

COMPARISON OF DEHYDRATION AND HYDRATION OF
TWO GENERA OF FROGS (*HELEIOPORUS* AND
NEOBATRACHUS) THAT LIVE IN AREAS OF
VARYING ARIDITY

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(Received 8 February 1958)

INTRODUCTION

Water conservation presents special problems to terrestrial Amphibia because: (a) evaporation from the naked skin is largely uncontrolled, and (b) no frog has been described which can secrete hypertonic urine.

Since frogs of different kinds do inhabit a variety of ecological situations with irregular rainfall and high rates of evaporation, the mechanism of survival has been the focus of attention and speculation.

An analysis of this problem demands answers to the following physiological questions:

- (i) Rate of dehydration in dry environments?
- (ii) Degree and duration of dehydration consistent with survival?
- (iii) Rate of hydration when desiccated animals are exposed to moisture?

If the answers to (i), (ii) and (iii) indicate that different species have different capabilities, the question arises as to whether the physiological responses correlate with habitat. If this is not so one must go beyond physiology and see whether natural history observations indicate the existence of behavioural responses which allow the animal to avoid the stress.

With regard to the three physiological points enumerated above there is scant evidence.

(i) Thorson (1955), working on a variety of species from different environments, showed that water loss was always the same per unit area of skin.

(ii) Thorson & Svihla (1943), working with several species, showed that there was correlation between ability to withstand desiccation and degree of aridity of the habitat.

(iii) Ewer (1952) showed that water uptake after desiccation was greater in terrestrial species of *Bufo* than in the aquatic *Xenopus*. Thorson (1955) compared water uptake after desiccation in the ecologically dissimilar species *Rana pipiens*, *R. clamatans* and *Scaphiopus hammondi* but could find no correlation between rate of water uptake and aridity of the habitat.

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If meanwhile we accept, admittedly on meagre evidence, that the only physiological differences between different frog species are (a) ability to withstand a high degree of desiccation, and (b) rate of water uptake when presented with water, then the question of mechanism arises. There is no data with regard to (a), but there is abundant evidence (Jørgensen, 1950; Ewer, 1952) that the posterior pituitary influences both uptake of water and urinary flow while Ewer (1952) has shown that forms which hydrate quickly exhibit a greater response to injection of posterior pituitary extracts than others which hydrate slowly.

The frogs of Western Australia, because of the number of species and the variety of habitats, offer special opportunities for a more complete analysis of mechanism in relation to environment than has hitherto been possible. In the present paper two endemic Australian genera *Neobatrachus* and *Heleioporus* are considered, *Neobatrachus* with four species and *Heleioporus* with five. It has thus been possible to make interspecies as well as intergeneric comparisons in relation to the considerations outlined above.

METHODS

Collection and preliminary treatment. Jørgensen, (1950) has pointed out the difference in water uptake that may occur at different times of the year. All the species used in these experiments were collected between February and May, i.e. summer and early autumn, when rain and temperature conditions were favourable for breeding, and so they were all presumed to be in a similar physiological condition. Weights of the various species were measured at the beginning of the experiments and are set out in Table 1.

Table 1. *Size of all the species of Neobatrachus and Heleioporus used in the experiments*

Species	Mean body weight (B.W.) \pm standard deviation (g.)	Surface area (cm. ² /g./B.W.)
<i>N. pelobatoides</i> (19)	6.0 \pm 1.22	3.30
<i>N. centralis</i> (11)	12.1 \pm 4.05	2.61
<i>N. sutor</i> (6)	6.7 \pm 1.33	3.18
<i>N. wilsmorei</i> (6)	27.1 \pm 3.69	2.00
<i>H. psamophilus</i> (13)	10.2 \pm 2.05	2.78
<i>H. inornatus</i> (14)	18.9 \pm 6.51	2.26
<i>H. eyrei</i> (17)	19.7 \pm 6.26	2.22
<i>H. australiacus</i> (6)	34.8 \pm 12.50	1.88
<i>H. albopunctatus</i> (13)	49.5 \pm 20.92	1.63

In parentheses: number of animals.

The animals were predominantly males, though a few non-gravid females were included in the experiments. There was no evidence of any statistically significant difference ($P > 0.05$) in water-uptake response between the sexes.

The frogs, kept in beakers containing tap water in a constant temperature room at $21^{\circ} \pm 1^{\circ}$ C., were not fed, and were allowed 3-4 days in the beakers containing

tap water before experiments were commenced. This minimized any chance of sudden weight changes due to defaecation.

The surface area of the frogs was calculated using the formula of Rey (1937).

Ability to withstand dehydration. After the urinary bladder of each animal had been emptied by catheterization, dehydration was carried out in wire cages at $21^{\circ} \pm 1^{\circ}$ C. and 55% relative humidity. As the survival limits of water loss were approached the frogs were weighed at hourly intervals. The last measurement before death was taken as the limit of water loss that could be withstood. Owing to shortage of animals no systematic attempts were made to see if recovery from this penultimate degree of dehydration was possible. A few experiments on each species suggested, however, that recovery was frequently possible.

Dehydration and water uptake. Animals were dehydrated as described above. When about 25% of the body weight had been lost by evaporation, they were placed in tap water and weight increases were measured at half hourly intervals. A weight loss of 25% was found to be always consistent with survival. Water uptake in the first hour after catheterization was found to give a representative picture, and was used as the parameter for comparison between species.

Water uptake with posterior pituitary extracts. After the bladder had been emptied the frogs were weighed to 0.1 g. A solution of Pitocin (Park, Davis and Co.) made up to appropriate strength with distilled water, was injected into a dorsal lymph sac. This had been previously found to be the most active posterior pituitary fraction affecting the two genera under observation. The injected volume amounted to 1% of the body weight at a dosage of 1 u./100 g. The animals were placed in tap water, catheterized 3 hr. later and weighed, increase in weight being taken to indicate water retained. Obviously, what was being measured was not the absolute water uptake through the skin but that portion that was retained due to an anti-diuresis. This would appear to be the important part of the whole response that would effect the survival of an animal with an intermittent water supply in nature.

RESULTS

No data are available on rate of water loss, the work being confined to the following:

Degree of dehydration consistent with survival

All species died after losing 40–45% of their body weight. An analysis of variance failed to show up any differences in the ability to withstand desiccation (Table 2) between any of the species of the two genera tested.

Water uptake after dehydration

The results are given in Table 3. An analysis of variance showed that there were no significant differences between the different species of *Heleioporus*. However, a similar analysis of the results from *Neobatrachus* indicated there were highly significant differences between the species. *N. centralis* took up water more rapidly than *N. pelobatoides*, while *N. sutor* and *N. wilsmorei* each took up water more

Table 2. *Ability of frogs of the genera Neobatrachus and Heleioporus to withstand desiccation*

Species	% loss of body weight at death	Species	% loss of body weight at death
<i>H. psamophilus</i>	41.3 (13)	<i>N. pelobatoides</i>	38.8 (17)
<i>H. inornatus</i>	40.1 (12)	<i>N. centralis</i>	41.7 (5)
<i>H. eyrei</i>	40.7 (18)	<i>N. sutor</i>	44.1 (5)
<i>H. australiacus</i>	38.9 (6)	<i>N. wilsmorei</i>	43.9 (6)

Error variance from the analysis of variance 1201.2747 (g. 100 g.)² on 83 D.F., variance ratio 1.84 on 8 and 83 D.F. which is not significant.

Results are the means of the numbers in parentheses.

Table 3. *Water uptake in species of the genera Neobatrachus and Heleioporus after dehydration*

	Water uptake (mg./cm. ² /hr.)		Water uptake (mg./cm. ² /hr.)	Significance of differences
<i>H. psamophilus</i>	44.0 (12)	(a) <i>N. pelobatoides</i>	33.3 (20)	(a)-(b) $P < 0.01$
<i>H. inornatus</i>	55.0 (13)	(b) <i>N. centralis</i>	55.7 (11)	(b)-(c) $P < 0.01$
<i>H. eyrei</i>	52.5 (18)	(c) <i>N. sutor</i>	84.8 (6)	(c)-(d) N.S.
<i>H. australiacus</i>	60.0 (6)	(d) <i>N. wilsmorei</i>	99.4 (6)	—
<i>H. albopunctatus</i>	57.7 (13)			

Error variance from analysis of variance: (i) *Heleioporus* 286.3448 (mg./cm.²/hr.)² on 57 D.F. variance ratio 1.40 on 57 and 4 D.F. which is not significant; (ii) *Neobatrachus* 393.4775 (mg./cm.²/hr.)² on 39 D.F. variance ratio 22.58 on 39 and 3 D.F. $P < 0.001$.

Results are the means of the numbers in parentheses.

rapidly than *N. centralis*. The difference in rate of water uptake between *N. sutor* and *N. wilsmorei* was not found to be statistically significant. The water uptake in the four species of *Neobatrachus* shows close correlation with the increasing aridity of the environment measured not by the annual rainfall but by the number of days in a year in which rain falls. Thus the species occupying regions with fewer days on which rain falls have the greatest rate of water uptake.

Small frogs have a greater surface area relative to body weight than large frogs (Table 1), and if water uptake per unit surface area is the same, small frogs would be expected to gain water more rapidly per unit body weight than large frogs. The possibility cannot be ruled out that this may place the larger frogs at a disadvantage and selective factors may be expected to produce a greater water uptake per unit surface area in the larger animals. It is therefore surprising that *N. sutor* and *N. wilsmorei*, which differ markedly in size and occupy the same geographical territory, are the two species that take up water most rapidly. Furthermore, *N. pelobatoides* and *N. sutor*, which are similar in size, take up water at markedly different rates. From Table 1 it can be seen that *N. wilsmorei* has only 61% the surface area per unit body weight of *N. pelobatoides*. *N. wilsmorei* takes up water at a rate of 19.75 ± 0.62 (S.E.) g./100 g./hr., while *N. pelobatoides* takes it up at

10.80 ± 0.507 (s.e.) g./100 g./hr. The difference is highly significant ($t=4.237$, $P<0.001$). *N. wilsmorei* thus takes up water faster than *N. pelobatoides* per unit body weight as well as per unit surface.

Effects of posterior pituitary extracts

In view of the demonstration by Brunn (1921) of an increase in water uptake by frogs injected with posterior pituitary extracts and subsequent repeated confirmation of these observations (see Heller, 1945, 1950; Sawyer, 1956) it was of interest to see if differences observed in the rate of water uptake after dehydration were also seen after the injection of posterior pituitary extracts. The results are given in Table 4. In the genus *Heleioporus* no statistically significant differences in response were observed to injection of Pitocin. In the genus *Neobatrachus* differences similar to those observed after dehydration were seen.

Table 4. Water uptake in species of the genera *Neobatrachus* and *Heleioporus* injected with 1 u. Pitocin/100 g. body weight

	Water uptake (mg./cm. ² /3 hr.)		Water uptake (mg./cm. ² /3 hr.)	Significance of differences
<i>H. psamophilus</i>	51.4 (12)	(a) <i>N. pelobatoides</i>	31.7 (20)	(a)-(b) $P<0.01$
<i>H. inornatus</i>	40.1 (14)	(b) <i>N. centralis</i>	52.9 (11)	(b)-(c) N.S.
<i>H. eyrei</i>	44.1 (17)	(c) <i>N. sutor</i>	56.6 (6)	(c)-(d) $P<0.01$
<i>H. australiacus</i>	58.8 (6)	(d) <i>N. wilsmorei</i>	98.6 (6)	—
<i>H. albopunctatus</i>	46.2 (13)			

Error variance from analysis of variance (i) *Heleioporus* 195.3298 (mg./cm.²/3 hr.)² on 58 D.F. variance ratio 2.43 on 58 and 4 D.F. which is not significant. (ii) *Neobatrachus* 309.6640 (mg./cm.²/3 hr.)² variance ratio 22.48 on 38 and 3 D.F. $P<0.001$.

Results are the means of the numbers in parentheses.

Because of the above findings, that desert species rehydrate more rapidly than non-desert forms, it was decided to make a comparison of two populations of *N. pelobatoides* from geographical extremes of the species range. A sample of eleven *N. pelobatoides* from the eastern limit of the range was compared with the *N. pelobatoides* mentioned in Table 4 which were from the western extreme of the range. The water uptake of the eastern animals when injected with 1 u./100 g. Pitocin was 28.1 ± 3.01 mg./cm.²/3 hr. is very similar to the water uptake of the western animals (Table 4) injected with the same dose of Pitocin. A comparison of the animals' ability to take up water after dehydration unfortunately could not be made as these animals were particularly sensitive to captivity and died.

DISCUSSION

Fig. 1 shows the geographical distribution of the two genera in Western Australia. Although a large territory is common to both, the results given above show that the two genera in no way parallel each other except in an ability to withstand

desiccation. This apparent contradiction can be resolved by considering the ecology of the genera.

All species of the *Heleioporus* are burrowers and usually frequent sandy or friable soil. During summer, burrows reach the damp subsoil and some specimens of *H. albopunctatus* have been dug from burrows 33 in. deep. The animal buries itself but does not remove the soil from the burrow, which is thus effectively sealed. Of the *Heleioporus* species the range of *H. albopunctatus* extends farthest into the region of low and uncertain rainfall; this species aestivates, during summer, in a micro-environment very similar to that occupied by all other species of the genus.

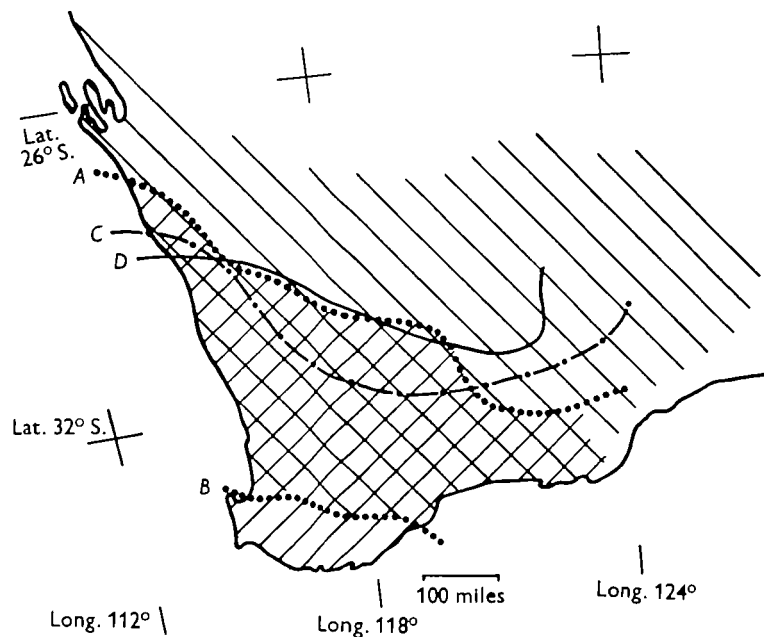


Fig. 1. Geographical distribution of (a) *Neobatrachus*, \\\; (b) *Heleioporus*, ///. A, 60 wet days a year; B, 120 wet days a year; C, south-western boundary of *N. sutor*; D, south-western boundary of *N. wilmorei*.

The genus *Neobatrachus* frequents clayey soils in which deep burrows are not possible. These burrows do not give the humid micro-environment provided by the burrows of *Heleioporus*. The water uptake in the various species of this genus closely follows the dryness of the habitat. Thus *Neobatrachus pelobatoides* is common between the isopleths of 120 wet days/year and 60 wet days/year (wet day defined as when 0.01 in. of rain falls in a day). In the drier part of the range *N. pelobatoides* is restricted to localized habitats adjacent to large rock outcrops, where additional run-off ameliorates the aridity. At the other extreme, *N. sutor* and *N. wilmorei* persist into regions where there are less than 40 wet days/year. The inland penetration of these two species is not known, but as *N. wilmorei* has the lowest surface area per unit body weight and the fastest water uptake per unit

surface area it might be expected that this species would persist in more arid areas than *N. sutor*.

Rate of water uptake would be under positive selective pressure if water supplies became available only sporadically and for limited periods. Dew may be present on cold mornings in inland areas occupied by *Neobatrachus*, but at these low temperatures the animals are inactive. The sporadic rains indicated by the number of wet days (see above), combined with the rapid disappearance of surface water, would seem to define the conditions under which rapid uptake of water would be an advantage.

Thorson (1955) found that *Scaphiopus hammondi*, from drier areas than any other species tested, actually took up water more slowly. As shown above with *Heleioporus*, rate of water uptake need not necessarily correlate with aridity of the environment if a behaviour pattern is present by which risk of serious desiccation is avoided.

The species tested in the present experiments showed no difference in ability to withstand desiccation. All fall about midway in the pattern described by Thorson & Svihla (1943) being inferior to *Scaphiopus*, but far more able to withstand water loss than the aquatic and semi-aquatic species of *Rana* used by Thorson & Svihla.

The response of water uptake after injection of Pitocin in the genus *Neobatrachus* was paralleled by the rate of water uptake after dehydration. This observation, together with the demonstration of active posterior pituitary principles in frogs and toads (see Jørgensen, 1950), seems to indicate that water uptake in dehydrated frogs is potentiated by release of endogenous posterior pituitary hormone. The objection to this view is the observation of Jørgensen, Wingstrand & Rosenkilde (1956) that neurohypophysectomized *Bufo bufo* take up water as rapidly after dehydration as intact animals. It is felt, however, that before these experiments are taken to invalidate this conclusion the experiments of Jørgensen *et al.* (1956) should be repeated on a genus such as *Neobatrachus* which, because of the species differences in rate of water uptake, offer comparative material more likely to show significant differences between intact and neurohypophysectomized animals if in fact the posterior pituitary is physiologically significant in this respect.

SUMMARY

1. Ability to take up water in response to dehydration and injection of posterior pituitary extract was measured in two genera of frogs, *Heleioporus* and *Neobatrachus*, that live in areas of varying aridity in south-west Australia.
2. Species of *Neobatrachus* from dry areas took up water more rapidly than those from less dry ones. No such correlation could be seen in the species of *Heleioporus*.
3. No differences could be seen in the ability of any of the animals to withstand desiccation.
4. These findings are discussed in relation to the animals' behaviour in its natural environment.

This work was carried out with the aid of research grants from the University of Western Australia. Mr N. Stenhouse of C.S.I.R.O. kindly performed a statistical analysis of the results.

REFERENCES

- BRUNN, F. (1921). Beitrag zur Kenntnis der Wirkung von Hypophysenextrakt auf den Wasserhaushalt des Frosches. *Z. ges. exp. med.* **25**, 170-5.
- EWER, R. F. (1952). The effects of posterior pituitary extracts on water balance in *Bufo carens* and *Xenopus laevis*, together with some general considerations of anuran water economy. *J. Exp. Biol.* **29**, 429-39.
- HELLER, H. (1945). The effect of neurohypophyseal extracts on the water balance of lower vertebrates. *Biol. Rev.* **20**, 147-57.
- HELLER, H. (1950). The comparative physiology of the neurohypophysis. *Experientia*, **6**, 368-76.
- JØRGENSEN, C. B. (1950). The amphibian water economy, with special regard to the effect of neurohypophyseal extracts. *Acta. physiol. scand.* **22**, suppl. 78.
- JØRGENSEN, C. B., WINGSTRAND, K. G. & ROSENKILDE, P. (1956). Neurohypophysis and water metabolism in the toad, *Bufo bufo* (L.). *Endocrinology*, **59**, 601-10.
- REY, P. (1937). Recherches expérimentales sur l'économie de l'eau chez les Batraciens. Dissertation, Paris.
- SAWYER, W. H. (1956). The hormonal control of water and salt-electrolyte metabolism with special reference to the amphibia. *Mem. Soc. Endocrinol.* **5**, 44-56.
- THORSON, T. B. (1955). The relationship of water economy to terrestriality in amphibians. *Ecology*, **36**, 100-16.
- THORSON, T. B. & SVIHLA, A. (1943). Correlation of the habitats of amphibians with their ability to survive the loss of body water. *Ecology*, **24**, 374-81.