February 1992 *Asiatic Herpetological Research* Vol. 4, pp. 68-75

Investigations on Ranid Larvae in Southern Sakhalin Island, Russia

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Abstract. -Using a special chemical technique, four breeding sites of two brown frog species, *Rana amurensis* and *Rana chensinensis*, were investigated on Sakhalin Island, Russia. They were living together with other amphibian species under very different conditions. A puddle with *Rana chensinensis* larvae was found adjacent to the Pacific coast with a salinity of 1.2%. The description of the mouth part morphology was completed by SCAN-investigations on the micromorphology of larval teeth, horny jaws and the warty epithelium.

Key words: Amphibia, Anura, Ranidae, *Rana amurensis*, *Rana chensinensis*, tadpoles, breeding sites, water chemistry, salinity, micromorphology, Russia, Sakhalin Island.

Introduction

In July and August of 1989 the authors visited the southern part of the Russian Far East island, Sakhalin. We found in many places the only two ranid frog species inhabiting the island, *Rana amurensis* and *Rana chensinensis*. In most habitats they coexisted with the other amphibian species of Sakhalin Island, *Salamandrella keyserlingii, Bufo gargarizans* and *Hyla japonica*. The latter was only found near Kostromskoe. In other papers (Hermann and Kabisch, 1990, 1991; Kabisch et al., 1990) the authors report on the herpetofauna of Sakhalin Island. The present paper is concerned with the life and environmental conditions of ranid frog larvae. Some micromorphological data for the species diagnosis are presented.

Methods

Tadpoles of *Rana amurensis* and *Rana chensinensis* were observed in many breeding sites on the island. Some of them were caught and fixed in 70% ethanol. According to a technique of W. Pietsch (Dresden) one liter samples of water from four breeding sites were obtained from: (1) brook in the city park of Jushno-Sachalinsk (Fig. 1), (2) pools near Pjatyretske with *Lysichiton camtschatcense* as the main botanical element, (3) pond near Kostromskoe with *Alisma orientale* as the dominant water plant, (4) puddle at the Pacific coast on the Krilon Peninsula (Fig. 2). The water samples were analyzed in the laboratory of W. Pietsch. The fixed larvae (24 specimens of each species, representing stages of the beginning of hind leg formation) were also prepared for investigation of their micromorphology (for technique see Herrmann, 1989). The preparations were investigated with a scanning electron microscope TESLA B 300 in the Technical College of Ilmenau (Thuringia).

Results

1. Characterization of the Breeding Sites

The water analysis data of the four investigated breeding sites are shown in table 1. From this information it can be concluded that the chemical nature of the water bodies is very different. In the first one, in a brook in the city park of Jushno-Sachalinks, we found larvae of *Rana chensinensis* in addition to those of *Bufo gargarizans*. *Rana chensinensis* prefers running water as breeding sites on Sakhalin Island. The water can be characterized as follows: slightly acidic, oligotrophic, poor in humic material,

FIG. 1. Tadpoles of *Rana chensinensis* in a brook in the city park of Jushno-Sachalinsk.

nutrient poor, poor in electrolytes, rich in iron, and of a low total hardness. The only vegetation at the brook consisted of some grasses and rushes.

The second water body was a breeding pool of *Rana amurensis*. It is one of four permanent pools near the Naitsa River in the Pjatyretske River system. The four pools (area about 550 m^2) are slightly acidic, very poor in humic material, nutrient poor and of a low total hardness.

In the third breeding site all amphibian species living on the island were found: *Salamandrella keyserlingii, Rana amurensis, Rana chensinensis, Hyla japonica* and *Bufo gargarizans*. Typical species of the very rich vegetation of this small pond (area about 80 m^2) were *Alisma orientale, Juncus bufonius, Epilobium palustre, Oenanthe decumbens, Phleum pratense,* and *Equlsetum palustre*. The water was slightly acidic, oligomesohumic, rich in phosphates and carbonates, but of a low total hardness.

The fourth habitat was a very small puddle (area 1.5 m^2) at the Pacific coast.

FIG. 2. Puddle with *Rana chensinensis* larvae adjacent to the Pacific coast on the Krilon Peninsula.

Splashing salt water contacted this puddle during the time of high tide. It was a breeding site of *Rana chensinensis*. The vegetation consisted of *Lemna* and *Juncus* only. We found many pieces of old wood in the water. The water can be characterized as follows: slightly alkaline, poor in humic material, nutrient poor, rich in electrolytes and of a medium total hardness. A typical feature of this breeding site is the NaCl content of 1.2%. Because of the high content of salt, the puddle can be classified as a ß-mesohalobien water representing the upper limit of brackish water. The 24 larvae represented an earlier stage of development compared to tadpoles of the same species living in other breeding places at the same time.

2. Micromorphology Investigations

The schematic drawings of the larval mouth of *Rana amurensis* and *Rana chensinensis* show clear differences in their morphology (Fig. 3). With the SCAN, the small conical teeth on the upper and on the lower horny jaw could be visualized in both species. In the literature (Bannikov et al., 1977) only

teeth on the lower jaw were shown for *Rana amurensis*. We found 69-73 in the upper jaw of *Rana chensinensis* and 51-55 in *Rana amurensis*. On the lower jaw 52-54 were counted in *Rana chensinensis* and 46-49 in *Rana amurensis* (Fig. 4-6). The labial teeth were identical in all rows in both species. Fan-like tips, 6-13 on each tooth, could be seen (Fig. 7). The warty epithelium around the mouth field of the larvae consisted of a fleshy skin. At the margin of this structure, labial teeth have been found (Fig. 8). The epithelium of the larvae was composed of hexaedric or octaedric plate cells.

Discussion

Some papers on the effects of low pH and other chemical variables on amphibian larvae were published by Freda (1986) and Freda and Dunson (1985a, 1985b, 1986). A lot of data on acid tolerance in amphibians are summarized in the paper of Gebhardt et al. (1987). The lowest pH tolerated by a brown frog, as described for *Rana sylvatica*, was 3.0. Other conditions were observed by Freda and Dunson (1984) in experiments with *Rana catesbeiana, Rana clamitans* and *Rana pipiens* in the laboratory. An increasing of the external calcium concentration extended the survival time in acid water by slowing the loss of sodium. So it is possible to survive in salt water under special conditions. There was a regulatory principle for the magnitude of larvae populations in breeding sites with such special conditions obtained by Kovaltsyk (1981). The main factors were acidity and the contents of cat ions in the water. Another role was played by the temperature of the water in connection with the photo period (Dupre and Petranka, 1985; Floyd, 1985).

The long days in spring and summer, as typical of Sakhalin Island, enable the amphibian larvae to develop under extreme conditions. These are characterized by extreme temperature differences, reaching minimum and maximum values in very short intervals, limiting the survival of larvae. The opportunity to survive in salt water was described for urodeles by Jones and Hillman (1978), (*Batrachoseps*), and Romspert and McClanahan (1981), (*Ambystoma tigrinum*). In the papers of Power (1937), Andren and Nilson (1979), Herrmann (1982), Dunson (1977), and Guix and Lopes (1989) some anuran species are listed as breeding and developing in brackish waters. Fljaks (1985) recorded some brackish breeding sites (1.5-9%) of *Bufo gargarizans* and *Rana amurensis* on the island of Sakhalin. Fljaks (1985, 1986) also reported on the mortality of the tadpoles of *Rana amurensis* and *Rana chensinensis* on this island. He observed the highest mortality in the first stages of larval development: 18-73% in

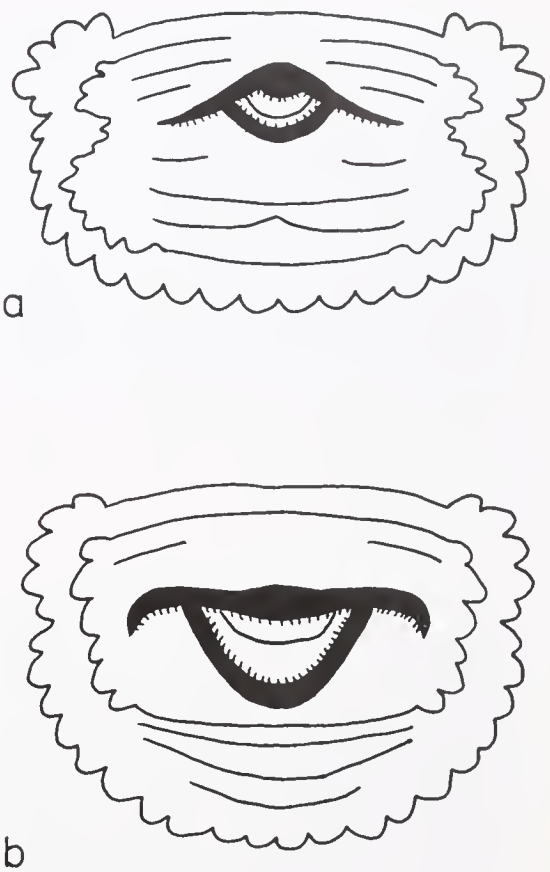

FIG. 3. Schematic drawings of the larval mouth parts of *Rana amurensis* (a) and *Rana chensinensis* (b).

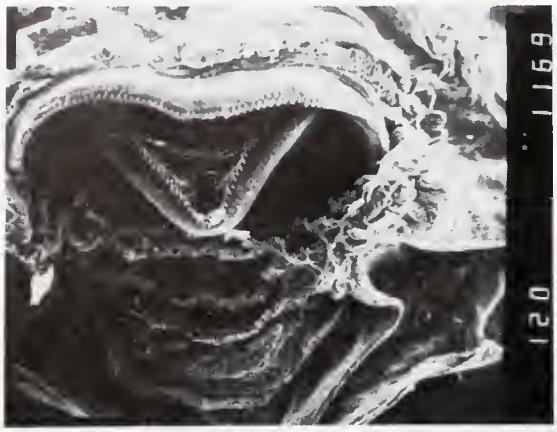

FIG. 4. Mouth parts of the tadpole of *Rana chensinensis* (x 120).

FIG. 7. Labial teeth of the tadpole of *Rana chensinensis* (x 2500).

FIG. 5. Teeth of the upper jaw of the tadpole of *Rana chensinensis* (x 1500).

FIG. 8. Warty epithelium of the tadpole of *Rana amurensis* (x 500).

FIG. 6. Teeth of the upper jaw of the tadpole of *Rana amurensis* (x 300).

Rana amurensis and 15-66% in *Rana chensinensis*. In a puddle with 9% salinity the *Rana amurensis* larvae had a mortality rate of 99.8%. An increased mortality was also found in acidic water. The slow development of ranid tadpoles in puddles at the ocean coast was also observed by Kopein (1973) in southern Sakhalin Island.

Micromorphological data on larvae of *Rana amurensis* and *Rana chensinensis* were found to be absent in the literature. Besides the macromorphology, form and topography of nostrils, spiracle, vent tube, lateral-line pores and buccal papillae according to Johnston and Altig (1986), the jaw sheaths and labial teeth can also

TABLE 1. Chemical/physical data of the breeding sites.

criterion	brook in Jushno-Sachalinks (1)	pool near Pjatyretske (2)	pond near Kostroms-koe (3)	puddle at the Pacific coast (4)
pH	6.8	6.6	6.9	7.2
pV ($KMnO_4$ mg l^{-1})	33.8	22.9	48.0	28.2
total hardness (°dH)	2.3	1.6	4.0	18.4
carbonate hardness (°KH)	2.1	1.5	4.0	11.9
nitrate (mg l^{-1})	3.2	2.4	4.0	3.8
sulfate (mg l^{-1})	0.9	1.6	7.6	32.5
ammonium (mg l^{-1})	0.12	0.06	6.40	0.08
iron (mg l^{-1})	1.52	0.08	0.56	0.02
calcium (mg l^{-1})	11.4	8.4	21.2	26.2
nitrite (mg l^{-1})	0.01	0.01	0.03	0.02
chloride (mg l^{-1})	7.6	9.5	34.8	76.5
magnesium (mg l^{-1})	3.1	1.8	4.3	7.8
CO_2 (mg l^{-1})	24.0	18.0	33.0	22.0
SiO_2 (mg l^{-1})	14.1	3.2	27.4	28.0
phosphate (mg l^{-1})	0.1	0.2	1.8	0.2
lime (mg l^{-1})	38.3	32.0	87.2	11.6
O_2 (mg l^{-1})	9.6	10.4	12.2	11.2
residue of evaporation (mg l^{-1})	94.0	94.0	230.0	864.0
residue of combustion (mg l^{-1})	50.0	49.0	104.0	376.0

be used for identification of anuran tadpoles. Wassersug (1980) and Viertel (1982) favored micromorphology for taxonomic classification. The present data support the necessity to add micromorphological data for species diagnosis in anuran larvae.

Acknowledgments

For their excellent scientific assistance we have to thank Dr. K. Pfeifer, Technical College of Ilmenau, and Dr. sc. W. Pietsch, Dresden. Furthermore we are very grateful to Doz. Dr. sc. K. Brauer, Paul Flechsig Institute for Brain Research, University of Leipzig, for critical reading of the manuscript.

Literature Cited

ANDREN, C., AND G. NILSON. 1979. On stinkpaddans *Bufo calamita* utbredning och ekologi pa den svenska västkusten. Fauna och Flora 74:121-132.

BANNIKOV, A. G., I. S. DAREVESKY, V. G. ISCHENKO, A. K. RUSTAMOV, AND N. N. SCHERBAK. 1977. [Field guide of the USSR amphibians and reptiles]. Prosveschenye Publishing, Moscow. 414 pp. (In Russian).

DUNSON, W. A. 1977. Tolerance to high termperature and salinity of tadpoles of the Philippine frog, *Rana cancrivora*. Copeia 1977: 375-378.

DUPRE, R. K., AND J. W. PETRANKA. 1985. Ontogeny of temperature selection in larval amphibians. Copeia 1985:462-467.

FLJAKS, N. L. 1985. Vyzhivaemost amfibij na rannych etapach rasvitija na ostrove Sachalin. Voprosy gerpetologii, Leningrad 6:214-215.

FLJAKS, N. L. 1986. Vlijanie pH sredy na vyzhivaemost i razvite beschvostngch amfibia Sachalina. *In* Sistematika i ekologija amfibij i reptilij. Leningrad.

FLOYD, R. B. 1985. Effects of photoperiod and starvation on the temperature tolerance of larvae of the giant toad, *Bufo marinus*. Copeia 1985:625-631.

FREDA, J. 1986. The influence of acid pond water on amphibians: a review. Water, Air and Soil Pollution 30:439-450.

FREDA, J., AND W. A. DUNSON. 1984. Sodium balance of amphibian larvae exposed to low environmental pH. Physiological Zoology 57:435-443.

FREDA, J., AND W. A. DUNSON. 1985. Field and laboratory studies on ion balance and growth rates of ranid tadpoles chronically exposed to low pH. Copeia 1985:415-423.

FREDA, J., AND W. A. DUNSON. 1985. The influence of external cation concentration on the hatching of amphibian embryos in water of low pH. Canadian Journal of Zoology 63:2649-2659.

FREDA, J., AND W. A. DUNSON. 1986. Effects of low pH and other chemical variables on the local distribution of amphibians. Copeia 1986:454-466.

GEBHARDT, H., K. KREIMES, AND M. LINNENBACH. 1987. Untersuchungenzur Beeinträchtigung der Ei- und Larvalstadien von Amphibien in sauren Gewässern. Natur und Landchaft 62:20-23.

GUIX, J. C., AND R. M. LOPES. 1989. Occurence of *Hyla geographica* Spix and *Bufo crucifer* Wied tadpoles in brackish water environmemts in the Juréia Region (São Paulo, SE Brasil). Amphibia-Reptilia 10:185-189.

HERRMANN, H. J. 1982. Amphibien, Reptilien, Fische. *In* Biologischer Exkursionsfuhrer durch die Umgebung von GreifsNald (Il). Wissenschaftliche Beiträge der Ernst-Moritz-Arndt-Universität.

HERRMANN, H. J. 1989. Die mikromorphologische Differenzierung di- und tetraploider grüner Kröten des *Bufo viridis*-Komplexes. Veröffent Naturhistorischen Museums Schloss Bertholdshüng Schleusingen 4:15-21.

HERRMANN, H. J., AND K. KABISCH. 1990. Die Herpetofauna der Insel Sachalin. Herpetofauna 12:6-10.

HERRMANN, H. J., AND K. KABISCH. 1991. Biological investigations of *Bufo gargarizans*

Cantor 1842 in Southern Sakhalin. (In press).

JOHNSTON, G. F., AND R. ALTIG. 1986. Identification characteristics of anuran tadpoles. Herpetological Review 17:36-37.

JONES, R. M., AND S. S. HILLMAN. 1978. Salinity adaptation in the salamander *Batrachoseps*. Journal of Experimental Biology 76:1-10.

KABISCH, K., H. J. HERMANN, AND G. MATJUSHKOV. 1990. Beobachtungen an Amphibien und Reptilien Sachalins. Aquarien Terrarien 37:235-248.

KOPEIN, E. I. 1973. 0 razmnozhenii amfibij na juge Sachalina. Voproby gerpetologii, Leningrad 3:100-102.

KOVALTSYK, L. A. 1981. 0 roli energitseskogo litsinok zemnovodnych v mechanizmack podderzhanija optimalnoj tsisllennosti populjazii. Voprosy gerpetologii, Leningrad 5:66-67.

POWER, J. H. 1937. Tadpoles in the transition zone between fresh and sea water. Cape Naturalist 1:127-128.

ROMSPERT, A. P., AND L. L. MCCLANNAHAN. 1981. Osmoregulation of the terrestrial salamander, *Ambystoma tigrinum*, in hypersaline media. Copeia 1981:400-45.

VIERTEL, B. 1982. The oral cavities of Central European anuran larvae (Amphibia). Morphology, ontogenesis and generic diagnosis. Amphibia-Reptilia 4:327-360.

WASSERSUG, R. J. 1980. Internal oral features of larvae from eight anuran families: functional, systematic, evolutionary and ecological considerations. Miscellaneous Publications of the Museum of Natural History of the University of Kansas 68:1-146.