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SALINITY TOLERANCE AND PREFERENCE IN THE FROG *RANA RUGULOSA* WIEGMANN

JOHN DAVENPORT[1] AND KHOO KHAY HUAT[2]

[1] *University Marine Biological Station Millport, Isle of Cumbrae, Scotland KA28 0EG, UK*

[2] *School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia*

Amphibians are predominantly characteristic of freshwater and damp terrestrial habitats. Early tolerance and physiological studies indicated that amphibians could not survive for more than a few hours in external media more concentrated than about 10 °/$_{oo}$, corresponding to about 30% sea water or 300-350 mOsm l^{-1}, because of osmotic dehydration and diffusional uptake of salt (see Gordon *et al.*, 1961 for review). In the past four decades, a number of anuran species have been discovered living in brackish-water. The most famous of these is the crab-eating frog *Rana cancrivora* which has been extensively studied by physiologists (Gordon *et al.*, 1961; Gordon & Tucker, 1965; Uchiyama *et al.*, 1987, 1990; Uchiyama & Yoshizawa, 1992) and can survive indefinitely in 18 °/$_{oo}$. The frog *Rana rugulosa* is similar in size and appearance to *Rana cancrivora*, while both species live in similar coastal habitats in the northern part of Peninsular Malaysia (K.B. Heang, pers. comm.), and are often collected together by commercial frog hunters. *Rana rugulosa* is a very common frog, widely sold in markets for food; it is not endangered and the bulk of the animals used in the study reported here were held in captivity for a short period before being released to the wild. Firstly, the salinity tolerance of *Rana rugulosa* was investigated for comparison with *Rana cancrivora*. Secondly, the behavioural response of frogs to environmental salinity was assessed. Finally, experiments were conducted to assess the basis of salinity discrimination.

Adult frogs were collected from tidal irrigation channels delivering water to paddy fields near Sungai Petani, Kedah, Malaysia, and used immediately in experiments. At high tide (on 15-9-96) these channels were found to contain water of 5 °/$_{oo}$, though frogs are known to be found in water bodies nearer the sea; comprehensive data for the full range of salinities that they can encounter are unavailable. To test salinity tolerance, 10 frogs were held in each of the following salinities for 72 hr: 0 °/$_{oo}$, 4 °/$_{oo}$, 8 °/$_{oo}$, 16 °/$_{oo}$, 24 °/$_{oo}$ and 32 °/$_{oo}$. Frogs were held in aquaria so that the legs and belly were immersed. At the end of the 72 hr period they were assessed for mortality. To investigate salinity preference, the apparatus shown in Fig. 1 was employed. Made of wood, with a loose-fitting, but light-tight lid, this choice box offered frogs a choice between two media. To conduct an experiment, the two chambers of the box were filled with different media, two frogs were placed in each chamber and the lid applied. After 30 min the lid was opened and the distribution of frogs recorded. The 30 min period was decided upon after pilot trials that demonstrated that no difference in response was evident after longer periods. Each experiment was repeated until a total of 20 frogs had been offered each choice. Between replicate trials, the box was cleaned and the media switched between chambers. Individual frogs were only used once in experiments, and then released to the wild. First, a series of salinities between 8 and 38 °/$_{oo}$ were offered to frogs, the alternative being rain water (0 °/$_{oo}$; ionic concentration unknown). In view of the results obtained, three further choices were offered: 0 °/$_{oo}$ vs. 1000 mOsm NaCl, 0 °/$_{oo}$ vs. 1000 mOsm mannitol, and 1000 mOsm NaCl vs. 1000 mOsm mannitol. These were designed to determine whether *Rana rugulosa* was sensitive to the ionic strength of the outside medium, or to medium osmolarity. During pilot trials, six salinity preference experiments (choice offered; between 0 and 34 °/$_{oo}$) were carried out without a lid on the choice box, allowing direct observation of frog behaviour.

Probit analysis of the salinity tolerance data (Finney, 1971) established that the median lethal salinity (72 hr) for *Rana rugulosa* was 10.2 °/$_{oo}$ (95% confidence limits 8.1 °/$_{oo}$ and 12.0 °/$_{oo}$). Although this frog inhabits broadly similar habitats to *Rana cancrivora*, it is far less tolerant of saline conditions than the latter species, and clearly cannot spend long periods in media more concentrated than 5 °/$_{oo}$. Table 1 displays salinity preference

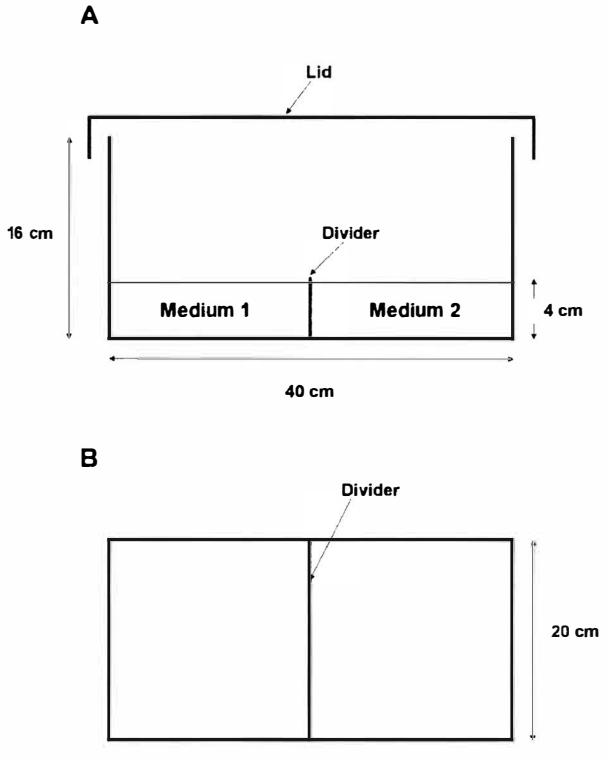

FIG. 1. Apparatus employed in study of salinity choice in *Rana rugulosa*. A, from side. B, plan view.

TABLE 1. Salinity choice in *Rana rugulosa*. 20 different frogs were offered each choice between two media. χ^2_{adj} = χ^2 with Yates' correction for small sample sizes. NS indicates that choice was not statistically significant (P>0.05).

	Available choice			χ^2_{adj}	P
1.	0‰	vs.	38‰		
	17		3	8.45	<0.005
2.	0‰	vs.	36‰		
	16		4	6.05	<0.025
3.	0‰	vs.	34‰	18.05	<0.001
	20		0		
4.	0‰	vs.	32‰	6.05	<0.025
	16		4		
5.	0‰	vs.	28‰		
	16		4	6.05	<0.025
6.	0‰	vs.	26‰		
	13		7	1.25	NS
7.	0‰	vs.	22‰		
	8		12	0.45	NS
8.	0‰	vs.	8‰		
	11		9	0.05	NS
9.	0‰	vs.	1000 mOsm NaCl		
	19		1	14.45	<0.001
10.	0‰	vs.	1000 mOsm mannitol		
	14		6	2.45	NS
11.	1000 mOsm mannitol	vs.	1000 mOsm Na Cl		
	18		2	11.25	<0.001

data for *Rana rugulosa* and it may be seen that the frog has a well developed ability to avoid high salinities (>26‰). Continuous visual observation during pilot trials showed that salinity choice (between 0 and 34‰) started within 20-30 seconds of contact with media and was complete within 3 min. It took place without the frog immersing the snout or head; contact of the medium by the belly and legs was sufficient to initiate choice, and there was no sign of cutaneous transport of fluid to the snout area by 'wicking'. The data for choices involving 1000 mOsm mannitol and 1000 mOsm NaCl were illuminating. These media are both approximately isosmotic with sea water (1000 mOsm≡ 31‰; Rankin & Davenport, 1981). However, while 1000 mOsm NaCl was avoided to a highly significant extent (when the alternative was fresh water), 1000 mOsm mannitol was not. In addition, when frogs were offerred a choice between 1000 mOsm mannitol and 1000 mOsm NaCl, they showed pronounced avoidance of the salt solution, even though both solutions will lead to similar rates of osmotic dehydration. Taken together these data indicate that salinity choice behaviour by the frogs is determined by ionic concentrations, not by osmolarities.

It is evident that *Rana rugulosa* does not have the tolerance of salinity exhibited by *Rana cancrivora*. However, it does have the ability to recognize and escape from deleterious salinities, a useful attribute in a mobile species inhabiting environments where some bodies of water will be more saline than others. Salinity discrimination is by cutaneous response, and is rapid, presumably because of the high permeability of frog skin to salts and water.

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