EXPERIMENTAL STUDIES ON THE SEXUAL CYCLE OF THE SOUTH AFRICAN CLAWED TOAD (*XENOPUS LAEVIS*). I.

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(With Three Text-figures)

I. INTRODUCTION

FOR the purpose of experimental analysis three distinct phases may be distinguished in the female sexual cycle of the South African clawed toad (*Xenopus laevis*): (*a*) ovarian growth, (*b*) oviposition and ovulation, and (*c*) ovarian retrogression. It has been shown elsewhere that the growth and retrogressive phases may be produced by increasing or withdrawing from the circulation a hormone elaborated by the anterior lobe of the pituitary body (Hogben, 1930; Hogben *et al.* 1931) and that they are dependent upon the available food supply of the animal (Alexander & Bellerby, 1935). In nature these two phases alternate in half-yearly periods, and contrast with the comparatively short period in which mating and ovulation occur. The anterior lobe of the pituitary is also essential for the two processes last named which apparently result from a higher level of pituitary secretion consequent upon some unknown external stimulus (Hogben, 1930; Bellerby, 1933).

Previous experiments of Alexander & Bellerby (1935) showed that the sensitivity of ovarian response to the ovulation-producing hormone in the laboratory is directly related to the degree of nutrition of the toad. Feeding twice a week or once a fortnight was sufficient to allow the animals to grow at approximately the same rate, but in the series which were fed less frequently the ovaries became desensitized and failed to respond to a standard dose of extract. These experiments led to the suggestions that ovulation could only occur under natural conditions if an abundant food supply had been available before the breeding season, and that the retrogressive and growth phases of ovarian activity were a direct result of the periodic fluctuation in food supply which normally occurs in nature as a result of ecological succession.

Another fact has to be considered in making an analysis of the agencies concerned in the control of the sexual cycle in *X. laevis*. Apart from variation in food supply its habitat usually undergoes periodic change in another way (Hutchinson *et al.* 1932). After the breeding season in July or August the ponds (vleis or pans) in which *Xenopus* lives begin to dry up as a result of evaporation, and when this process is completed the toads finally aestivate in the mud at the bottom. During

this period the vestigial ovary resulting from ovulation cannot grow, because of the absence of food, and it is not until the seasonal rains begin and the ponds fill up that redevelopment takes place. In consequence of an increasingly abundant food supply the ovaries then grow rapidly, and attainment of full maturity is followed once more by ovulation in June or July.

The present communication deals with the part that might be played in the regulation of the reproductive cycle of *X. laevis* by periodic fluctuation in water volume.

II. TECHNIQUE AND MATERIALS

All series of toads were kept in glass tanks which held different volumes of water, but which had approximately the same air-water interface when filled. Each group of experiments was carried out in the same room. The toads were thus subjected to the same variation in the duration and intensity of normal and artificial light and to fluctuations in room temperature. They were fed once a week on raw minced meat and were weighed in bulk every 5 weeks. One week was allowed to elapse between the last feeding and weighing. Water was changed once a week 24 hours after the animals had been given food. The physiological condition of the ovaries was determined as in previous experiments by ascertaining whether ovulation occurred after a single injection of anterior lobe pituitary extract. The method of preparation of the extract used has been described elsewhere (Bellerby, 1935; Young & Bellerby, 1935). A threshold dose was employed. All toads which did not ovulate were killed and the weights of the body and ovaries ascertained. Since previous experience has shown that desensitization or slight atrophic changes are not associated with a distinctive histological picture, no sections were cut, the visible state of maturity of the ovaries being determined by naked-eye examination alone. The weekly food intake was measured by weighing toads in bulk before and after feeding. Since the average animal consumes anything from 2 to 5 g. of food at one meal the food intake is easily determined by this simple method.

III. EXPERIMENTAL DATA

In Fig. 1 are shown the growth curves of three series of toads which had been kept for 7 months in 6 l. of water. The first series (A) consisted of twenty females and thirty males. The second (B) was made up of twenty females with a similar number of males. In the third series (C) twenty females alone were used. The growth curves given for series A and B only refer to the twenty females in each group. The results of injection of the three batches of toads are given with relevant data in Table I. The post-mortem results of the two groups of non-ovulating toads (A and B) are summarized in Table II. In series A the ovary was completely atrophic in three cases and partly atrophied in thirteen others. In four females the appearance was normal. In series B, eighteen toads had atrophic ovaries and in five of these atrophy was complete. In the remaining two animals no atrophic changes were visible. The growth curve of the thirty male toads used in series A is shown in Fig. 2 and is compared with a standard growth curve for a similar number of males.
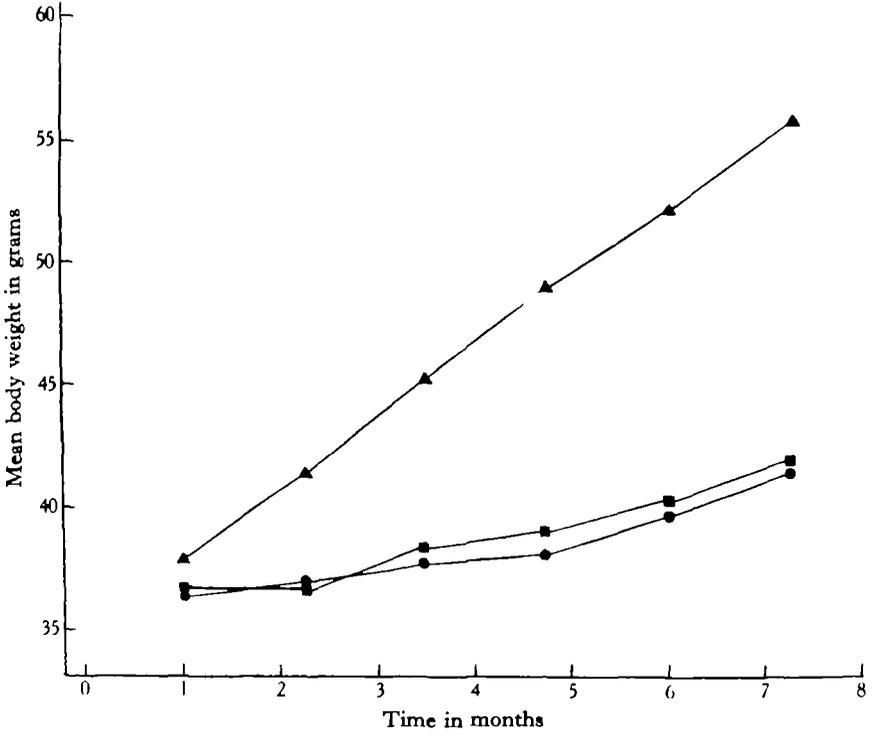
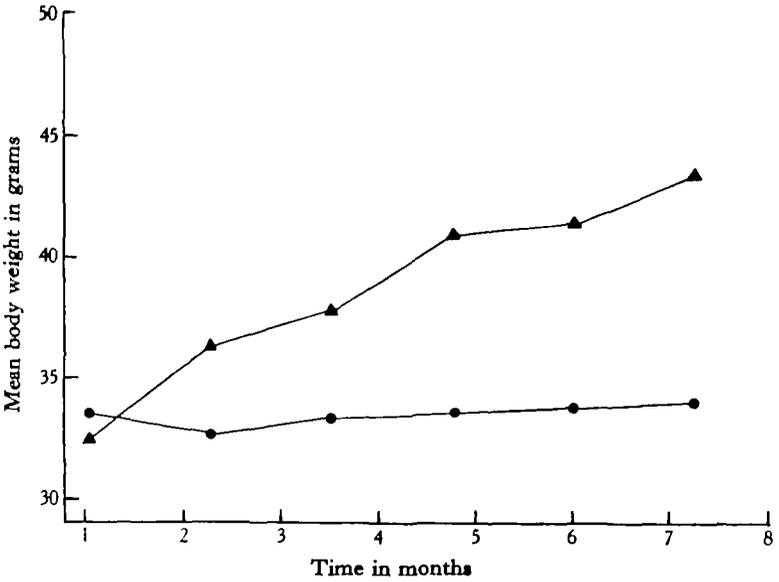Fig. 1. ■ series A, 50 toads; ● series B, 40 toads; ▲ series C, 20 toads.

Fig. 2. ▲ control ♂♂; ● overcrowded ♂♂

Table I

No. of series	Total no. of toads	Mean body weight in g.		% increase in body weight	No. of toads ovulating after injection	% response
		Initial	Final			
A	50	36 3	41·7	14·8	0	00·0
B	40	36·5	41·2	12·8	0	00·0
C	20	37·6	55·7	47·9	17	85·0

Table II

Series	Mean weight of ovary in g (O.W.)	Mean body weight in g. (B.W.)	$\dfrac{\text{O.W.} \times 100}{\text{B.W.}}$
A	2·41	36·33	6·30
B	1 76	34·66	4·93

It was apparent from this first group of experiments that toads could be kept under conditions which prevent them from growing at a normal rate in spite of regular feeding and change of water. In both series a large number of females had atrophic ovaries. A former study (Alexander & Bellerby, 1935) has shown that ovarian atrophy occurred to an extent of 44 per cent in a series of females which had been insufficiently fed at the rate of once a month. In the above two series definite atrophy (as opposed to desensitization) occurred in 80 and 85 per cent of animals. Since the only difference between the three series of females and the two series of males was in the number of toads kept in a given volume of water it is evident that overcrowding can be responsible for a greater extent of ovarian atrophy than that which occurred in toads fed only once a month.

In Fig. 3 are given the growth curves of three groups of thirty female toads which were kept for 7 months under the same conditions as before but in different volumes of water (3, 9 and 12 l.). The results of injection and other data are shown in Table III. A summary of the post-mortem results of the toads which did not respond to injection of extract is presented in Table IV. Out of this series thirteen toads had atrophic ovaries. In two instances the gonad appeared to be normal. In five it was completely atrophic.

Results identical with those of the first series of experiments were obtained. Only those toads which had been kept in the greatest volumes of water grew at a normal rate and responded to injection at the end of 7 months. A final series of experiments was carried out to see whether the diminished growth rate in the over-crowded series was associated with a decrease in food consumption. Four groups of thirty female toads were kept in 4, 6, 8 and 12 l. of water respectively and their food intake measured at weekly intervals for a period of 15 weeks. The results are given in Table V. The ovulation response and other data are summarized in Table VI.

In this last experimental group the 12 l. series of toads grew at the most rapid rate. The lowest growth rate occurred in the 4 l. series. The food-intake figures for
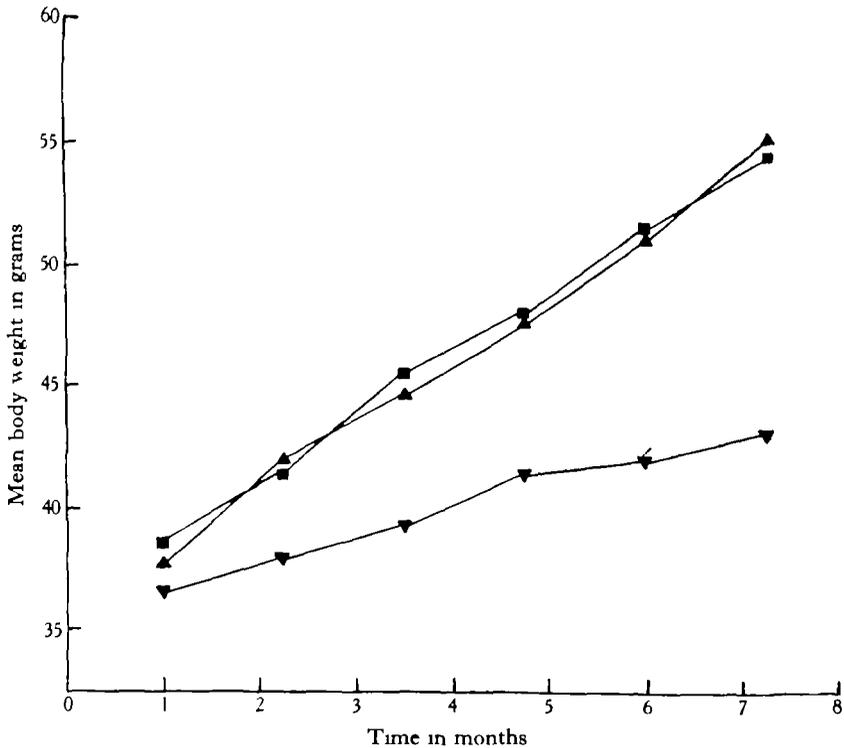
Fig 3. ▲ 3-litre series of ♀♀; ■ 9-litre series of ♀♀ , ▼ 12-litre series of ♀♀.

Table III

Volume of water in l	Mean body weight in g		^o_o increase in body weight	No. of toads ovulating after injection	^o_o response
	Initial	Final			
3	36·5	43·0	17·8	o	oo·o
9	37·8	55·2	46·0	26	80·6
12	38·4	54 5	41·9	27	90 0

Table IV

Mean weight of ovary in g	Mean body weight in g	$\dfrac{O.W. \times 100}{B.W.}$
1·66	38 63	4 33

these two groups were also a striking contrast, the mean food intake for the 4 l.
group being approximately half that of the first series. In the two intermediate
series the mean values for the food-intake and growth-rate readings were very
nearly the same. In the 6 l. group, however, the sensitivity of response to injection
was much less. From this it appears that diminished food intake alone cannot be

Table V

Mean weekly food intake in g.

4 l.	6 l.	8 l	12 l
1·10	1 23	1·33	2·63
0 07	0·37	0·83	2·17
1 20	1·63	1·83	2·60
0 83	0·73	1·30	1·53
0 63	1·36	1·50	1·83
1·66	1·26	1·44	2·21
0 90	1·06	1·35	2·15
1 93	1·73	1·50	2·60
1·00	1·52	0 83	1·83
1·70	2·26	2·60	2 53
1 33	1 40	1 60	2·23
1·93	2·40	2·66	2·16
1·66	2·33	1·86	2·86
1 53	2·00	2·26	3·30
1 70	2·50	2·36	2·43
1·60	2·30	2 04	3·36

Table VI

Volume of water in l	Mean food intake in g.	Mean body weight in g		% increase in body weight	No. of toads ovulating after injection	% response
		Initial	Final			
4	1 30±0·03	31·8	37·6	17 2	3	10·0
6	1 69±0 04	31·8	39·3	23·5	7	23·3
8	1·70±0·03	33·6	40·9	21 5	22	73·3
12	2·40±0 03	33·1	42 5	28·6	26	86·6

solely responsible for the degree of ovarian atrophy observed. The sum total of the above results strongly suggests that the marked decrease in the growth rate in the series of overcrowded toads may be attributed to diminished food consumption. The data are therefore complementary to the previous enquiry (Alexander & Bellerby, 1935) into the significance of diminished ovarian sensitivity and atrophy following a decrease in food supply.

IV. DISCUSSION

Emphasis has been laid on the fact that in nature *X. laevis* lives under conditions which are essentially periodic. In consequence seasonal variation occurs not only in the volume of water in the ponds but in the nature and number of the flora and fauna inhabiting them. Marked variation also occurs in the qualitative and quantitative character of the saline constituents, and in the opacity and oxygen content of the water. In view of the number of variables involved and the small amount of information available as to fluctuation in these factors under natural conditions it would be premature to select any one as having a primary influence in controlling the reproductive cycle of *X. laevis*. Furthermore, in the case of the larger vlei or pans, it is by no means certain that periodic changes occur to the same extent as in the smaller ponds. With this reservation the present data permit us to advance the suggestion that decrease in volume of the natural waters in which *Xenopus* lives

would contribute to the maintenance of the retrogressive phase of the ovarian cycle which occurs after ovulation, or even induce retrogression of the gonads in the absence of any periodicity in food supply. Whether this is correct or not, a definite result of the present experiments is that a maximum population density limits the fertility of *Xenopus* when it is kept under laboratory conditions. That population density has a marked influence upon the rate of growth is a conclusion that applies to a variety of animals, terrestrial as well as aquatic, and a number of investigations have been carried out on the nature of the mechanism involved (Bilski, 1921; Dywina & Bohn, 1921; Legendre, 1908; Semper, 1890; de Varigny, 1894).

Less attention has been paid to the relation of population density to decreased fertility. Pearl & Surface (1909) found that with fowls reduction of floor space without any diminution in food supply led to decreased egg production. Later Pearl *et al.* (1926) showed that population density had a definite effect on the fertility rate in cultures of *Drosophila ampelophila*. Similar results were obtained by Crew (1931, 1932) on mice. He noted that overcrowding had a marked influence on the general condition of his experimental animals resulting in a lowering of the reproductive rate. None of these investigators showed how overcrowding actually affected the growth rate and fertility of the animals studied. The present experiments also fail to explain exactly how decreased water volume causes the diminution of food intake in *Xenopus laevis*.

They do show, however, that in the case of this amphibian decreased fertility may be attributed to actual changes in the gonads resulting in the non-production of mature ova or viable sperm. They also suggest that the lowering of fertility which has been observed in other experimental animals under crowded conditions of existence may be directly due to diminished activity of the ovaries and testes consequent upon an insufficient level of food consumption and rate of growth.

V. SUMMARY

1. Retrogression of the ovaries of *Xenopus laevis* occurs if toads are kept for long periods in an insufficient volume of water.

2. Atrophy takes place in spite of regular feeding and change of water and occurs more frequently and to a greater extent than in toads which are under-fed.

3. Overcrowding is followed by a diminution in food intake; the weekly consumption of food being halved in the case of females subjected to the maximum population density.

4. Under natural conditions the seasonal decrease in water volume of the ponds in which *Xenopus* lives may induce or at least contribute to the maintenance of the retrogressive phase of ovarian activity which normally occurs.

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