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GROWTH INHIBITION AND RETARDATION OF GONADAL DEVELOPMENT IN CROWDED TADPOLES

By

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Introduction

It has been noticed by many investigators that individuals of various aquatic species were retarded in growth by confinement in small space or by the presence of many other individuals. In respect to tadpoles the space factor has been adequately recognized or evaluated, and a number of studies have shown that crowding in tadpoles resulted inhibition of their growth rate (Bilski 1921, Adolph 1931a, 1931b, Rugh 1934, Lynn & Edelman 1936, Richards 1958, Rose 1960, West 1960, Rose & Rose 1961, Akin 1966). Various explanations have been made to elucidate the reduced growth rate under crowded condition such as too little volume or too little space per individual, lack of oxygen, and frequent collisions between tadpoles. An important explanation was presented by Richards (1958) from the experimental results with *Rana pipiens* that a cell in the feces of tadpoles has consistently associated with inhibited growth in those tadpoles.

On the other hand, there has been few studies on how much intrinsic organs could be affected to the development under crowded conditions. The purpose of this study is to clarify the nature of the spatial factor in tadpole growth which may be of significance in the culture of tadpoles in the laboratory. Further investigation was designed to clarify the correlation which may exist between the growth inhibition and the retardation of development of an internal organ under the crowded conditions, especially of the cell differentiation in tadpole gonads.

Materials and Methods

Rana nigromaculata eggs were secured after the fertilization in which all were at 2- to 4-cell stages. All eggs in comparable experiments were derived from a single clutch, and all brood of the animal were maintained in a water tank of the laboratory.

Two days after their emergence from the egg jelly, the larvae were separated into four experimental groups. Each group of tadpoles were cultured in glass finger bowls 18 cm in diameter containing one liter of water. A series of the population density were varied in number of individuals as 2, 5, 10, and 50 per bowl and each experimental group was make up of 4 to 6 bowls. These groups were numbered from Groups I to IV,

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respectively. All test tadpoles were carefully chosen so that the individual sizes were as nearly alike as possible in the beginning of the experiment. Commercial spinach paste was mainly supplied as a food twice a week, with a sufficient quantity being given them to last until the next water change. No attempt was made to control the temperature at which experiments were run, that generally varied between 21°−28°C.

The growth of the test tadpoles in each experimental group was measured at intervals of 6 to 12 days and examined their morphological features. For this purpose calipers were used. Representative animals of each group being average in size were measured the total length being taken from the tip of the head to the tip of the tail and the body length.

For histological preparations a couple of tadpoles were selected from each group and fixed with the Bouin's fluid. Fixed gonads were then treated by a routine micro-technique providing to the histological observations of developmental gonads.

In the course of the experiment such tadpoles as being the protrusion of the fore limbs was taken out from a bowl due to recognizing the occurrence of metamorphosis.

Results

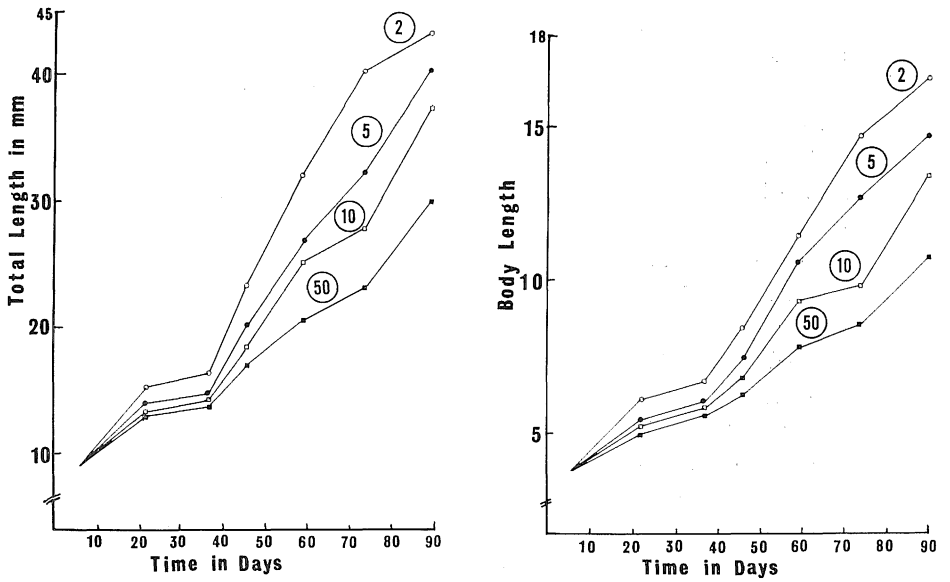
Changes in Growth Rate by Crowding

Every representative tadpoles from a total of four complete sets of experimental groups (Groups I-IV) were measured at 22, 36, 45, 59, 73, and 89 days after hatching. As shown in Table 1, tadpole sizes both of total length and body length increased depend on the variable density of the space. During first two weeks crowding has little

TABLE I. Changes in growth rate of tadpoles affected by crowding. The number indicating average length (mm) attained by individuals of each group. B. L.: body length. T. L.: total length.

Group No. (No. of Tadpoles per Bowl)	I (2)		II (5)		III (10)		IV (50)	
Measurement	B. L.	T. L.	B. L.	T. L.	B. L.	T. L.	B L.	T. L.
Age in Days								
22	6.0	15.3	5.4	14.0	5.2	13.3	5.0	13.2
36	6.6	16.4	6.0	14.7	5.8	14.2	5.6	13.7
45	8.5	23.4	7.5	20.4	7.0	18.5	6.3	17.0
59	11.6	32.1	10.6	26.9	9.3	25.1	7.9	20.6
73	14.8	40.4	12.8	32.2	9.8	27.9	8.5	23.1
89	16.7	43.6	14.8	40.6	13.6	37.6	10.9	30.0

effect upon the rate of growth. At 22 days after hatching a little differences have appeared in measurements between Group I and other groups, and the growth rate of Group IV has obviously affected. Group I of minimum population, consisting of two



Figures 1 and 2. Growth curves of body size of tadpoles which were cultured under different population densities. The number of individuals cultured in a bowl containing one liter of water are 2, 5, 10, and 50, respectively. A plot indicates average length of 2 to 4 tadpoles in each experimental groups.

individuals in 1 liter of water showed rapidly growth than other groups. Four individuals in a bowl, Group II, grow slower but not seemed to be repressed strongly. There were little differences in the measurement between Groups II and III during 60 days, to the contrary remarkable repression in growth rate occurred in the highly crowded population of Group IV. Afterwards these discrepancies have further expanded between four groups. Figures 1 and 2 represented very clearly differential growth affected by available space. Figure 3 indicated the ultimate sizes attained by the animals which were affected to a considerable degree by this so-called "space factor".

Well developed individuals of Group I has emerged the bud of hind-limbs at 59 days, and same characters were observed in Groups II and III at 73 days, while Group IV has shown any feature in their body at all. In 89 days after hatching, the hind-limb formation were complete or were nearly complete in Groups I and II, most individuals of these groups entered in premetamorphosing stage. In contrast to this development tadpoles of Group IV were still keeping the limb-bud form at this stage.

Histological Features of Developmental Gonads Affected by Crowding

In order to examine the correlation which may exist between crowding effects upon body growth and possible retardation of development of an internal organ, histological features of gonads were observed in the process of the experiment.

Two to 4 tadpoles per group were sacrificed for the histological preparations. From cross sections of gonads being 10 micron thick, a number of cells was counted and the

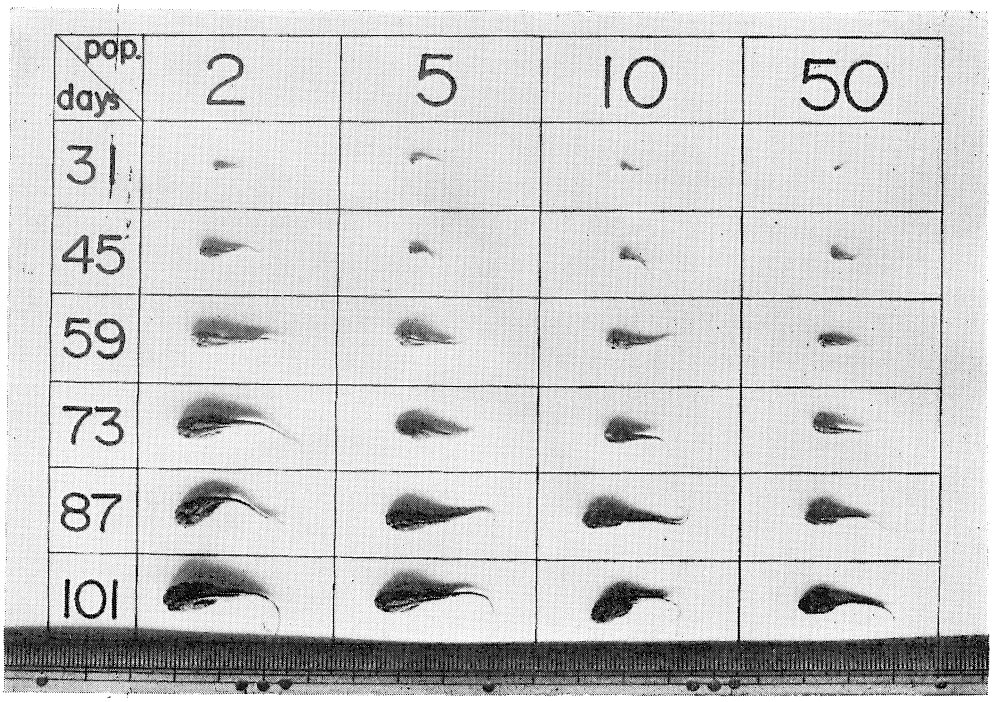


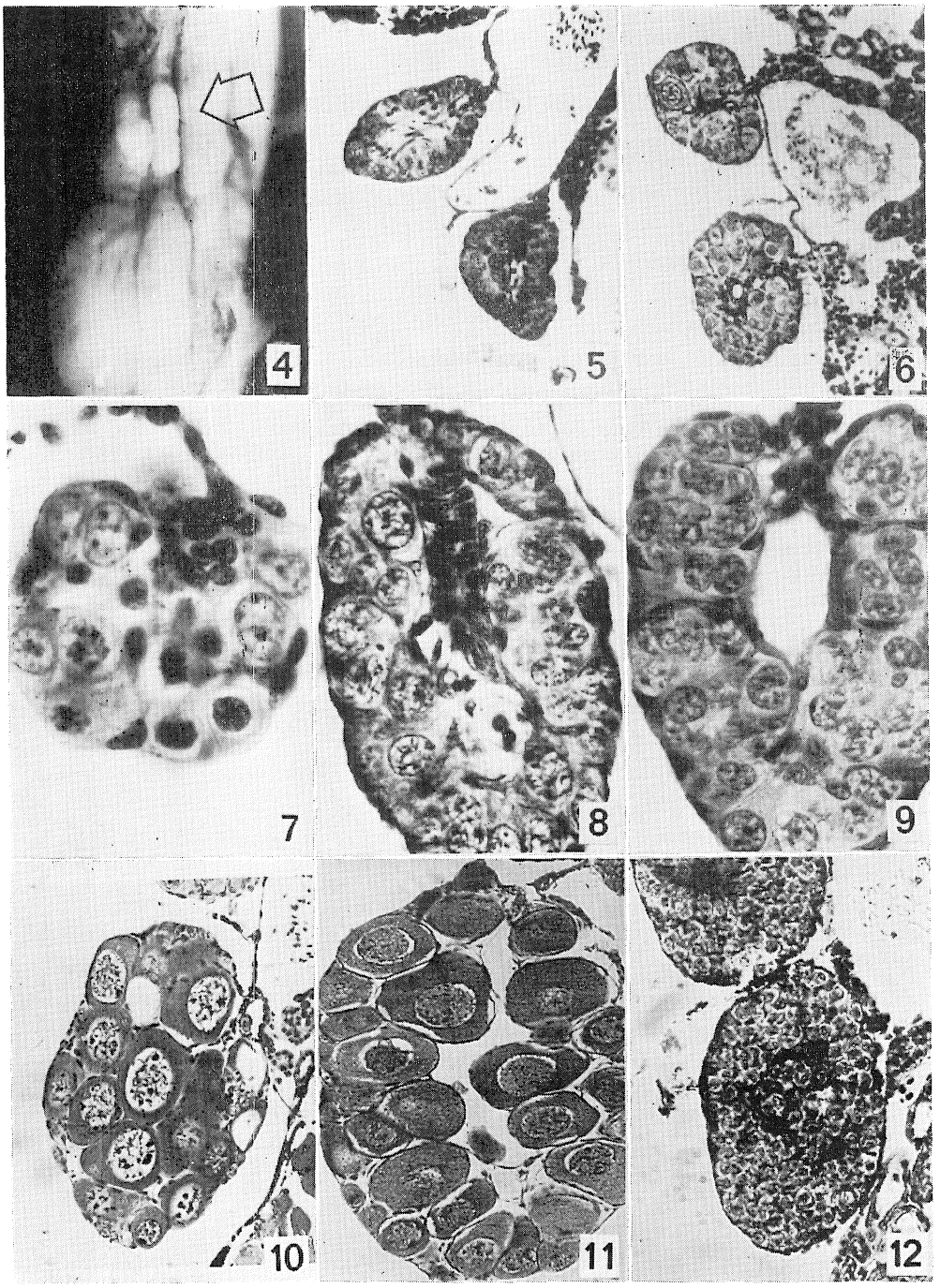
Figure 3. Comparative appearance of body size in representative tadpoles from different populations ; Group I (2 individuals), Group II (5 individuals), Group III (10 individuals), and Group IV (50 individuals). All animals were fixed at the date indicating in the first column.

morphological feature of cells organizing the gland was observed in same intervals of the above measurements.

Within first two to three weeks, no characteristic differences in development of gonads and gonadal tissue has observed in any experimental groups. Slight differences in the tissues have detected at 24 days after hatching between groups of lower-density (Groups I & II) and of higher-density (Groups III & IV). The gonads of Group IV were such minute and thin as hardly detected by naked eyes at this stage. At 36 days after hatching gonads of Groups I and II were externally getting thicker and more extended than those of other groups, and several cellular elements in a gonadal fold were counted at a cross section of the tissue. At this age well-grown tadpoles of Group I have already established an ovarian-type gonad consisting of a typical double structure of oogonial nests in cortex and medullary cord (Fig. 5).

At 45 days ovarian-type gonads of Groups I and II have shown frequently mitotic figures of gonial germ cells in the cortical portion (Fig. 7), while there has been still poor existence of cellular components in crowded group (Group IV). Well-developed gonads of Group I have 40 to 50 primordial germ cells (Fig. 6), and cellular elements of Groups II and III were counted about a half of the above cells.

In passing through the process from 45 days to 59 days after hatching, gonads of every group have rapidly developed. Group I showed the formation of ovarian sac in accordance with more development of cortex, which contained occasionally oocytes in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meiotic prophase (Fig. 8). Gonads of Groups II and III have increased in number of oogonial cells at cortex, and some have proceeded to the form of oocytes. Tadpoles of Group IV still have poor developed gonads, number of oogonial nests were a few in cortex and most cells have shown the features of primordial germ cells.

At 73 days after hatching, gonads of Group I have well differentiated, which were typically of the ovarian type containing auxocytes or of the musculine type having full of medullary cords. In Group II gonads have enlarged their ovarian sac and a few oocytes appeared in the cortex (Fig. 9), while in Group III no oocyte has observed, most of cellular elements were still remained in the stage of oogonium. There have been observed less developed gonads in Group IV, number of gonial cells were a few and no enlarged ovarian sac existed.

In 89 to 101 days after hatching most gonads of Groups II and III became corpulent type, which containing full of auxocytes in differentiated gonads (Figs. 10 & 11). Gonads of Group IV have emerged primary oocytes in the cortex, but they have obviously retarded in increasing gonadal cells and the rate of differentiation (Fig. 12).

In general, these differences in gonadal development were more expanded with tadpole age. However these variation in gonadal differentiation among Groups I, II and III were not strictly proportional with body growth rate as shown in Figures 1-3.

Discussion

The present study has shown that when different numbers of tadpoles all from the same egg mass were cultured in containers of the same size the average growth rate was inversely proportional to the number of individuals. Development of internal organs, as demonstrated with gonads, have also affected by the crowding effects, the degree of gonadal differentiation descended with increase in population density.

Many considerations have been given to explain the reduced growth rate under crowded conditions, such as too little volume or too little space per individual, lack of oxygen, and frequent collisions between tadpoles. Rugh (1934) pointed out the principal factors which directly and independently affect the growth rate of tadpoles. According to him, those are, (1) temperature of the medium, (2) amount of available food, (3) amount of radiant energy through light, (4) amount of surface and available oxygen, (5) amount of accumulated excretory wastes, (6) amount of bacterial growth, (7) amount of available volume per individual, (8) amount of available space per individual, (9)

(*Explanations of Figures*)

Figure 4. Appearance of well developed gonads (*arrow*) of *Rana nigromaculata* larvae in Group I on 73 days.

Figures 5-12. Cross sections of gonads of experimental animals. Figs. 5 & 6, ovarian-type gonads of Group I on 36 days and on 45 days, the later consisted of 40 to 50 cellular elements. 150×. Fig. 7, less developed gonad of Group II on 45 days, mitotic figures are seen in gonial cells. 450×. Fig. 8, early stage of ovarian development in Group I on 59 days, a few oocytes are present in cortex. 400×. Fig. 9, a female gonad of Group II on 73 days, an ovarian cavity developed. 400×. Fig. 10, ovary of Group II on 89 days. 400×. Fig. 11, ovary of Group I on 89 days, the ovarian cavity is almost filled with auxocytes. 400×. Fig. 12, testicular structre of Group IV on 89 days, which has not developed seminiferous tubules. 250×.

amount of forced or stimulated exercise, (10) presence of growth inhibiting substances. The present study has been attempted to recognize or evaluate in respect to the space of tadpole growth rate. Therefore all of these variables of (1) to (9), except (8), could be well controlled in the present experiment.

On the subject of crowding and growth in the tadpoles, early works have been done by Bilski (1921) and by Adolph (1931a). Bilski (1921) believed a factor which seems to be of most importance in the retardation of growth rate in crowded tadpoles is the increased movement in the crowding culture due to over stimulation of the animals as a result of frequent contacts. Adolph (1931a) invetisgated on the basis of periodic weight measurements of tadpoles raised in different volumes of water and found that although crowding has little effect upon the rate of growth during the first two weeks, the later growth rate is greatly affected, declining rapidly in crowded cultures. The present investigation based on the periodic measurements of body size and a organic development have shown the agreement with Adolph's result.

On the growth inhibition substances it is clear from the data presented by Richards (1958), Rose (1960) and West (1960) that large rather specifically inhibitory particles appeared in the culture water of growing tadpoles and the crowding effect could be wholly explained as a consequence of the production of these particles. According to the Richards' description, a peculiar type of cell which is present in the gut of the tadpole and its culture water has been postulated that this cell is responsible for growth inhibition. Licht (1967) has also found the 'alga-like' cells in the fecal material and intestinal tracts of inhibited tadpoles. As this study, however, has not planned to prove the presence of growth inhibiting substances in the culture water no comparable datum on this problem has been presented here.

Experimental evidences presented here indicate the gonadal development was also greatly affected. The frog used in this examination belongs to a sexually differentiated race (Kawamura 1949). Individuals of crowded group (Group IV) have not shown perfectly differentiated gonad even after 90 days. Obvious retardation was occurred to a certain degree in respect to obtain the characteristic morphology of gonads of either sex in Groups II and III. Although some individuals presented a ovarian feature in gonads, there was hardly detected a typical male gonad in affected animals. It is probable that several factors besides the body size are responsible for the retardation of gonadal differentiation.

It has to be explained the effect of crowding upon metamorphosis. In the present study the percentage of individuals which have metamorphosed successfully was unusualy lower than other data (Lynn & Edelman 1936). Only a few tadpoles of Groups I and II have started to metamorphosis at the end of this experiment. As Adolph (1931b) has described that body size is a tangible quantitative factor in the complex of conditions which regulate the onset of metamorphosis, the fact is very closely correlated with the space available per individual. The space factor, however, can not entirely wipe out questions by itself concerning the retardation of metamorphosing. There must be a complex set of factors at work and the effect upon metamorphosis can not be explained on the basis of the size difference alone.

Summary

Tadpoles of *Rana nigromaculata* were cultured in different population densities ranging from 2 to 50 animals per bowl of one liter of water. Animals raised in crowded bowls inhibited their growth rate as compared with uncrowded groups.

The indication of inhibitory effect was made by measurements of body size and by examinations of gonadal development with respect to representative tadpoles from each experimental group which has variable population density.

The crowding of many individuals together brought on the decline in growth rate and retardation of gonadal development as well as failure in occurrence of metamorphosis.

However the differences in gonadal development among four groups were not strictly proportional with the variation of their body sizes as a result of growth inhibition.

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