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STARVATION EXPERIMENTS ON TADPOLES AND NEWLY METAMORPHOSED OF SOME SPECIES OF ANURA

(Bombina variegata, Bufo viridis, Hyla arborea, Rana dalmatina).

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Abstract: *Laboratory starvation tests on newly-hatched tadpoles and newly-metamorphosed individuals of the sympatric Anura Bombina variegata, Bufo viridis, Hyla arborea and Rana dalmatina showed that they could survive for several weeks without any external food and several of them may even recover when they are given food after a severe starvation.*

The resistance to starvation of the newly-hatched tadpoles is related with the size of eggs interspecifically and, up to a point, intraspecifically.

The survival of the starved tadpoles and newly metamorphosed individuals is depended on the time food is given interspecifically; intraspecifically, it is mainly depended on the ability to get food.

INTRODUCTION

Although food is considered to be an important factor for the duration of larval period (Kaltenbach, 1968; Goin and Goin, 1971) and the morphology of tadpoles and newly metamorphosed frogs and toads (Shimozawa, 1967), it has not been cleared up whether it plays a decisive role to the survival of them. Lack of food may be important to the regulation of the populations of other vertebrates (Lack, 1954; Emlen, 1973) but for the tadpoles and frogs which live in ponds and marshes, where food is abundant, there is only a small possibility that this lack causes death to the tadpoles (Calef, 1973; Licht, 1974). Evidence which supports the argument that starvation is not a principal factor of mortality is given by starvation experiments on tadpoles and newly metamorphosed Anurans in the laboratory (Shimozawa, 1967; Cafel, 1973; Licht, 1974; Sofianidou, 1977).

The main purpose of the present study is a resistance test to com-

plete lack of food on tadpoles and newly metamorphosed of some species of Anura *(Bombina variegata, Bufo viridis, Hyla arborea, Rana dalmatina)* and moreover, an attempt to learn something about intraspecific and interspecific effect of food on their survival. Experimenting on many species of tadpoles of widely varying phylogenies may provide us with useful information on the survival of populations.

MATERIAL AND METHODS

The four species of tadpoles and newly metamorphosed frogs and toads used in this study represent 4 genera, 4 families and 3 suborders.

The species *Rana dalmatina, Hyla arborea, Bombina variegata* and *Bufo viridis* are sympatric in riverside ponds of Gallikos river. During the breeding period (February - April) of 1976, eggs of the first three species were collected from the above breeding ponds and were carried to the laboratory for hatching. In one case, eggs of *Bufo viridis* were collected from the same region of Gallikos river and in the rest cases from the pool near Meteorological Building in the University area, Thessaloniki. The eggs were always recent (undivided or 2-8 blastomeres). In every group of eggs their mean diameter was measured with a micrometric climax adjusted to a stereoscope; the total number of eggs of each eggmass which gave the experimental egg specimens were calculated with direct count.

After hatching, which took place in water brought from the biotopes and in temperatures similar to those of the environment (in a cold room near open windows), 50 newly-hatched tadpoles of each species* were placed in similar glass aquariums with a proportion of 10 tadpoles per liter of water. We followed the experimental procedure from data of the works by Licht (1974) and Sofianidou (1977). As the beginning of each experiment was considered the stage of covering of the external gills (stage 25, Shumway, 1940) because the various species are hatched during different stages of their embryonic development. The tadpoles were given no food at all, and the water was changed every three days, to avoid the development of microor-

* For *Bombina variegata* a smaller number of tadpoles was used. This species lays its eggs in small groups or solitarily on aquatic plants *(Ranungulus trichophilus)*, for that reason it is difficult to find a large number of eggs and be sure that they come from the same individual. For the same reason it is difficult to count the total number of eggs of each eggmass.

ganisms. The number of days the tadpoles lived was recorded daily. When 50% of the tadpoles of each species died the remaining ones were given food (boiled lettuce) and the subsequent survival time and behavior were recorded.

Similar experiments were carried out on newly metamorphosed frogs and toads of each species. Groups of each species of the newly metamorphosed individuals in the laboratory were raised without food from the beginning of metamorphosis. The frogs and toads were placed in terrariums with a layer of moist sand on the bottom, in room temperature. The number of days until death was recorded. When 50% of each species in every group died, food was given to the remaining ones (drosophilae and pieces of small earthworms). The behavior and survival of the frogs and toads were recorded.

In order to assess the effects of resistance to starvation among populations of the same species, at least two series of experiments were carried out on different eggmasses of each species (except the newly-metamorphosed of *Bombina variegata* and *Hyla arborea*).

RESULTS

The results of starvation experiments on the various species of tadpoles and newly metamorphosed Anura are summarised in Table 1 and 2 and a more detailed analyses of them is given below.

TABLE 1

Resistanee to starvation of the tadpoles of some species of Anura.

Species	Without food		After feeding		
	Initial number of tadpoles	Mean life in days of the 50% of tadpoles	Initial number of tadpoles	Number of dead tadpoles	Number of survived tadpoles
Bombina variegata	18	25.8 (23-28)	9	3 or 33.3 %	6 or 66.6%
» »	15	26.3 (25-27)	8	3 or 37.5 %	5 or 62.5%
Bufo viridis	50	25.5 (23-27)	25	18 or 72 %	7 or 28 %
» »	50	22.7 (13-27)	25	18 or 72 %	7 or 28 %
» »	50	21.8 (20-26)	25	16 or 64 %	9 or 36 %
Hyla arborea	50	25.8 (14-31)	25	15 or 60 %	10 or 40 %
» »	40	18.9 (15-21)	20	6 or 30 %	14 or 70 %
» »	20	18.1 (17-20)	10	4 or 40 %	6 or 60 %
Rana dalmatina	50	30.1 (23-41)	25	12 or 48 %	13 or 52 %
» »	50	23.3 (14-26)	25	19 or 76 %	6 or 24 %
» »	50	25.5 (12-27)	25	16 or 64 %	9 or 36 %

Bombina variegata. In the first experiment, 9 of the 18 starved tadpoles died in 23 - 28 days ($\bar{x}$=25.8) from the day the external gills were covered (beginning of the experiment). Three of the remaining 9 individuals that were fed died in 2-5 days after food was given, while the remaining 6 survived and metamorphosed regularly.

In the second experiment, 7 of the 15 starved individuals died in 25-27 days ($\bar{x}$=26.3). Three of the remaining 8, to which food was given, died in 1 - 5 days after the beginning of feeding. The remaining 5 individuals survived and metamorhosed regularly.

Bufo viridis. Twenty five of the 50 starved tadpoles, in each of the 3 experiments that were carried out, died in 25.5(23-27), 22.7(13-27) and 21.8 (20-26) days in average in the first, second and third experiments respectively. After food was given to the remaining 25 tadpoles of each experiment, 18, 18 and 16 individuals died respectively, while the remaining ones survived.

Hyla arborea. During the first experiment, 25 of the 50 starved tadpoles died in 14-31 days ($\bar{x}$=25.8) from the beginning of the experiment. Fifteen of the remaining 25 individuals, which were fed, died in 1 - 5 days from the day of feeding. The remaining ones survived.

During the second experiment, 20 of the 40 starved tadpoles died in 15-21 days ($\bar{x}$ = 18.9) from the beginning of the experiment. Six of the remaining 20 individuals, to which food was given, died in 2-5 days from the beginning of feeding. The remaining 14 tadpoles survived and metamorphosed. Two of them, which were considerably larger during hatching, metamorphosed in 60 and 63 days, respectively, since egg-laying (regular metamorphosis time for normally developed tadpoles of this species) ; six metamorphosed in 72-80 days and the last 4, which were considerably smaller than the others during their development, metamorphosed with a singificant delay in 105-110 days.

In the third experiment, 10 of the 20 starved tadpoles died in 17-20 days ($\bar{x}$=18.1). Four of the remaining 10 individuals died in 2 days while the rest survived and metamorphosed. The metamorphosis of the last 2 tadpoles took place with a significant delay, such as in the previous experiment.

Rana dalmatina. Twenty five of the 50 starved tadpoles in each of the three experiments died in 23-41 ($\bar{x}$=30.1), 14-26 ($\bar{x}$=23.3), and 12-27 ($\bar{x}$=25.5) days respectively. After food was given to the remai-

ning 25 tadpoles of each experiment, 19, 12 and 16 individuals died respectively, while the rest survived and metamorphosed regularly.

NEWLY - METAMORPHOSED INDIVIDUALS

Starvation experimets on newly-metamorphosed frogs and toads showed the following results for each species.

TABLE 2

Resistance to starvartion of the newly-metamorphosed individuals of some species of Anura.

Species	Without food		After feeding		
	Initial number of individ.	Mean life in days of 50 % of individ.	Initial number of individ.	Number of dead individ.	Nnmber of survived individ.
Bombina variegata	20	30.4 (28-35)	10	4 or 40 %	6 or 60 %
Bufo viridis	50	26.7 (14-28)	22	22 or 100 %	0
» »	50	26 (15-26)	25	23 or 92 %	2 or 8 %
Hyla arborea	20	30 (22-38)	10	8 or 80 %	2 or 20 %
Rana dalmatina	50	12 (9-15)	24	13 or 54 %	11 or 46 %
» »	50	17.3 (15-21)	25	19 or 76 %	6 or 24 %

Bombina variegata. Ten of the 20 starved individuals since memorphosis lived 28 to 35 days ($\bar{x}$=30.4). Of the remaining 10 individuals that were given food later, 4 died in 5-10 days from the day of feeding, in other words, they lived 40 to 45 days from the beginning of the experiment. The remaining 6 individuals survived and developed regularly.

Bufo viridis. Twenty-eight of the 50 srarved individuals died in 14-28 days ($\bar{x}$=26.7). Twenty-one of the remaining 22 individuals which were given food later died in 1 - 4 days ($\bar{x}$=2.24) from the beginning of feeding or 28-32 days from the beginning of the experiment. The only remaining individual which was considerably larger since metamorphosis lived for 45 days altogether, but finally died. The twenty-two individuals which were given food, were in very poor condition and although most attempted to catch flies, they could not.

In the second experiment* 25 of the initial 50 individuals lived

* In this experiment the newly metamorphosed individuals came from tadpoles of a population from nature, which were carried to the laboratory when they were in the stage that the fore limbs appeared (stage xx Taylor and Kollros, 1946).

from 15 to 26 days ($\bar{x}$=26) without any external food since metamorphosis. Ten of the remaining 25 individuals which were given food later, died within the next two days and 13 died in 5-8 days after the beginning of feeding. Two individuals finally survived and they were raised in the laboratory for 5 months, but their development was very slow.

Hyla arborea. Ten of the 20 starved newly-metamorphosed individuals lived from 22 to 38 days ($\bar{x}$=30) and 8 of the remaining 10 lived from 40 to 46 days. Two individuals survived and developed normally. Most of the 8 individuals which died after feeding, showed a light edema before food was given, and they almost could not take any food.

Rana dalmatina. Twenty-six of the 50 starved individuals died in 9 - 15 days ($\bar{x}$=12). Thirteen of the remaining 24 individuals which were given food later died in 1 - 22 days ($\bar{x}$=12,8) from the beginning of feeding, or in 15-37 days from the beginning of the experiment. The rest 11 individuals survived and developed.

In the second experiment, 25 of the 50 individuals died in 15-21 days ($\bar{x}$=17.3). Five of the remaining 25 individuals which were given food, died the next day without taking any food, and 14 died in 3-8 days from the day of feeding. The remaining 6 individuals survived and are still raised in the laboratory.

TABLE 3

Diameter of eggs in mm, number of eggs in each egg-mass, and mean life in days of the starved tadpoles.

Species	Diameter of eggs in mm.	Total number of eggs in each eggmass	Mean life in days of the starved tadpoles
Bombina variegata	1.90	—	25.8
» »	2.00	—	26.3
Bufo viridis	1.35	13,956	25.5
» »	1.20	15,309	22.7
» »	1.17	17,956	21.8
Hyla arborea	1.11	55	18.9
» »	1.10	—	18.1
» »	1.23	85	25.8
Rana dalmatina	1.99	1,300	30.1
» »	1.49	545	23.3
» »	—	—	25.5

TABLE 4

Lenght in mm of the newly metamorphosed, mean life in days of the starved newly-metamorphosed, and percentage survival after feeding.

Species	Length in mm of the newly-metamorphosed	Mean life in days of the starved newly metamorphosed	Survival % after feeding
Bombina variegata	13.7	30.4	60
Bufo viridis	12.8	26.7	0
» »	12.7	26	8
Hyla arborea	15.3	30	20
Rana dalmatina	15.2	11.9	46
» »	15	17.3	24

DISCUSSION

The results of the starvation tests on tadpoles (Table 1), showed that the newly-hatched tadpoles of the Anura *Bombina variegata, Bufo viridis, Hyla arborea* and *Rama dalmatina* could survive for several weeks without external food. Several of them may even recover soon, when they are given food after a severe starvation. Similar experiments which were carried out on other species of Anura also showed a high resistance to starvation. Thus, it has heen proved that *Pelobates syriacus* resists to starvation for an average time of 29.4 and 32.5 days respectively, in two populations of 100 and 150 tadpoles (Sofianidou, 1977). Licht (1974) also reports a resistance to starvation for an average time of 25 and 24 days for the species *Rana pretiosa* and *Rana aurora* among populations of 20 tadpoles for each case. Shimozawa (1976) reports that the tadpoles of *Bufo vulgaris tormosus* lived without food from the stage of covering the external gills 41.7 days in average, but tbe estimation was on the whole population and not on the 50% of it.

Since in nature it is impossible to occur a complete lack of food, the possibility that starvation acts as a mortality factor of tadpoles is very small. Brockelman (1969), in a study on growth and survival of *Bufo americanus* tadpoles, considers that food shortage is very important, while from laboratory starvation tests by Licht (1974) and Sofianidou (1977) as well as from our experiments is shown that even a small amount of food aids to the survival of tadpoles and newly metamorphosed. Moreover, there are various mechanisms in nature which work for the supply of food (Licht, 1967) and if food is not so plen-

tiful, natural selection favours maximum efficiency in feeding (Emlen, 1973).

The resistance of tadpoles to starvation seems to be characteristic for each species as it is fluctuating in a variance related to the range of variation of size of the different eggmasses. As it is shown in Table 3, tadpoles which come from the larger eggs of the same species indicate a higher resistance to starvation. This relation is obvious in the case of *Rana dalmatina* and *Hyla arborea* where the eggs of two eggmasses differ significantly in size.

The size of the eggs seems to be related with the resistance to starvation not only interspecifically but, up to a point, intraspecifically. For example, the tadpoles of *Hyla arborea* which derive from the smaller eggs show a lower resistance to starvation compared with the tadpoles of *Bufo viridis*, which have an average size of eggs, and with those of *Rana dalmatina*, with a larger size of eggs. However, this relation between the various species is only indicative, because declinations have been noticed, as in the case of *Bombina variegata* where tadpoles which come from the same size of eggs with those of *Rana dalmatina*, show a lower resistance to starvation (Table 3). Tadpoles of *Pelobates syriacus* whose eggs have a mean diameter of 1.60 mm have also showed a very high resistance for an average of 29.4 and 32.5 days in two populations (Sofianidou, 1977).

In some cases, the survival of the starved tadpoles after feeding seems to be affected by the time food was given. So, the significantly higher percentage of the survived tadpoles of the species *Hyla arborea* (Table 1) may be explained by the fact that food was given earlier than to the other species, and the harmful effect of starvation was possibly smaller. Another case of higher survival, due to the different time of feeding, was noticed between two populations of *Pelobates syriacus* (Sofianidou, 1977). However, there are more factors which affect the survival of the tadpoles after food is given. Thus, the higher percentage of the survived tadpoles of *Bombina variegata*, compared with that of *Bufo viridis* which were fed at the same time, may be due to the increased ability of *Bombina variegata* to get food. It is known that, for most species, selection favours maximum efficiency in feeding (Emlen, 1973), and the species *Bombina variegata* which lays relatively fewer eggs than other Anurans was possibly favoured by it. The same explanation may be given in the case of *Hyla arborea*. This species lays a small number of extremely small eggs and consequently has small newly-hatched individuals. These should show

a higher capacity for feeding in order to survive and to grow quickly, so that they avoid the antagonism with the larger tadpoles of the other species among which they coexist at the same time.

The results of the starvation experiments on the newly metamorphosed individuals (Table 2) show that they generally present a higher resistance to starvation in relation to that of their tadpoles (Table 1). An exception to that, are the newly-metamorphosed of the species *Rana dalmatina* which showed a significantly low resistance to starvation. Licht (1974) reports a higher resistance of the newly-metamorphosed, in relation to that of the tadpoles, for the species *Rana aurora* and *Rana pretiosa* (34 and 35 days for the newly-metamorphosed to 24 and 25 days for the tadpoles, respectively). Sofianidou (1977) also reports, about the species *Pelobates syriacus*, 38.5 and 40.4 days for the newly-metamorphosed to 29.4 and 32.5 days for the tadpoles, respectively. The higher resistance to starvation of the newly-metamorphosed, compared to that of the tadpoles, may be due to the fact that the opportunities for feeding are fewer in land than in water; thus, selection favours the higher resistance for that critical stage of life (Sofianidou, 1977).

The survival of the newly-metamorphosed individuals after feeding is surely affected by the time of food supply, as it is shown in the results for the species *Rana dalmatina* and *Bufo viridis* (Table 2). The extremely high survival of the newly-metamorphosed of *Bombina variegata* (Table 2) - although food was given for a long time (35 days) after metamorphosis - was due to their remarkable capacity for feeding in relation to those of the same size and larger newly-metamorphosed individuals of other species. So, they are the only relatively small newly-metamorpbosed individuals which can be fed immediately after metamorphosis with small pieces of earthworms. Most of the individuals were capable of getting food immediately after a severe starvation and some even recovered in a few days. The species *Hyla arborea* also had a high percentage of survival, although food was given after a long time. These two species are referred to be the most resisting to the crowding effects (Heusser, 1972). On the contrary, the starved newly-metamorphosed individuals of *Bufo viridis* could not recover after food was given; they were generally very weak and they were not able to get any food at all.

All the above aspects are problematic and in order to have answers more studies are needed.

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ΠΕΡΙΛΗΨΗ

ΑΝΤΟΧΗ ΣΤΗΝ ΠΕΙΝΑ ΤΩΝ ΓΥΡΙΝΩΝ ΚΑΙ ΝΕΟΜΕΤΑΜΟΡΦΩΜΕΝΩΝ ΑΤΟΜΩΝ ΜΕΡΙΚΩΝ ΕΙΔΩΝ ΑΝΟΥΡΩΝ

(Bombina variegata, Bufo viridis, Hyla arborea, Rana dalmatina)

Ὑπὸ

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Δοκιμὲς πείνας στὸ Ἐργαστήριο σὲ νεοεκκολαπτόμενους γυρίνους καὶ νεομεταμορφωμένα ἄτομα τῶν συμπατρικῶν Ἀνούρων *Bombina variegata, Bufo viridis, Hyla arborea* καὶ *Rama dalmatina* ἔδειξαν ὅτι αὐτὰ ζοῦν ἀρκετὲς ἑβδομάδες χωρὶς καθόλου ἐξωτερικὴ τροφὴ καὶ μερικὰ μποροῦν ἀκόμα καὶ νὰ ἀναλάβουν μὲ χορήγηση τροφῆς ὕστερα ἀπὸ αὐστηρὴ πείνα.

Ἡ ἀντοχὴ στὴν πείνα τῶν νεοεκκολαπτόμενων γυρίνων σχετίζεται μὲ τὸ μέγεθος τῶν αὐγῶν μέσα σ' ἕνα καὶ τὸ αὐτὸ εἶδος καί, ὡς ἕνα βαθμό, μεταξὺ τῶν διαφόρων εἰδῶν.

Ἡ βιωσιμότητα τῶν πεινασμένων γυρίνων καὶ τῶν νεομεταμορφωμένων ἀτόμων σ' ἕνα καὶ τὸ αὐτὸ εἶδος ἐξαρτᾶται ἀπὸ τὸ χρόνο χορήγησης τῆς τροφῆς· μεταξὺ τῶν διαφόρων εἰδῶν ἐξαρτᾶται κυρίως ἀπὸ τὴν ἱκανότητα λήψης τῆς τροφῆς.