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GROWTH, DEVELOPMENT AND BEHAVIOUR OF TADPOLES OF XENOPUS LAEVIS DAUDIN UNDER CROWDED CONDITIONS[1]

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1 This study is dedicated to the 60th birthday of Professor Dr. Hans ADAM

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Abstract

In two experiments tadpoles of *Xenopus laevis* Daudin either of the same total length (experiment # 1 and part of experiment # 2) or the same developmental stage (part of experiment # 2) were studied under well defined crowded conditions in respect to growth, development and behaviour whereby environmental factors were kept as constant as possible. Crowding conditions in experiment # 1 were established by housing 25, 100, 200 and 400 tadpoles per rearing compartment (section) of 6 liters of tapwater (27,8 cm × 17,8 cm × 11,7 cm) or 25, 12 or 6 tadpoles per 6 liters of tap water and 6 or 3 tadpoles per 3 liters and 3 tadpoles per 0,72 liter of tap water in experiment # 2. All sections in an experiment were interconnected with each other and the rearing water was recirculated without any treatment 6 times per hour.

Experiment # 1 clearly revealed that 25 tadpoles in 6 liters grew and developed significantly faster ($p < 0,01$ or 0,05) than those housed together in the same space in higher numbers. Thus an individual space of 240 ml per tadpole was jugded as optimal for tadpole growth and development. Mortality of tadpoles during experiment # 1 was highest in section 2 (100 individuals) with 11% and lowest in section 1 (25 individuals) with no dead tadpole. While tadpoles in section 1 preferentially gathered along solid walls tadpoles in the other sections distributed rather evenly all over the section.

Experiment # 2 made evident that 3 tadpoles in 0,72 liter of tap water (section 4/2) developed slowlier than their conspecifics in section 1–4/1 although they had the same individual space (240 ml) availiable. Tadpole mortality in experiment # 2 was almost zero. Tadpoles in all sections rather preferred edges than free walls.

It is concluded that 1) a "chemical factor", 2) insufficient food, 3) competition for food, 4) external factors, 5) interference competition, 6) individual space and 7) geometry of living space per se can be ruled out as sole cause for retardation of growth and development of tadpoles in experiments # 1 and # 2. At the present moment therefore only stress exerted upon the tadpoles by the specific experimental conditions (different numbers of tadpoles per section) remain as the factor most probably causing the effects found.

Introduction

Crowding and resulting effects are of great importance in the animal kingdom (for review see 69) and in human life (22). As far as the effects of crowding on growth and development of larval amphibians are concerned in generally they are attributed to four causes, namely 1) to stress created by the increase of physical contacts between individuals which in turn lead to a decrease in food consumption (1, 2, 26, 33) or in food utilisation resulting in a decrease of accumulated energy availiable to the individual (12); 2) to the excretion of "factor(s)" by the large individuals of the crowded community which then inhibit the smaller members of the crowd (24, 25, 37, 47, 48, 49, 56, 57, 70); 3) to an occurance of algae-like cells (45, 46) or yeast cells in the rearing water and in turn in the alimentary tract of tadpoles (60, 61, 62, 63, 64) which then influence food utilisation and 4) to an increased competition for food amongst individuals (10, 13, 54, 64, 71, 72, 73, 74, 75).

The general conclusions to be drawn from crowding studies clearly indicate that with increasing larval densities a decrease in growth and development occures leading to longer larval periods and smaller sizes at metamorphosis (75). The growth-based model of amphibian metamorphosis (75) implies that for species which temporarly live in ponds the danger of pond drying increases with longer larval periods and that these species are thus exposed to a higher selection pressure. To overcome this danger and to metamorphosize in time and thereby to become land-living before pond drying happens amphibian metamorphosis, especially its fine timing reveals a high plasticity (59).

Most of the studies done on metamorphosis of amphibians in generally and on metamorphosis and crowding in particularly are done in randis, bufonids and hylids (for reviews see 20, 21). With few exceptions only (14) these genera leave the aquatic environment after metamorphosis and become terrestric with a return to water at the spawning season only. The complex life cycles these animals reveal greatly influence community structure and organization. Pond drying as an external stimulus for shorter larval periods and enhanced metamorphosis as actually suggested for facultativly paedogenetic urodels (5, 29) are therefore events of great importance for survival of these genera. In a permanent, secondarely aquatic spe-

cies like in the pipid *Xenopus laevis* Daudin the selection pressure of this environmental stimulus in terms of enhancement of metamorphosis has to be called in doubt since in this species pond drying in any case results in animals abolishment.

The aim of the present study is 1) to demonstrate the extent of crowding effects in terms of growth, development and behaviour in tadpoles of a permanent aquatic anuran species, the South African Clawed Toad, *Xenopus laevis* Daudin under standardized laboratory conditions; 2) to gain further information on the individual space necessary for optimal growth and development of this species during larval life and thus 3) to improve the raising conditions for that amphibian tadpole which nowadays is used in so many laboratories all over the world doing research work in the fields of immunology, molecular biology and genetic engeeniering just to name some disciplines in which tadpoles of *Xenopus laevis* Daudin have proofed to be an ideal study object (16, 17, 51).

Materials and Methods

1 Experiment # 1

For experiment # 1 which was run from May 17th to July 7th, 1982 a total of 725 tadpoles of the South African Clawed Toad, *Xenopus laevis* Daudin was used.

1.1 Life history of tadpoles prior to the experiment

Tadpoles came from an own breed which was induced on April 17th, 1982 by injecting male and female adult Xenopus with a single dose (600 IU) each of PG 600 (WERFFT-CHEMIE, Vienna, Austria) dissolved in 1 ml sterile physiological saline. For further details we refer to (36). The fertilized eggs which were deposited into a plastic tank with 10 liters of a two day old tap water from the local water supply were aerated until April 22nd, 1982 when 1500 free swimming tadpoles hatched. The next day 900 respectively 600 tadpoles were transferred to white plastic tanks containing 60 respectively 40 liters of two day old tap water and feeding was started. Till May 3rd, 1982 tadpoles were fed each forth day by adding 3,6 liters respectively 2,4 liters of nettle-juice resulting in 4 ml juice per tadpole. For purposes of standardization the nettle-powder juice was adjusted photometrically (nephelometrically) to a transmission of 12% using a wavelength of 580 nm. From May 3rd to May 15th, 1982 tadpoles were fed each second day with the same amount of juice as before. During this rearing period both tanks were placed into a light chamber having a light cycle of 14 hours bright (6 am to 8 pm) and 10 hours dark (8 pm to 6 am; local time). Each tank was aerated by one air-bubble stone placed in the center of the tank. The rearing water was changed each second weak. On May 17th, 1982 a total of 725 tadpoles having stage 49, 49/50 and 50 according to (41) and a total length (tip of the head to tip of the tail) from 16 mm to 32 mm was removed from both rearing tanks and transferred into the experimental set-up to start experiment # 1.

1.2 Experimental set-up

The experimental set-up described in full detail elsewhere (35) briefly consists of a white plastic tank subdivided by net-frames into four equal-sized rearing compartments – further termed sections 1–4 – each having a size of 27,8 cm × 17,8 cm

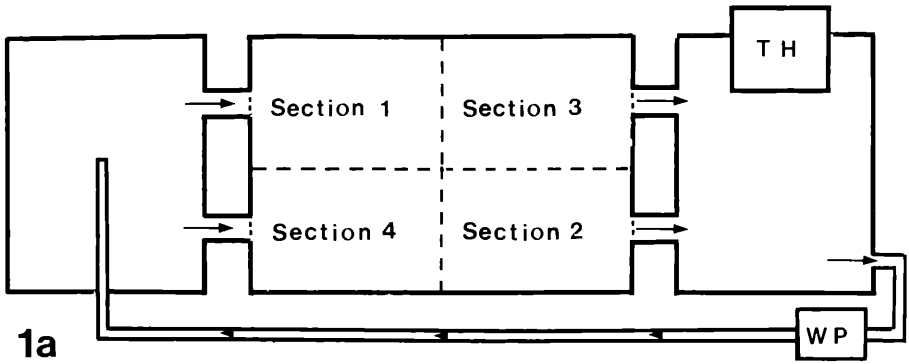


Fig. 1a: Diagram of the experimental set-up used in experiment # 1. TH thermostat, WP water pump. Arrows and arrowheads mark direction of water circulation.

(ground area). Bilaterally the tank is connected to a smaller plastic tank by two tube-lines in a manner shown in Fig. 1a. In the small right tank a thermostat is installed coupled with a water pump which circulates the rearing water without any treatment from the right tank to the small left tank via a tube line. Thus the water temperature is adjusted to 24 ± 0,1° C and the water circulates with about 6 liters per minute. By adjusting the water depth within sections 1–4 to 11,7 cm the whole system (experimental set-up) contains 60 liters. Thus every ten minutes the rearing water becomes recirculated and homogenous water conditions within the four sections are created. Each section was aerated by one air-bubble stone positioned as shown in Figs. 24 and 25. The water within the whole system was renewed each second week by two day old tap water as described above.

1.3 Experimental conditions

On May 17th, 1982 the experimental set-up was loaded with 725 tadpoles and placed into a light chamber containing various control elements (for details see 35). Fig. 2 gives the numbers of tadpoles placed into sections 1–4 as well as the individual space (volume of rearing water in the section/number of tadpoles per section) resulting from. Fig. 3 again shows the numbers of tadpoles having a particular developmental stage (41) at the beginning of experiment # 1.

Temperature, water transmission at 580 nm, strength of illumination 1 cm below water surface in section 2 (lux) and pH were recorded twice a day (9 am and 4 pm).

The experimental tadpoles were fed each second day with 2,9 liters nettle-powder juice resulting in 4 ml per tadpole. The nettle-powder juice was poured in equal portions into sections 1–4. For standardization procedure see above.

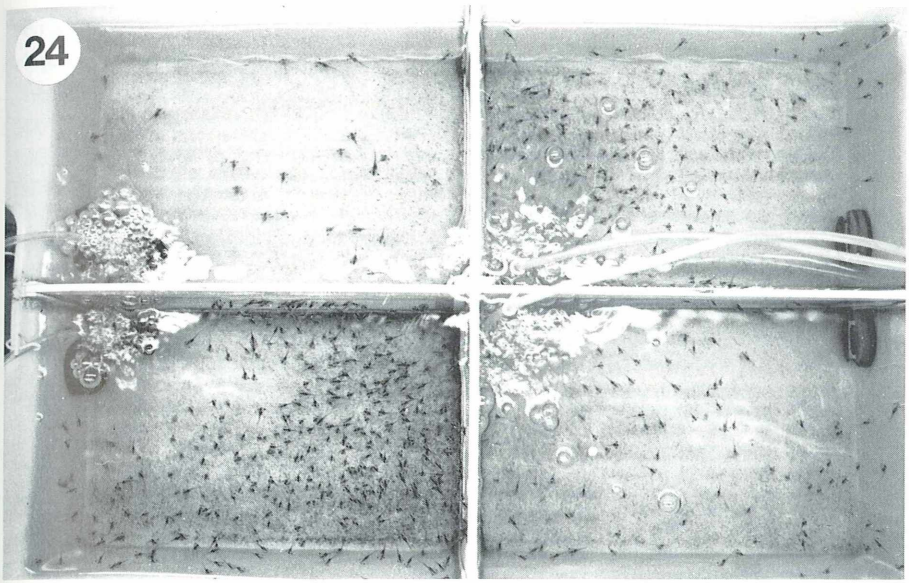


Fig. 24: Experiment # 1. Spatial distribution pattern of tadpoles of *Xenopus laevis* Daudin in the experimental set-up with aeration.

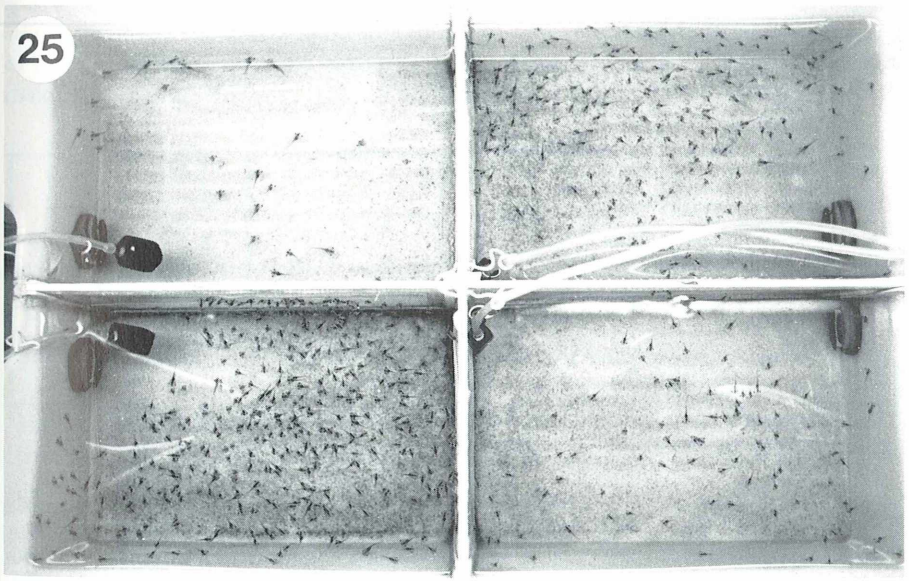


Fig. 25: Same as Fig. 24, but without aeration. Micrograph taken 30 Sconds after Fig. 24. Note the similiar distribution patterns under both conditions.

2	Number of tadpoles	Individual space (ml)
Section 1	25	24o
Section 2	1oo	6o
Section 3	2oo	3o
Section 4	4oo	15

Fig. 2: Experiment # 1. Number of tadpoles in sections 1–4 and resulting individual space (= water volume per section/number of tadpoles per section).

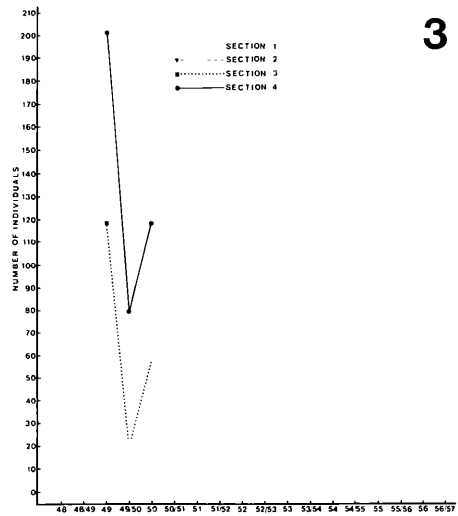


Fig. 3: Experiment # 1. Number of tadpoles having a particular developmental stage at the begin of the experiment.

Prior to feeding from each section 7 tadpoles were sampled randomly and total length and developmental stage were recorded.

As soon as the first tadpole metamorphosized within sections 1–4 which happened on July 3rd, 1982 experiment # 1 was finished.

2 Experiment # 2

Experiment # 2 was performed to study the importance of the number of tadpoles per section on growth, development and behaviour when having the same individual space availiable. In the four series run a total of 196 tadpoles was used.

2.1 Life history of tadpoles prior to the experiment

Tadpoles were bred the same way as described above, but instead of PG. 600 Gonadoplex® (LEO PHARMACEUTICAL PRODUCTS, Ballerup, Denmark) (500 IU in series 1–3) and Gonadoplex® (500 IU) plus Pregnyl® (ORGANON) (150 IU) dissolved in 1 ml of sterile physiological saline were used for induction of spawning in parent animals.

Fig. 4 summarizes further data on the life history of tadpoles till they were brought into the experiment.

4

	Induction of spawning on	Hormone(s) used/ dose per animal	Date of fertilization	Begin of the experiment	End of the experiment	Duration of the experiment
Series 1	April 19th, 1983	Gonadoplex® 500 I.U.	April 20th, 1983	May 10th, 1983	June 27th, 1983	48 days
Series 2	June 3rd, 1983	Gonadoplex® 500 I.U.	June 4th, 1983	June 29th, 1983	August 29th, 1983	61 days
Series 3	August 14th, 1983	Gonadoplex® 500 I.U.	August 15th, 1983	October 7th, 1983	November 8th, 1983	32 days
Series 4	March 12th, 1984	Gonadoplex® 500 I.U. + Pregnyl® 150 I.U.	March 13th, 1984	May 25th, 1984	July 4th, 1984	40 days

Fig. 4: Data on the life history of tadpoles of *Xenopus laevis* Daudin used in experiment # 2.

2.2 Experimental set-up

The experimental set-up used basically was the same as described for experiment # 1 with the exception that 1) the sections were numbered different and 2) that section 4 was subdivided by a net-frame into two equal-sized subdivisons having a size of 13,7 cm×8,9 cm. Into one of them a net-enclosure (7,7 cm×7,7 cm) was centered making up section 4/2 (Fig. 1b). By adjusting the water level to 11,7 cm the whole

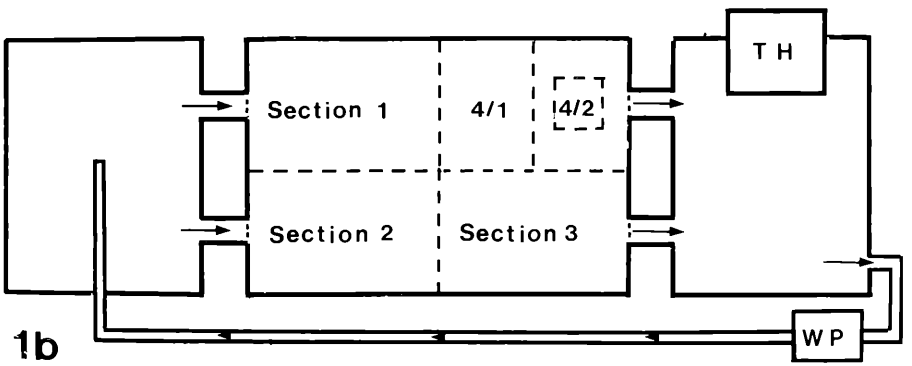


Fig. 1b: Diagram of the experimental set-up used in experiment # 2. Abbreviations and symbols see Fig. 1a.

rearing system again contains 60 liters like in experiment # 1. Further details – with the exception that during short periods in series 1 and 2 because of a failure of

the thermostat the water temperature ranged between 21–24°C – were identical to those given in experiment # 1. The air-bubble stone however was placed outside section 4/2 while that in the other sections was placed in the center of the section (Fig. 31).

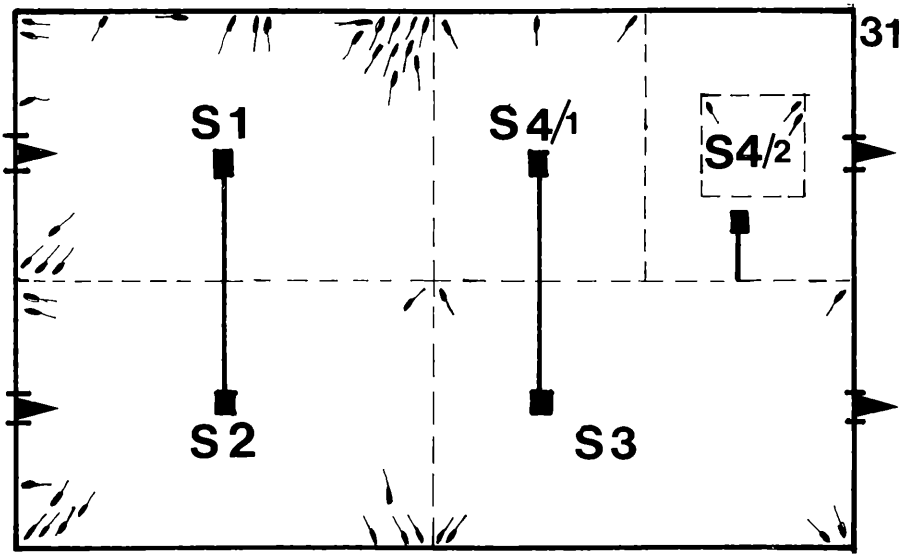


Fig. 31: Experiment # 2. Spatial distribution pattern of tadpoles of *Xenopus laevis* Daudin in the experimental set-up with aeration. S section, arrowheads indicate direction of water circulation, black rectangles represent air-bubble stones.

2.3 Experimental conditions

For each series of experiment # 2 a total of 49 tadpoles was used. Fig. 5 gives the

5

	volume in ml	number of tadpoles	individual space in ml
Section 1	6000	25	240
Section 2	6000	12	500
Section 3	6000	6	1000
Section 4/1	3000	3	1000
Section 4/2	720	3	240

Fig. 5: Experiment # 2. Series 1–4. Number of tadpoles in sections 1–4/2 and resulting individual space.

numbers of tadpoles per sections and the individual space resulting from. Additionally to the parameters recorded during experiment # 1 also oxygen content of the rearing water was measured just prior and shortly after water changes as well as

after each addition of nettle-powder juice using WINKLERs method (7). Tadpoles were fed each second day with 686 ml nettle-powder juice, i. e. 14 ml per tadpole. For further details we refer to experiment # 1. Prior to feeding seven tadpoles were randomly selected from sections 1 and 2 for measurement of total length and for staging; from the other sections all animals were recorded.

In experiment # 2 series 1 and 3 were started with tadpoles of stage 48 respectively 53 (41), series 2 and 4 with tadpoles having a total length of 14–17 mm respectively 29–32 mm. Experiment # 2 was run until in one of the sections a tadpole metamorphosized. Thus series 1 was run for 48 days, series 2 for 61 days, series 3 for 32 days and series 4 for 40 days.

3 Statistics

Sample data (total length and developmental stage) were used for histograms and after testing for fitting a normal distribution (KOLMOGOROV-SMIRNOV-test; 58) they were subjected to a non parametric test (multiple comparison according to NEMENYI; 53) in experiment # 1 and to the KRUSKAL-WALLIS-test (58) in experiment # 2 since data did not fit a normal distribution.

Results

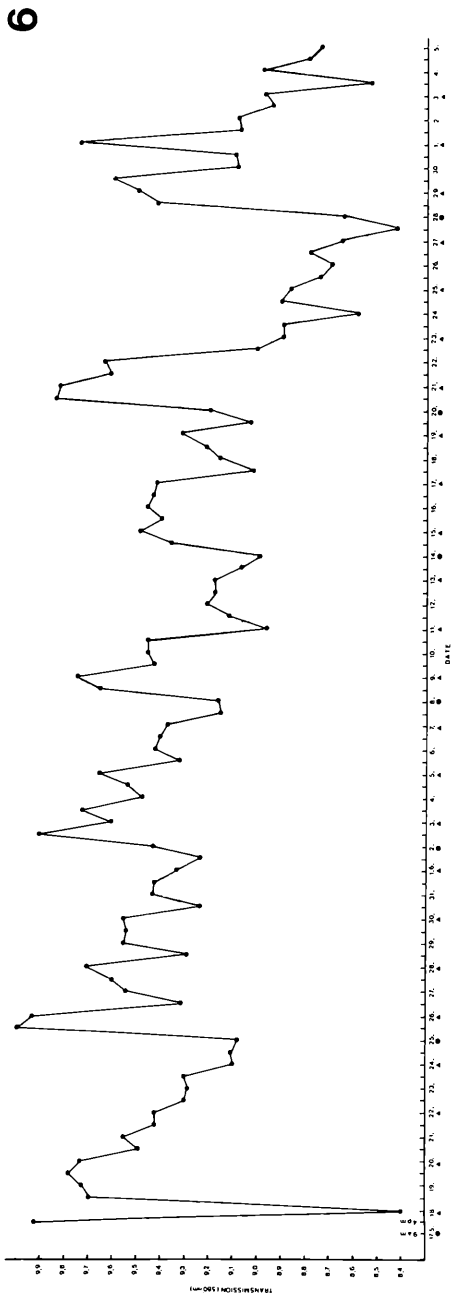
1 Experiment # 1

1.1 External parameters

The experimental set-up used held the temperature of the rearing water very constant at 24 ± 0,1°C resulting in good conditions for tadpole rearing. The transmission of the rearing water ranged from 84% to 100% whereby for calibrating 100% transmission a two day old tap water was used. The periodical changes observed closely correlated with addition of food and water renewal (Fig. 6). pH values which also showed the periodical changes ranged from 7,7–8,3 (Fig. 7). The intesity of illumination was from 360–570 lux.

1.2 Tadpole growth and development

Measurements of total lengthes of tadpoles on the second day of the experiment already revealed significant differences between tadpoles in sections 1 and 4 with higher lengthes in section 1 (Figs. 8, 9). During the further course of the experiment significant differences in total tadpole lengthes also occure between animals in section 1 and sections 2, 3 and 4. But differences between tadpoles in section 1 and 4 gradually become more pronounced (Figs. 11, 12, 14, 15, 17, 18, 20 and 21). There occured, however, no differences in total tadpole length between individuals in sections 2, 3 and 4 (Figs. 9, 12, 15, 18 and 21).



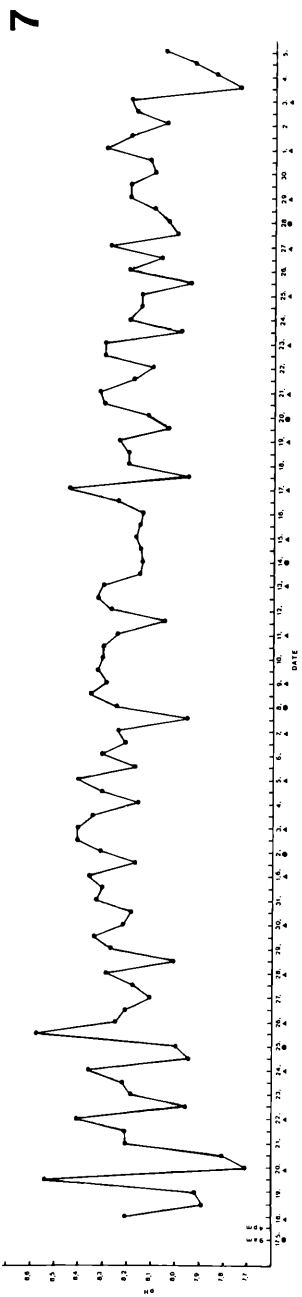
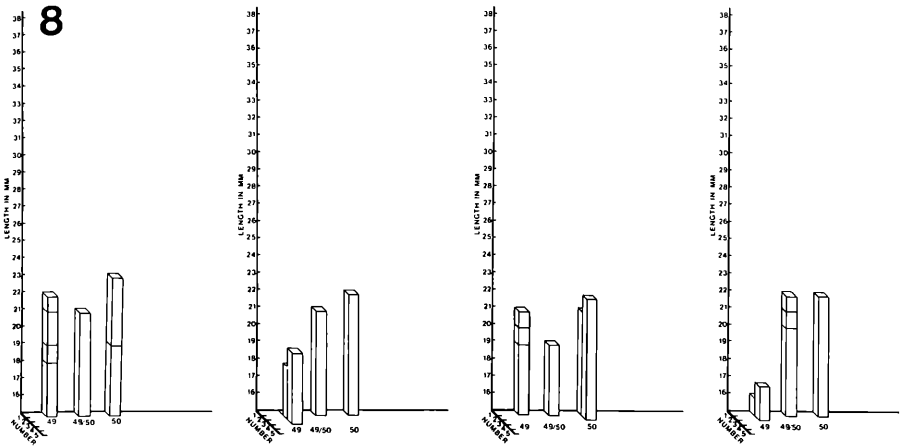


Fig. 6: Experiment # 1. Time course of the transmission of the rearing water in % at wavelength 580 nm.

Fig. 7: Experiment # 1. Time course of the pH of the rearing water.



9

Section		1	2	3
	Rank sum	159,5	86,0	90,5
86,0		63,5	x	45,0
90,5		69,0		
		89,5		

10

Section		1	2	3
	Rank sum	100,5	83,5	117,5
83,5		17,0	x	34,0

Fig. 8: Experiment # 1. Histogram of tadpole total lengthes and developmental stages sampled (N = 7) on the 2nd day of the experiment from sections 1–4.

Fig. 9: Experiment # 1. Second day. Multiple comparison of tadpole total lengthes sampled in sections 1–4 (according to NEMENYI [53]).

Fig. 10: Experiment # 1. Second day. Multiple comparison of tadpole developmental stages sampled in sections 1–4 (according to NEMENYI, 53).

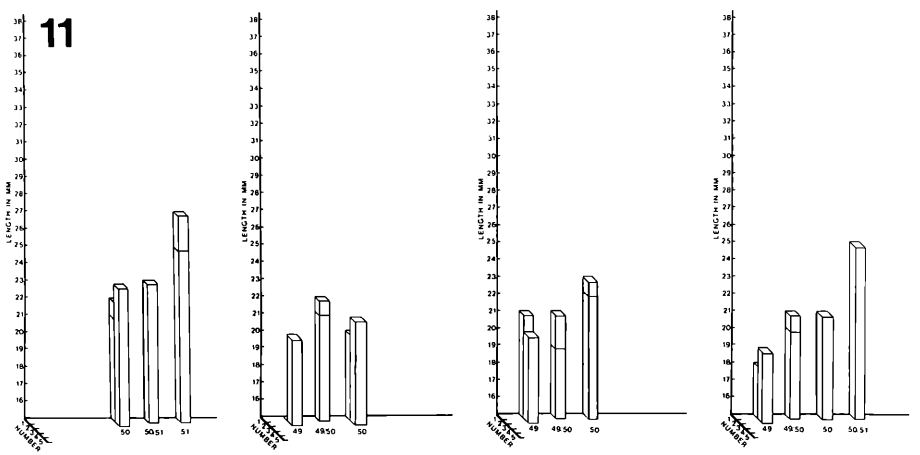


Fig. 11: Same as Fig. 8, but 8th day.

Section		1	2	3
	Rank sum	160,5	90,5	93,5
		70,0	\	3,0

12

Fig. 12: Same as Fig. 9, but 8th day.

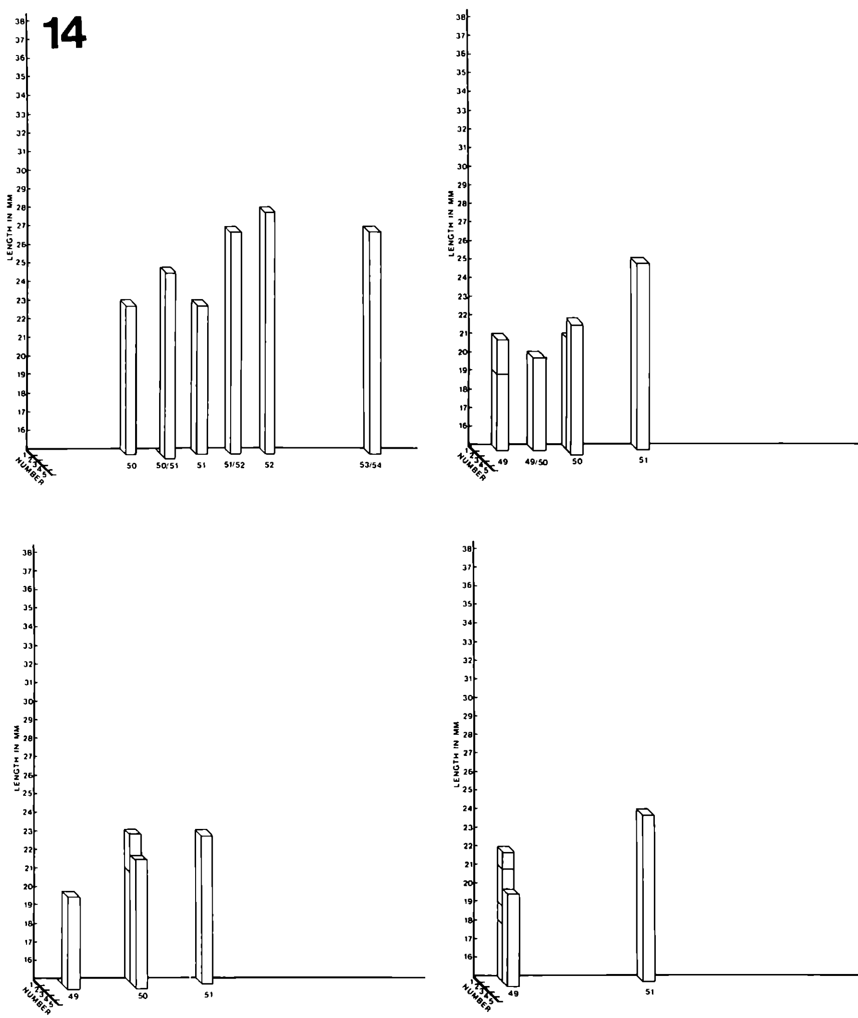


Fig. 14: Same as Fig. 8, but 20th day.

Section		1	2	3
	Rank sum	168,0	83,5	91,5
	83,5	84,5*	x	8,0
	91,5			
			20,5	28,5

15

Fig. 15: Same as Fig. 9, but 20th day.

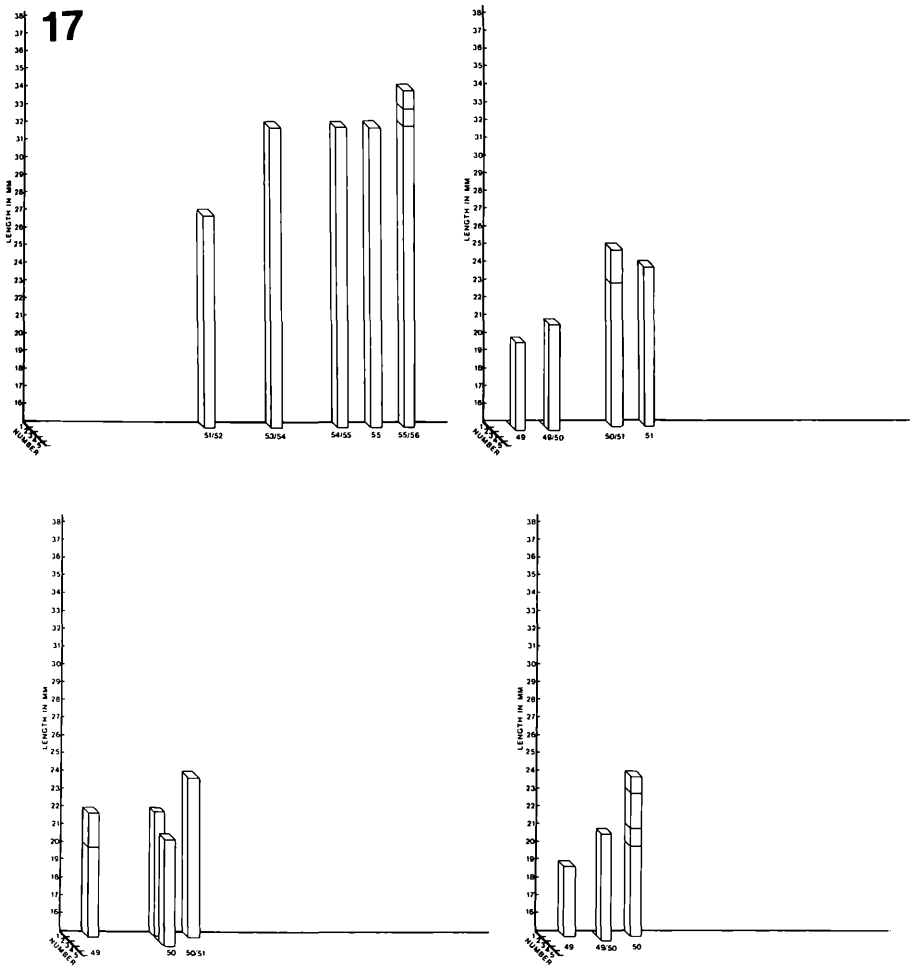


Fig. 17: Same as Fig. 8, but 32rd day.

Section		1	2	3
	Rank sum	175,0	83,5	77,5
	83,5	91,5*	x	
		97,5**	6,0	
		105,0**		

18

Fig. 18: Same as Fig. 9, but 32rd day.

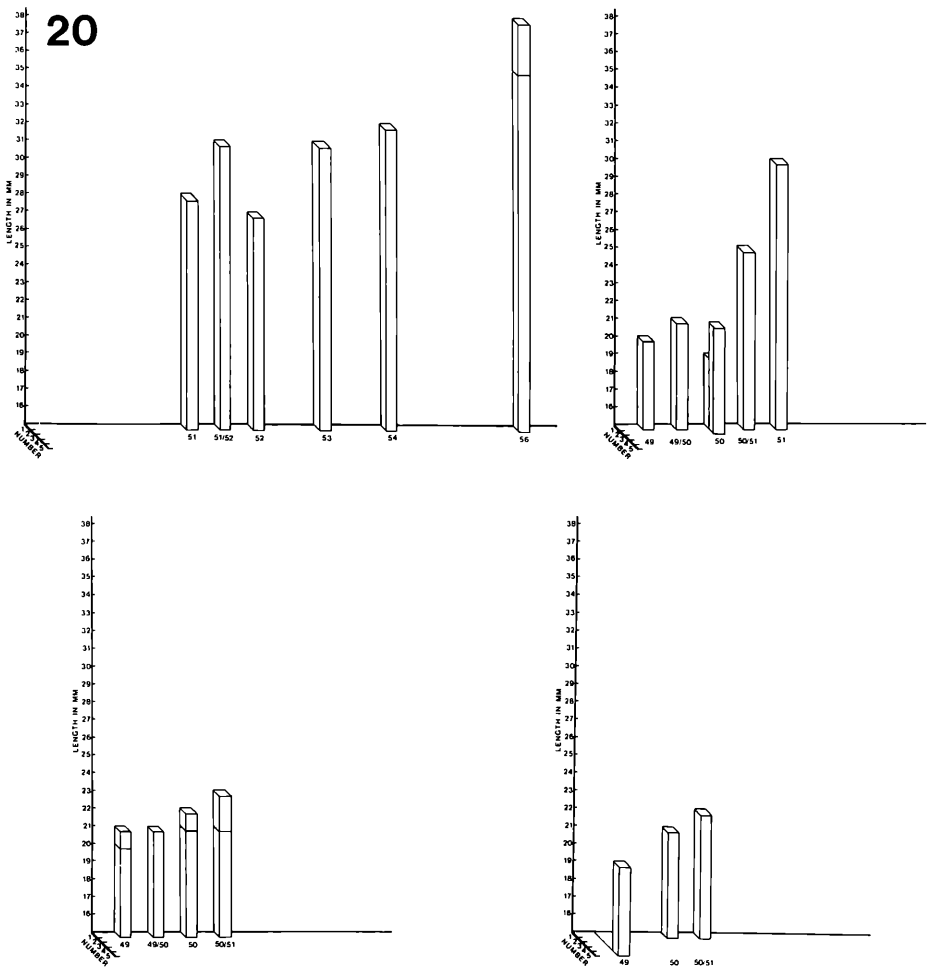


Fig. 20: Same as Fig. 8, but 48th day.

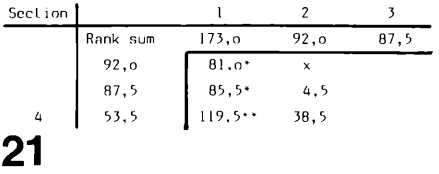


Fig. 21: Same as Fig. 9, but 48th day.

In respect to tadpole development differences between animals in section 1 and sections 3 and 4 occure as soon as on the 8th day of the experiment (Figs. 11 and 13). Again the greatest differences in development exist between individuals in sec-

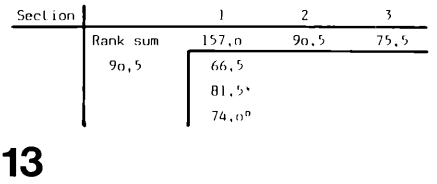


Fig. 13: Same as Fig. 10, but 8th day.

tion 1 and 4 with the fastest developing tadpoles in section 1. During the further course of the experiment the same happens as reported above for tadpole total lengthes (see above and Figs. 14, 16, 17, 19, 20 and 22). Again there are no differences in

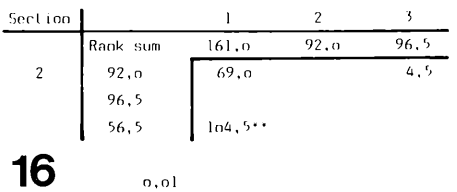


Fig. 16: Same as Fig. 10, but 20th day.

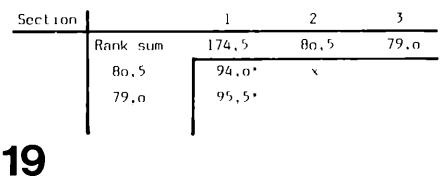


Fig. 19: Same as Fig. 10, but 32rd day.

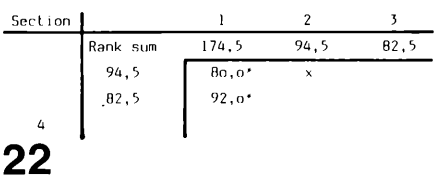


Fig. 22: Same as Fig. 10, but 48th day.

the developmental rate between tadpoles in sections 2, 3 and 4 which generally developed slowlier than those in section 1.

At the end of experiment # 1 in section 1 developmental stages ranged from stage 51 to stage 56/57, in section 2 and 3 from stage 49–52 and in section 4 from stage 49 to stage 53 (Fig. 23). In sections 2–4 the higher stages, i. e. stages 51 to 53

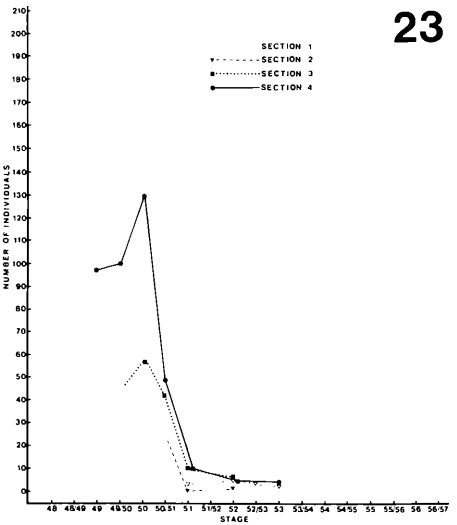


Fig. 23: Experiment # 1. Number of tadpoles having a particular developmental stage at the end of the experiment. Compare with Fig. 3. In section 1 all tadpoles have developed further.

comprised only 3% in section 2, 8% in section 3 and 4% in section 4 (Fig. 23). The majority of tadpoles belonged to lower developmental stages. This clearly indicates that in sections 2–4 the majority of tadpoles was arrested in its development (compare Fig. 3 with Fig. 23). It is obviously the high number of individuals in these sections which has led to a severe arrest of individuals. The critical density causing the observed effects is found between 25 animals per section (section 1) and 100 animals per section (section 2). In terms of individual space this means that effects occured somewhere between individual spaces of 240 ml (section 1) and 60 ml (section 2).

From experiment # 1 we therefore conclude that for tadpoles of *Xenopus laevis* Daudin an individual space of 240 ml is optimal if 25 tadpoles are raised together under well standardized conditions. If individual spaces of less than 240 ml to 60 ml cause a gradual or an abrupt arrest of tadpoles growth and development cannot be answered from experiment # 1. But if the number of individuals per se exerts an influence upon growth and development of tadpoles although having the same individual space available then experiment # 2 will show.

1.3 Tadpole mortality

At the end of experiment # 1 mortality was highest in section 2 with 11% dead individuals (11 tadpoles). In section 3 mortality was 3% (6 tadpoles), in section 4 it was 2,5% (10 tadpoles). In section 1 all tadpoles survived.

1.4 Behaviour of tadpoles

The main spatial distribution pattern of tadpoles during the experiment is depicted in Fig. 24 (with aeration) and in Fig. 25 (without aeration). Tadpoles were found to behave the same way under both conditions. This indicates that aeration does not influence the spatial distribution of individuals within sections 1–4. A zone around air-bubble stones as well as around water inlets (Figs. 24, 25; left side) and water outlets (Figs. 24, 25; right side) remained free of tadpoles. Tadpoles within section 1 preferentially gathered along solid walls and in the lower right quadrant while those in section 2 were more evenly distributed all over the section with some slight tendency to prefer the right half of the section. 200 tadpoles in section 3 swam in the upper half of the section when aeration was stoped for 30 seconds (Fig. 25) with some shifting to the left side when aeration was on (Fig. 24). Tadpoles in section 4 were distributed all over the section with the exception of those areas listed above.

2 Experiment # 2

2.1 External parameters

The external parameters transmission, pH and illumination of the rearing water revealed similar periodic changes as reported for experiment # 1. Because of a transient failure of the thermostat the water temperature temporarly ranged from 21–24°C. There was, however, no influence on growth and development of tadpoles. Oxygen content of the two day old tap water used for the weekly water change was 8 ± 0,5 mg O_2 ($\bar{x}$ ± S.E.M.) per liter at 19 ± 1°C. During the next two days a decrease in the oxygen content of the rearing water to 3 ± 0,3 mg O_2 per liter occured which remained at this level till the next water change.

2.2 Tadpole growth and development

During series 1, starting with tadpoles of stage 48 no significant differences were observed in growth and development between animals in sections 1–4/2 (Fig. 26). Starting the experiment with tadpoles having a total length of 14–17 mm (series 2) resulted in a significant different tadpole growth ($p < 0,05$), but not in a significant different tadpole development (Fig. 27). In series 3 when the experiment was started with tadpoles of stage 53 a statistics on the total lenghtes of tadpoles cannot be done because in this series tadpoles entered metamorphosis and thus inspite of increasing development tadpoles because of tail resorption show a decrease in total length (Fig. 28). For series 4 the same is due. Fig. 29 reveals the histograms of growth and development of tadpoles in these series.

Summarizing the development of tadpoles in experiment # 2 we find that animals in section 4/2 are in generally slowlier in their development than those in sections 1–4/1 (Fig. 30).

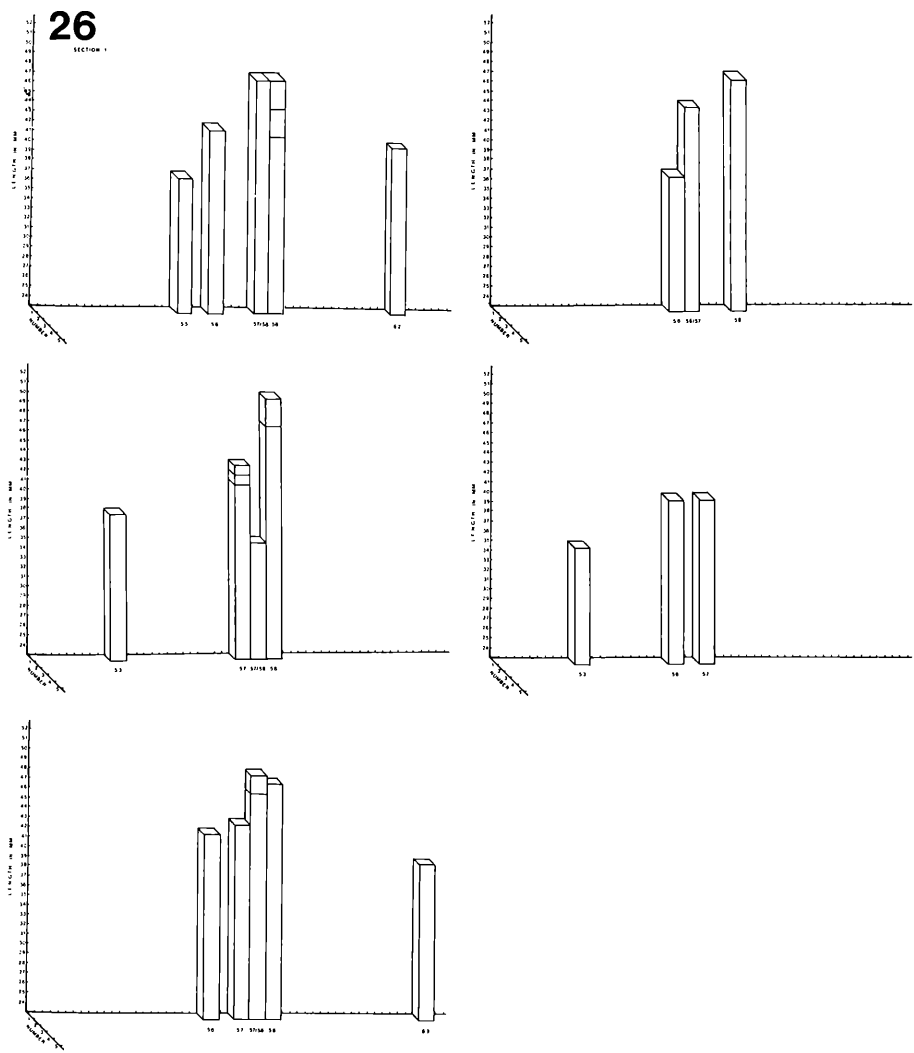


Fig. 26: Experiment # 2. Histogram of tadpole total lengthes and developmental stages sampled at the end of series 1 from sections 1–4/2.

Concluding it has to be mentioned that growth and development of tadpoles of *Xenopus laevis* Daudin in both experiments (# 1 and # 2) was generally slowlier than given in the normal table (41).

2.3 Mortality of tadpoles

With the exception of series 1 where 1 tadpole died out of section 1 there was no mortality during experiment # 2.

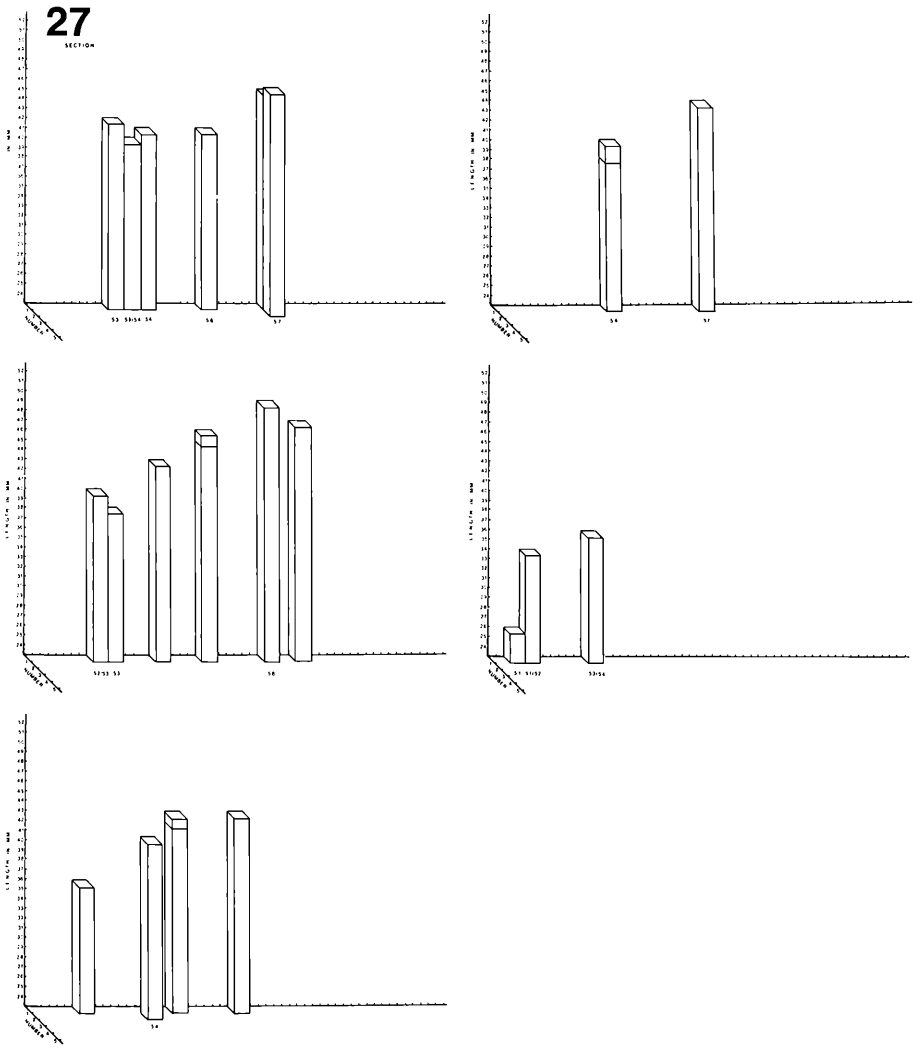


Fig. 27: Same as Fig. 26, but series 2.

2.4 Behaviour of tadpoles

In all five sections tadpoles preferred edges and to a less extent solid walls. In section 1 tadpoles aggregated predominantly in the right upper edge of the section. A representative spatial distribution pattern of tadpoles for experiment # 2 is given in Fig. 31. Tadpoles tended to form strong aggregates especially in corners in all sections whereby low interactive distances occure between individuals. The interactive distances ranged at about the half interocular distance of a tadpole. Tadpoles

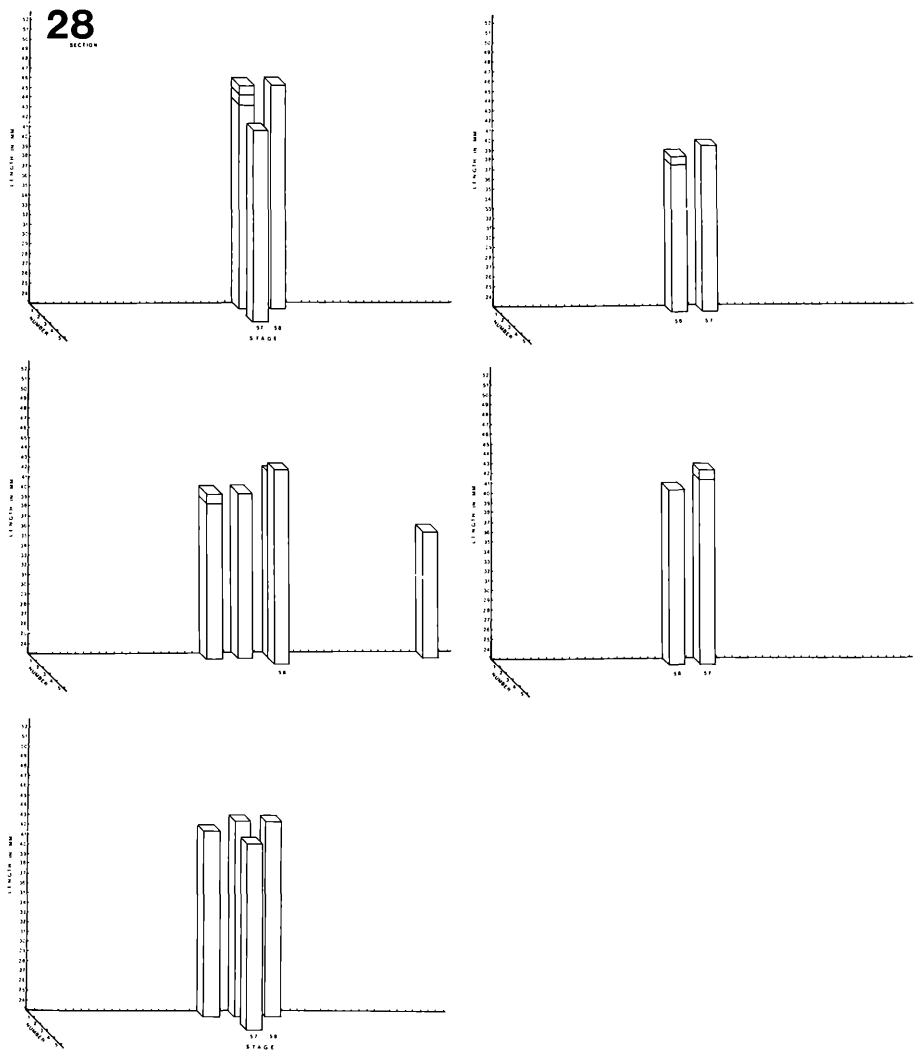


Fig. 28: Same as Fig. 26, but series 3.

in corners revealed little swimming activities. They stand quietly at midwater level and reveal a high degree of parallel orientation to each other. Along solid walls tadpoles are 1 to 1,5 interocular distances apart from each other. They swam actively, showed high filtering activities and revealed little parallel orientation if compared with those tadpoles being in corners.

Tadpole interactive distances were larger in section 4/1 than in section 4/2. Both sections housed 3 individuals, but they have different size and geometry and thus

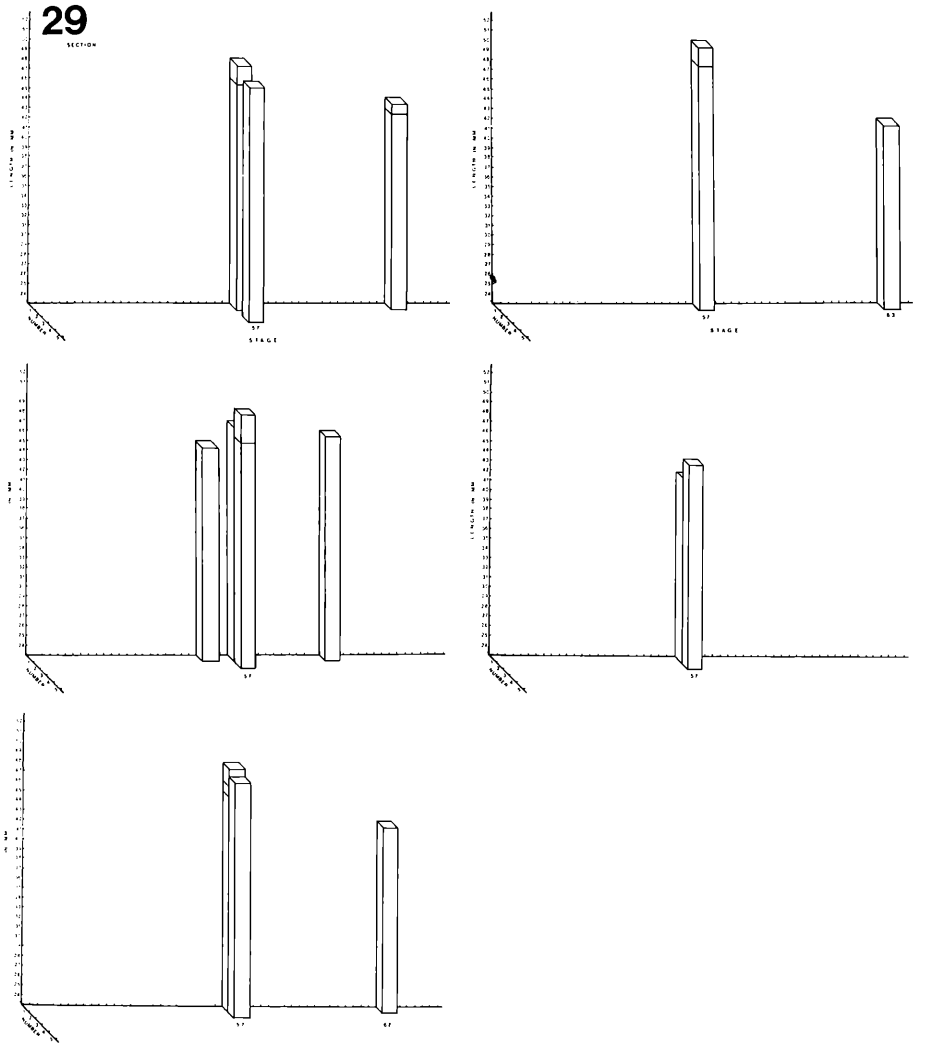


Fig. 29: Same as Fig. 26, but series 4.

tadpoles have different individual space availiable. Direct contacts between individuals occured very rarely.

Like in experiment # 1 tadpoles traversed the zones around the air-bubble stones and the areas in front of water inlets and water outlets only if changing their position within the section. They however never remain in these zones for a longer period of time.

30

	Section 1	Section 2	Section 3	Section 4/1	Section 4/2
Series 1	55 62	53 66	56 63	56 58	53 57
Series 2	52 66	52/53 59	52 57	54 57	51 53/54
Series 3	57 58	55 63	56 58	57 57	56 57
Series 4	57 - 62	56 - 60	57 62	57 63	56

Fig. 30: Experiment # 2. Range of developmental stages within sections 1–4/2 at the end of series 1–4. Note the low stage of tadpoles in section 4/2 during series 2–4.

Discussion

1 Arguments against the action of a "chemical factor" upon growth and development of tadpoles of *Xenopus laevis.*

The results of experiment # 1 indicate that a "chemical factor" released by large individuals cannot cause directly the significant decrease in larval growth and development of tadpoles of *Xenopus laevis* Daudin in sections 2–4. Two facts are in favour of this. 1) At the begin of experiment # 1 in all four sections a homogenous composition of larval stages, comprising large and small individuals, is given. 2) If a "chemical factor" is released by the larger members it will be distributed within a few moments all over the rearing water and tadpoles in all four sections will be exposed to the same concentration of this "chemical factor". From this we have to conclude that also smaller individuals present in section 1 should reveal retarded growth and development as actually is given in sections 2–4. But in contrary in section 1 all tadpoles develope further. This occures although two thirds of the initial number of tadpoles at the begin of experiment # 1 had stage 49, a low stage very capable to a retarding action of a given "chemical factor"

Thus from the facts given at the present time we have no evidence for a direct action of a "chemical factor" – if at all given – upon tadpoles of *Xenopus laevis* Daudin as described for other anuran tadpoles (49, 59).

We of course cannot rule out the possibility – since we have not made the attempt to remove a present "chemical factor" by pumping the circulating rearing water through a char-coal filter which is said to remove the "chemical factor" – that such a "chemical factor" is given, but then we have to state that it plays only a role as co-factor together with other factors. Stress, increased competition for food and increased interference are candidates for such factors. Since however these factors are believed to be absend or to be minimal in section 1 the role of a "chemical factor" as a co-factor does not become established. Thus in section 1 no retardation of growth and development of tadpoles occures.

2 Insufficient food as a cause of retarded growth and development of tadpoles of *Xenopus laevis* Daudin?

The retardation or even arrest of growth and development of tadpoles in sections 1–4 during experiment # 1 might be also attributed to an insufficient amount of

food availiable to tadpoles of these sections, while tadpoles in section 1 receive enough food. If we assume a homogenous suspension of the nettle-powder juice (nettler-powder particles) in the rearing water entering at first section 1 and section 4 (Fig. 1a) there will be more food particles availiable to each of the 25 individuals in section 1 than to each of the 400 tadpoles in section 4. Given that each tadpole filters a certain amount of food particles during a unit of time while the rearing water passes through section 1 and section 4 there will be more food particles left from animals in section 1 than from those in section 4. In turn the food particles left will enter sections 3 and 2 (Fig. 1a). Thus a higher amount of food particles will be avialiable to the 200 individuals present in section 3 than to those 100 tadpoles housing section 2. Rearing water leaving sections 2 and 3 will be mixed up by the stirring wheel of the thermostat in the small right plastic tank and will be repumped to the left small plastic tank to reenter sections 1 and 4. From the different amount of food particles availiable to tadpoles in sections 4, 3 and 2 we therefore should expect a gradual decrease of tadpole growth and development in sections 2, 3 and 4. But no such differences are found (Figs. 8–22).

Since food was added each second day we have to consider that because of the filtering activity of tadpoles with each recirculation the total amount of food particles entering sections 1 and 4 decreases. Thus it might happen that the number of particles availibale to tadpoles in section 1 is still high enough to enable normal growth and development while that in sections 2, 3 and 4 is too low and leads to an undernourishment of tadpoles resulting in the effects observed.

In respect to a possible undernourishment of tadpoles coprophagy is of interest (26, 60). It generally is assumed that some tadpoles under conditions of a low availiablity of food tend to perform coprophagy to cover their energetic demands (26, 60). Since in both our experiments tadpoles of *Xenopus laevis* Daudin never were found feeding on their feces we have to consider 1) that these tadpoles in any case cannot use their feces as a source of food and 2) that the amount of feces present in each section (Figs. 24 and 25) indeed reflects the amount of food particles actually ingested by tadpoles in these sections. The observation that in sections 4, 3 and 2 a proportionally higher amount of feces was found than in section 1 is therefore not in favour of an undernourishment of these tadpoles by simply too less food availiable to them. Lack of sufficient food alone therefore cannot be claimed having caused retardation or even arrest of growth and development of tadpoles in sections 4, 3 and 2.

3 Competition for food as a cause of retarded growth and development of tadpoles of *Xenopus laevis*?

At the first glance the experimental conditions given during experiment # 1 are very indicative for the occurance of serious competition for food between animals in sections 2–4, but not in section 1. We therefore conclude that in section 1 also the smaller members of the community could cover their energetic demands without any serious competition for food with the larger individuals. So also the initial smaller tadpoles have normal rates of growth and development. If one trans-

fers this assumption to the situation given in sections 2–4, one has to expect that here only the initially larger individuals can cover their energetic demands while the majority of tadpoles cannot. As a consequence few large tadpoles will suppress the sufficient supply of the small conspecifics which then under these conditions respond with retardation or even with an arrest of growth and development as actually found during experiment # 1 in sections 2–4 (Figs. 8–22).

But if competition for food is a matter of fact one also has to suggest that it will increase with higher numbers of tadpoles per section. Our data however reveal no differences in total lenghtes and developmental stages of tadpoles from section 2 to section 4.

Series 1 of experiment # 2 documents that competition for food is not alone a matter of numbers of tadpoles per section. If so tadpoles from section 3 of this experiment (# 2) should have least competition and therefore should reveal optimal growth and development. But they reveal the same growth and development as tadpoles in sections 1 and 2.

From our results we therefore conclude that competition for food also cannot be the main reason for the crowding effects observed in experiment # 1.

4 Are external parameters responsible for slow down of growth and development of tadpoles of *Xenopus laevis?*

Temperature, pH and osmolarity of the rearing water as well as light regimes (duration, intensity) are some of the external parameters which are known to influence growth and development of tadpoles. So pH-values below 4,8 are reported to accelerate the metamorphosing action of thyroxine (40, 50). But since during experiment # 1 pH-values ranged between 7,7 and 8,3 and between 6,1 and 8,3 in experiment # 2 they were well between critical values and therefore an influence upon thyroxine action has to be excluded as a cause of retardation of tadpole growth and development.

Different illumination times (light cycles) are also known to influence growth and development of larval amphibians (15, 18, 19 and 67). The action hereby is thaught to be via the hypothalamo-hypophysial-thyroidal axis. Since – with the possibility of minor deviations in the strength of illumination in section 4/2 of experiment # 2 which could be given by the additional net-enclosure leading to a little shadowing in some spots of this section (4/2), illumination was the same for all animals and therefore this external parameter also have to be eliminated as possible cause for the effects found. It further is known that tadpoles of *Xenopus laevis* Daudin raised under permanent darkness or permanent light reveal no differences in development (11). Therefore a possible little shadowing in section 4/2 during a light cycle from 14 hours bright and 10 hours dark cannot cause retardation of growth and development to an extent found in experiment # 2. Concluding this point of discussion it has to be kept in mind that all external parameters were the same for all tadpoles. They therefore should have acted upon all of them with the same intensity and all of them should have suffered from them provided however all tadpoles responded in the same manner.

5 Does interference competition act upon growth and development of tadpoles of *Xenopus laevis?*

Having excluded a series of factors as directly causing retardation or even arrest of tadpole growth and development the question concerning the importance of interference between tadpoles in the sections arises. Tadpoles in sections 1–4 (experiment # 1) have different individual space availiable. Those in section 1 obviously have enough space to fullfill their activities without being bordered by their conspecifics if the individual space – for definition see above – is 240 ml. In sections 2–4 only 60 ml, 30 ml or 15 ml of individual space are availiable. This in consequence leads to an increase in interference and animals increasingly need more energy to cover their needs from less and less food availiable. Only few larger animals will be able to cover their needs under these circumstances, but this already will be at costs of smaller members of the community. So the smaller animals gradually will have to struggle and as a consequence they will slow down growth and development or they even will stop it. How interference between tadpoles in detail acts upon growth and development, i. e. physically, psychically, neurologically or endocrinologically or in a combination remains subject of further studies.

6 Does the size of the individual space confine crowding effects of tadpoles of *Xenopus laevis?*

From experiment # 1 it is deducible that animals in section 1 having 240 ml of individual space have optimal conditions for growth and development. But with this conclusion one has to be very carefully because of the definition given for the individual space before (volume of rearing water per section divided by the number of tadpoles housing the section). So by simply multiplying or dividing both terms with any number new sections can be constructed, which offer the same individual space but with different numbers of tadpoles living together in different sized sections. Thus additional information to the data received from experiment # 1 are necessary. These data can be received from experiment # 2 which was designed to study this particular matter. Data received clearly show that in series 2 and 4 tadpoles in sections 1 and 4/2 reveal different development (Fig. 30) although they had the same individual space to live in. From this we have to conclude that the size of the individual space per se does not allow to draw conclusions as done before. We further have to keep in mind, that inspite of the same individual space, i. e. its volume, great differences are given in interference, competition for food, physical and social interactions if tadpole numbers and section volumes are increased or decreased.

Summarizing we have to state, that the decrease in tadpole growth and development in section 4/2 (experiment # 2) cannot be caused by the individual space per se if compared with the situation in section 1, were the same individual space is given, but where tadpoles grew and developed further.

Discussing the importance of the individual space necessary for tadpole raising one generally has to ask about the smallest and largest sizes of individual spaces which enable tadpoles to have normal growth and developmental rates. In this

respect one further has to ask if different species of tadpoles need different sized spaces to gain optimal conditions for growth and development. As far as this question is concerned, it is reproted that a single tadpole of *Rana pipiens* can be raised in 8 ml water (2 cm×2 cm×2 cm) up to a mass of 3,09 grams (32). This tadpole was reported to have a mass of about 40% of the living space availiable to the tadpole. The conclusion drawn is that for a tadpole of *Rana pipiens* growth is not inhibited by physical confinement provided the dimensions of the growth chambers (sections in our study) do not seriously hamper the tadpole movement (32). The author of the study further states that "At the same time that physical confinement depressed growth rates, it caused moderate acceleration of developmental rates and thereby advanced the onset of metamorphic climax" (32). If we compare the individual space of 240 ml given to tadpoles in section 1 during experiment # 1 and that of 240 ml, 500 ml and 1000 ml given to tadpoles in experiment # 2 with the individual spaces reported for tadpoles of *Xenopus laevis* Daudin in literature (Fig. 32) we will find, that with the exception of two studies (6, 23) the individual space availiable to tadpoles were considerably smaller then in our experiments. Reported individual spaces range from 5,1 ml – 166,6 ml (23) but there are also similar spaces like those given in our experiment # 1 in sections 2–4 (3, 23, 34). The number of tadpoles per section (compartment, growth chamber, experimental enclosure) however is different to those given in our experiment # 1.

If we compare the results of previous authors (32) with ours we have to conclude that physical confinement in none of the sections during experiments # 1 and # 2 can have influenced tadpoles in that manner as reported (32). As far as the growth of tadpoles in section 4/2 in experiment # 2 is concerned, we here have a similar sized section as JOHN (32) used for his control animals. But since we have found a decrease in tadpole growth within this section one should test, if for a tadpole of *Rana pipiens* an individual space of 8 cm×8 cm×8 cm indeed represents optimal living conditions (free swimming distances, mobility). Provided a similar behavioural mode of *Rana pipiens* tadpoles and *Xenopus laevis* tadpoles our data on growth and development of Xenopus tadpoles urge to test also larger individual spaces for *Rana pipiens* tadpoles as actually done (32).

In the literature data concerning the size of the individual space come mainly from laboratory work, while those from field studies are still very rare. Data published sofar primarely concern ranids, bufonids, hylids and pipids. From Fig. 32 it becomes evident that in previous studies done with tadpoles of *Xenopus laevis* Daudin 3 to 200 individuals per sections were tested (6, 23) resulting in an individual space from 5,1 ml to 5678 ml.

Having in mind the results from experiment # 1 where tadpoles having an individual space of 240 ml revealed normal growth and development (section 1) only one study worked without serious crowding (6). All other studies which primarely did not focuss their attention to growth and development of tadpoles but rather to their behaviour have therefore to be considered as done under crowded conditions, a fact which should be kept in mind in interpretating the results received on tadpoles behaviour.

Concerning the individual space we generally have to consider that a tadpole performs various activities which obviously need a certain amount of space. If the individual space becomes smaller than the space needed for tadpole activities the animal in any case will be irritated, either by other members of the community or by the physical enclosure, i. e. by the walls of the sections. But since tadpoles of *Xenopus laevis* Daudin are social individuals forming schools and aggregates their individual space has to be considered as being interwoven in a hitherto unknown manner. So the pure mathematical size of the individual space has to be considered as not that important as it might look like. This point of view becomes very important if experiments with large numbers of tadpoles and larger total space availiable to the tadpoles are compared with experiments using low numbers of individuals and low total space but with still having the same individual space. The difference comes from the fact that tadpoles in the larger space can fullfill their activities inspite of their higher numbers per section, while few tadpoles in a small section cannot do so because for example their free swimming distances are longer than the confinement is. This fact is very likely in section 4/2 of experiment # 2 where tadpoles are forced to contact the net-walls which they in the larger sections were never found to touch for a longer period of time. Concerning the retardation of growth and development of tadpoles in section 4/2 (experiment # 2) one also might argue, however, that the additional net-enclosure making this section prevented food particles from entering the section in a sufficient amount. To overcome this pitfall 1) food was dropped right into the net-enclosure whenever feeding was done and 2) special care was given to the cleaning of the net-enclosure to facilitate optimal food entrance and to keep contamination of the net-enclosure minimal. Thus it seems very unlikely, that undernourishment of tadpoles in section 472 of experiment # 2 has caused the effects found.

7 Do too large individual spaces retard growth and development of tadpoles of *Xenopus laevis?*

From a series of studies it is known that sensual deprivation influences development of animals. In experiment # 2 tadpoles in section 4/1 have an individual space of 1000 ml. The question if this causes a decrease in social contacts ("loneliness") which in turn may lead to a slow down of growth and development can be answered in as far as tadpoles in section 4/1 have the same growth and development as those in sections 1–3 of experiment # 2. From this we conclude that a low number of tadpoles of *Xenopus laevis* Daudin in a large rearing section is not affected negatively. As shown in Fig. 31 tadpoles in any case tend to be as close together as possible. The retarded growth and development of tadpoles in section 4/2 of experiment # 2 therefore cannot be explained by sensual deprivation.

8 Does the geometry of the individual space influence growth and development of tadpoles of Xenopus laevis?

As early as in 1885 (76) it was suggested, that aquatic animals grow faster in a shallow broad tank than in a small high one with the same volume of rearing

medium. Authors who studied this matter in detail, concluded that different shapes (geometries) of rearing tanks greatly influence the number of collisions between members of a tadpole community (24, 32). These collisions in turn lead to the effects of crowding, i. e. retardation of growth and development. Section 4/2 of our experiment # 2 is a rather small, but high section and might thus influence tadpoles in a manner similar as described elsewhere (32). The observation, that during experiment # 2 tadpoles in sections 1–4/1 kept off the net-walls, those in section 4/2 in contrast were found frequently on the nets indicates that the different geometry of section 4/2 forced the tadpoles to contact the "unwanted" nets. As a result mechanical lesions of the skin of tadpoles are likely to occure. These lesions irritate tadpoles and create physical stress to the animal. This stress at the present time seems to be a realistic candidate causing retardation of growth and development of tadpoles during experiment # 1 in sections 2–4 and during experiment # 2 in section 4/2.

Aknowledgements

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32

Species	Number of tadpoles per compartment	Individual space in ml (volume of compartment/ number of tadpoles)	Reference(s)
Rana pipiens	3, 25, 1oo	162,5; 19,5; 4,87	ROGH (1962)
	1o	8o; 15o; 2oo	WASSERSUG and SEIBERT (1975)
	1	6ooo	ROSE (1959)
	1, 2, 3	1ooo; 5oo; 333,3	ROSE (196o)
	4, 5, 6	25o; 2oo; 166,6	ROSE (196o)
	7, 39, 4o	143; 25,6; 25	ROSE (196o)
	66	16,6	ROSE (196o)
	14, 16	857; 75o;	ROSE (196o)
	37, 53	324; 226	ROSE (196o)
	1, 2, 4	25o; 125; 125	ROSE (196o)
	1o	3oo	WEST (196o)
	5	8oo; 4oo; 2oo; 1oo	GROMKO et al. (1973)
	5	5oo; 25o; 125; 62,5	GROMKO et al. (1973)
	5	11oo; 55o; 275; 137,5	GROMKO et al. (1973)
	1	8; 512	JOHN (1981)
	6, 4	166,6; 25o	RICHARDS (1958)
	2o, 32	285; 2o6	JOHN and FENSTER (1975)
	4	25o	RICHARDS (1958)
	6, 1, 1	83; 21 237; 15 927	AKIN (1966)
	12	83	LICHT (1967)
Rana utricularia	16o	237,5	ALFORD and CRUMP (1982)
	1, 2 , 8	2oo; 1oo; 25	STEINWASCHER (1978)
Rana clamitans	1	2oo	STEINWASCHER and TRAVIS(1983)
	1, 2, 8	2oo; 1oo; 25	STEINWASCHER (1978)
	75	13,3	AKIN (1966)
Rana arvalis	4o	unknown	PYASIOLOVA and SHVARTS(1976)
Rana sylvatica	1	15o	SEALE and WASSERSUG (1979)
	1, 2, 4, 8	25o; 125; 62,5; 31,25	WILBUR (1977)
	5o, 75, 1oo	18 16o; 12 1o6; 9 o8o	WILBUR and COLLINS (1973)
	125, 15o, 3oo	7 264; 6 o53; 3 o26	WILBUR and COLLINS (1973)
	6oo, 12oo	1 513; 756	WILBUR and COLLINS (1973)
Rana esculenta	2o	6oo; 27o; 93; 57	BILSKI (1921)
	13, 1o, 5	88; 114; 228	BILSKI (1921)
	2, 4, 8	37,5; 18,7; 9,35	GOETSCH (1924)
	2, 4, 8	12,5; 6,25; 3,12	GOETSCH (1924)
	2o	9o; 1o	GOETSCH (1924)
	1, 2, 5	1ooo; 5oo; 2oo	POURBAGHER-MOHTADI (1968)
	1o, 15	1oo; 66,6	POURBAGHER-MOHTADI (1968)
Rana temporaria	14o, 14	5o; 5oo	HODLER (1958)
	1, 1o	1ooo; 1oo	POURBAGHER-MOHTADI (1968)
	2, 5, 6,	5oo; 2oo; 166,6	POURBAGHER-MOHTADI (1968)
	15, 25	66,6; 4o	POURBAGNER-MODIADI (1968)
Rana catesbeiana	75	13,3	AKIN (1966)
	2, 12	5oo; 83	LICHT (1967)
Rana dalmatina	1, 2, 5	1ooo; 5oo; 2oo	POURBAGHER-MOHTADI (1968)
	8, 1o	125; 1oo	POURBAGHER-MOHTADI (1968)
Rana palustris	12	83	LICHT (1967)
Rana camerani	5o	4o	STEPANOVA (1974)

Fig. 32: Reference data concerning experimental parameters (number of tadpoles per section and individual space resulting from) used by various authors, who have studied crowding effects or tadpole behaviour.

Species	Number of tadpoles per compartment	Individual space in ml (volume of compartment/ number of tadpoles)	Reference(s)
Bufo woodhousei	1o	8o; 15; 2oo	WASSERSUG and SEIBERT (1975)
	36, 7o, 1o7	32; 16; 11	BREDEN et al. (1982)
	1o, 35	1oo; 28,5	LICHT (1967)
Bufo americanus	1oo	4 46o; 1 68o; 63o	BROCKELMAN (1969)
	1oo	4 46o; 1 11o; 27o	BROCKELMAN (1969)
	1oo	66; 4o	BROCKELMAN (1969)
	4	25o	ROSE (196o)
Bufo boreas	1oo	18	HUEY (198o)
Bufo valliceps	35	28,5	LICHT (1967)
Bufo speciosus	1o, 35	1oo; 28,5	LICHT (1967)
Bufo bufo	1, 2, 5, 1o	1ooo; 5oo; 2oo; 1oo	POURBAGHER-MOHTADI (1968)
	15, 25	66,6; 4o	POURBAGHER-MOHTADI (1968)
Bufo sp.	12	45o; 15o; 11o; 95	STEINWASCHER (1978)
Xenopus laevis	3	93,7; 166,6	GERBES (1957)
	6 8	62,5 46,8	GERBES (1957)
	6 8	62,5 83,3	GERBES (1957)
	4 6	62,5 93,7	GERBES (1957)
	4 6	83,3 125	GERBES (1957)
	3 4	93,5 125	GERBES (1957)
	3 4	125 166	GERBES (1957)
	1	375; 5oo	GERBES (1957)
	1	15o	SEALE and WASSERSUG (1979)
	1oo	1o	AKIN (1966)
	47, 91, 182	72,7; 37,5; 18,7	KATZ et al. (1981)
	1o 2o	5 678 2 839	ARONSON (1944)
	1oo 2oo	567,8 283,9	ARONSON (1944)
Hyla chrysoscelis	2, 6	1oo; 33,3	STEINWASCHER (1981)
	1	2oo	STEINWASCHER and TRAVIS(1983)
	15	66,6	LICHT (1967)
Hyla crucifer	1o, 2o	7oo; 35o	CRUMP (1981)
Hyla gratiosa	5o, 8, 16, 1	6oo; 75o; 375; 4oo	TRAVIS (1983)
Litoria ewingi	1o, 4o	236; 59	SOKOL (1984)
	5, 2o	5oo; 125	SOKOL (1984)
	1o, 35	25o; 71	SOKOL (1984)
Alyes obstetricans	2, 1	4oo	GUYETANT (1977a, 1977b)
	5, 2	4oo	GUYETANT (1977a, 1977b)
	1	8oo	GUYETANT (1977a, 1977b)

Fig. 33: Fig. 32 continued.

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