THE FECUNDITY AND PREFERRED BREEDING PERIOD OF THE AFRICAN CLAWED TOAD *Xenopus laevis* FROM ROCKWATER FISH FARM, JOS PLATEAU STATE

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ABSTRACT

The preferred breeding period and fecundity of *Xenopus laevis* sampled from Rockwater fish farm were studied within ten months. The analysis of gonadosomatic index (GSI) showed peak gonadal activity in August for males (1.51) and November for females (15.0). The number of eggs produced per female organism increases with body mass. Large individuals produced as much as 6,000 eggs. The length-weight correlation coefficients of males (r = 0.105) and females (r = 0.089) were not significantly different (P> 0.05). The influences of temperature and rainfall on the reproductive cycle are also discussed as well as the effect of water depth.

Key words: Xenopus laevis, breeding period, fecundity, Rockwater fish farm.

INTRODUCTION

The reproductive behaviour in most animals has been shown to be regulated in such a way that reproduction occurred under the most favourable environmental conditions which are necessary for the survival of the new generation of animals, Lawan (1989). After maturity animals in most cases, exhibit periodicity in their reproductive activities. Consequently, these animals have seasons induced by external environmental conditions and internal physiological changes such that favourable seasonal cycles always coincide with the reproductive cycle. Smith (1955) reported that gonads in animals like the amphibians undergo seasonal cycles involving changes in their size. Deucher (1975) observed that in the African clawed toad, moisture, more than any single factor plays a leading role in the timing of reproduction. And Savage (1961) discovered that the date of spawning in common frogs was significantly correlated with the rainfall and temperature experienced by the frogs several months before spawning. He suggested that temperature and rainfall act indirectly on spawning by stimulating the growth of pond algae. High rainfall causes additional plant

nutrients to be washed out of the land into ponds, thus accelerating the growth of pond algae (Beatle, 1985). Experiments with *X. laevis* led Savage (1961) to the conclusion that spawning was controlled by specific olfactory stimuli provided by certain chemical products of the algal flora in the breeding ponds.

Beurden (1979) reported that xeric anurans lack a well defined breeding season and that they exhibit opportunistic breeding patterns. Spawning is said to be the culmination of the adult reproductive cycle. Rastogi *et al* (1983) reported that during the non-breeding season the frog, population is dispersed over a wide area. While in early spring, at the time of a rising moon and/or following a heavy rainfall and a rising environmental temperature, the frogs move to the breeding water site.

In comparison to fishes, Lowe-McConnel (1975) noted that majority of fish species in the equatorial regions breed during the peak rainfall periods. It is said that many fish species breed all year round with peak reproductive activity during the wetter months. Jorgensen (1981) observed that

in anuran females that exhibit a pattern of ovarian cycle, recruitment of oocytes to vitellogenic growth phase takes place during a restricted period of time early in the annual period of body growth. Whereas the period of vitellogenic growth presumably coincides with the period of body growth.

X. laevis is a great waster of fish fry and a big threat to any meaningful fish farming efforts. A good knowledge of its reproductive dynamics will facilitate better control measures in the field.

MATERIALS AND METHODS

Samples of the African clawed toad, *X. laevis* were collected every week from the ponds of Rockwater fish farm over a period of ten months. Forty (40) samples were collected each month using seine nets. They were preserved in 10% formaldehyde and transported to the laboratory.

Identification of Sexes and Length-Weight Measurements

Identification of the sexes was done according to Brown (1970). The female is usually plumb and swollen with eggs, she can be distinguished from the male, not only by her greater size but by the presence of three labia, two of which are dorsal and one ventral to the cloaca.

The snout to vent length of each specimen was measured using a standard metre rule by the method of Rastogi *et al* (1982) and Haris *et al* (1987). The weight was measured using a Mettler balance (Model P1210). Both length and weight of the specimens were measured to the nearest centimeter (cm) and gramme (g) respectively.

Determination of Gonosomatic Index (GSI) And Estimation of Fecundity

Gonads of matured specimens were excised and weighed to the nearest milligramme (mg) and the GSI computed following Nikolsky (1963):

GSI = Gonad wt. In g

. . .

This formula was used to determine the GSI of both male and female toads.

Fecundity may be defined in broad terms

as the number of eggs produced by an individual in its lifetime (Lowe-McConnel, 1975). Various methods of estimating fecundity have been described. These include direct egg count, volumetric, gravimetric and van Bayer methods, (Moore 1975, Bagenal and Brown, 1978). The volumetric method was used in this work as follows:

- (i) The entire load of eggs in the ovary was dried after removal and the volume obtained by water-displacement in a graduated cylinder for each matured and gravid female.
- (ii) Three randomly selected aliquots of 100 eggs each were taken.
- (iii) The volume of each sample was then obtained by water displacement in a graduated cylinder.

The fecundity was finally estimated thus:

$$\begin{array}{c} X = & \underline{Vn} \\ V \end{array} \qquad \qquad \text{where} \\ \end{array}$$

- X = unknown number of eggs in ovary (fecundity)
- V = volume of the entire ovary
- n = number of eggs in a sample
- v = volume of eggs in the sample.

Determination Of Physico-Chemical Parameters

Physico-chemical parameters are usually determined for the biological monitoring of important aquatic organisms to assess environmental stress and physiological effects of such factors on populations. This work monitored dissolved oxygen (DO), temperature, pH and rainfall on monthly basis throughout the study period.

Dissolved Oxygen (DO) And Hydrogen-Ion Concentration (pH)

This was assessed monthly using the Alsterberg (Azide) method immediately after collection. DO values were calculated and expressed in milligrammes per litre (mg/l).

Oxygen concentration

entration = <u>V(D) x N(D) x 8 x 1000</u> Volume of sample

Where

 $V(D) = \text{volume of sodium azide } (\text{Na}_2\text{S}_2\text{O}_3) \label{eq:VD}$ used in titration

 $N(D) = normality of Na_2S_2O_3$ (0.025N)

pH gives a measure of the alkalinity or acidity of water.

Water samples were measured soon after collection using a corning pH metre (Model 220). Measurements were spread out throughout the duration of this research.

Temperature And Rainfall Data

Temperature was measured using a mercury thermometer daily from March to December. The average was computed for each month and the values expressed in degrees celcius (°C). Rainfall data was collected from the Meteorogical station of the farm where this work was carried out. Data was collected from the beginning to the end of the rains. Rainfall totals and cumulative totals were computed for each month and the results expressed in milimetres (mm).

Water Depth

Fluctuations in water level of the ponds where samples were collected was monitored by measuring the water depth using a metre rule. This was necessary in view of the rains and sometimes more water was allowed into the ponds resulting in changes in water depth.

Statistical Analysis

This was carried out so as to help interpret the data obtained during the study.

Chi-square test was used to assess the monthly sex ratio of *X. laevis* collected during the entire period of the investigation by the method of Kelly *et al* (1992) as follows:

J:K =	<u>(KA – Ja)</u> ²
	JKn
Here: a =	number of males in the sample
A =	number of females in the sample
J =	the ratio of males
K =	the ratio of females

n = the sum of a + A

The degree of association between mean length and weight of the specimens was measured by the correlation coefficient (r). Given by the formula.

$$r = \underline{\Sigma xy - \Sigma x\Sigma yn}_{\Sigma x^2 - (\Sigma x)^{2/n}} (\Sigma Y^2 - (\Sigma Y)^{2/n})$$

Mean length was represented by X and mean weight by y. Correlation co-efficient was also used to test the association between mean GSI of males and females used in the study. The mean GSI of males was represented by X and that of females by Y.

Analysis of variance (ANOVA) was used to test variations in the mean GSI of males and females during the months of sampling. The various months of the study period represented the treatments whereas the sexes (males & females) were taken as the replicates.

RESULTS

Size Groups

The length grouping of *X. laevis* showed six (6) size classes as in Table 1 below. Specimens having the highest frequency were those in the size group 5-5.9cm which had a percentage frequency of 35. This group seems to form the base line for matured individuals from the results of determination of GSI and fecundity. Immature stages fell in the lower size class ranges whereas the largest individuals encountered fell in the range 6-6.9 & 7-7.9 cm.

Sex Ratio

High proportions of males in the 5-5.9 cm class range were caught during this study. However, the monthly analysis of sex ratio of males to females showered a statistically significant ratio in the March samples. But the overall ratio within monthly

Table - 1: Size classes & frequency of occurrence of *X. Laevis* in Rockwater fish farm

Size Class (cm)	Frequency	% Frequency				
2-2.9	20	5				
3-3.9	68	17				
4-4.9	82	20.5				
5-5.9	140	35				
6-6.9	58	14.5				
7-7.9	32	8				

samples was not significant at the 5% level of probability (P > 0.05). The various sex ratios within each month are shown in Table -2.

Length – Weight Relationship

Weight measurements ranged from 1.85-43.37g. Computed values of correlation co-efficient of length against weight in matured individuals indicated a highly significant correlation in both males (r : calculated = 0.105 tabulated = 0.897, n=10) Fig. -1. The regression line gives a positive association between length and weight and the regression co-efficient is -0.433. This results show that length is positively correlated with body mass in both males and females, therefore length increases with body mass in both sexes.

Variations in Gonosomatic Index (GSI)

The GSI of both males and females in the size range 5-7.9 cm most of which were matured and some gravid was investigated (Fig. -2)

There was a peak in male GSI in August followed by September. Peaks in female GSI occurred in October and November. There was a strong correlation between the male and female mean monthly GSI (r=0.006, n=10). Analysis of variance of the mean monthly GSI of males and females was not significantly different at the 5% level of probability (P>0.05). This points to the fact that gonadal activity in both males and females is synchronized during the breeding season. That is, testicular development and vitellogenic growth are both active in the breeding period.

The correlation co-efficient of mean length and weight of matured females show a significant positive relationship; (r=0.089, n=10).

Months	No. of Toads Sexed	No. of Males	No. of Females	Sex ratio Male:Female	X ²
March	40	17	23	1:1.4	4.02*
April	40	19	21	1:1.1	0.38
May	40	20	20	1:1	0
June	40	22	18	1.2:1	1.61
July	40	21	19	1.1:1	0.38
August	40	19	21	1:1.1	0.38
September	40	19	21	1:1.1	0.38
October	40	18	22	1:1.1	1.47
November	40	19	21	1:1.1	1.38
December	40	19	21	1:1.1	0.38

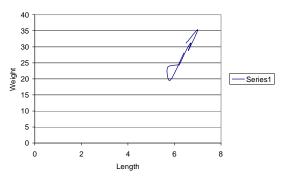
Table - 2: Monthly sex ratio of X. laevis

 X^2 - tabulated = 3.841

* = Significant at 5% level of probability (P<0.05)

Fecundity

Result of gravid females investigated is shown in Table -3 indicating the relationship between length, weight and fecundity. There is a positive correlation in the mean length of both male and female toads (r=0.105, and 0.089 respectively). Fecundity tends to increase with the length of individual females. The same trend was observed for body mass. Fecundity values ranged between about 450 to almost 6,000 eggs per female depending on the size.



Temperature and rainfall

The temperature record during this work

Fig. - 1: Length –weight relationship of matured *X. laevis*

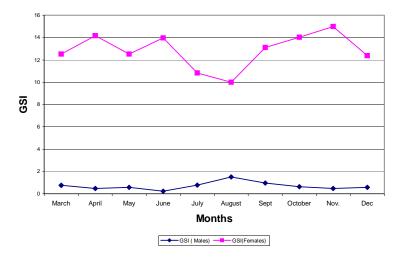


Fig. - 2: Mean monthly gonadosomatic indices of male and female X. laevis

shows a peak in September followed by June. The colder months of the harmattan period have lower temperature values (Table -4). The altitude of the farm (which is about 1331m) above sea level and its environs generally encourages cold weather resulting in low temperatures. Rainfall data for the entire study period is presented in Table -4 and Fig. -3.

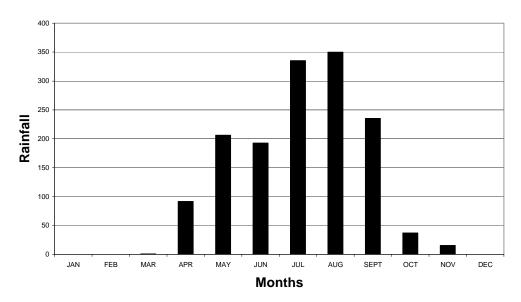
DISCUSSION

The migration of *X. laevis* to Rockwater Fish Farm is a fascinating phenomenon. The toads are rarely found in the farm during the cold months of the harmattan period. This observation corresponds with those of Koskela and Pasanen (1974) and Rastogi *et al* (1983) that toads and frogs hibernate during the cold months of winter. Beatle (1985) also reported that during the winter, frogs use places like pond and river bottoms as well as crevices in the ground for hibernation.

This implies that the migration of the African clawed toad to Rockwater Fish Farm takes place from the surrounding areas and/or hibernating toads in the farm emerge when environmental conditions become favourable. The period of breeding migration agrees with those observed in the common toad, *Bufo bufo* by Gittins (1983) and Reading (1984). Beatle (1985) stated that the reproductive cycle in the common frog *Rana temporaria* begins with hibernation and culminates in spawning. The timing of the reproductive cycle appears to be largely controlled by endogenous factors as reported by Juszazyk and Zamachowski (1965). In the present study *X. laevis* were fewer at the beginning of sampling as evidenced by low numbers encountered initially. The relatively large number of juveniles found in the early samples point to the fact that the toads bred as soon as they were in the ponds. This observation is similar to that of Beatle (1985) in his studies on *R. temporaria.* However, Savage (1961) reported that frogs are found in ponds several weeks before they breed in other years.

Results of the length-weight measurements showed that length increased with body weight. There was a positive correlation between these two parameters. This trend was observed by Dixon & Staton (1976) in *Heptodactylus macrosternum*, another anuran species.

In the size classes and frequency of occurrence of *X. laevis*, (Table -1) size group 5-5.9cm had the highest percentage frequency of occurrence. This group also appears to form the nucleus of the breeding population. Dixon and Staton (1976) also reported the same result in *H. macrosternum*. This phenomenon of positive association of length with weight has also been reported in fishes. Ikomi (1990) found a highly significant correlation between total length and the body weight in the grey mullet, *Mugel cephalus*.



Mean Monthly rainfall(mm)

Fig. - 3: Mean monthly rainfall data

Months	М	Α	М	J	J	Α	S	0	Ν	D
No. of Toads	10	10	10	10	10	10	10	10	10	10
Mean length (cm)	6.41	5.82	5.7	6.2	6.2	6.6	6.71	6.6	7.0	6.5
Mean Weight (g)	28.1	19.6	23.6	24.4	24.3	30.2	31.2	28.9	35.3	31.1
Mean Fecundity	2475	1725	2079	3333	4090	4260	4018	3155	3119	1696

Table - 4: Mean monthly record of physico-chemical parameters

 Table - 3: Relationship between length, weight & fecundity of females

Month	J	F		М	Α	М	J	J	Α	S	0	Ν	D
Parameters													
Temperature	17.7	18.1	21.5	22.8	23.4	23.6	22.8	23.0	25.5	22.0	18.5	16.0	
Dissolved													
Oxygen (mg/l)	-	-		3.0	3.8	4.1	5.0	6.1	6.8	5.6	5.1	4.3	5.9
рН	-	-		5.9	6.5	6.2	7.2	7.4	7.1	6.9	5.8	6.9	6.3
Rainfall (mm)	0	0		0.7	91.6	206.2	192.6	335.2	349.8	235.4	37.0	15.6	0.0

The gonadosomatic index (GSI) of female *X. laevis* showed peak gonadal activities in November while those of males occurred in August. These peaks fall within the months of rainfall.

Analysis of variance (ANOVA) for GSI and the months of rain showed that there was no significant difference at 5% level of probability (P>0.05). This points to a possible influence of rainfall on the

gonadal activity of both males and females. Rastogi et al (1983) also observed a sharp increase in the ovary weight of *R. esculenta* during the months of September to October. When Jorgensen (1981) expressed mean ovarian mass as percentage total body mass in the frog *R. temporaria*, he observed that it was lower in the September samples than in the October to November samples suggesting that, vitellogenic growth had not yet finished in the ovaries of some or all of the frogs constituting the September samples. The onset of vitellogenic growth may therefore be an important factor in the determination of peak gonal activity.

Deucher (1975) reported that females of the African clawed toad do not shed all their eggs from the ovaries in a breeding season. While Toft and Duellman (1979) observed that in the majority of anurans they examined, the peak of breeding activity (number of females with fully developed eggs) occurs in the rainy season, although gravid females of some species can be found throughout the year. Beurden (1979) also found out that in the frog *Cyclorana platycephalus*, mature females (over 5cm) and males (regardless of size) have eggs and mature sperms throughout the year.

The above findings corroborate the incidence of many gravid females in the December samples as shown in Fig. 2. The highest mean fecundity occurred between July and September with a peak in August (Table 3). This incidentally coincides with the peak period of rainfall (Fig. - 3, Table - 4). Individuals of the size 7 – 7.9cm had the highest number of eggs (table 3). The number of eggs was observed to increase with body mass. Eggs produced by individuals of this size group varied between 5,000 to almost 6,000. Small and medium size toads had about 500 to 4000 eggs. Jorgensen (1981) observed a similar trend in *R. temporaria* with a maximum of 9,000 eggs in a 35g frog.

Data on physico-chemical parameters showed that the highest mean temperature (24.5°C) was recorded in the month of September with the least in December. Between March and August, the temperature varied from 21.5°C to 23.4°C.

Gittins (1983) stated that studies have

been directed at the effects of weather on the breeding migration and spawning dates of *B. bufo.* He observed that low temperature disrupted the inward migration of toads. The rare nature of occurrence of *X. laevis* in Rockwater Fish Farm in December (the lowest temperature date during this study) is an indication that temperature plays an important part in the control of breeding migration in these toads.

Beattle (1985) reported that the frog, R. temporaria does not spawn at high attitudes during the months of January and February due to cold temperatures until March and April. He attributed this observation to a possible temperaturedependent reaction in the timing of emergence. In this study, samples collected in March had quite a number of juveniles. This suggests that toad migration to the ponds started some time in February. The first rain was recorded in March and if individuals of the size - class 2 - 2.9 cm appeared in the March samples, it is unlikely that rainfall is a governing factor in the timing of reproduction in X. laevis as observed by Deucher (1975). The observations in this work point to the fact that breeding started prior to the onset of the rainfall.

The findings of Dixon et al (1976) showed that in Leptodactylus macrosternum, individuals which bred in May and June had reached maturity by the end of six months. It's possible from this trend to assume that individuals of X. laevis, which bred early in February, had reached a size range of 2 - 2.9cm by March. Rastogi et al (1983) reported from their observations on *R. esculenta* that under optimal environmental and nutritional conditions, the development from egg to froglets takes about two months. Although data obtained from the studies of GSI and fecundity showed peaks in the wetter months of this study, Rastogi et al (1983) see temperature and rainfall as the determining factors in the breeding period of X. laevis. From these findings, it can be said that the inter-play of temperature, rainfall and nutritional conditions influence to a large extent the breeding period of the African clawed toad.

The effect of depth which varied between 85cm to 1.85m during the dry and wet months respectively was not obvious in this investigation.

REFERENCES

- Bagenal, T. B. and Braum, E.: Eggs and early life history, In: Methods for assessment of fish production in fresh water. Blackwell Scientific Publication, Oxford: 165 – 201 (1978)
- Beattle, R.C.: The date of spawning in population of the common frog *R. temporaria* from different altitudes in Northern. *Journal* of the Zoological Society of London: 205, 137 – 154 (1985)
- Beurden, E.V.: Gametic development in relation to season, moisture, energy source and size in the Australian water-holding frog, *Cyclorana platycephalus. Herpetotologia*, 35 (4) 370 – 374 (1979)
- 4. Brown A.L.: The African clawed toad. Butherworth Publ. London, 1 -40 (1970)
- Deucher E. M.: Xenopus: The South African clawed toad. William Clowes and Sons Ltd., London, 1 – 97 (1975)
- Dixon R. J. and Staton M.A.: Some aspects of the biology of *Heptodactylus macrosternum*, Miranda Ribeiro (Anura, Heptodactylidae) of the Venezuela Wanos . *Herpetologica*, **32**, 227-232 (1976)
- Gittins S.P.: The breeding migration of the common toad, *Bufo bufo* to a pond in Mid-Wales, *Journal of the Zoological Society of London.* 199, 555 – 562 (1983)
- Harris P.D. and Tinsley R.C.: The biology of *Gyrodactylus galleni* (Gyrodactylidae) an unusual viviparous monogenaen from the African clawed taod, *X. laevis Journal of the Zoological Society of London* 212: 325-346 (1987)
- Ikomi, R.B.: On the ecology of the grey mullet mugil cephalus (L.) in Kumala Creek Niger Delta, Nigeria). ACTA Hydrobiologia, 32: 237 – 249 (1990)
- 10. Jorgensen C.B.: Ovarian cycle in a temperate zone frog *Rana temporaria* with special reference to factors determining number and size of eggs. *Journal of the Zoological society of London*, **195**, 449 458 (1981)
- 11. Juszczyk, W. and Zamachowski W.: Terms of ovulation and oviposition of the grass frog

Rana temporaria (L.) in condition of an artificial and prolonged hibernation. *Acta Biol. Cracov. (Zool.)* **8:** 211 – 223 (1965)

- Kelly H.G. and Onyeka J.O.A.: Introduction to Statistics and Experimental design for Life Sciences. ABIC Publ. Enugu, Nigeria 1-186 (1992)
- Koskela, P. & Pasanen, S.: The wintering of the common frog *Rana temporaria* (L.) in Northern Finland. *Aquilo* (Zoology) **15:** 1-17 (1974)
- Lawan M. K.: Production of toads for teaching and research purposes. M. Sc. Thesis, Ahmadu Bello University, Zaria, Nigeria 1-77 (1989)
- Lowe McConnel R.H.: Fish communities in tropical fresh waters. Longman, London: 1-64 (1975)
- Moore, J.W.: Reproductive biology of anadromous arctic char *Solvelinus alpinus* (L.) in the Cumberland sound area of Baffin Island. *Journal of Fish Biology*, 7: 143 – 151 (1975)
- Nikolsky, G.V.: The ecology of fishes (translated from Russian) Academic Press Limited, London: 1-50 (1963)
- Rastogi R.K., Izzo Vitiello I., Dimaglio M., Dimalteo, L., and Chieffi, G.: Ovarian activity and reproduction in the frog, *Rana* esculenta. Journal of the Zoological Society of London 200: 233 – 247 (1983)
- 19. Reading C.J.: Interspecific spawning between common frogs, *R. temporaria* and common toads, *B. bufo. Journal of the Zoological Society of London* **203:** 95-101 (1984)
- 20. Savage R. M.: The ecology and life history of the common frog *Rana temporaria*. Sir Isaac Pitman and Sons Limited, Pitman House; Parker StreetKingsway, London 1-256 (1961)
- 21. Smith C.L.: Reproduction in female amphibian. *Men. Soc. Endocr.* **4:** 39-56 (1955)
- Toft, C.A. and Duellman W.E.: Anurans of the lower Rio Lullepichis, Amazonian Peru. A preliminary analysis of community structure. *Herpetologica*, **35** (1): 71 – 77 (1979)