BASIS OF NATIVE POND FIDELITY IN ANURAN AMPHIBIANS: THE CASE OF CHEMICAL LEARNING

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INTRODUCTION

Anuran amphibians come to breed to the native pond (Blair, 1953; Breden, 1987). Chemical stimuli from the pond could be among cues that guide them. That these cues are learnt early in ontogeny is often discussed but hasn't been tested yet (Grubb, 1973). Rana lessonae seems to learn the native pond odor during larval stages and is attracted by it after metamorphosis while keeping near the pond (Bastakov, 1986). We tried to find out whether other species possess the same type of reaction and whether sensitive periods to memorize chemical stimuli exist in larval development.

MATERIAL AND METHODS

Juveniles of 5 species (*Rana lessonae*, *R. ridibunda*, *R. temporaria*, *Bufo viridis*, *B. bufo*) were collected near the ponds of Moscow Oblast' (Russia) within 3 days after metamorphosis. They were tested in a plastic chamber $76 \times 12 \times 15$ cm divided into 5 sections by walls of 5 mm height (Fig. 1). A transparent glass cover had ventilatory holes at both ends. A 40-W incandescent lamp put 40 cm from the middle of one of the longest walls provided low illumination. A pair of odorants was positioned in Petri dishes at both ends. After each test the chamber was washed with tap water. 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. A subgroup was placed in the center of the chamber and left walking freely for 40 min while each 5 min we counted the number of individuals in sections. Results of subgroup tests were combined in accordance with the position of stimuli thus obtaining 8 sequential observations on the distribution of a group.

To describe frogs distribution in sections with stimuli we compared sequential observations with random-effect model using a classical procedure (Gotelli, 2000). We in-

troduced a distribution stability coefficient (S). For each test we calculated a sequence of 8 differences between the number of frogs in utmost sections. The differences were ranked (including "0" differences), and sums of ranks of "+", "–", and "0" differences were computed separately. The part that sum of ranks of "+" differences constitutes from the total sum of ranks is the coefficient S for the section with the stimulus of interest ("–" differences describe S for the opposite section). S varies from 0 to 1. The more often individuals are observed in the section of interest and the more is the difference in the number of frogs in that section than in the opposite one the larger is the S value for it.

We used program Microsoft Excel 2000 (Microsoft Corp., 1985 – 1999) to generate a sequence of 8 random numbers taken with equal probability from $-n$ to $+n$ (n, group size). It models 8 differences between the number of frogs in the utmost sections. S value for "+" differences was then calculated. We chose one-tailed null hypothesis: experimental S does not exceed critical value. If the value is too low than S for the opposite section as well as S for "0" differences could exceed critical level. We used 95% and 99% percentiles to calculate confidence interval. For groups of 3 and more individuals and 225,000 iterations critical values of S come to 0.83 ($p = 0.05$) and 0.94 ($p = 0.01$). If experimental S is larger than the critical value (distribution is stable in time and nonrandom), we speak about preference of one of the stimuli (Fig. 2A). If it is lower than the critical value (frogs move at random), we speak about indifference (Fig. 2B).

To reveal a sensitive period we reared *R. lessonae* tadpoles with a natural marker (pond water) during one of the following stages of larval development (Gosner, 1960): till
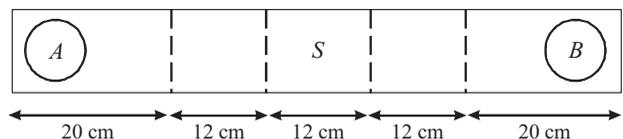
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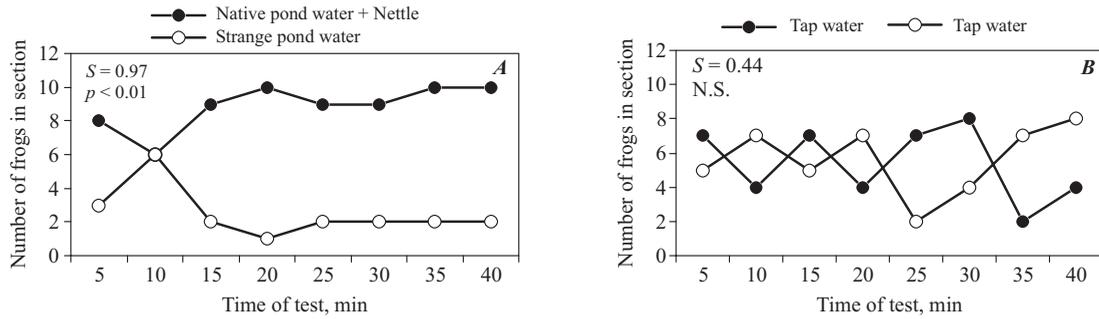
Fig. 1. Scheme of test-chamber (view from above): *A*, *B*, Petri dishes with chemical stimuli; *S*, starting position of froglets.

Fig. 2. Types of reaction: *A*, preference of *R. lessonae* froglets (*n* = 12) to familiar marker (native pond water with boiled nettle) compared to unfamiliar stimulus; *B*, indifference to two identical stimuli (dechlorinated tap water) in the same group.

hatching on stages 1 – 18 (Group 1), till the beginning of active feeding on 1 – 21 (Group 2), from the disappearance of external gills till the spade-shape hind limbs on 25 – 31 (Group 3), from the later one till the complete formation of hind limbs on 31 – 41 (Group 4). Group 4 received boiled nettle as a foodstuff during exposure. The water was changed once in 1 – 2 days. For the tests of Group 1 and 2 we used one pair of ponds ("native" vs. strange), and Group 5 that had passed the whole larval development in the native pond served as a control. For Group 3 and 4 we used another pair of ponds. Their "Control" group had no contact with pond water. Ponds treated in tests as "native" and strange were located within 0.7 – 1 km from each other.

RESULTS AND DISCUSSION

A long-lasting group test models the situation when juveniles keep near the pond before dispersal: they move back and forth from the native pond and keep their neighbors in view. According to our observations this period occupies from 3 – 7 days in terrestrial species (*R. tempora-*
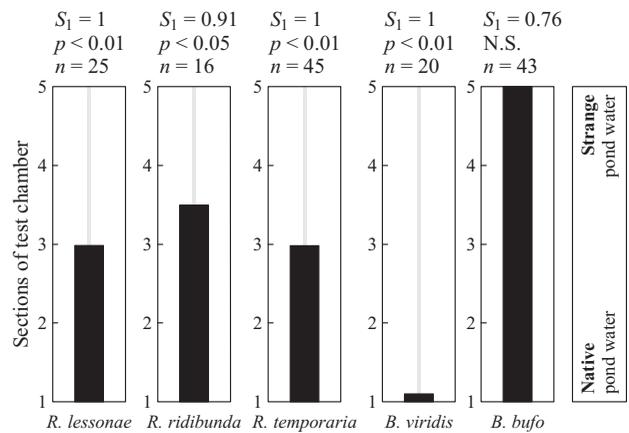

Fig. 3. Discrimination between water from the native pond and water from a strange one in juveniles of 5 anuran species soon after metamorphoses. Black rectangle, 25 and 75% percentiles (median 50% of individuals) of the median distribution of frogs in a chamber during a test (view from above); $S_1 - S$; value for section 1; *p*, difference from random distribution; *n*, number of frogs in a group.

ria, *B. viridis*) to 1 – 1.5 months in semiaquatic (*R. lessonae*). Juveniles of 4 species (*R. lessonae*, *R. ridibunda*, *R. temporaria*, *B. viridis*) caught near the native pond soon

TABLE 1. Influence of the Period of Exposure at Larval Stages on the Reaction of *R. lessonae* Froglets to the Native Pond Water

Group	Stages of exposure	Stimulus treated as "native"	Distribution between sections with stimulus, median (min – max)		Binomial *p*	*S*	Random model *p*	*n*
			native	strange				
Control	not exposed	pond water	6 (3 – 9)	5 (2 – 6)	N.S.	0.70	N.S.	12
Group 1	1 – 18	pond water	6 (1 – 8)	6 (3 – 9)	N.S.	0.39	N.S.	17
Group 2	1 – 21	pond water	17 (5 – 24)	9 (6 – 14)	<0.05	0.94	<0.05	37
Group 3	25 – 31	pond water	8 (6 – 10)	8 (5 – 9)	N.S.	0.68	N.S.	17
Group 4	31 – 41	pond water	6 (3 – 7)	6 (3 – 8)	N.S.	0.41	N.S.	12
		boiled nettle	5 (1 – 7)	4 (4 – 7)	N.S.	0.41	N.S.	12
		pond water + boiled nettle	9 (6 – 10)	2 (1 – 6)	<0.05	0.97	<0.01	12
Group 5	1 – 46	pond water	14 (11 – 18)	6 (4 – 7)	<0.05	1.00	<0.01	25

Note. *n*, Group size. *S* is given for section with "native" stimulus. Water from an unfamiliar pond was used as a strange stimulus, except for the test with boiled nettle where we used dechlorinized tap water.

after metamorphosis demonstrated preference to the native pond water. But *B. bufo* showed indifference to the same stimulus (Fig. 3). For *R. lessonae* our group tests gave the same results as individual tests (Bastakov, 1986).

R. lessonae tadpoles exposed to pond water on stages 1 – 21 (8 days) revealed preference to the "native" pond water as did Group 5 of natural froglets. As Group 1 did not form preference on stages 1 – 18, we consider learning to occur on stages 18 – 21 (4 days). Group 3 (exposed for 20 days) and Group 4 (48 days) showed no preference as did the Control group. But in Group 4 it appeared that only a complex stimulus actually used for exposure – a mixture of "native" pond water and boiled nettle (1 leaf stayed for 30 min in 200 ml of water), but not single components alone caused preference (Table 1, Fig. 2*A*). Video analysis of tests showed that movements of *R. lessonae* froglets were independent (they did not move in groups or follow each other). Thus we tried binomial test for time averaged (median) distribution, that gave us the same results (Table 1). They speak for the existence of 2 sensitive periods of chemical learning during larval development. We obtained the same results with artificial chemical markers: morpholine and β-phenylethanol (Ogurtsov and Bastakov, 2001).

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