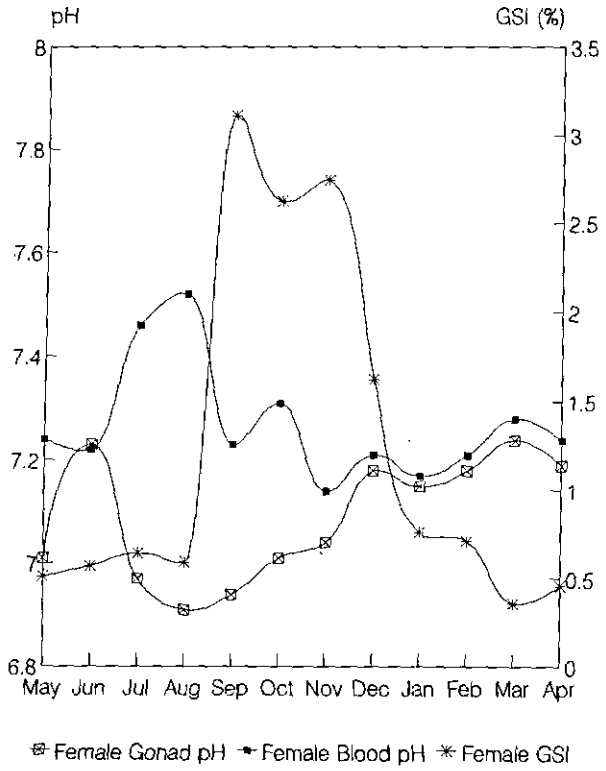


***Figure 3***
*Mean monthly blood and gonadal pH and GSI in female* O. mossambicus

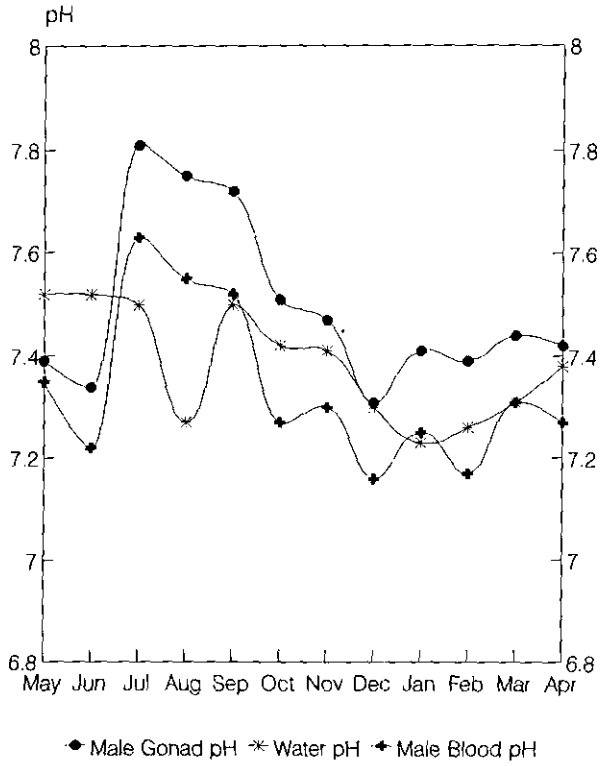


***Figure 4***
*Mean Syferkuil Dam water pH and blood and gonadal pH in male* O. mossambicus

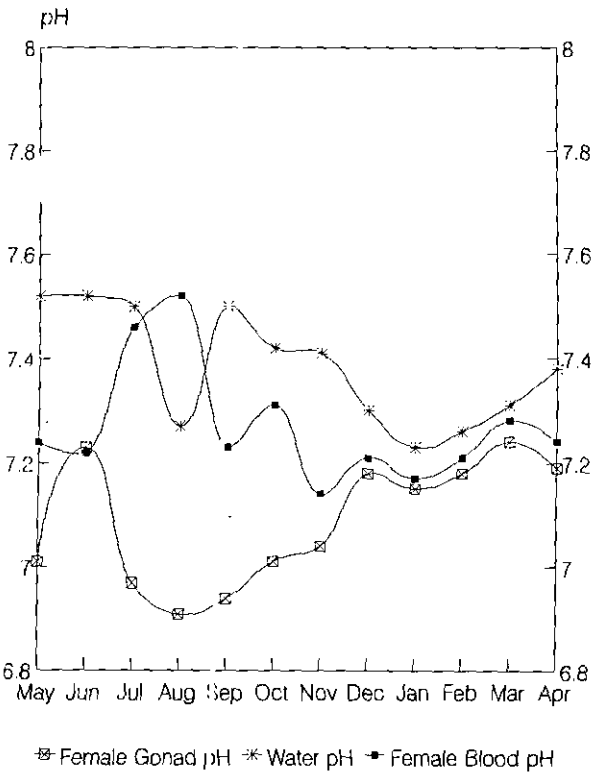


***Figure 5***
*Mean Syferkuil Dam water pH and blood and gonadal pH in female* O. mossambicus

declined sharply from February. Thereafter a period of gonad quiescense was observed. Male GSI values increased significantly ($p < 0.1$) from the months of September to January, reaching a peak during November. Male GSI therefore suggests a three-phase cycle, with the GSI peaking during November. Figure 2 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 was arrested until a period of maturation and ovulation related to size frequency distribution was observed during September, October, November and December. February and March are considered to be the gonadal resting period.

Figure 3 shows that in female *O. mossambicus*, there appears to be an inverse relationship between blood pH and gonadal pH, notably from June till November. Female GSI reached a maximum of $3.11 \pm 0.72$ during September and remained high till November whereafter it declined markedly. During this period, four different peaks were recorded, i.e. September, October, November and December. The period from March to July showed a gradual increase in female GSI. GSI remained significantly ($p < 0.01$) higher during September to December with an intermediate phase following during January and February. Females experienced 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 was slightly higher than the August levels from October to November. Thereafter gonad pH increased significantly up to April. This clearly indicates a three-phase gonadal pH cycle in females.

Figure 4 indicates that, as water pH declined in July and August, so both male blood and gonadal pH increased and thereafter followed a similar pattern for the remainder of the

experimental period. This probably indicates a relative alkalinity in the males. Figure 5 shows that as water pH began to decline, so the blood pH in females increased. However, at this same time, the gonadal pH declined markedly resulting in an "acidic female".

There appears to be an inverse relationship between male and female plasma glucose concentration from June until December (Fig. 6). Female plasma glucose concentration reached a minimum of 1.09 ± 0.14 mg/100 mℓ during September whilst male plasma glucose peaked at 12.57 ± 2.24 mg/100 mℓ in October. Both males and females displayed a biphasic cycle. In males, it extended from July to October whereafter a sharp decline occurred. Female plasma glucose levels showed a steady decline from June to reach their lowest value in August. Thereafter it increased markedly until December whereafter a sharp decline occurred. In general, male glucose levels were higher than female values with a direct relationship between the two observed throughout the year.

Female gonad glucose levels were generally double those for males over the entire period. Female gonad glucose levels showed a bimodal cycle which peaked during August and November. Males displayed a three-phase gonad glucose cycle; a low in August followed by a high in October and another low in January. There appeared to be a close relationship between male plasma and female gonadal supernatant glucose levels. A similar relationship was seen in the female plasma and male gonadal supernatant. Furthermore, in both males and females, there appeared to be an inverse relationship between plasma and gonadal supernatant glucose concentrations. Male plasma glucose levels increased sharply from July and remained high until October. Thereafter they 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. 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.

There was a good correlation (r = 0.87) in lipids between male and female plasma lipid concentrations with maximal values of 838.25 ± 21.52 and 1280.63 ± 50.20 mg/100 mℓ respectively being observed in September (Fig. 7). Thereafter, both male and female plasma lipid concentrations levelled out between November and December whereafter they declined. A slightly weaker correlation (r = 0.55) between male and female gonadal supernatant lipid concentrations existed. In this case, a single sharp increase was observed for both males and females during September. However, female levels remained high until November whereas male levels declined sharply in October to remain low thereafter. As was noted in the plasma, gonadal supernatant lipid concentration reached maximal values of 1143.88 ± 49.63 and 2222.05 ± 30.63 mg/100 mℓ respectively in males and females during the month of September. However, it may be seen that in the female, gonadal supernatant lipid concentration remained relatively high till November, whereafter it declined markedly. 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 was higher than in the male throughout the experimental period. In general gonad levels for both males and females were higher than plasma levels, except in males from October to April where the situation was reversed.

Figure 8 represents the total protein concentration (mg/100 mℓ) in male and female *O. mossambicus* plasma and gonadal super-

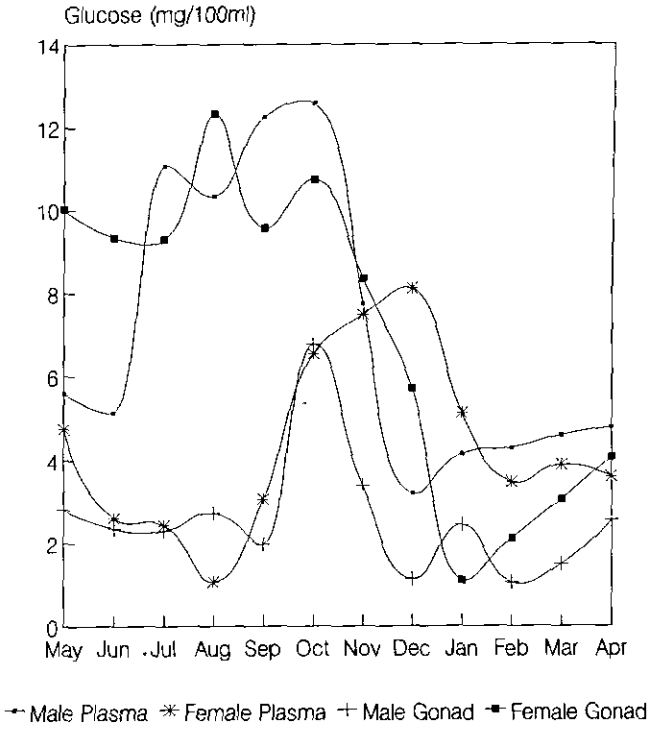


***Figure 6***
*Mean monthly plasma and gonadal glucose levels in male and female* O. mossambicus

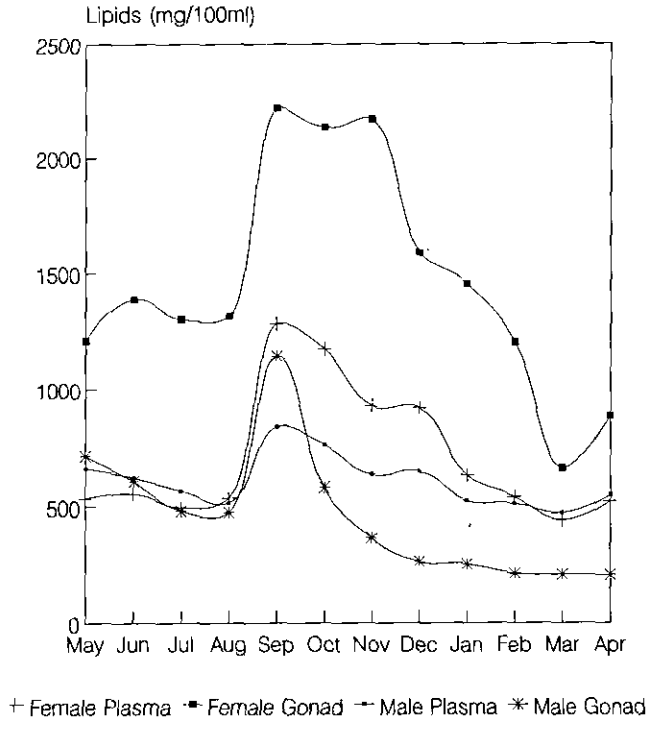


***Figure 7***
*Mean monthly plasma and gonadal lipid levels in male and female* O. mossambicus

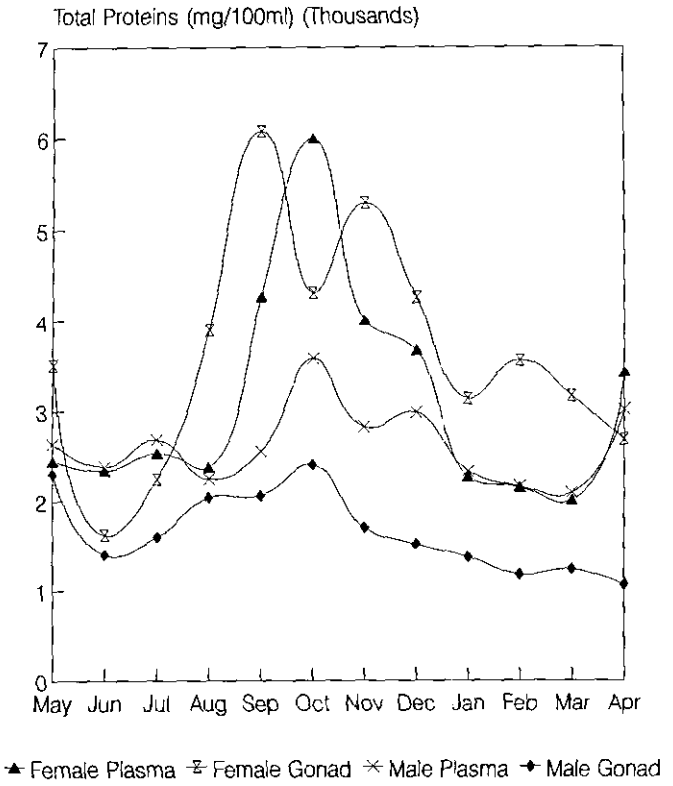


***Figure 8***
*Mean monthly plasma and gonadal protein levels in male and female* O. mossambicus

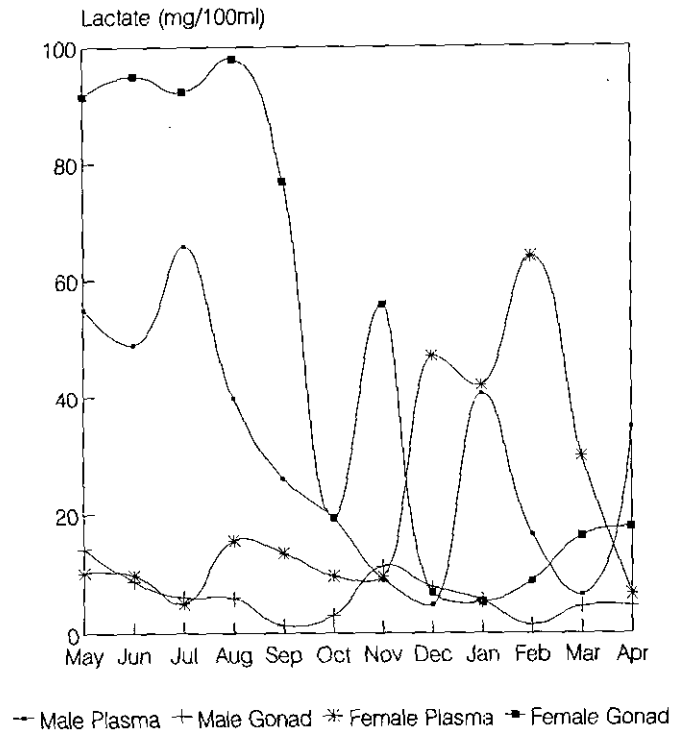


***Figure 9***
*Mean monthly plasma and gonadal lactate levels in male and female* O. mossambicus

natant. 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/100 mℓ respectively being observed in October. In both cases, a biphasic cycle was observed in October and December. During this period, female plasma levels were higher in females. Apart from the period from September till December, the plasma protein concentrations in *O. mossambicus* appeared to be much the same in both males and females. Female levels were significantly higher than male values. Female values displayed 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 reached a maximum of 6082.11 ± 247.28 mg/100 mℓ, whereas the male reaches its peak of 2403.63 ± 34.24 mg/100 mℓ 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 appeared to remain at a higher level than that observed in the male throughout the experimental period. It may be noted that in the male, both plasma and gonadal supernatant total protein concentration reached a peak during October, although the plasma concentration was higher than that measured in the gonadal supernatant. This contrasted with the situation in the female, where both plasma and gonadal supernatant total protein concentration peaked at a similar concentration, but the gonadal concentration peaked in September, whereas the plasma concentration reached its maximum one month later in October. Furthermore, the gonadal supernatant was generally higher than the corresponding plasma concentration for each month which was opposite to that recorded for males.

Figure 9 represents the lactate concentration (mg/100 mℓ) in male and female *O. mossambicus* plasma and gonadal supernatant. In male plasma, there appeared to be a peak in lactate concentration of 66.64 ± 1.65 mg/100 mℓ during July and a second peak of a lower magnitude (40.41 ± 2.15 mg/100 mℓ) during January. In female plasma, the lactate concentration increased markedly in December and remained relatively high until it reached a maximum of 63.92 ± 6.34 mg/100 mℓ in February whereafter it declined again. Female plasma lactate levels showed a three-phased increase in August, December/January and February whereafter a noticeable decline was observed. From May to September, male plasma levels were significantly higher ($p < 0.001$) than female values. From November to March, this position was reversed where female levels were significantly higher. There appeared to be a virtual year-round inverse relationship between male and female *O. mossambicus* gonadal supernatant lactate concentrations. The male lactate level attained a maximum of 11.13 ± 0.68 mg/100 mℓ during November. In general, levels displayed a four-phase cycle during August, November, January and March. Females, on the other hand, had high lactate levels from May until September, whereafter the levels fell off markedly. Although a maximum of 97.21 ± 3.90 mg/100 mℓ was reached in August, a second, smaller peak of 56.78 ± 2.83 mg/100 mℓ may be noted during November, thus indicating a biphasic cycle. However, both males and females showed two corresponding peaks during August and November.

Male plasma lactate 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 also varied with male GSI. Gonad levels were, however, considerably lower than plasma levels. Female plasma lactate levels 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 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.

Figure 10 represents the urea concentraion (mg/100 mℓ) in male and female *O. mossambicus* plasma and gonadal supernatant. There is a significant correlation ($r = 0.99$) between male and female plasma urea concentrations with maximal values of 148.76 ± 21.08 and 168.55 ± 10.86 mg/100 mℓ respectively being observed in July, which is during mid-winter in the Northern Province. 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 was recorded. A much weaker correlation ($r=0.54$) between male and female gonadal supernatant urea concentration exists. In both males and females, peak urea levels were reached later in the year. Male gonadal supernatant urea concentration reached a maximum of 93.67 ± 23.06 mg/100 mℓ during October. Female gonadal supernatant urea levels showed a marked increase in September, reaching a maximum of 196.39 ± 12.49 mg/100 mℓ 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. 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.

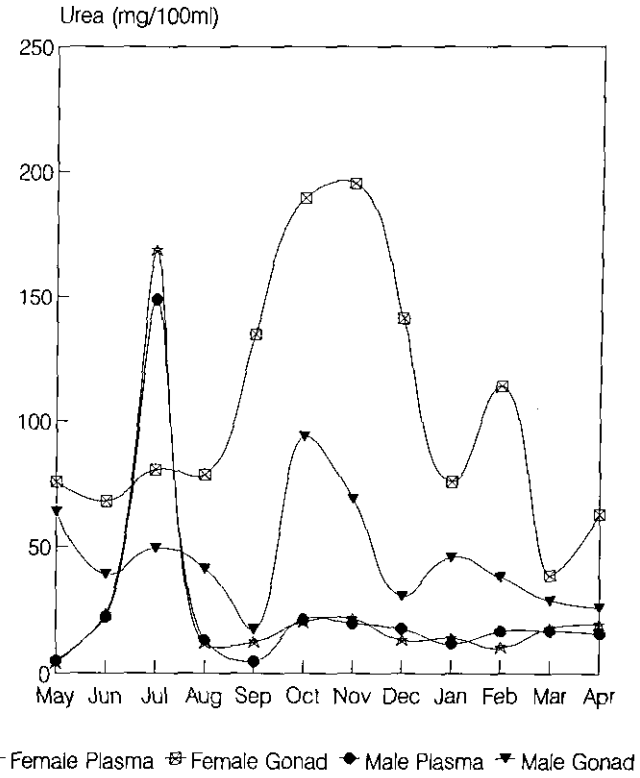


***Figure 10***
*Mean monthly plasma and gonadal urea levels in male and female* O. mossambicus

## Discussion

*O. mossambicus* is a mouthbrooder. During the period of this investigation, females with fertilised eggs or larvae in their mouths were not used for sampling, which probably explains the observation of the larger specimens breeding earlier than the smaller ones. Toward the end of August and during September, larger males and females were therefore more sexually active. As the breeding cycle continued, the smaller, younger specimens matured and joined the breeding cycle.

Through the months of September till January, there was a great fluctuation in male GSI. At the onset of the female breeding cycle in September, male gonad mass showed an initial inverse relationship with fish length. Thereafter it changed to a more direct relationship for those males observed. This implies that smaller male fish experience a greater increase in gonad mass relative to their size, when compared with larger males. This could explain the differences in the relationship between male gonad mass and fish length in the smaller specimens. From February the males collected were predominantly of a smaller size. However, it is clear that the mass increase in male gonads did not occur to the same significant extent as it did 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. Hydration in males did not show the same significant changes that were noted in females. This contributed 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. 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.

The female reached reproductive maturity (increased GSI) prior to the male, and therefore, in addition to the environmental cues such as temperature, photoperiod and rainfall (Cornish and Smit, 1995), female maturity may be a stimulus for the commencement of male gonadal development.

Figures 4 and 5 show 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. 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). The dam water pH increase could be associated with algal blooms at the end of the winter season as a result of increased relative humidity and the first rainfall after winter (Cornish, 1993).

The general decrease in dam water pH (Figs. 4 and 5) 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, may also be a factor at Syferkuil Dam with females. The decline in water pH was such that it would not have had an effect on inhibiting breeding activity, since water pH levels did not drop below a value of seven.

Figure 1 shows an inverse relationship between male and female *O. mossambicus* gonadal pH. During the month of August, which was prior to the surge in GSI, the male gonad became more alkaline (there is an increase in relative alkalinity) and the female gonad became more acidic.

Figures 2 and 3 show an inverse relationship between pH and

GSI in both male and female *O. mossambicus*, which, together with the fact that the female gonads reached 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 gonadal 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 suggests that during the breeding cycle, females showed a greater blood buffering capacity than males, indicating that the nature of the metabolic activities in females is different to males.

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, i.e. 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.

The changes in both blood and gonadal pH (Figs. 2 and 3) could be a further stimulus to at least the initiation of gonadal development in both male and female *O. mossambicus*. Reproductive development and metabolism were associated with changes in blood and gonadal pH as well as an increase in GSI values which corresponded with the state of gonadal maturity.

The marked increase in the concentration of glucose (Fig. 6) in male plasma during September and October and a much lesser increase in the gonad at the same time coincided with the increase in male GSI. Thus 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 fertilisation may occur. 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), who indicated that this increase was associated with increased plasma cortisol levels in both males and females.

In female *O. mossambicus*, there was a surge in glucose concentration in the plasma during the months subsequent to spawning and fertilisation. As a mouth brooder, this was when the fish required greater energy reserves, which could explain the increase in glucose during November until January. The female gonad showed a similar trend to the male plasma levels of glucose. Thus the female gonad, which was generally very immature during the months of June and July, showed a high glucose concentration, which may be used as an energy source for the ova which were beginning to develop. The inverse relationship between plasma and gonad glucose suggests a major shift from the plasma to the gonads to provide the nutritional and energy needs. Thus after spawning, a major increase in the plasma glucose level was 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* (Fig. 7) in the plasma and gonads. Male gonad lipid levels 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 energy reserves. Seasonal variations of phospholipid fatty acid contents are often related to changes in cell membrane properties. Thus the high concentration of lipids is 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 gluconeogenesis 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 specific role of protein in fish semen is unknown. It has been suggested that protein fulfils protective and nutritional roles (White and Macleod, 1963; Mann, 1964; Cruea, 1969). The present study showed a much greater quantity of total plasma protein (Fig. 8) 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 reflect 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 explains the increases in proteins and lipids associated with gonad maturation. The non-phosphorus lipids may be made up of neutral lipids. It was shown that in *Oreochromis niloticus* vitamins play an important role in transport of egg yolk proteins and the induction of steroidogenesis in the gonads.

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. Mobilisation of

glucose is a function of cortisol by the inter-renal tissues which results 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 fertilising 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 fertilisation. Furthermore, there appears to be an interdependence between lactate levels and pH in both the blood and gonads in this investigation.

Urea is considered in relationship to protein metabolism and total protein, particularly in animals having a seasonal sexual development. *Oreochromis mossambicus* has a defined breeding season (it is a summer spawner in a temperate climate). This study showed 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. There is a surge in urea 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 metabolised as reproductive maturity is taking place.

An apparent relationship between urea and protein 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 mobilisation 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 is associated with a decrease in the pH levels of the blood and gonads. Thus the mobilisation 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. It is also possible that such changes may be gonad-size related, since male GSIs are significantly lower than female values. The increase in lactate levels in females may occur as a result of a 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. Firstly, it may be related to closure of oocyte membranes and the formation of glucuronides responsible for fertilisation to occur. Secondly, 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 fertilisation and larval development to occur.

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