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DO FROGS DRINK?

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SUMMARY

Drinking in response to dehydration and exposure to saline solutions was measured in adult *Rana pipiens*, *Bufo marinus* and *Xenopus laevis*, and tadpoles of *Rana catesbeiana*. These animals normally occupy quite different types of environments where the possible advantages of drinking may differ. Small amounts of the external media were swallowed by all the animals and the quantity usually increased when they were placed in hyperosmotic NaCl solution. However, no relationship between the oral intake of water and the particular conditions, such as the degree of dehydration, was observed. Usually 80–90% of the total water uptake occurred by absorption across the skin. Thus although 'secondary' type drinking takes place, 'primary' drinking did not appear to occur. These results directly confirm the general popular impression that amphibians, in contrast to other tetrapods and marine teleosts, do not drink in order to rehydrate.

INTRODUCTION

Drinking plays a vital role in the regulation of body fluids of most vertebrates, but there are some interesting exceptions, especially among the freshwater teleosts, elasmobranchs and amphibians. Regulatory or 'primary' drinking occurs in response to thirst (see Fitzsimons, 1972). A further type of drinking, 'secondary' drinking, is not a regulatory response to a deficit of body water; it may take place during feeding or in response to stress. In mammals, primary drinking involves the stimulation of the thirst centre in the hypothalamus as a result of cellular dehydration or a decrease in the volume of the extracellular fluids. This type of response has also been observed in birds and reptiles (Fitzsimons, 1975; Fitzsimons & Kaufman, 1977). Drinking by marine teleost fish appears to be of the primary type, but the mechanism appears to be a reflex that does not involve the hypothalamus (Hirano, 1974).

It is generally believed that amphibians do not normally drink but rehydrate by absorbing water across their permeable skin (see Adolph, 1943; Bentley, 1971). Few species have been examined, however, and the observations are largely based on hearsay evidence, usually not substantiated by actual measurements of the water that is swallowed. When frogs are placed in hyperosomotic saline solutions, they have been observed to drink the solution (Krogh, 1939; Bentley & Schmidt-Nielsen, 1971; Katz, 1975) but it is not clear whether this is a regulatory response or a non-specific reaction to a stressful situation. It is, however, interesting that the tadpoles of the crab-eating

Table 1. *The contribution of drinking to water uptake in amphibians*

	ml 100 g^{-1}h^{-1}					
	Normal*			Dehydrated†		
	Total	Drinking	Drinking as % of total	Total	Drinking	Drinking as % of total
Rana pipiens (5)	1·94 ± 0·11	0·11 ± 0·02	5·4 ± 1·25	13·4 ± 1·46	0·19 ± 0·05	1·4 ± 0·28
Bufo marinus (6)	2·56 ± 0·35	—	—	20·0 ± 2·28	0·02 ± 0·01	0·1 ± 0·36
Xenopus laevis (5)	0·67 ± 0·13	0·11 ± 0·01	18·1 ± 3·0	2·4 ± 0·24	0·21 ± 0·05	8·4 ± 2·1

* The difference between the total water uptake and that due to drinking represents that accumulated across the skin. The determination was made over a 15 h period, with the cloaca ligated, for *Rana* and *Bufo*, and 8 h for *Xenopus*.

† Dehydration equivalent to a loss in body weight of 25–30% was performed in a beaker in the laboratory. Water uptake and drinking were measured over a 2 h period; the cloaca was not tied. The results are as means ± S.E. of the number of experiments in parentheses.

frog, *Rana cancrivora*, which live in sea water, normally drink this solution (Gordon & Tucker, 1965); behaviour which is reminiscent of that of marine teleosts.

In this paper, drinking is examined in four types of amphibians that normally occupy different types of environment, where the possible advantages of drinking may differ.

METHODS

Rana pipiens, *Rana catesbeiana* tadpoles, *Bufo marinus* and *Xenopus laevis* were obtained from commercial suppliers and kept, as appropriate, in aquaria or terraria in the laboratory at 21 °C. Drinking was estimated by measuring the accumulation of a 'non-transportable' marker in the gastrointestinal tract of the animals. Evans (1973) has used ^{125}I-iothalamate (Glofil-I-125, Abbott Laboratories, Chicago) to measure drinking in fish. This marker (about 0·2 μCi/ml) was placed in the bath of tap water or saline containing the animal. At the end of the measuring period, the animal was rinsed in tap water. Adults were then killed by double pithing, and tadpoles were anaesthetized with MS222. The entire gastrointestinal tract was then dissected free, after closing it at each end with a thread suture. If necessary, it was divided into segments suitable for measurement of its contained radioactivity in a gamma counter (Beckman, Biogamma II). Standard samples from the outside bathing medium were taken at the beginning and end of each experiment. The experiments were usually of a short duration, less than 2 h, so that the cloaca was not tied. For longer experiments, the cloaca was closed with a purse-string suture. It is important to know if a significant amount of the ^{125}I-iothalamate may be absorbed from the gut, thus reducing the apparent rate of drinking. This possibility seems unlikely (see Evans, 1973) but to test it, arterial blood samples were collected from six *Rana pipiens* after they had been exposed to the ^{125}I-iothalamate in tap water for 24 h. In samples of 0·3–0·6 ml, radioactivity significantly in excess of the background level was not detected.

Table 2. *Effects of immersion in hyperosmotic* 3·3 % *NaCl solutions on drinking in* Rana pipiens *and* Bufo marinus

	ml 100 g^{-1} h^{-1}	
	3·3 % NaCl* for 30 min drinking	Tap water† for 30 min drinking
Rana pipiens	1·68 ± 0·40	0·27 ± 0·08
Bufo marinus	2·65 ± 0·33	0·39 ± 0·28

Means ± S.E. for six experiments.

* Under these conditions, the Na concentration of the body fluids rises markedly due to osmotic water loss and the accumulation of Na (see Bentley & Schmidt-Nielsen, 1971).

† This group of amphibians was first exposed to the 3·3 % NaCl and then placed in the tap water.

RESULTS

Rana pipiens (*Ranidae*)

The North American leopard frog is an amphibious anuran which divides its time between dry land and fresh water. When the experimental animals were confined to a beaker of tap water, water uptake due to drinking was about 0·1 ml 100 g^{-1} h^{-1} (Table 1). The total water uptake under these conditions, which also includes that absorbed across the skin, was 1·9 ml 100 g^{-1} h^{-1} so that the drinking only made up 5·4 % of the total.

Five frogs were dehydrated by placing them in a beaker without water for 18 h. At this time, they had lost water equivalent to about 30 % of their body weight. When placed in tap water, these frogs rapidly regained this lost water but only 1·4 % of the total was taken up by drinking (Table 1), an even smaller proportion than in the normally hydrated frogs.

It has previously been shown that leopard frogs drink when placed in NaCl solutions equivalent in concentration to sea water (3·3 %) (Bentley & Schmidt-Nielsen, 1971) and these observations were confirmed in the present series of experiments (Table 2). To see if this drinking behaviour is perpetuated or even increased when fresh water is subsequently made available, we transferred frogs treated in this way to a beaker of tap water. Drinking, however, was not much greater than that observed when they are normally hydrated.

Bufo marinus (*Bufonidae*)

The 'marine' toad is a cosmopolitan tropical species that spends most of its life on land and, despite its name, does not lead an oceanic existence. When sitting undisturbed in beakers of tap water these animals did not submerge their heads so that drinking could not occur. These toads were dehydrated by leaving them in air without access to water for 48 h at which time they had lost about 30 % of their body weight. When placed in tap water, they rapidly rehydrated and regained nearly all the lost water in 2 h (Table 1). Drinking was negligible during this period, so that virtually all the water was regained by cutaneous absorption. When *Bufo marinus* were placed in 3·3 % NaCl solution for 30 min, they drank small amounts of fluid (Table 2). Replacement of tap water, following exposure to the saline, resulted in less drinking.

Table 3. *Drinking in tadpoles* (Rana catesbeiana) *immersed in NaCl solutions*

	ml 100 g^{-1} h^{-1}
Tap water*	0·18 ± 0·020
0·3 % NaCl	0·24 ± 0·028
0·6 % NaCl	0·26 ± 0·053
0·9 % NaCl	0·23 ± 0·041
2 % NaCl for 30 min	0·85 ± 0·075
2 % NaCl *then* tap water 30 min	0·22 ± 0·030

Results as means ± S.E. for eight experiments.

* For 2 h except for 2 % NaCl.

Xenopus laevis (*Pipidae*)

The African clawed toad is an aquatic anuran. When the ponds and lakes were they normally live dry up, they burrow into the remaining mud where they aestivate until the next rainy season (Alexander & Bellerby, 1938). During this period, they accumulate metabolites, principally urea, which they excrete in large amounts when water again becomes available (Balinsky *et al.* 1967). Compared to other species, this anuran only takes up water across its integument relatively slowly (Heller & Bentley, 1965; Yorio & Bentley, 1978). It thus appears to be a species in which water uptake by drinking would be especially appropriate. Local hearsay evidence suggests that they do this in the south-west of Cape Province in South Africa when they emerge from aestivation following rain (T. W. Mittag, personal communication).

In its normal aquatic type of environment water uptake by drinking by *Xenopus* was found to be equivalent to 0·1 ml 100 g^{-1} h^{-1} (Table 1). This quantity is 18% of the total water accumulated. The toads were dehydrated by leaving them in a beaker without water for 15 h when they lost water equivalent to about 26% of their body weight. When replaced in fresh water, they drank fluid equivalent to 0·2 ml 100 g^{-1} h^{-1} over a 2 h period which is similar to the rate observed in the normally hydrated toads and 8% of the total uptake. The *Xenopus* only regained their lost weight very slowly and after 2 h in tap water, had only reaccumulated one-fifth of the water which they lost. Thus, although drinking ought to have facilitated their rate of rehydration, they did not resort to it. This observation, however, does not exclude the possibility that these animals may drink in their native habitat.

Rana catesbeiana *tadpoles*

The tadpoles of the crab-eating frog, *Rana cancrivora*, have been observed to drink (Gordon & Tucker, 1965). This species lives in sea water, and drinking appears to play a vital role in their osmoregulation. We measured the drinking of bullfrog tadpoles in fresh water and a series of NaCl solutions (Table 3). In tap water, drinking amounted to 0·18 g 100 g^{-1} h^{-1} and it was similar in 0·3, 0·6 and 0·9 % NaCl solutions. When they were placed in 2 % NaCl solution, the tadpoles drank more rapidly: 0·85 ml 100 g^{-1} h^{-1}. The time period for the last measurement was 30 min and if left in the solution, the animals all died within 45–60 min so that the conditions can be considered to constitute 'stress'. When tadpoles which had been kept in the 2 % NaCl solution for 30 min were replaced in fresh water, they survived but did not drink more rapidly.

DISCUSSION

Primary drinking, in response to dehydration or immersion in hyperosmotic saline solutions, was not observed in *Rana pipiens*, *Bufo marinus* or *Xenopus laevis*. The tadpoles of *Rana catesbeiana* also did not display such a response. Most of the amphibians swallowed small amounts of water compared to that taken up across the skin even when they were normally hydrated in fresh water and this increased only slightly when they were placed in hyperosomotic saline. The fluid uptake thus appears to constitute 'secondary' drinking, which is not directly related to osmotic need. This type of water uptake has also been observed in aquatic urodele amphibians (Bentley, 1973).

It is interesting that frogs can apparently 'taste' water and can distinguish between salt solutions of different concentration (Andersson & Zotterman, 1950; Araike & Saito, 1976). This ability, however, does not seem to subserve the usual type of thirst mechanism. Some amphibians appear to seek water in which to lie in order to rehydrate. It has been suggested that this process may constitute a 'primeval thirst' (Fitzsimons, 1975) which is slaked by the absorption of water across the integument, instead of by more conventional oral drinking. Some of the physiological prerequisites, such as a 'thirst' mechanism, may thus be present. Amphibians are a diverse group of vertebrates and it remains possible that a drinking response like that seen in the amniotes may yet be identified among them. Their 'cutaneous drinking', however, appears to be a satisfactory process which has not hampered their occupation of even quite arid habitats.

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REFERENCES

Adolph, E. F. (1943). *Physiological Regulations*, pp. 110–116. Lancaster: The Jacques Cattell Press.

Alexander, S. S. & Bellerby, C. W. (1938). Experimental studies on the sexual cycle of the South African toad (*Xenopus laevis*). *J. exp. Biol.* **15**, 74–81.

Andersson, B. & Zotterman, Y. (1950). The water taste in the frog. *Acta physiol. scand.* **20**, 95–100.

Araike, N. & Sato, M. (1976). Water response in frog taste cells. *Comp. Biochem. Physiol.* **54A**, 149–156.

Balinsky, J. B., Choritz, E. L., Coe, C. G. L. & Van Der Schans, G. S. (1967). Amino acid metabolism and urea synthesis in naturally aestivating *Xenopus laevis*. *Comp. Biochem. Physiol.* **22**, 59–68.

Bentley, P. J. (1971). *Endocrines and Osmoregulation. A Comparative Account of the Regulation of Water and Salt in Vertebrates*, pp. 168. New York, Heidelberg, Berlin: Springer Verlag.

Bentley, P. J. (1973). Osmoregulation in the aquatic urodeles *Amphiuma means* (the Congo Eel) and *Siren lacertina* (the Mud Eel). Effects of vasotocin. *Gen. comp. Endocrinol.* **20**, 386–391.

Bentley, P. J. & Schmidt-Nielsen, K. (1971). Acute effects of sea water on frogs (*Rana pipiens*). *Comp. Biochem. Physiol.* **40A**, 547–548.

Evans, D. (1973). Sodium uptake by the sailfin molly, *Poecilia latipinna*: a kinetic analysis of a carrier system present in both freshwater-acclimated and seawater-acclimated individuals. *Comp. Biochem. Physiol.* **45A**, 843–850.

Fitzsimons, J. T. (1972). Thirst. *Physiol. Rev.* **52**, 468–561.

Fitzsimons, J. T. (1975). Thirst and sodium appetite in the regulation of body fluids. In *Control Mechanisms of Drinking* (ed. G. Peters, J. T. Fitzsimons and L. L. Peters-Haefeli), pp. 1–7. Berlin, Heidelberg, New York: Springer Verlag.

Fitzsimons, J. T. & Kaufman, S. (1977). Cellular and extracellular dehydration, and angiotensin as stimuli to drinking in the common iguana *Iguana iguana*. *J. Physiol., Lond.* **265**, 443–463.

Gordon, M. S. & Tucker, V. A. (1965). Osmotic regulation in the tadpoles of the crab-eating frog (*Rana cancrivora*). *J. exp. Biol.* **42**, 437–445.

HELLER, H. & BENTLEY, P. J. (1965). Phylogenetic distribution of the effects of neurohypophysial hormones on water and salt metabolism. *Gen. comp. Endocrinol.* **5**, 96–108.

HIRANO, T. (1974). Some factors regulating water intake by the eel, *Anguilla japonica*. *J. exp. Biol.* **61**, 737–747.

KATZ, U. (1975). NaCl adaptation in *Rana ribibunda* and a comparison with the euryhaline toad *Bufo viridis*. *J. exp. Biol.* **63**, 763–773.

KROGH, A. (1939). *Osmotic Regulation in Aquatic Animals*. Cambridge: University Press.

YORIO, T. & BENTLEY, P. J. (1978). The permeability of the skin of the aquatic anuran *Xenopus laevis* (Pipidae). *J. exp. Biol.* **72**, 285–289.