OSMOREGULATION OF THE CANE TOAD, *BUFO MARINUS, IN* SALT WATER

G. W. LIGGINS and G. C. GRIGG
School of Biological Sciences, The University of Sydney, NSW, Australia, 2006. Telephone: (02) 692-2433

Abstract-1. Adult cane toads, *B. marinus,* survived in salinities up to 40% sea-water (SW).

2. Pre-exposure to 30, then 40% SW, increased the survival time of toads in 50% SW.

3. Plasma from toads acclimated to salt water is hyperosmotic to the environment - a result of increased plasma sodium, chloride and urea concentrations.

4. When toads were placed in tap-water and 20% SW, all significant changes to plasma sodium, chloride, urea and osmotic pressure occurred within the first 2 days of exposure.

5. When toads were placed in 30 and 40% SW environments, the increases in plasma sodium and chloride concentrations occurred within the first 2 days of exposure while urea and total osmotic pressure continued to rise until some time between 2 and 7 days exposure.

INTRODUCTION

In general, semi-terrestrial and freshwater amphibians maintain their body fluids at an osmotic pressure approx. 25% of that of sea-water (i.e. 25% SW) and in all species so far studied it is apparent that for adults hyperosmoticity to the environment is essential for survival (Balinsky, 1981). Accordingly, salinities greater than 25% SW provide challenging environments for an amphibian.

Although amphibians are generally regarded as freshwater animals, adults of a surprisingly large number of species have been reported to inhabit or tolerate some degree of brackish water (for a review see Balinsky, 1981). The most striking is *Rana cancrivora* which can tolerate an environmental salinity as great as 80% SW (Gordon et al., 1961). Species such as *Xenopus laevis, Bufo viridis, Rana temporaria* and *Bufo bufo* are intermediate in their ability to tolerate brackish water while species such as *Rana tigerina* are incapable of surviving an environmental salinity of 25% SW or above (Romspert, 1976; Katz, 1973; Ackrill et al., 1969; Ferreira and Jesus, 1973; Gordon et al., 1961).

Species that can tolerate ambient salinities higher than normal body fluids (i.e. approx. 25% SW) do so by increasing their plasma osmotic pressure to a level above that of the environment, thereby avoiding dehydration as a result of water loss to the environment by osmosis. Increases in plasma osmotic pressure are achieved by abandoning homeostasis and increasing the concentration of plasma sodium, chloride and urea, the proportional contributions of each of these solutes to the increased osmotic pressure varying among species.

Henderson et al. (1972) and Garland and Henderson (1975) investigated the influence of environmental salinity on renal function in *B. marinus* but limited their experiments to environments of distilled water and 0.9% sodium chloride (28% SW), and a single exposure time of at least 3 weeks.

Bufo marinus (Linnaeus), originally a native of Central and South America, was introduced to Australia from Hawaii in 1935 and since then has been successful in establishing itself in a wide range of environments. These environments include the frontal dunes of ocean beaches, the inland border of coastal mangroves, tidal mud-flats, wet and dry creek beds, as well as pools of brackish water (van Beurden and Grigg, 1980; Covacevich and Archer, 1975).

The aim of this investigation was to describe the time-course of any changes in plasma and urine osmotic pressure, concentrations of their major contributing solutes, as well as hydration state of *B. marinus* placed in aqueous solutions at various salinities. In addition to describing the acclimation of *B. marinus* to these environments, the range of environmental salinities tolerable to *B. marinus* under laboratory conditions was identified. Finally, the ability of *B. marinus* to increase its salinity tolerance in saline environments following a gradual rather than rapid increase in salinity was investigated.

MATERIALS AND METHODS

Maintenance of animals

A commercial supplier in southern Queensland provided adult *B. marinus* of both sexes between 35 and 100 g in weight. The animals were kept in 56 x 36 x 27 cm plastic tanks tilted to provide the animals with a choice of sitting in shallow tap-water or on the dry plastic substratum ("pre-treatment conditions"). Lids with small ventilation holes prevented escape of the animals. The tanks were kept in a room temperature of 24 ± 2°C and the tank water was changed daily. The toads were not fed.

Experiments were carried out between July and October 1982 by exposing *B. marinus* to aqueous environments of various salinities, achieved by filling the plastic tanks with an appropriate mixture of tap-

water and sea-water to a depth of 2.5 cm. The tanks were not tilted so that the toads had no choice but to sit with their ventral surfaces immersed:

Collection and storage of plasma and urine

Urine was obtained by gently inserting a glass tube (4 mm OD) into the animal's cloaca and pushing it up through the muscular sphincter into the bladder. Urine then flowed freely into a plastic vial. Blood was obtained by cardiac puncture of a double-pithed animal using a 26-gauge needle attached to a heparinized 2 ml syringe. Approximately 75 µl of this blood were transferred to a micro-hematocrit capillary tube for hematocrit determination. The remainder was centrifuged and the plasma transferred to a plastic vial. Plasma and urine were kept refrigerated pending determination of osmotic pressure (within 3 hr). Samples were then frozen for later determinations of sodium, potassium, chloride, urea and, in the case of urine, ammonia concentrations.

Analytical techniques

Osmotic pressures were determined on duplicate 150 µl samples by measuring freezing point depression with a Knauer semi-micro osmometer. Sodium and potassium concentrations were measured on samples of approx. 60 µl using a Corning 435 flame photometer. Plasma and urine urea and urine ammonia concentrations were determined on duplicate 200 µl samples of diluted plasma or urine by performing an enzymatic colorimetric assay (Boehringer Mannheim Kit No. 124788), the concentration of urine ammonia being assayed by substituting distilled water for urease. Absorbance of the standards and unknowns were measured in a Varian Techtron 635 spectrophotometer.

Condition factors as a measure of hydration

Relative states of hydration of *B. marinus* in different treatments were assessed by comparison of condition factors. An animal's condition factor is defined as equalling (weight (g)/length (cm)) x 100 where *a* is the slope of the regression line of log weight on log length for that batch of animals. The condition factor calculated for an individual animal is dependent on its weight and length. By comparing the condition, factors, or changes in condition factor, of animals in experimental treatments relative to animals in control conditions it can be deduced whether the treatment animals have become more or less hydrated as a result of the experimental treatment.

Experiment 1: The time-course of acclimation to salt water

Sixteen *B. marinus* were assigned randomly to each of four tanks and maintained under pre-treatment conditions for 8 days. The weight and snout-urostyle length of each toad was then measured so that the relationship between weight and length for this batch of toads could be determined. The toads were marked individually by means of colour-coded pieces of string tied to one of the front legs and were returned to their respective tanks. The tanks were then emptied and three filled respectively with tap-water, 20% and 40% SW. The fourth tank was set up as it had been originally, as a control.

Three *B. marinus* were sampled at random from each of these tanks initially and after 3 hr, 12 hr, 2 days and 7 days. The following were then determined for each animal: hematocrit, change in condition; osmotic pressures of plasma and urine, concentrations of sodium, potassium, chloride and urea in the plasma and urine as well as the concentration of ammonia in the urine. The survival of animals in each tank was also monitored.

Experiment 2: Late stages of the acclimation process

Sixty-two *B. marinus* were allocated randomly to four tanks so that one tank received 11, one 12, one 21 and one 18 toads. After 7 days under pre-treatment conditions the weight and snout-urostyle length were measured to establish the weight-length relationship for these animals. The tanks were then emptied of water and those containing 11, 12, 21 and 18 toads were filled with 20, 30, 40 and 50% SW respectively. Different numbers of animals were allocated to each tank as only 62 *B. marinus* were available and considerably higher mortality was expected in the 40 and 50% SW treatments than in the others. Five *B. marinus* were sampled at random from each tank after 2 and 7 days. Measurements were made for each animal as described for Experiment 1 except that "absolute" condition and not change in condition was measured so that the need to mark individual animals could be avoided. From the weight and length data obtained from the animals initially, five were selected randomly from each treatment so that the calculated initial condition factors could be compared with those after 2 and 7 days. A sample of the aqueous environment was also taken from each tank at days 2 and 7 for determination of environmental sodium, potassium and chloride concentrations as well as osmotic pressure.

Experiment 3: Survival in high salinity environments-the effect of pre-exposure to lower salinities

Fourteen *B. marinus* were transferred from pre-treatment conditions to 30% SW for 3 days and then 40% SW for 4 days (the experiment pre-exposure treatment). Three animals died in the 40% SW leaving 11 animals. Concurrently with the above, 10 *B. marinus* were maintained in a tapwater/terrestrial environment for 7 days (the control treatment). Three *B. marinus* were selected randomly from each of these treatments for determinations of plasma osmotic pressure and plasma sodium, potassium, chloride and urea concentrations (the initial data). Each tank was then filled with 50% SW. The survival of animals in each treatment was monitored daily and it was intended to select three animals randomly from each treatment after 2 days and then after 7 days of exposure, for determinations of plasma osmotic pressure and the concentrations of sodium, potassium, chloride and urea.

All statistical tests performed on the results of this and the previous experiments used $P = 0.05$ as the level of probability at which significance was recognized.

RESULTS

Experiment 1: The time-course of acclimation to salt water

No deaths occurred in the control, tap-water, or 20% SW treatments over the 7 day experimental period. In the 40% SW treatment two *B. marinus* died between the 12-hr sampling and day 2 of the experiment with the remaining animals surviving until day 7.

There was no significant difference in the hematocrits of *B. marinus* in any of the treatments or at any of the sampling times (2-factor ANOVA).

There was a significant regression of log wt on log length with the slope of the line being 2.28. Thus, the condition of each animal at each stage of the experiment was calculated as $(\text{wt}/\text{length}^{2.28}) \times 100$. No condition data were collected at the 2- and 7-day samplings for the 20% SW animals as there was a high loss of identification tags in this treatment. Accordingly an analysis of variance was not performed on the condition data. However, it appears that *B. marinus* placed in the 40% SW environment dehydrated initially and then regained more than the control level of hydration after 2 days while the animals placed in the tap-water environment became more hydrated initially but by day 2 had returned to the level of hydration of control animals (Fig. 1A).

Significant differences in plasma osmotic pressure and the concentrations of plasma sodium, potassium, chloride and urea for *B. marinus* among the treatments and exposure times were detected using 2- or 3-factor analyses of variance followed by StudentNewman-Keuls (SNK) multiple comparison tests. There was no significant difference in the plasma osmotic pressures of *B. marinus* in the four treatments initially and no difference in the control animals over the 7 days. While the plasma osmotic pressure of the tap-water animals decreased initially and then stabilized after 12 hr there was no significant difference in the plasma osmotic pressure of the 20% SW animals initially and after 7 days. The plasma osmotic pressure of *B. marinus* in the 40% SW treatment increased over the first two days then levelled off. The changes in plasma sodium and chloride followed the same pattern as plasma osmotic pressure (see Figs lb-d).

There was a difference in the concentration of plasma potassium of animals in the four treatments independent of time but the SNK comparison could not detect the source. Plasma potassium also increased between days 2 and 7 of the experiment, independent of treatment (see Fig. le).

There was no difference in the concentration of urea in the plasma of *B. marinus* in the four treatments initially and no significant change over the 7 days for the control, tapwater and 20% SW animals. The plasma urea concentration of the toads in the 40% SW increased until day 2 of the experiment and did not increase significantly after that (see Fig. 1f).

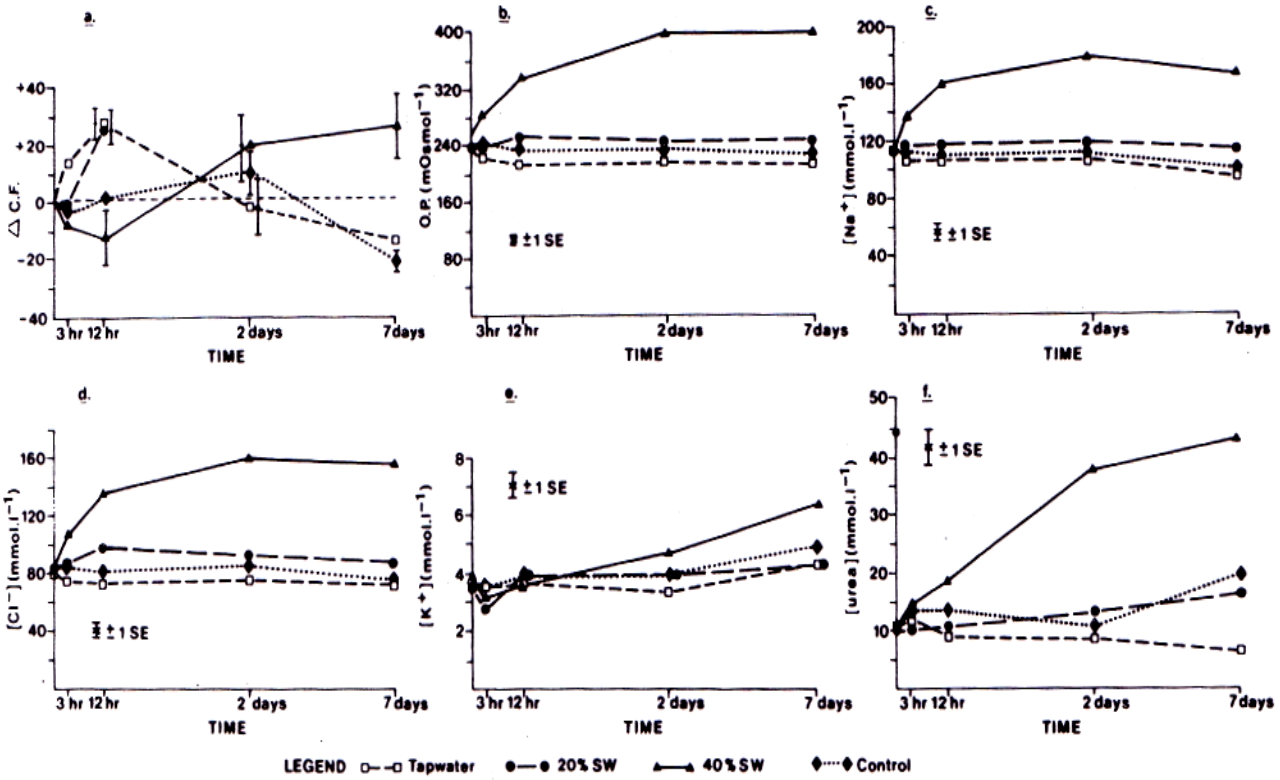

Fig. 1. Mean change in condition (a), plasma osmotic pressure (b), and plasma sodium (c), chloride (d), potassium (e) and urea concentrations for *B. marinus* exposed to tap-water, 20, 40% SW and control environments for 0, 3, 12 hr, 2 and 7 days. Experiment 1 data.

Experiment 2: Late stages of the acclimation process

The survivorship of *B. marinus* in each treatment is plotted against time in Fig. 2. After 2 days, *B. marinus* in the 50% SW treatment clearly experienced a much higher mortality than animals in the other three treatments. The 50% SW treatment terminated at this point as the two surviving animals were sampled. After 4 days it is also clear that the 40% SW animals experienced a much higher mortality than the 20 and 30% SW animals.

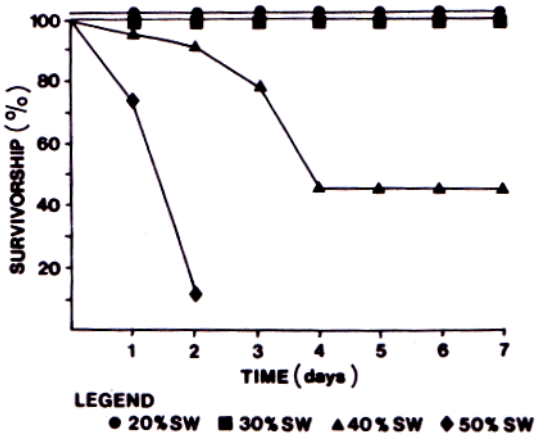

Fig. *2.* Survivorship of *B. marinus* in *20, 30, 40* and 50% SW environments. Experiment *2* data.

The relationship between weight and length for this batch of toads was calculated prior to the experiment as: wt (g) a length $(cm)^3$. There was no significant difference in the condition of *B. marinus* for any salinity (20, 30, 40% SW) or exposure time (initially, 2 days, 7 days) (2-factor ANOVA). Thus it appears that *B. marinus* in 20, 30 and 40% SW were in the same state of hydration after 2 and 7 days of exposure as they were initially.

There were no significant differences between the hematocrits of *B. marinus* in 20, 30 or 40% SW or either exposure time (2-factor ANOVA).

Significant differences in plasma osmotic pressure and the concentrations of plasma sodium, potassium, chloride and urea for *B. marinus* in each treatment at 2 and 7 days exposure were detected using the same tests as described for Experiment 1. *B. marinus* in the 20% SW treatment experienced no significant change in the plasma osmotic pressure or in the concentrations of sodium, chloride or urea between days 2 and 7 of the experiment. There was a significant decrease in the concentration of potassium of 1.3 ± 0.2 mOsmol l^{-1}, However, this represents less than 0.5% of the total osmotic pressure. In both the 30 and 40% SW treatments there was no significant change in the concentrations of plasma sodium, potassium or chloride but a significant increase in the concentration of urea and consequently a corresponding increase in plasma osmotic pressure (see Table 1).

For *B. marinus* in the 20% SW treatment the urine osmotic pressure was much less than that of the plasma while for animals in 30% SW the urine was hypo-osmotic to the plasma after 2 days but isosmotic with the plasma after 7 days. For *B. marinus* in 40% SW after 2 and 7 days and in 50% SW after 2 days the urine was isosmotic with the plasma (see Tables 1 and 2). Sodium, potassium, chloride, urea and ammonia accounted for a minimum of about 87% of the total urine osmotic pressure for *B marinus* in the treatments after 7 days.

Combining data from Experiments 1 and 2 it is apparent that sodium, potassium, chloride and urea account for a minimum of around 85% of the total plasma osmotic pressure of animals in tap-water, 20, 30 and 40% SW environments (see Fig. 3). Note that for animals in 40% SW the concentration of plasma sodium was 1.9 ± 0.1 times greater than that of the tap-water animals. The plasma chloride concentration of the 40% SW animals was 2.3 ± 0.1 times greater and the urea concentration was 10.5 ± 3.5 times greater than that of the tap-water animals. Overall, the plasma osmotic pressure of the 40% SW animals was 2.0 ± 0.1 times that of the tap-water animals.

Table 1. Mean (±1 SE) plasma osmotic pressure and concentrations of plasma sodium, potassium, chloride and urea for *B. marinus* after 2 and 7 days exposure to environments of 20, 30, 40 and 50% SW. * Indicates a significant difference between adjacent means (50% SW data not included). Experiment 2 data.

	O.P. (mOsmol.l^{-1})		Na^+ (mmol.l^{-1})		K^+ (mmol.l^{-1})		C1 (mmol.l^{-1})		Urea (mmol.l^{-1})	
Time (days)	2	7	2	7	2	7	2	7	2	7
20% SW	242	245	123	120	4.1	2.8	104	97	22	15
n=5	±1	±2	±3	±2	±.2 *	±0	±4	±2	±3	±1
	*	*	*	*		*	*	*		*
30% SW	310	325	149	144	4.9	4.3	126	121	23	42
n=5	±1	±3	±3	±3	±.3	±3	±4	±2	±4	±5
	*	*	*	*		*	*	*		*
40% SW	386	425	177	178	5.0	5.7	161	166	29	61
n=5	±2	±2	±4	±2	±.4	±4	±4	±4	* ±2	±4
50% SW	477	-	205	-	7.1	-	187	-	48	-
n=2	±5		±12		±.8		±7		±4	

Table 2. Mean (± 1 SE) urine osmotic pressure and concentrations of urine sodium, potassium, chloride, urea and ammonia for *B. marinus* after 2 and 7 days exposure to environments of 20, 30, 40 and 50% SW. * Indicates a significant difference between adjacent means (50% SW data not included). Experiment 2 data

	O.P. ($mOsmol.l^{-1}$)		Na^+ ($mmol.l^{-1}$)		K^+ ($mmol.l^{-1}$)		Cl ($mmol.l^{-1}$)		Urea ($mmol.l^{-1}$)		Ammonia ($mmol.l^{-1}$)	
Time (days)	2	7	2	7	2	7	2	7	2	7	2	7
20% SW	124	147	40	54	1.6	7	37	49	39	22	6	2
n=5	±19	±6	±10	±6	±.2	±.2	±11	±7	±9	±6	±3	±0
	*	*	*	*		*	*	*		*		*
30% SW	300	319	123	105	8.7	4.9	126	113	26	40	6	16
n=5	±3	±5	±3	±19	±2	±2	±4	±19	±3	±5	±2	±6
	*	*										*
40% SW	346	433	123	130	4.2	14.3	131	169	19	45	8	46
n=5	±15	±3	±11	±12	±1.5	±3	±8	±11	* ±3	±8	±2	±9
50% SW	462	-	154	-	3.9	-	156	-	38	-	2	-
n=2	±8		±22		±.1		±15		±8		±0	

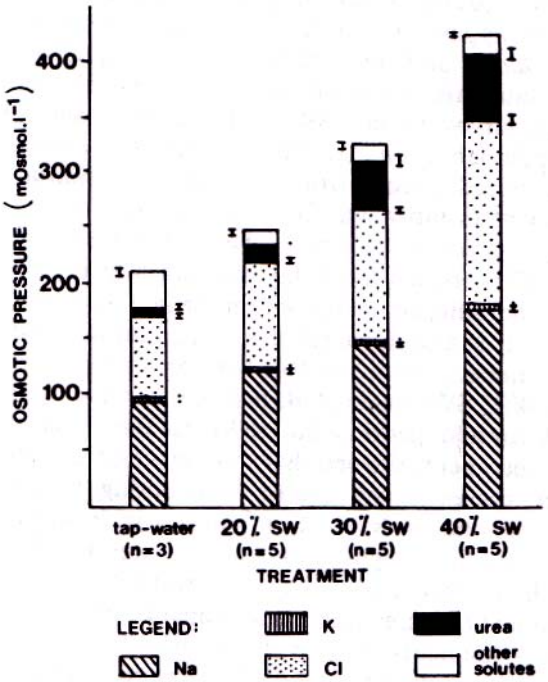

Fig. 3. Contribution of sodium, potassium, chloride and urea to the total plasma osmotic pressure of B. marinus exposed to tap-water, 20, 30 and 40% SW environments for 7 days. ± 1 SE is indicated for total osmotic pressure and for each solute (tap-water data from Experiment 1; 20, 30 and 40% SW data from Experiment 2).

Plasma osmotic pressure of *B. marinus* is plotted against environment osmotic pressure for both 2 and 7-day exposure times in Fig. 4a. Significant differences between plasma and environmental osmotic pressure were detected using Student's t-tests. Those animals placed in tap-water and 20% SW were initially hyper-osmotic to the environment and remained so after 2 and 7 days exposure. *B. marinus*

placed in 30% SW were clearly hypoosmotic to the environment initially. Then, after 2 days, the toads were isosmotic before becoming hyperosmotic by 7 days. The animals placed in 40% SW were initially hypoosmotic and were still hypoosmotic after 2 days. However, by day 7, the osmotic pressure of the plasma had risen to a level significantly above that of the environment. The plasma osmotic pressure of the animals in 50% SW after 2 days appears to be below that of the environment (no t-test performed as N = 2). Note that after 7 days all surviving animals in all salinities were hyperosmotic to the environment.

The concentration of plasma sodium exceeded the environment sodium concentration for animals in environments between 0 and 30% SW while the reverse applied for 40% SW and probably 50% SW (no t-test performed on 50% SW data as N = 2). The plasma chloride concentration of *B. marinus* became less than that of the environment at an environmental concentration slightly less than 20% SW. Concentrations of plasma sodium, potassium and chloride are plotted against the environmental concentration of these ions in Figs 4b-d.

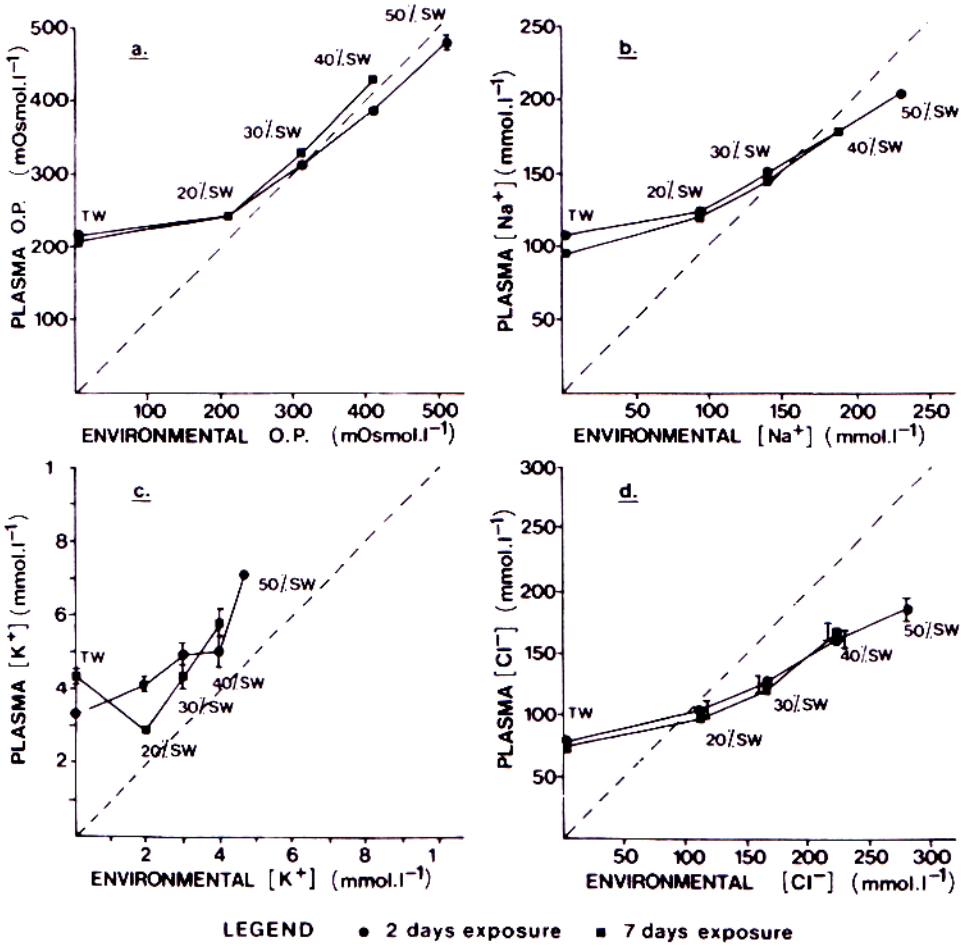

Fig. 4. Mean (± 1 SE) plasma versus environmental osmotic pressure (a) and sodium (b), potassium (c) and chloride (d) concentrations after 2 and 7 days exposure (tap-water data from Experiment 1; 20, 30, 40 and 50% SW data from Experiment 2). Dashed line is line of equality.

Experiment 3: Survival in high salinity environments-the effect of pre-exposure to lower salinities

Survival time of *B. marinus* increased in 50% SW as a result of pre-exposure to 30% then 40% SW in contrast to the standard pre-treatment conditions (see Fig. 5). After 2 days of exposure to 50% SW there was a significantly better survival of animals in the pre-exposure treatment than in the control treatment (x^2 test). At this stage, only one out of eight experimental animals had died while all seven control animals had died. After 7 days exposure, the remaining experimental animals had also died.

Table 3 summarizes the plasma osmotic pressure and plasma solute concentration data obtained in this experiment. Note that, initially, concentrations of plasma sodium, potassium, chloride and urea as well as plasma osmotic pressure were significantly higher in the pre-exposure animals than in the controls. The plasma osmotic pressure increased significantly over the first 2 days of exposure to 50% SW for the pre-exposure

animals. This was due to the significant increase in plasma sodium and chloride. The concentrations of potassium and urea did not increase. It appears that 50 mmol l^{-1} was the highest plasma urea concentration obtained by the pre-exposure animals.

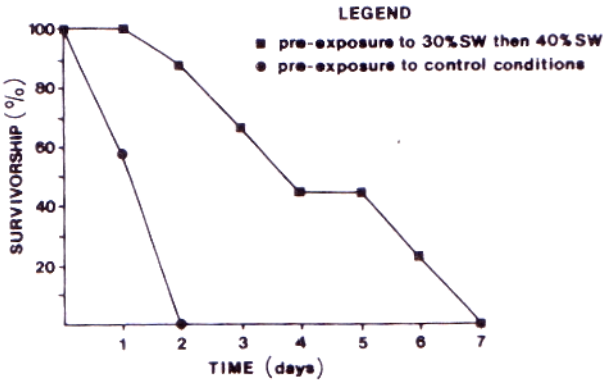

Fig. 5. Survivorship of *B. marinus* in 50% SW after pre-exposure to 30 then 40% SW and pre-exposure to control conditions. Experimental 3 data.

Table 3. Mean (± 1 SE) plasma osmotic pressure and plasma solute concentrations of *B. marinus* pre-exposed to 30 then 40% SW and *B. marinus* pre-exposed to control conditions. * Indicates a significant difference between the adjacent means. Experiment 3 data

	Controls			Pre-exposure				
	Initially n=10			Initially n=11			2 days n=7	
O.P. mOsmol.l^{-1}	284	±4	*	418	±2	*	472	±2
Na^{+} mmol.l^{-1}	114	±3	*	171	±2	*	202	±7
K^{+} mmol.l^{-1}	3.8	±3.0	*	6.1	±.2		6.8	3.4
$C1^{-}$ mmol.l^{-1}	82	±2	*	151	t3	*	179	±5
UREA mmol.l^{-1}	12	tl	*	50	±2		46	±5

DISCUSSION

B. marinus can tolerate salt-water environments in the 0-40% SW range and thus can be considered as one of the more euryhaline amphibians. However B. *marinus is* apparently inferior to *Rana cancrivora, Xenopus laevis* and *Bufo viridis* in the ability to tolerate salt-water environments as *B. marinus is* unable to survive in 50% SW for longer than about 6 days under laboratory conditions. When *B. marinus* was transferred directly from a tap-water/terrestrial environment to tap-water, 20 or 30% SW there was no mortality. Just under half of the toads transferred to 40% SW survived the 7-day experimental period with the mortality occurring within the first 4 days of exposure. It is also apparent that a gradual increase in environmental salinity to 50% SW (pre-exposure to 30 then 40% SW) enabled *B. marinus* to survive in 50% SW for a longer time but no longer than 7 days.

The experiments showed that sodium, chloride and urea are the principal solutes contributing to the plasma osmotic pressure of *B. marinus* in environmental salinities ranging from fresh water to 50% SW. When transferred from a tap-water/terrestrial environment (pre-treatment conditions) to these saltwater environments the concentrations of these solutes as well as the total osmotic pressure changed.

For animals transferred to tap-water and 20% SW environments (which are hypoosmotic to *B. marinus's* plasma before acclimation) all significant changes to plasma sodium, chloride and urea concentrations, as well as osmotic pressure, occurred within the first 2 days of exposure. When *B. marinus* was placed in 30 and 40% SW environments (which are hyperosmotic to *B. marinus* plasma before acclimation) the increases in plasma sodium and chloride concentrations occurred in the first 2 days of exposure while it is apparent from the second experiment that plasma urea and therefore also osmotic pressure continued to rise after this time.

In contrast to sodium and chloride, the rate of build up of plasma urea appears to be fairly constant over the first 2 days of exposure (see Figs lc, d and f). The urea concentration probably stops increasing between 2 and 7 days exposure as it appears from Experiment 3 that 50 mmol l^{-1} is the maximum achievable plasma urea concentration for *B. marinus* and this level was achieved within 7 days by animals in the 40% SW treatment in Experiment 2. The peak urea concentration obtained by animals in Experiment 3 was 50 ± 2 mmol l -' after exposure to 30% SW then 40% SW and this was not increased when the animals then experienced 50% SW. Thus it appears that this is the plateau level.

It is clear that plasma potassium is a minor contributor to plasma osmotic pressure and it is unclear how the concentration of this ion changed with time for animals placed in each environmental salinity.

The results of Experiments 1 and 2 support the hypothesis that *B. marinus* became hydrated when placed in tap-water but by 2 days had returned to the control state of hydration. Correspondingly, it also appears that *B. marinus* placed in 40% SW dehydrated initially then regained the control level of hydration by day 2. These results appear to be similar to those obtained for *Bufo viridis* by Gordon (1962) and dissimilar to those obtained for *Rana cancrivora* by Gordon et al. (1961).

Qualitative evidence from the first experiment in which there was difficulty in obtaining urine from *B. marinus* in 20 and 40% SW environments, is consistent with the idea that the rate of urine production is reduced considerably when the toads are placed in saline environments. Henderson et al. (1972) studied this aspect of *B. marinus'* physiology and found that this antidiuresis was due to a reduction in the glomerular filtration rate and an increase in tubular resorption.

No adult amphibian is known to survive in high salinities by hypo-regulating. All *B. marinus* subjected to 30% SW became hyperosmotic to their environment and there was no mortality within the 7 day experimental period. In contrast, approximately 90% of the toads placed in 50% SW died within 2 days, the 2 remaining animals having plasma osmotic pressures of 470 and 485 mOsmol l^{-1}. *B. marinus* placed in 40% SW became hyperosmotic to the environment some time between days 2 and 7 of the second experiment. Hyperosmoticity to the environment was probably achieved after about 4 days as the mortality of *B. marinus* in this treatment occurred within the first 4 days of exposure.

It is apparent from Experiment 2 that the urine of *B. marinus* is hypoosmotic to the plasma at low environmental salinities and that, as salinity is increased to about 30% SW, the osmotic pressure of the urine approaches that of the plasma. These results are entirely consistent with the findings of Henderson et al. (1972) and Garland and Henderson (1975) who studied the renal function of *B. marinus.* As is the response of other amphibians, *B. marinus* produces copious quantities of very dilute urine when in freshwater. When in salinities above about 30% SW *B. marinus* produces less urine, which is isosmotic with the plasma.

It appears from Fig. 4b that there is less need for the inward active transport of sodium ions across the integument of *B. marinus* when salinity is elevated. For example, in 40% SW the electrochemical gradient between *B. marinus* and the external environment favours a diffusion of sodium and potassium from the environment into the animal. Although a sodium pump is necessary for the survival of amphibians in fresh water a decrease in its activity when animals are in highly saline environments would place less burden on the kidneys and bladder to excrete excess electrolytes. A decrease in sodium uptake has been shown in several species when acclimated to high salinities (Ferreira and Jesus, 1973; Katz, 1975).

REFERENCES

Ackrill P., Hornby R. and Thomas *S. (1969)* Responses *of Rana temporaria* and *Rana esculenta* to prolonged exposure to a saline environment. *Comp. Biochem. Physiol. 38, 1317-1329.*

Balinsky J. B. *(1981)* Adaptation *of* nitrogen metabolism to hyperosmotic environment in amphibia. *J. exp. Zool. 215, 335-350.*

Covacevich J. and Archer *M. (1975)* The distribution *of* the cane toad *Bufo marinus* in Australia and its effects on indigenous vertebrates. *Mem. Qd. Mus. 17, 305-310.*

Ferreira H. G. and Jesus C. H. *(1973)* Salt adaptation in *Bufo bufo. J. Physiol. 228, 583-600.*

Garland H. O. and Henderson *I. W. (1975)* Influence *of* environmental salinity on renal and adrenocortical function in the toad, *Bufo marinus. Gen. comp. Endocr. 27, 136-143.*

Gordon M. S., Schmidt-Nielsen K. and Kelly H. M. *(1961)* Osmotic regulation in the crab-eating frog *(Rana cancrivora). J. exp. Biol. 38, 659-678.*

Henderson I. W., Edwards B. R., Garland H. O. and Chester-Jones *I. (1972)* Renal function in two toads, *Xenopus laevis* and *Bufo marinus. Gen. comp. Endocr. Suppl. 3, 350-359.*

Katz *U. (1973)* Studies on the adaptation *of* the toad *Bufo viridis* to high salinities: oxygen consumption, plasma concentrations and water content *of* the tissues. *J. exp. Biol. 58, 785-796.*

Katz *U. (1975)* Salt-induced changes in sodium transport across the skin *of* the euryhaline toad, *Bufo viridis. J. Physiol. 247, 537-550.*

Romspert A. *P. (1976)* Osmoregulation *of* the African clawed frog, *Xenopus laevis,* in hypersaline media. *Comp. Biochem. Physiol. 54A, 207-210.*

van Beurden E. K. and Grigg G. *C. (1980)* An isolated and expanding population *of* the introduced toad *Bufo marinus* in New South Wales. *Aust. Wild Life Res. 7,305-310.*