THE ROLE OF THE NATIVE POND ODOR IN ORIENTATION OF THE GREEN TOAD (*Bufo viridis* LAUR.) YOUNGS-OF-THE-YEAR

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INTRODUCTION

It is known that froglets of the pool frog *Rana lessonae* keep near the native pond for 1 – 1.5 months after metamorphosis and this pattern of behavior is, at least partly, based on the preference for native pond odor that is learnt during larval development on stages 18 – 21 and 32 – 43 (stages are given by Gosner, 1960). Preference gradually changes to indifference or rejection with the onset of dispersal (Bastakov, 1986; Ogurtsov and Bastakov, 2001). Unlike the pool frog that belongs to a semiaquatic ecological group of Anura representatives of terrestrial ecological group, e.g., *Bufo viridis*, start to disperse from the native pond much earlier — within a few days. Does the dynamics of the reaction to the native pond odor in terrestrial species resemble that in *R. lessonae*? Does learning of the native pond odor in toads occur as a two-staged process as it is in the pool frog?

MATERIAL AND METHODS

The subject of our study was the green toad *B. viridis* obtained from the pond (~600 m^2) that was located 60 km west from Moscow. Each day from the onset of metamorphosis in the pond we calculated the average density (mean calculated from minimum and maximum number of individuals per 1 m^2 observed at the place of the largest emergence of toadlets, total area of 10 m^2) of tadpoles, toadlets near the water edge and toadlets at a distance of 5 – 8 m from the native pond in vegetation. The day when toadlets moved further than 50 m away from the pond was regarded as the start of dispersal (explanations in the text). To reveal the reaction of wild individuals (ind.) to native pond water we caught 10 groups 5 – 8 m away from the pond (5 groups, 179 ind., before and 5 groups, 148 ind. after the start of dispersal) and 5 groups (138 ind.) 50 – 500 m from the pond in the open field.

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Juveniles were tested under pair choice conditions in a plastic chamber 76 × 12 × 15 cm divided into 5 sections. Tests were conducted under low illumination conditions. A pair of odorants (for natural toadlets - native pond water vs strange pond water, for artificially reared toadlets - tap water solution of chemical marker vs. tap water) was positioned in Petri dishes at the corners of the chamber. Each experimental group was divided into 2 or 4 subgroups of 6 – 10 individuals. Each subgroup was tested separately with an altered position of stimuli. At the start of a test a subgroup was placed in the center of chamber. For 40 min toadlets were left walking freely in a chamber and every 5 min we counted the number of individuals in sections. Then results of subgroups' tests were combined in accordance to the position of the familiar stimulus thus obtaining 8 consecutive observations on the distribution of a group as a whole. To analyze the repeated observations we compared our results with the model of random process using a distribution stability coefficient (S). It changes from 0 to 1. The more often toads locate themselves in a section with a native odor, the higher is the difference between the number of toads in the opposite sections the larger is the S for the section with the native odor ("$+S$"). If S obtained in a test is larger than 0.83 the distribution of toads is regarded as stable with $p < 0.05$. For "$+S$" distribution is interpreted as "preference" (Fig. 1*A*), for "$–S$" as "rejection." If neither "$+S$," nor "$–S$" exceed critical value of 0.83 the distribution is regarded as "indifference" (Fig. 1*B*) (for details of the method see Ogurtsov, 2005). In addition we used Wilcoxon matched pairs test to compare the number of individuals visiting the opposite sections during the test. From each group we obtained a pair of numbers, average numbers of toads that visited the opposite sections with native and strange stimuli during a test.

To reveal the learning period in larval development we reared tadpoles in the presence of a chemical marker (Table 1). After metamorphosis toads were tested with a chemical marker compared to dechlorinated tap water. For the tests we used morpholine in concentrations 10^{-8}, 10^{-7}, 10^{-6} mole/liter.

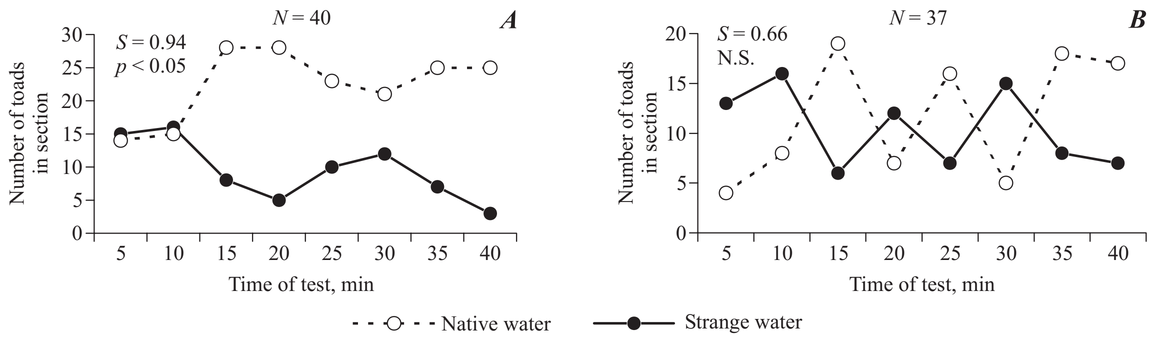

Fig. 1. Types of reaction to familiar water: ***A***, preference (caught before start of disperse); ***B***, indifference (caught after start of disperse).

RESULTS AND DISCUSSION

When moving from water to land, metamorphosing individuals did not stay long near the water edge, rather they all moved 5 – 8 m away to vegetation and formed a large aggregation there (Fig. 2). We called the zone of large aggregation a "buffer" zone as the density of aggregation there remains stable for a long time due to income of tailed toadlets and outcome of dispersing individuals. Zone with moist soil and plenty of shelters seems to be favorable for toadlets to complete their physiological changes associated with metamorphosis. When toadlets start to leave the "buffer" zone we talk about the beginning of dispersal. After a week from the beginning of dispersal the majority of toadlets were found 500 m away from the pond.

Individuals from the "buffer" zone prefer the native pond water during a week before the beginning of dispersal (as described by a distribution stability coefficient *S*; Fig. 3). Soon after the start of dispersal some groups of toads caught in the "buffer" zone could still demonstrate preference to the native pond water, while others show indifference or rejection. The latter are, probably, individuals ready for dispersal. At the same time some groups of toads caught 50 m away from pond could also reveal preference to the native pond water. These could be individuals that make their first exploratory routes but have not decided to leave the pond yet. Later toadlets caught at far larger distances from the pond demonstrate indifference to the native pond water. Wilcoxon's matched pairs test reveals significant differences between the number of individuals visiting native and strange stimuli only for toadlets caught in the "buffer" zone before dispersal (Fig. 4).

In the current study we investigate only temporal but not causal relations between toads behavior near the pond and behaviour in laboratory. Could toadlets simply forget the native pond odor with time thus revealing indifference

TABLE 1. Groups of Tadpoles Reared in Different Chemical Markers

Group	Exposition period, stages	Period characteristics	Marker (10^{-7} mole/liter)
1	19 – 21 (first sensitive period)	From hatching till the beginning of active feeding	Morpholine
2	31 – 41 (second sensitive period)	From formation of knee joint till appearance of forelimbs	Morpholine
3	31 – 41 (second sensitive period)	From formation of knee joint till appearance of forelimbs	Phenylethanol + isoamyl acetate + geraniol
Control	1 – 46		No marker

TABLE 2. Results of Tests with Chemical Markers

Group	*N*	Marker concentration, mole/liter	Distribution between sections with stimulus, median (min – max)		*S* for sections with stimulus		Random model *p*
			native	strange	native	strange	
Control	19	$A10^{-8}$	8 (7 – 11)	8 (6 – 10)	0.55	0.39	N.S.
	32	$B10^{-8}$	14 (6 – 18)	13 (10 – 19)	0.44	0.56	N.S.
1	18	$A10^{-8}$	12 (9 – 14)	5 (2 – 8)	1.00	0.00	<0.01
2	20	$A10^{-8}$	6 (3 – 7)	4 (3 – 6)	0.96	0.04	<0.01
	20	$A10^{-6}$	4 (0 – 6)	6 (4 – 7)	0.03	0.97	<0.01
3	20	$B10^{-8}$	5 (1 – 11)	2 (1 – 3)	0.94	0.06	<0.05

Note. A, morpholine; B, phenylethanol + isoamyl acetate + geraniol.

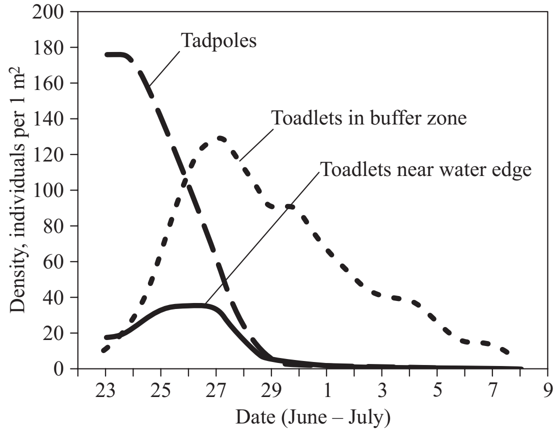

Fig. 2. Dynamics of toads' density.

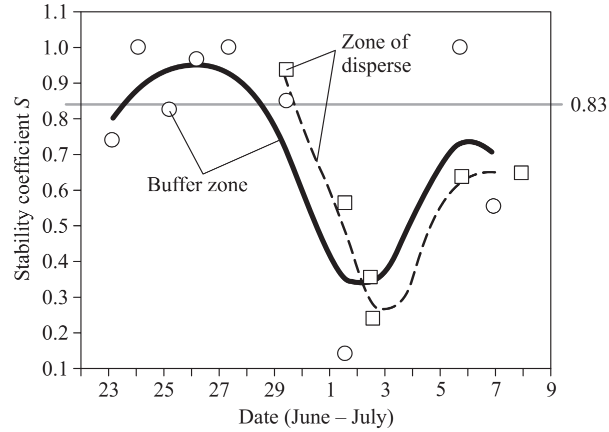

Fig. 3. Dynamics of stability coefficient *S* (least squares approximation).

to it during dispersal? A special study showed that the main parameters determining the type of reaction to the native pond odor in *B. viridis* and *R. temporaria* are the age of juveniles (but not the time spent on land after metamorphosis) and, probably, temperature and moisture conditions that determine the onset of dispersal (Arhipova et al., in press). Thus groups of wild toads hold in laboratory for 9 – 14 days still preffered the odour whereas newly caught individuals from natural conditions started to disperse and were indifferent to the same stimulus (unpublished data). So it seems that toads could switch the reaction off rather than forget it.

Toadlets that were reared in a chemical marker at first (Group 1) and at second exposition period (Groups 2, 3) showed preference to it (Table 2). Concentration of the marker more than that used during exposition caused rejection. One could assume that toadlets interpret high concentration of familiar odor as excessive proximity to native pond and try to keep some distance away from the odorant, as probably do wild toads from the "buffer" zone. Control group reacted indifferently to all chemical markers offered.

Like semiaquatic species, such as *R. lessonae*, *B. viridis* toadlets stay for a certain period near the native pond and prefer its odor. After the onset of dispersal they become indifferent or reject this odor. But unlike randomly distributed froglets of *R. lessonae*, green toads form dense aggregation in a "buffer" zone where they undergo physiological maturation. Like *R. lessonae* the green toad seems to have two periods of learning of chemical stimuli marking the native environment during larval development. The type of reaction to native chemical marker could depend on its concentration, the fact also known for the pool frog (Ogurtsov and Bastakov, 2001). Thus, representatives

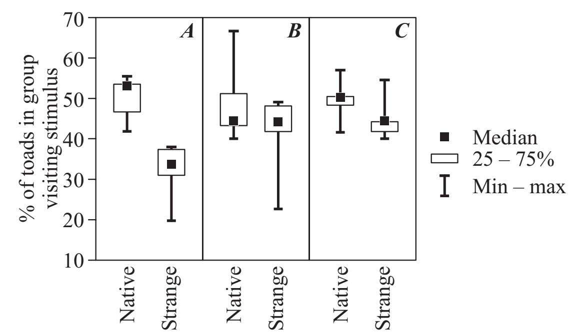

Fig. 4. Reaction to the native pond odor in different zones and in different stages of dispersal: ***A***, buffer zone before dispersal, $p < 0.05$; ***B***, buffer zone after starting of disperse; ***C***, zone of disperse.

of two ecological groups of Anura (*R. lessonae* and *B. viridis*) have much in common concerning the periods of learning and dynamics of reaction to the native pond odor.

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