Salt tolerance of *Rana temporaria*: spawning site selection and survival during embryonic development (Amphibia, Anura)

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Abstract. Spawning site selection of *Rana temporaria* is dependent on the salinity of the water. In the nature reserve 'Salzwiesen von Münzenberg' (Germany) the frogs avoided increased conductivity values, Cl^- ion concentrations and salinities and selected lower values for spawning. In the laboratory Gosner stages 20/21 to 22/23 were more sensitive to sodium than the earlier G stages 8 to 20/21 (exposition for 72 h) though they were exposed for the shorter time period of 24 h. The 'no observed effect concentration' (NOEC) between G stages 8/9 and 20/21 was 648 ppm (3350 μS, 2.2‰ salinity) for Na^+ and 1872 ppm (6500 μS, 4.0‰ salinity) for K^+. The NOEC between G stages 20/21 and 22/23 was 1490 ppm (7400 μS, 4.5‰ salinity) for Na^+ and for K^+ also 1872 ppm (6500 μS, 4.0‰ salinity). All developmental stages tolerated much higher ion concentrations and conductivity values in the laboratory than the adult frogs selected for spawning.

Introduction

Salinity is one limiting abiotic factor for successful breeding and development of amphibians. Some species and their developmental stages tolerate elevated salinities as measured in brackish waters and, exceptionally, sea water. Salinity of sea water reaches a level of 32 to 37.5‰ (10.77 g/kg Na^+, 0.399 g/kg K^+, 19.354 g/kg Cl^- at a salinity of 35‰), brackish water 0.5 to 32‰ and freshwater 0 to 0.5‰ accordingly (Wangersky, 1980; Bogenrieder et al., 1986). Mathias (1971) found the upper lethal limit for adults of *Bufo calamita* in saline water for four days 16 to 17‰. Breeding of a south coastal *Bufo calamita* population in Sweden occurred at a salinity of 4‰ sea water, while the larvae of this population died in the surrounding Baltic Sea (about 28‰ salinity) within 2 hours (Andrén and Nilson, 1985). However, breeding in the Baltic Sea was reported by Giselen and Kauri (1959). Berglund (1976) mentioned successful breeding on the southwest and west coast of Scania in pools and logoons on beaches (Skanör, Falsterbo and the meadows of Saxtorp) or even in the Öresund (4‰ salinity) itself. Beebee (1985a) demonstrated survival of spawn of *Bufo calamita* from an inland (southern England) and a coastal (north-west England) population

up to 4.2‰ salinity (NaCl) and of the larvae up to 7‰ salinity. Tests by Lönnberg showed salt tolerance of adult *Bufo viridis* up to 15 ‰, explaining the distribution of that species around the Baltic Sea (Kauri, 1948). In the experiments of Gordon (1962) salt tolerance in adult *Bufo viridis* was up to 19 ‰ (equals 50% sea water) and exceptionally 23‰, after acclimatization in 15‰. Ruibal (1959) studied the distribution of *Rana pipiens* at the San Felipe Creek, Imperial County in California, with salinities ranging from 1.75 to 34‰. The frogs were encountered at salinities of 1.75 to 9.0‰ and mating and spawning occurred only within a range of 2.0 to 2.75‰. Uchiyama et al. (1990) captured the euryhaline *Rana cancrivora* (Crab-Eating Frog), the anuran species with the highest salt tolerance, around ponds with a salinity of 33‰ in the mangrove swamps near Bangkok. Gordon and Tucker (1968) collected *Rana cancrivora* larvae in ponds with salinities of 6 to 24‰ (19 to 75% of sea water) and described them as good osmoregulators in all salinities from freshwater to 32 ‰ sea water, whereas embryogenesis and metamorphosis took place at salinities of not higher than 20‰ (see also Uchiyama et al., 1990).

Breeding *Rana temporaria* are found in puddles, pools, ponds, lakes, flooded gravel pits, banked up waters, spring waters, swamps, ditches, brooks and small rivers (Cooke, 1975; Beebee, 1985b; Schlüpmann and Günther, 1996). For a species capable of breeding in such a broad range of environments the ability to discriminate higher from lower salinities to select a suitable level for reproduction would be an advance in overcoming the scarcity of breeding sites.

No evidence was found in literature with regard to the sodium (Na^+), potassium (K^+) and chloride (Cl^-) tolerance of gametes, oocysts, cygotes and early developmental stages of *Rana temporaria*. The aim of the present work is to explore the influence of conductivity, salinity and chloride concentrations on breeding site selection in respect to the ability of the frogs to mate and of the embryos to develop successfully. Another aim of the study is to define the upper limit of embryonic salt tolerance (NaCl and KCl).

Materials and Methods

Field study

In the nature reserve 'Salzwiesen von Münzenberg' (Germany) the conductivity, salinity and concentrations of chloride ions as well as the pH values, dissolved oxygen concentrations and water temperature of the actual and potential spawning sites of *Rana temporaria* were measured (table 1). Conductivity, salinity and Cl^- concentrations were taken as salt parameters representative for enrichment of the water with NaCl and KCl. Spawning site selection of *Rana temporaria* is defined by the following criteria: 1. Depth of water between 10 and 30 cm; 2. Slight water flow preferred but not absolutely necessary (Lemmel, 1977; Strijbosch, 1979; see also Cooke, 1975). The density of vegetation is a minor factor. Site fidelity of the population to the different spawning sites could superimpose effects of increased salinity on spawning site selection and cause a problem in statistical plau-

Table 1. Field study. Parameters demonstrating salt concentration in potential spawning sites of *Rana temporaria* in the Natural Reserve 'Salzwiesen von Münzenberg' and spawning site classes as indicators of actual reproduction in the last column, spawning site class 0 = no clutches found, spawning site class 1 = 1 clutch, spawning site class 2 = more than 1 clutch, embryos = G stage 24-25, already free of jelly.

Date	spawning site No.	pH	temp. [°C]	oxygen [ppm]	conductivity [μS cm^{-1}]	salinity [‰]	chloride [ppm]	*Rana temporaria* spawning site classes
11.03.78	1	7.9	11.9	12.5	1700	1.5	770	0
13.04.80	1	8.2	16.1	–	2020	0.9	684	1
11.03.78	2	7.9	14.0	10.0	470	0.4	46	2
02.04.78	3	8.3	14.0	–	450	0.3	30	2
30.03.79	4	7.5	6.0	11.5	580	0.3	194	1
02.04.78	5	7.9	15.8	–	1200	0.8	405	1
02.04.78	6	7.7	15.7	–	1200	0.8	405	2
02.04.78	7	7.9	15.0	–	1200	0.8	470	1
02.04.78	8	7.6	14.1	–	415	0.3	60	1
02.04.78	9	7.6	12.7	–	440	0.3	46	2
30.03.79	10	8.0	5.3	11.5	1820	1.5	850	0
22.03.80	10	7.1	4.4	14.4	610	0.1	172	1
13.04.80	11	8.2	12.7	–	1220	0.5	384	2
30.03.79	12	7.5	6.0	11.5	600	0.3	200	2
30.03.79	13	7.7	6.8	12.5	425	0.2	34	2
22.03.80	14	7.0	4.6	3.7	530	0.1	210	1
15.03.80	15	7.6	4.0	6.9	1550	1.0	600	0
22.03.80	16	7.3	5.6	3.9	505	0	41	2
13.04.80	17	8.0	20.9	–	700	0	56	1 (embryos)
29.03.80	18	7.5	9.5	–	5600	4.5	2600	0

sibility. The return of *Rana temporaria* after experimental displacement to the breeding environment is described by Blab (1986) for Central European populations. In general this behaviour does not lead to site fidelity within the reproductive environments (Elmberg, 1986). In the present study no site fidelity to the different spawning sites occurred during the three years registration period (see table 1).

Conductivity and salinity were measured with equipment provided by Yellow Springs Instruments Co., Inc. (YSI Mod. 33). For the registration of the dissolved oxygen concentrationsa Clark electrode (oximeter WTW, accuracy 1%) was applied and for the pH values an electronic pH meter (Schott CG 818). Temperature was measured with a mercury thermometer accurate to 0.2°C. Free chloride (Cl^-) was analyzed by titration (Aquamerk, accuracy 2 ppm).

For statistical evaluation of the data in the field study the potential spawning sites were divided into three classes: class 0 with no spawn, class 1 with one clutch and class 2 with more than one clutch (see table 1, last column). This classification was chosen to avoid the influence of the number of clutches on the statistical evaluation of the spawning site selection. The occurrence of only one clutch could be a consequence of the urge

to spawn after ovulation or could be induced by only one male, incidentally calling at higher salinities and attracting a female conspecific. Therefore, discrimination between spawning sites with one clutch and with more than one clutch was made to exclude single or incidental events. Evaluation of the salt parameters and the spawning site selection of the frogs was limited to the period between March 11 and April 13, when the embryonic stages were actually found inside the jelly or as hatchlings glued to the outer surface of the jelly (until Gosner stage 23; Gosner, 1960). To exclude phenological effects on statistics only the values and concentrations of the sample date at which clutches were seen for the first time per year in the spawning site classes 1 and 2 were included in the calculation and for spawning site class 0 accordingly. Statistical analysis was performed according to Snedecor and Cochran (1968). For all statistical calculations the validated EDV-system BIDAS was applied. All tests were two-tailed. A probability of $P < 0.05$ was considered statistically significant for all statistical comparisons.

Laboratory experiments

Gosner (G) stages 8/9 (early/mid cleavage; Gosner, 1960) from 8 clutches of *Rana temporaria* collected from a spawning site class 2 in the 'Salzwiesen von Münzenberg' were exposed within intact egg capsules to a series of 19 different concentrations of NaCl and KCl dissolved in aquaria with 10 litres of water (tables 2 and 3). 122 to 180 embryos were assigned randomly in 4 to 6 small clutches to the aquaria, i.e. to two controls and the 19 NaCl and 19 KCl concentrations and were positioned near the bottom of the aquaria. Effects of this position on development were excluded. 1. A decrease of oxygen concentration at the bottom of the aquaria was avoided by air input and in all aquaria oxygen was near saturation at the surface and at the bottom. 2. The position of the small clutches was changed twice daily by increasing the air input suspending the clutches for few minutes. Concentrations of sodium and of potassium were calculated according to the atomic weight of NaCl and of KCl on the basis that the 19 levels of chloride ion concentration were the same in the NaCl and the KCl tests (see tables 2 and 3). All experiments were carried out in AOAC (Association of Official Analytical Chemists)-water according to Ashworth and Crozier (1972; physico-chemical parameters: 10 mOsmol, 10° dH equivalent to 3.57 mval, 650 μ S, pH 7.3). The two control groups were held in aquaria with AOAC-water exclusively. The embryos were tested in the NaCl and KCl concentrations in one trial. The survival of the embryos was controlled twice daily and the carcases of the death specimens were separated from the survivors. Both the ranges of concentration and the conductivity were stable throughout the experiment, as explained in 'Results' (tables 2 and 3) below. For all aquaria temperature and pH values remained constant within a range of 19 to 22° C and at pH 7.2 to 7.5 respectively and were normal (Cummins, 1986; Mittmann, 1989; Schlüpmann and Günther, 1996; Breuer and Viertel, 1993). Physico-chemical parameters were measured as described for the field experiments.

Table 2. Laboratory experiment. Survival and mortality of embryonic stages under influence of increased sodium concentration.

Chloride[a] [ppm]	sodium[a] [ppm]	osmolarity [mosmol ltr^{-1}]	conductivity [μ S cm^{-1}]	salinity [‰]	chloride[b] [ppm]	sodium[b] [ppm]	number st. 8/9	number st. 20/21	number st. 22/23	percent st. 20/21	percent st. 22/23
0	0	10	670	0.2	168	0	150	148	148	1.3	1.3
0	0	10	700	0.2	164	0	155	151	151	2.5	2.5
100	64.8	5.63	920	0.4	270 (+ 4.0)	70 (+ 8.0)	149	147	147	1.3	1.3
200	129.6	11.56	1200	0.6	360 (– 3.0)	180 (+ 38.9)	151	149	149	1.3	1.3
300	194.4	16.90	1480	0.8	474 (+ 2.6)	290 (+ 49.2)	147	145	145	1.4	1.4
400	259.2	22.53	1730	1.0	570 (+ 1.0)	380 (+ 46.6)	158	157	155	0.6	1.9
500	323.9	28.17	2100	1.2	690 (+ 4.8)	490 (+ 51.3)	174	173	173	0.6	0.6
600	388.8	33.80	2340	1.4	800 (+ 5.6)	610 (+ 56.9)	165	163	163	1.2	1.2
700	453.5	39.43	2700	1.6	910 (+ 6.3)	780 (+ 72.0)	179	175	175	2.2	2.2
800	518.3	45.07	2890	1.9	1020 (+ 6.8)	860 (+ 65.9)	143	140	140	2.1	2.1
900	582.2	50.70	3110	2.1	1240 (+ 19.3)	920 (+ 58.0)	159	157	157	1.3	1.3
1000	648.0	56.34	3350	2.2	1320 (+ 15.4)	990 (+ 52.8)	151	147	147	2.6	2.6
1100	747.3	61.97	3800	2.3	1360 (+ 7.6)	1190 (+ 59.2)	164	131	131	20.1	20.1
1300	842.3	73.24	4350	2.8	2096 (+ 48.5)	1290 (+ 53.2)	144	122	122	15.3	15.3
1500	971.8	84.51	4900	3.1	2188 (+ 34.8)	1450 (+ 49.2)	157	138	138	12.1	12.1
1700	1101.4	95.77	5800	3.5	2318 (+ 26.6)	1500 (+ 36.2)	155	100	100	35.5	35.5
1900	1231.0	107.04	6300	3.9	2480 (+ 21.8)	1660 (+ 34.8)	144	113	113	21.5	21.5
2100	1360.6	118.31	6800	4.1	2532 (+ 12.6)	1710 (+ 25.7)	122	72	72	41.0	41.0
2300	1490.1	129.58	7400	4.5	2760 (+ 12.9)	1800 (+ 20.8)	180	138	138	23.3	23.3
2500	1619.7	140.85	7900	4.9	2960 (+ 11.8)	1900 (+ 17.3)	156	86	31	44.9	80.1
2700	1749.3	152.11	8400	5.2	3140 (+ 10.1)	1990 (+ 13.8)	143	75	0	47.6	100.0

[a] = concentration according to initial weight of NaCl (initial concentration), [b] = concentrations measured at the end of trial (atom emission spectrometry, ICP-AES), in brackets the difference from the initial concentration (%, mean of controls = 166 ppm subtracted from the initial concentration), number = number of embryos, st. = Gosner (1960) stage, percent = mortality in percent related to the initial number of st. 8/9 embryos.

Table 3. Laboratory experiment. Survival and mortality of embryonic stages under influence of increased potassium concentration.

Chloride[a] [ppm]	potassium[a] [ppm]	osmolarity [mosmol ltr^{-1}]	conductivity [μS cm^{-1}]	salinity [‰]	chloride[b] [ppm]	potassium[b] [ppm]	number st. 8/9	number st. 20/21	number st. 22/23	percent st. 20/21	percent st. 22/23	P-values[c] st. 20/21	P-values[c] st. 22/23
0	0	10	650	0.2	168	0	155	147	147	5.1	5.1	–	–
0	0	10	650	0.2	170	0	205	191	191	6.8	6.8	–	–
100	110.1	5.63	1060	0.5	302 (+ 33)	153 (+ 67.2)	143	140	140	2.1	2.1	0.67	–
200	220.3	11.26	1380	0.8	390 (+ 10.5)	356 (+ 61.6)	188	182	182	3.2	3.2	0.30	–
300	330.4	16.89	1730	1.0	510 (+ 13.7)	507 (+ 53.5)	188	179	179	4.8	4.8	0.12	–
400	440.6	22.52	2100	1.2	623 (+ 13.5)	638 (+ 44.8)	151	144	144	4.6	4.6	0.03	–
500	550.7	28.16	2300	1.4	700 (+ 6.2)	732 (+ 32.9)	147	140	140	4.8	4.8	0.02	–
600	660.8	33.79	2780	1.7	660 (– 18.2)	808 (+ 22.3)	142	138	138	2.8	2.8	0.42	–
700	771.0	39.42	3180	2.0	995 (– 3.7)	890 (+ 15.4)	177	175	175	1.1	1.1	0.68	–
800	881.1	45.05	3490	2.2	1100 (+ 16.4)	957 (+ 8.6)	138	130	130	5.8	5.8	0.13	–
900	991.3	50.68	3910	2.4	1260 (+ 21.2)	1030 (+ 3.9)	189	180	180	4.8	4.8	0.07	–
1000	1101.4	56.32	4020	2.6	1350 (+ 18.1)	1250 (+ 13.9)	163	155	155	4.9	4.9	0.38	–
1100	1212.0	61.95	4350	2.8	1480 (+ 6.9)	1740 (+ 43.6)	151	137	137	9.3	9.3	<0.01	–
1300	1431.8	73.21	5200	3.1	2103 (+ 48.8)	1980 (+ 52.3)	194	185	185	4.6	4.6	<0.01	–
1500	1652.1	84.48	6000	3.7	2220 (+ 36.7)	2240 (+ 35.6)	165	155	155	6.1	6.1	0.07	–
1700	1872.4	95.74	6500	4.0	2345 (+ 28.0)	2440 (+ 30.3)	176	163	163	7.4	7.4	0.00	–
1900	2092.7	107.00	7500	4.5	2510 (+ 23.2)	2680 (+ 28.1)	188	165	151	12.2	19.7	0.02	0.02
2100	2312.9	118.27	8000	4.9	2575 (+ 14.6)	2880 (+ 24.5)	159	143	80	10.1	49.7	0.00	0.00
2300	2533.2	129.53	8500	5.3	2790 (+ 14.0)	3060 (+ 20.7)	117	97	6	17.1	94.9	0.24	0.24
2500	2753.5	140.80	9200	5.8	3050 (+ 15.2)	3220 (+ 16.9)	220	185	0	15.9	100.0	0.00	0.00
2700	2973.8	152.11	9900	6.2	3175 (+ 11.3)	3400 (+ 14.3)	170	120	0	29.4	100.0	<0.01	1.00

[a] = concentration according to initial weight of KCl (initial concentration), [b] = concentrations measured at the end of trial (atom emission spectrometry, ICP-AES), in brackets the difference from the initial concentration (%, mean of controls = 169 ppm subtracted from the initial concentration), number = number of embryos, st. = Gosner (1960) stage, percent = mortality in percent related to the initial number of st. 8/9 embryos; [c] = Fisher-Exact test, comparison of survivors between the K^+ and the Na^+ enriched solutions (survival G stages 8/9 to 20/21 and G stages 20/21 to 22/23).

Sodium and potassium concentrations were measured by atom emission spectrometry (ICP-AES) at the end of the test (after 96 h).

Results

Field study

Variances of pH values, temperature and oxygen concentration were homogeneous within the three spawning site classes (Bartlett-test, P-values: 0.548, 0.548, 0.725). Significant differences between the three spawning site classes existed for conductivity, salinity and chloride (Duncan-test as global test, all three P-values: < 0.01), whereas mean pH values, mean temperature and mean oxygen concentration were comparable (P-values: 0.785, 0.374, 0.968). Thus analysis of variance showed the relationship of the spawning site classes to the conductivity values, the salinity and the chloride and their independence on pH values, dissolved oxygen and water temperature.

Test of linearity by means of a polygonal model demonstrated linearity, i.e. linear correlation between conductivity and chloride (P-values: 0.09 and 0.06, F-values: 0.03 and 0.04) and a nearly linear correlation between salinity (P-value: 0.03, F-values: 0.06) and the spawning site classes 0, 1 and 2. The linear regression was significant (P-values for conductivity, salinity and chloride: < 0.01). The correlation was negative (r: conductivity -0.58, salinity -0.59, chloride -0.62). These statistical evaluations indicate that the distribution of spawning site classes 1 and 2 was linked to lower values of conductivity, salinity and lower chloride concentration when compared with spawning site class 0 and that the distribution of the spawning sites was independent of pH value, temperature and oxygen concentration.

Laboratory experiments

Statistical analysis showed a high correlation between the parameters. The dependence of osmolarity on Na^+ ion concentration was expressed by a saturation curve ($y = -74.7 + 23.11 \times x - 0.065 \times x^2$, $r = 0.99$) and on K^+ by linear regression ($y = 76.25 + 23.18 \times x$, $r = 0.99$). Though in a strong sense the mode of relationship between conductivity and the Na^+ and K^+ concentrations was not comparable, their dependence was equally high, as demonstrated by the coefficient of correlation. The dependence of conductivity and osmolarity on Na^+ ($y = 579.8 + 52.19 \times x$, $r = 0.99$) and on K^+ ion concentration ($y = 714.66 + 60.9 \times x$, $r = 0.99$) was linear, while for Cl^- and osmolarity it was non linear ($y = -12.47 + 27.87 \times x - 0.047 \times x^2$, $r = 0.99$; $y = 8.86 + 28.07 \times x - 0.047 \times x^2$, $r = 0.99$). These relations were expected and were understood as proof and confirmation of the reliability of the test system. The results suggest that parameters in the aquaria, i.e. in the test system, were in principle comparable through the different concentrations of sodium, potassium and chloride thus supporting the aim of the study. However, during the

96-hour experiments Na^+ and K^+ ion concentration was found to increase (tables 2 and 3, see 'Discussion').

Embryonic survival between G stages 8/9 and 20/21 (late tail but stages with gill circulation/transparent cornea, after 72 h) in concentrations of 64.8 to 648.0 ppm Na^+ was in the range of 99.4 to 97.3%, decreasing from 87.9 to 35% between 747.3 ppm Na^+ and the highest Na^+ concentration of 1749.3 ppm (table 2). In the K^+ solutions survival between G stages 8/9 and 20/21 ranged from 90.7 to 98.9% in concentrations of 110.1 to 1872.4 ppm. From 2092.7 ppm to 2973.8 ppm survival was reduced in the range of 89.9 to 70.6% (table 3). From 747.3 ppm survival in Na^+ was significantly lower than in K^+ (P-values see table 3). No mortality occurred between G stages 20/21 and 22/23 (late embryonic stages with tail fin circulation/initial opercular fold, after 96 h) up to a Na^+ concentration of 1490.1 ppm and to a K^+ concentration of 1872.4 (tables 2 and 3). From 1619.7 ppm Na^+ and 2092.7 ppm K^+ survival decreased dramatically (P-values see table 3).

Discussion

Field study

The results clearly indicated that the selection of spawning sites in *Rana temporaria* was dependent on conductivity values, salinity values and chloride concentration but not on pH values, dissolved oxygen or water temperature which were normal in respect to the requirements of the species. The pH values of 7.0 to 8.3 in the present study were within the range of 4.0 to 8.9 described by Cummins (1986), Gebhardt et al. (1987), Mittmann (1989) and Schlüpmann and Günther (1996) for spawning sites and larval biotopes of *Rana temporaria*. Dissolved oxygen was near saturation except in sample sites 14, 15 and 16. Also the lowest oxygen concentrations of 3.7 and 3.9 ppm were no criterion of exclusion for spawning site selection as demonstrated by the occurrence of the one and the 15 clutches respectively. The temperatures were comparable with those measured by Breuer and Viertel (1993) in *Rana temporaria* spawning sites. In the study of Mittmann (1989) 50% of the small ponds and pools with a conductivity of up to 140 μS were inhabited by *Rana temporaria* tadpoles but only 34% with a conductivity of 140 to 220 μS, which were the majority. The mean conductivity of the spawning sites selected in the present study was 664 μS in spawning site class 2, thus placing it almost within the range of Lüttmann and Smolis (1983; 470 to 650 μS) and close to the mean of Beebee (1983; 606 μS) and was in spawning site class 1 (907 μS) within the range of Beebee (1983; 228 to 1080 μS). The selection of lower salinities in the field by *Rana pipiens* and *Bufo calamita* (see 'Introduction') for mating and spawning resembles the behaviour of *Rana temporaria*. For all these cases a selection of spawning sites dependent on salinity must be supposed.

Laboratory experiments

The increase of ionic content during the 96-hour experiment (see 'Results' and tables 2 and 3) could not be avoided because of the properties of the egg jelly (see Duellman and Trueb, 1986) and their change during development including hatching of the embryos and the ensuing decay in connection with the macerating carcases of the intercurrently died embryos though they were removed twice daily. Measurements of Na^+ and K^+ concentration after the end of the experiment, i.e after the first measurement, showed ion concentration to be around 10% lower than 48 h before. This was understood as evidence of the role of egg jellies and carcases as sources of the increased ionic content of the test solutions. In the experiments of Breuer and Viertel (1990) the spontaneous mortality of early larval stages of *Rana temporaria* was around 5% per day and was at least 10% until metamorphosis in the study of Mittmann (1989). Mortality in the control groups of the present study (NaCl experiment: 1.3 and 2.5%, KCl experiments 5.1 and 6.8%) conformed to these data and was understood as regular.

The susceptibility of G stages 8/9 to 20/21 to Na^+ was higher than in G stages 20/21 to 22/23 and was also higher than the susceptibility of G stages 8/9 to 20/21 and 20/21 to 22/23 to K^+. The 'no observed effect concentration' (NOEC) between G stages 8/9 and 20/21 was 648 ppm (3350 μS, 2.2‰ salinity) for Na^+ and 1872.4 ppm (6500 μS, 4.0‰ salinity) for K^+. The NOEC between G stages 20/21 and 22/23 was 1490.1 ppm (7400 μS, 4.5‰ salinity) for Na^+ and for K^+ also 1872.4 ppm (6500 μS, 4.0‰ salinity). Alteration of ion concentration during the 96-hour experiments may have increased the levels of the NOECs (G stages 8/9 to 20/21 for Na^+ 990 ppm, for K^+ 2440 ppm and for Cl^- 1320/2345 ppm; G stages 20/21 to 22/23 for Na^+ 1800 ppm, for K^+ 2440 ppm and for Cl^- 2760/2345 ppm). It is to be supposed that the alterations increased with time (see above). In that case the degree of alteration is less during development of the G stages 8/9 to 20/21 and greater between G stages 20/21 and 22/23. But the real course of the alterations during the trial is unknown. It is thus practically impossible to assess its influence on the NOECs and, therefore, the definition was based on the initial concentrations of Na^+, K^+ and Cl^-.

The NOECs were clearly above the means of conductivity, Cl^- and salinity avoided by *Rana temporaria* during spawning site selection (spawning site class 0: mean conductivity: 2668 μS, mean Cl^- concentration: 1205 ppm, mean salinity: 2.1‰, spawning site class 1: 907 μS, 274 ppm Cl^-, 0.4‰ salinity, spawning site class 2: 664 μS, Cl^- 148 ppm, 0.4‰ salinity), i.e. the safety margin between NOECs in the laboratory experiment and salt concentrations in the spawning sites selected in the field was remarkably broad.

All in all the salt tolerance of the embryonic stages of *Rana temporaria* is more limited than that of *Bufo calamita* (see 'Introduction'). Selection of the lowest salinities possible is the crucial consequence while the broad safety margin between the NOECs and the salt concentrations selected in the field provides the basis for the changing susceptibility of developmental stages and the fluctuating salinities associated with the possible distortion of ionic ratios in the breeding environment. This points to a key role of spawning site selection

in the prevention of salt embryotoxicity. On the one hand the availability of breeding sites may be limited from time to time by the broad safety margin (the broad safety margin beeing superfluous if no fluctuation occurs in the ionic concentration); on the other hand *Rana temporaria* makes optimal use of potential breeding sites with salinities near the tolerance limit of the embryos.

Acknowledgements. I am indebted to Dr. K. Kritsotakis (Johannes Gutenberg-University Mainz) for his measurements by atom emission spectrometry and to Dr. G. Trieb, Dr. J. Theinhardt and to Mr. G. Kürsammer (all Boehringer Ingelheim Pharma KG) for the statistical calculations. The field study was supported by the Hessische Gesellschaft für Ornithologie und Naturschutz (HGON) and was approved by the state of Hessen.

References

Andrén, C., Nilson, G. (1985): Habitat and other environmental characteristics of the natterjack toad (*Bufo calamita* Laur.) in Sweden. British J. Herpetol. **6**: 419-424.

Ashworth, R. de, Crozier, B. (1972): Standard waters: recommendations for the preparation of standard waters used for testing pesticidal and other formulations, and an FAO survey of naturally occuring waters. In: CIPAG Monograph I, p. 1-64. Raw, G.R., Ed., Cambridge, Collaborative Pesticides Analytical Council.

Beebee, T.J.C. (1983): Habitat selection by amphibians across an agricultural land-heathland transect in Britain. Biological Conservation **27**: 111-124.

Beebee, T.J.C. (1985a): Salt tolerances of natterjack toad (*Bufo calamita*) eggs and larvae from coastal and inland populations in Britain. Herpetol. J. **1**: 14-16.

Beebee, T.J.C. (1985b): Discriminant analysis of amphibian habitat determinants in South-East England. Amphibia-Reptilia **6**: 35-43.

Berglund, B. (1976): Skånes Sällsynta Groddjur. Statens Naturvårdsverk, PM 765.

Blab, J. (1986): Biologie, Ökologie und Schutz von Amphibien. Schriftenreihe für Landschaftspflege und Naturschutz **8**, Bundesforschungsanstalt für Naturschutz und Landschaftsökologie Bonn. Bad Godesberg, Ed., Kilda Verlag Greven.

Bogenrieder, A., Collatz, K.-G., Kössel, H., Osche, G. (1986): Lexikon der Biologie, vol. 8. Freiburg, Basel, Wien, Verlag Herder.

Breuer, P., Viertel, B. (1990): Zur Ökologie der Erdkrötenlarven (*Bufo bufo*) und Grasfroschlarven (*Rana temporaria*). I. Die Überlebensrate unter dem Einfluß von Regenbogenforellen (*Oncorhynchus mykiss*). Acta Biol. Benrodis **2**: 225-244.

Breuer, P., Viertel, B. (1993): Zur Ökologie der Erdkrötenlarven (*Bufo bufo*) und Grasfroschlarven (*Rana temporaria*). II. Wachstum und Entwicklung. Acta Biol. Benrodis **5**: 1-17.

Cooke, A.S. (1975): Spawn site selection and colony size of the frog (*Rana temporaria*) and the toad (*Bufo bufo*). J. Zool. Lond. **175**: 29-38.

Cummins, C.P. (1986): Effects of aluminium and low pH on growth and development in *Rana temporaria* tadpoles. Oecologia **69**: 248-252.

Duellman, W.E., Trueb, L. (1986): Biology of Amphibians. New York, St. Louis, San Francisco, Mc Graw-Hill Book Company.

Elmberg, J. (1986): Apparent lack of territoriality during the breeding season in a boreal population of Common Frogs. Herpetol. J. **1**: 81-85.

Gebhardt, H., Kreimes, K., Linnenbach, M. (1987): Untersuchungen zur Beeinträchtigung der Ei- und Larvenstadien von Amphibien in sauren Gewässern. Natur und Landschaft **62**: 20-23.

Giselen, T., Kauri, H. (1959): Zoogeography of the Swedish amphibians and reptiles. Acta Vertebratica **1**: 197-397.

Gordon, M.S. (1962): Osmotic regulation in the Green Toad (*Bufo viridis*) J. Exp. Biol. **39**: 261-270.

Gordon, M.S., Tucker, V.A. (1968): Further observations on the physiology of salinity adaptation in the Crab-Eating Frog (*Rana cancrivora*). J. Exp. Biol. **49**: 185-193.

Gosner, K.L. (1960): A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica **16**: 160-183.

Kauri, H. (1948): Über die Ausbreitung und die Ausbreitungsumstände der Wechselkröte (*Bufo viridis*) im Ostseegebiet. Lunds Universitets Årsskrift N. F. **44**: 1-30.

Lemmel, G. (1977): Die Lurche und Kriechtiere Niedersachsens. Grundlagen für ein Artenschutzprogramm. Naturschutz und Landespflege in Niedersachsen **5**, Veröffentlichungen des Niedersächsischen Landesverwaltungsamtes, Hannover.

Lüttmann, J., Smolis, M. (1983): Auswertung von Bestandsaufnahmen an Amphibien-Laichgewässern als Teil des ökologischen Beitrages zum Landschaftsplan. Verh. Ges. Ökol. **10**: 157-166.

Mathias, J. (1971): The comparative ecologies of two species of amphibia (*Bufo bufo* and *Bufo calamita*) on the Ainsdale Sand Dunes National Natural Reserve. Ph.D. thesis, University of Manchester.

Mittmann, R. (1989): Die Larvenbiologie von Grasfrosch (*Rana t. temporaria* Linnaeus, 1758) und Erdkröte (*Bufo b. bufo* Linnaeus, 1758) in verschiedenen Kleingewässern des Niederrheins. Dissertationsschrift am Fachbereich Biologie. Universität Köln.

Ruibal, R. (1959): The ecology of a brackish water population of *Rana pipiens.* Copeia **1959**: 315-322.

Schlüpmann, M., Günther, R. (1996): Grasfrosch — *Rana temporaria,* Linnaeus 1758. In: Die Amphibien und Reptilien Deutschlands, p. 412-454. Günther, R., Ed., Jena, Stuttgart, Lübeck, Ulm, Gustav Fischer.

Snedecor, G.W., Cochran, W.G. (1968): Statistical Methods. Ames, Iowa, The Iowa State University Press.

Strijbosch, H. (1979): Habitat selection of amphibians during their aquatic phase. Oikos **33**: 363-372.

Wangersky, P.J. (1980): Chemical Oceanography. In: The natural environment and the biogeochemical cycles, p. 51-77. Hutzinger, O., Ed., Berlin, Heidelberg, New York, Springer-Verlag.

Uchiyama, M., Murakami, T., Yoshizawa, H. (1990): Notes on the development of the Crab-Eating Frog *Rana cancrivora*. Zool. Sci. (Tokyo) **7**: 73-78.

Received: June 4, 1997. Accepted: September 28, 1998.