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

By C. W. BELLERBY

From the Department of Social Biology in the University of London

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(With Two Text-figures)

I. INTRODUCTION

A NUMBER of investigations (Rowan, 1926, 1929, 1930; Bissonette, 1930, 1931 *a*, *b*; Cole, 1933; Ivanova, 1935; Kirschbaum, 1933; Whetham, 1933; Witschi, 1935) have shown that seasonal variation in light intensity and in wave-length may play a not inconsiderable part in regulating the periodic changes that occur in the avian gonad. It has been similarly shown that light is involved in the control of the mammalian reproductive cycle (Bissonette, 1932, 1933, 1935 *a*, *b*; Baker & Ranson, 1932; Marshall & Bowden, 1934, 1936; Whitaker, 1936). In view of the generally accepted opinion that the anterior lobe of the pituitary is concerned in the regulatory mechanism of the sexual cycle of most animals, it is thought that the eye acts as the receptor organ by which the light stimulus evokes reflex secretion of the gland. This is borne out by several observations. In the course of their experiments Marshall & Bowden noted, for instance, that oestrus never occurred in a blind ferret during a period of 2 years. Likewise Ceni (1928) found that degenerative changes took place in the testes of blinded cocks. Hill & Parkes (1933) also state that oestrus could not be produced during the winter in the hypophysectomized ferret by irradiation with artificial light.

Among the experiments that have been cited as supporting the light-eye-pituitary hypothesis are those of Hogben *et al.* (1931) and Shapiro & Zwarenstein (1933) on the South African clawed toad (*Xenopus laevis*). The former noted that the mean weight of the ovaries of eyeless females was significantly lower than that of normal controls in experiments primarily concerned with the effect of hypophysectomy on the pigmentary effector system. The latter assert that in spite of regular feeding and change of water, this amphibian cannot be kept in captivity in Cape Town without undergoing drastic loss in body weight and ovarian atrophy. They attribute this "captivity effect" to insufficient illumination under laboratory conditions and suggest that the main factor influencing the reproductive cycle in nature is seasonal variation in the amount of solar radiation (Zwarenstein & Shapiro, 1933). The present experiments also deal with this question. They were undertaken to explore how far the light-eye-pituitary hypothesis is relevant to cyclical sexual activity in *X. laevis*, and have been carried out with special reference to earlier work on the influence of nutrition (Alexander & Bellerby, 1935).

Previous investigations have shown that *X. laevis* is a specially suitable species for enquiries into reproductive physiology on account of its remarkable viability under laboratory conditions and its low mortality consequent on major operations, but that striking disparities in reproductive activity and body growth are compatible with prolonged survival. As far as females are concerned this is partly due to a peculiarity described in a former communication (Alexander & Bellerby, 1935) in which it was shown that the ovary of *Xenopus* is an organ of food storage responding in size and activity to relatively small changes of available food supply. Thus a necessary prerequisite for the use of the toad as a reliable experimental animal is careful attention to the amount of food supplied. That this condition is not of itself sufficient to ensure a reliable standard is shown by the fact that considerable variation in growth and the condition of the gonads may also occur in males. Besides ensuring a minimum of food supply in excess of what is requisite for continued growth of the body, it is also necessary to keep the population density of experimental toads below a certain maximum (Alexander & Bellerby, 1937). These facts, which were unknown when earlier work on the relation of the eye to the reproductive cycle was carried out, led to this supplementary study. It includes the effect of removal of the eyes on the growth rates and activity of the gonads in both sexes of *X. laevis* as well as experiments on the influence of variation in light intensity.

II. TECHNIQUE AND MATERIALS

All series of toads were kept under the conditions described in previous papers. Twenty-five animals were used in each control and experimental group. They were all kept in the same-sized white enamelled tanks, in equal volumes of water, and at the same mean temperature. They were fed once a week on raw minced beef and were weighed in bulk at monthly intervals on the morning of a day on which they were to be fed. The water in the tanks was changed every week 24 hours after the toads had been given food.

The state of maturity of the ovaries was determined as described elsewhere by ascertaining whether ovulation would occur after a single injection of extract prepared from anterior lobe pituitary tissue. Male toads were killed and the body and testes weighed. The latter were then examined histologically. Bouin's fluid was used as a fixative and sections were stained with eosin and haemotoxylin.

Removal of the eyes was carried out under ether anaesthesia and was not followed by any adverse symptoms. Bleeding was slight and no disturbance of locomotion or feeding occurred after the operation. Completeness of removal of the eyes or possibility of the regeneration of visual elements was checked by taking readings of the melanophore index at intervals throughout the experiments.

No special apparatus was used for the experiments on the effect of darkness and different light intensities because the particular lay-out of the temporary building at present occupied by the Department allowed these phases of the enquiry to be investigated with ease and simplicity. The experiments on the influence of absence of light were carried out by keeping the toads in a small underground windowless chamber which formed an adjunct to the cellar now used as a dark room. This room was fitted with a thick close-fitting door and ventilated by a shaft which had its outlet some 40 ft. above, but which did not, however, allow the penetration of any light.

All manipulations such as feeding and water change were carried out with the door shut. The latter in turn was never opened without both doors of the dark room being closed. When the weight of the toads had to be determined this was done by picking them out of their tanks, counting them, allowing all superfluous water to drain off and then putting them into a weighed can with a well-fitting light-proof lid. All these operations were performed in the dark. The batches of toads were then weighed in the open laboratory and finally put back into their respective tanks. By rigidly adhering to these conditions during the experiment it is now possible to claim that no light ever reached the toads throughout the experimental period of 7 months even for a few seconds.

The experiments on the effect of different light intensities were also facilitated by the particular construction of the building and were carried out in a long corridor which, like the dark room, was also underground and completely cut off from daylight. The four different intensities were obtained by the simple method of placing the tanks of toads at specific horizontal distances from one source of light, provided in the present instance by a 100 W. "Daylight" lamp suspended over the first container at a height of 5 ft. The distance of each tank from the light source was then adjusted according to the reading given by a Weston photo-electric cell. Since one source of light was used all series of toads were subjected to the same degree of variation in light intensity or wave-length which might occur as the result of ageing or other change in the lamp. Each tank was kept covered with a sheet of ground glass to eliminate shadows. The initial intensity determinations to ascertain position of the tanks were made at the bottom of each container when empty of water and with the sheet of glass in place.

III. EXPERIMENTAL

In Fig. 1 are shown the growth curves obtained from the first series of experiments. They demonstrate that over a period of 7 months male and female *Xenopus* grow at a normal rate in the complete absence of light. The possibility that any atrophic changes produced in the gonads could be a secondary consequence of the cessation of growth rather than to the absence of light itself is thus eliminated. The flattening out of the growth curves in the last part of the experiment is probably due to the fact that the last weight determinations were carried out in the early winter when the growth rate of *Xenopus* begins to slow down as a result of the fall in temperature during the autumn.

The condition of the ovaries of the control and experimental females, as shown by sensitivity of response to injection of extract, is given with other relevant data in Table I. A summary of the post-mortem results of the male toads is presented in Table II. The subsequent histological examination of the testes of control and experimental animals showed that no significant differences could be detected between them. The sum total of these experiments indicates that the general action of light, as distinct from light acting locally upon the pituitary through the intermediary of the eye and optic nerves, is not essential for general growth or reproductive activity.

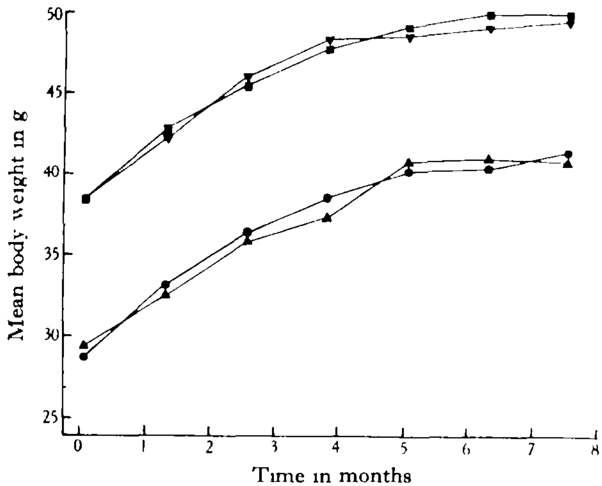

Fig. 1. ■ Females in dark; ▼ control females; ▲ males in dark; ● control males.

Table I

Series	Mean body weight in g		% increase in body weight	No. of toads ovulating after injection	% response
	Initial	Final			
Dark	38·4	50·0	30·2	22	88
Control	38·6	49·7	28·7	23	92

Table II

Series	Mean body weight in g. (B.W.)		% increase in body weight	Mean weight of testes in mg. (T.W.)	Mean $\frac{T.W.}{B.W.} \times 100$
	Initial	Final			
Dark	28·8	41·6	44·4	121	0·32 ± 0·04
Control	29·4	41·3	40·4	120	0·33 ± 0·05

The data obtained from a second group of experiments on the effect of removal of the eyes on the growth rates and condition of the gonads of both male and female toads are summarized in Fig. 2 and Tables III and IV. As in the first group, no difference in the growth rates of control and eyeless animals was observed, although this might be expected to arise if a diminution of food intake occurred in the case of the animals without eyes through the absence of a visual stimulus to feeding. As in the first group, no histological differences could be detected also in the testes of the control and experimental series of male toads.

The results of further series of experiments on the effect of different intensities of light are shown in Table V. Since they were carried out at the same time as the

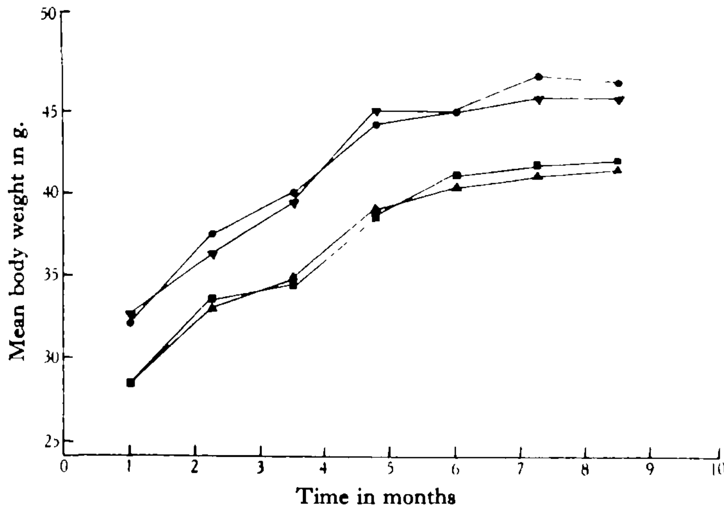

Fig. 2. ▼ Eyeless ♀♀; ● control ♀♀; ▲ eyeless ♂♂; ■ control ♂♂.

Table III

Series	Melanophore index / White background (M.I./W.B.)	Mean body weight in g. Initial	Final	% increase in body weight	No. of toads ovulating after injection	% response
Eyeless	2·8 ± 0·03	32·6	47·3	45·0	24	96
Control	1 5 ± 0·04	32·4	47·7	48·5	23	92

Table IV

Series	Mean $\frac{\text{M.I.}}{\text{W.B.}}$	Mean body weight in g Initial	Final	% increase in body weight	Mean weight of testes in mg.	Mean $\frac{\text{T.W.}}{\text{B.W.}} \times 100$
Eyeless	2 6 ± 0·04	28·4	41·5	46·1	130	0·37 ± 0·04
Control	1 4 ± 0 04	28·2	42·0	48·9	119	0 34 ± 0 05

other series they simply provide complementary evidence for the fact that *X. laevis* can grow and maintain its reproductive organs in a fully mature condition in the complete absence of daylight for long periods of time. The experiments also demonstrate that artificial light alone in the intensities used has no depressant effects upon somatic growth or reproductive development in the case of the female.

Two further sets of data are available for inclusion with the present results. In Tables VI and VII are summarized the findings at autopsy of two series of twenty-five male and female toads. These animals which had been employed in purely

Table V

Intensity of illumination	Mean weight of body in g.		% increase in body weight	No. of toads ovulating after injection	% response
	Initial	Final			
Full	37·4	49·6	32·6	22	88
$\frac{1}{4}$	41·5	53·4	28·6	23	92
$\frac{1}{16}$	38·0	51·8	36·3	19	76
$\frac{1}{20}$	39·3	53·9	37·1	23	92

observational work on colour change had been operated upon 18 months previously. In both series removal of the eyes had been carried out on immature specimens. As noted before, the operation had no effect either upon general bodily growth or upon the development of the testes and ovaries. All the females had large fully mature ovaries containing masses of ripe ova. Examination of the testes of the males showed that no signs of degeneration were present, that spermatogenesis was active and that their condition was normal in every respect. In both these series the figures for the gonad body weight percentage were, if anything, higher than usual

Table VI

$\frac{\text{M.I.}}{\text{W.B.}}$	Body weight in g. (B.W.)	Weight of ovary in g. (O.W.)	$\frac{\text{O.W.} \times 100}{\text{B.W.}}$
2 9 ± 0 03	57·6	8·2	13·73 ± 0·15

Table VII

$\frac{\text{M.I.}}{\text{W.B.}}$	Body weight in g. (B.W.)	Weight of testes in mg. (T.W.)	$\frac{\text{T.W.} \times 100}{\text{B.W.}}$
2·5 ± 0·03	38·8	188	0·48 ± 0 01

IV. DISCUSSION

The main facts that emerge from the above experiments are that growth of body and gonads in *X. laevis* can occur to a normal extent in the complete absence of light if the animals are well fed, and that removal of the eyes does not then lead to any degenerative changes in the ovaries and testes. Two alternative conclusions can be drawn from the experimental results. The first is that light plays no part whatsoever in the control of the reproductive cycle under natural conditions and that the occurrence of periodic phases of growth and retrogression in the gonads of *Xenopus* in its natural habitat may be more simply explained by assuming the primary influence of food supply and temperature. As far as this cycle of changes is concerned it is unnecessary at present to postulate for *Xenopus* a complex mechanism of control involving photo-receptors in the eyes or skin and the anterior lobe of the pituitary. There seems to be little doubt that this type of mechanism is involved in an animal such as the ferret, and there is no reason why it should not exist in other species of Anura. It must be remembered, however, that a means of control found in one species of animal must not necessarily be recognizable in others of a similar pattern. Physiological variation can be extremely varied even in closely related types and the experiments described in the present paper provide additional evidence for the view that *Xenopus* is anomalous not only in its morphology but in its reproductive mechanism. The suggestion made above as to the importance of extraneous factors is not new. The influence of environment in the control of reproductive activity in Amphibia was pointed out many years ago by Spallanzani (1784), whilst the importance of the part played by nutrition has been stressed in recent years by Heape (1931). That control of the reproductive cycle by variation in light intensity may not be a general phenomenon is shown by several investigations. Dempsey *et al.* (1934) found that alteration in the periods of light or darkness had no effect upon the oestrus cycle of the guinea-pig. The same is true of the sexual cycle in the spermophile (Moore *et al.* 1934). Recently Benoit (1934, 1935) has brought forward evidence to show that in the drake reflex stimulation of the anterior lobe of the pituitary by light acting through the eye and optic nerves must be regarded as questionable. Preliminary experiments favoured this possibility, but in later studies it was found that the pronounced growth of the testes after irradiation could also be induced in birds from which the eyes had been removed or the optic nerves severed. Hooding of the eyes or the region immediately surrounding them, however, had an inhibitory effect and Benoit concluded that some receptor organ other than the eye is involved in this species of water-fowl.

The second conclusion that can be drawn is that light only plays a part in the controlling mechanism under less favourable conditions of temperature and nutrition. In the present series of experiments toads were kept under conditions which at present are believed to be the optimum—at least as far as nutrition is concerned. There can be little doubt that food supply, temperature and light may all to a varying extent affect the seasonal rhythm of reproductive activity in vertebrates, and it is probable that the controlling mechanism is complex and contains the same elements in most allied types. It is also probable that different elements may predominate over all others in species which are closely related, just as similar species fully equipped with the basic mechanisms of photo-, geo- or stereo-orientation may be predominantly phototactic, geotactic or stereotactic in their general behaviour under certain conditions of existence. As stated before, the experiments described above do not disprove the effective role of light in the sexual cycle of other Amphibia nor do they disprove the suggestion that light might play a part in the control of the cycle of *X. laevis* itself at a lower temperature or nutritional level. If the light-pituitary-gonad mechanism of other Amphibia exists in a rudimentary form in *Xenopus* it is not improbable that the adverse effects which absence of light may exert on the growth of the reproductive organs could be demonstrated by keeping the food supply near the threshold for full development of the gonads or by keeping the toads themselves at a lower temperature. This would account for the small but consistent difference of ovary weight between eyeless and normal females recorded in the experiments of Hogben *et al.* (1931) since the possibility remains that their toads were maintained at a lower level of nutrition than that of the present experiments. The apparently insignificant role of light in the control of the sexual cycle of the rat and spermophile may also be explained on the grounds that in the laboratory these animals are kept under conditions which ensure an optimum food supply.

SUMMARY

1. When maintained at an optimum nutritional level both sexes of the South African clawed toad (*Xenopus laevis*) grow at a normal rate in the complete absence of light, or after removal of the eyes.

2. No detectable atrophic changes take place in the ovaries and testes of eyeless animals after 18 months or in the gonads of male and female toads kept in absolute darkness for 7 months.

3. No evidence is provided by the experiments to show that light is essential for the maintenance of reproductive activity in *X. laevis* or that seasonal variation in light intensity or wave-length plays any part in the control of the sexual cycle under natural conditions.

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