

# ARTIFICIAL BREEDING AND RAISING OF TADPOLES UNDER LABORATORY CONDITIONS\*

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Amphibian tadpoles for a long time have been favorite objects of experimentation in various biological fields. Quite often there arises the need for having laboratory animals the year around and tadpoles of specific ages up to the stage of metamorphosis may be required to permit the work to go on.

There has been much work done describing evocation of artificial ovulation in frogs during the winter-time [2,5,6,8 and others], the effect of light has been investigated [3,4], temperature [1,3,9], rations on growth [3,8,10,12] and the development of the tadpoles of different species.

We have not recounted all the studies touching on the problem but nowhere have we seen a unified description of how to procure and breed frog tadpoles.

For the last two and one-half years, because we were studying some peculiarities of the ontogeny of Rana temporaria, we had to work with the tadpoles of this species during the fall winter and spring seasons. In the present communication we have combined our observations on 18 series of tadpoles bred in this laboratory.

## EXPERIMENTAL METHODS

Ovulation was produced artificially by the method of Ya. M. Kabak [5]. In the October – January period the female received two injections (with an intervening day between them) into the subcutaneous lymph sac of a suspension taken from two frog hypophyses and then, 48 hours after the 2nd injection, artificial insemination was performed. In February one injection was sufficient while in March – one injection from a suspension taken from one hypophysis. In April and especially by May the female was ready to shed eggs naturally so that there was no longer any need for preliminary hypophyseal injections.

At the end of the period of natural egg laying, during June–September period, ovulation could no longer be induced. Living tadpoles can then be obtained no earlier than the middle of September.

When describing larvae, their age from the moment of fertilization may be used as a guide but with tadpoles differences are seen in succeeding series between tadpoles of the same age. As a consequence of this, it is more convenient to classify them by the various growth stages based on the identification of various internal and external structures. From the many classifications available [3,7,10 and others] the most practical is the one proposed by P. V. Terentyev [7]. This author describes a total of 33 stages from the unfertilized egg to the adult creature: further it is his grouping that we will use.

The fertilized roe, taken from one frog, was placed in two glass pans 5 cm deep with a diameter of 20–23 cm. The roe was covered with tap water which had stood for a day in the room so that temperature was

\* Although this subject is well covered in previous publications, the editorial board has considered this work of sufficient practical merit to warrant publication.

14-16°C. From then on the pans were cleaned and the water changed daily. Until the 25-26th stage was reached (formation of gill slit and appearance of rear limb rudiments) the water level was maintained at 2 cm level, after which the level was decreased to 1.5 cm. From then on, less and less water was added and the pans were slightly inclined so that during metamorphosis the young frogs could crawl out "on dry land". During this period the pans were carefully covered with gauze cloth as otherwise the little frogs could readily get out and scatter.

For the first 2-3 days after fertilization the roe was left at room temperatures but then the pans were placed in a plywood enclosure forming a chamber with a door and one glass wall. For lighting and heating a 96 watt electric globe was kept within this chamber, this being switched off at night thus creating a night and day fluctuation both in light and temperature. The temperature of the water in the pans usually was about 15.5-17.5°C.

On the 7-10th day after fertilization (20th stage) the larvae having a general body length of 8.6-11.2 mm were redistributed into different pans, 20-30 tadpoles in each.

The tadpoles began to be fed at the 22-23rd stage (opening of mouth, appearance of gill fold and teeth). For the first 4-6 days they received only vegetation food: scrapings from aquarium walls, chips of vegetation but later ground meat was added to the food. During metamorphosis (29-30th stage) the tadpoles do not eat but at its end the young frogs can consume only small, live, moving food. During the fall-winter season it is difficult to obtain small insects and such so that many frogs are lost at this stage of development.

It should be underscored that for the first 30-35 days after fertilization very few of our tadpoles die. Also, in the still later stages our losses are minimal.

If the conditions enumerated above are watched (light, temperature, feeding, water level) it is quite feasible to have during the fall-winter season fully grown frogs at the end of 60 days, although, as a rule, the time needed is longer - 87 to 140 days.

Under natural conditions, metamorphosis in *Rana temporaria* begins between 50 and 180 days after fertilization, the Leningrad region being about 150 days [7]. So it can be seen that our results are comparable with the natural. The size of our tadpoles and young frogs also are same as seen naturally: our largest tadpoles - 40 mm, frogs - 9.6 to 14.5 mm; natural frogs measure 46 and 11 to 15 mm [8].

Our method requires a minimum of effort and has been proven on an adequate series to give living tadpoles.

#### SUMMARY

A simple method for artificial breeding of frogs, *Rana temporaria*, is described. The water must be kept at 15.5-17.5°C; illumination maintained for 8 hours daily; water depth in the pans be maintained at precise levels and vegetable and animal feeding be guaranteed as required.

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