ELSEVIER

Evaporative Water Loss and Oxygen Uptake in Two Casque-Headed Tree Frogs, *Aparasphenodon Brunoi* and *Corythomantis greeningi* (Anura, Hylidae)

Denis Vieira de Andrade and Augusto Shinya Abe

DEPARTAMENTO DE ZOOLOGIA, UNIVERSIDADE ESTADUAL PAULISTA, C.P. 199, 13506-900, RIO CLARO, SP, BRASIL

ABSTRACT. Evaporative water loss (EWL) and oxygen uptake ($\dot{V}o_2$) was measured in two species of tree frogs with cranial co-ossification, *Aparasphenodon brunoi* and *Corythomantis greeningi*. Both species use their head to seal the entrance of bromeliads, tree holes or rocky crevices used as shelters. EWL was significantly reduced in sheltered individuals of both species as compared with those exposed nude to desiccation. EWL per unit area through the head surface was significantly lower than the body skin for A. *brunoi* but not for C. *greeningi*. EWL per unit surface area through C. *greeningi* body skin was about 50% that of A. *brunoi*, indicating a less permeable skin in the former species. The relationship between cranial coossification and EWL is discussed. $\dot{V}o_2$ in A. *brunoi* was comparable with other anurans of similar size, whereas in C. *greeningi*, it was lower than predicted from body mass. Moreover, $\dot{V}o_2$ in C. *greeningi* showed less sensitivity to temperature increase than in A. *brunoi*. C. *greeningi* occurs in a drier environment than A. *brunoi*, and this appears to be reflected in their EWL and $\dot{V}o_2$ characteristics. COMP BIOCHEM PHYSIOL 118A;3:685–689, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. Anura, *Aparasphenodon*, *Corythomantis*, cranial co-ossification, evaporative water loss, Hylidae, oxygen consumption, phragmosis

INTRODUCTION

Amphibians were the first vertebrates to invade terrestrial environments and hence to face the problems of living out of water (3,16). Among these problems, one of major importance was, and still is, dehydration, because of the highly permeable integument of the group (24). Notwithstanding, many modern species of amphibians have an array of behavioural, morphological and physiological modifications that enable them to live in relatively dry regions (e.g., 12,14,18–20,28). The correlation between some traits and water conservation is not always clear, for example, in the case of some species of casque-headed tree frogs (Hylidae), which use their co-ossified head to seal the opening between leaf axis of bromeliads, tree holes or rocky crevices where they shelter (1,10,11,26). Such a habit, known as phragmotic, and the cranial co-ossification have been suggested to be a device to decrease water loss (26) and to provide protection against predators (11). Although these functions are not mutually exclusive, no experimental evidence exists supporting the predation hypothesis. Moreover, their use-

fulness for water loss reduction was only demonstrated in two species (17).

Here, we report on the relationship between evaporative water loss (EWL) and cranial co-ossification in *Aparasphenodon brunoi* and *Corythomantis greeningi*, two hylid species with co-ossified head and phragmotic habits. C. *greeningi* occur in thorn-shrub dry forests (caatingas) of northeastern Brazil, whereas A. *brunoi* are found in sand dunes or forest bromeliads within the Atlantic rain forest domain in southeastern Brazil. A. *brunoi* use mainly bromeliads as shelters, whereas C. *greeningi* hide in tree holes and rock crevices. Specifically, we assessed the total water economy achieved by the phragmotic mode of life of C. *greeningi* and A. *brunoi* and whether cranial co-ossification decreases the rate of EWL through the head surface. We also measured the rate of oxygen uptake of the animals, because gas exchange involves the exposure of a relatively large, permeable and moist surface, lung and/or skin, to the gas phase and therefore causes considerable water loss by evaporation [see (21)].

MATERIALS AND METHODS
Animals

EWL and oxygen uptake ($\dot{V}o_2$) were measured in six adult A. *brunoi* collected at Aracruz (19° 49′ 08″ S; 40° 16′ 43″ W), state of Espírito Santo, southeastern Brazil, and in six

C. greeningi from Angicos (5° 39' 43" S; 36° 36' 18" W), state of Rio Grande do Norte, northeastern Brazil. They were maintained in glass aquaria provided with bromeliads, bricks and forest litter. The substrate was kept moist and the animals had free access to water. Food (cockroaches) was provided *ad libitum*, except for 48 hr before the experiments. They were kept at the experimental temperature for 24 hr before experimentation.

Evaporative Water Loss

The rate of water loss was measured under two conditions. First, we measured the water loss of fully exposed animals in a circular Plexiglas container (65 cm of diameter) attached to a shaft (same diameter of the Plexiglas container). In the second experiment, we measured the water loss of tree frogs sheltering inside bromeliads, with only the co-ossified top of their head exposed. To avoid the formation of a humidity gradient over the animals, we used a fan to produce a wind current of 95 m/min. The animals remained exposed to this wind current for 1 hr in the first series of experiments and for 2 hr in the second. In both series of experiments, the animals were not restrained and we waited until they fell quiescent before experimentation. In the experimental room, temperature was kept at 30 ± 0.1°C and the relative humidity at 65 ± 5%.

We estimated EWL indirectly by determining changes in body mass [see (21)]. The tree frogs were weighed on a scale with a precision of 0.001 g before and after experimentation. If the animals voided or moved during experimentation, the measurement was discarded. The change in mass of the animals was assumed to represent the total EWL. We expressed EWL as a percentage of body mass lost in an hour, and in mg · cm^{-2} · hr^{-1} that represents the rate of EWL per unit area of skin.

Frog surface area was estimated following the Heatwole *et al.* (9) formula: SA = 4.8M$^{0.72}$, where SA is the exposed surface area in the water-conserving posture (cm^2) and M is the body mass in g. To determine the co-ossified head area exposed to desiccation (HA), we made a cast of the frog's exposed head surface with aluminium foil and used the mass/surface area relationship for the foil to estimate HA.

Respirometry

Oxygen uptake was measured at 20 and 30°C from 8:00 to 18:00 hr using an intermittently closed respirometer. The respirometry unit was described in detail elsewhere (29). Basically, it consists of a computer-operated system that was programmed to cyclically flush the respirometer with fresh air, followed by a closed phase when the O$_2$ uptake was measured. The open phase lasted 9 min followed by 1 min of adjustment and 10 min of the closed phase. O$_2$ uptake was calculated from the rate of oxygen depletion in the

closed phase. Oxygen concentration was monitored by an O$_2$ analyser (Servomex 570A). Water vapour was trapped using a tube with silica gel placed between the respirometer and the O$_2$ analyser. Temperature inside the respirometer was kept constant using a thermostatic bath (Radiometer Copenhagen) with a precision of ±0.1°C.

Data Handling and Analysis

Our respirometry unit provided oxygen consumption measurements every 20 min that were used to calculate the mean $\dot{V}_{O_2}$ value at rest (SMR, standard metabolic rate). Whenever activity was detected during the experiments, indicated by a sudden increase in the oxygen uptake values, the reading was discarded (27).

To calculate the rate of EWL through the head, we assumed that water loss from tree frogs sheltered inside bromeliads occurred only through their head surface. These EWL values were divided by HA and hourly corrected to obtain the rate of EWL, in mg · cm^{-2} · hr^{-1}, through the tree frogs head surface. The rate of EWL through the body skin (in mg · cm^{-2} · hr^{-1}) was obtained by subtracting the water amount calculated to be lost by head from the total EWL measured and dividing the resulting value by body area (SA − HA). All comparisons between species were made by one-way ANOVA. Comparisons between treatments for the same individual were made by a paired *t*-test. Whenever necessary to comply with assumptions of normality and homoscedasticity, the data were log transformed. Unless otherwise noted, the data are presented as means ± SD. All statistical tests followed Sokal and Rolf's (22) procedures. Differences were considered significant at $P \leq 0.05$.

RESULTS

A. brunoi and *C. greeningi* did not differ in their behaviour when exposed to desiccation, and both species became quiescent a few minutes after the experimentation began. Animals with bodies exposed to desiccation adopted the water-conserving posture described by Heatwole (8) and Heatwole *et al.* (9), with the limbs held close to the body and the ventral body surface pressed against the substrate. Individuals subjected to desiccation inside bromeliads readily accommodated their body into the leaf tube and adjusted the top of their heads to totally block the opening of the plant.

The mean body mass of the *A. brunoi* (mean mass 19.13 ± 3.74 g; range 15.35–25.2 g) was not significantly different from that of the *C. greeningi* (mean mass 17.05 ± 4.85 g; range 11.6–25.6 g) ($F_{1,10} = 0.69$; $P = 0.42$). Similarly, no difference was found between the surface area of *A. brunoi* (SA 38.03 ± 5.06 cm^2; range 33.35–45.56 cm^2) and *C. greeningi* (SA 35.24 ± 6.8 cm^2; range 26.63–46.43 cm^2) in the water-conserving posture ($F_{1,10} = 0.8$; $P = 0.39$). However, head surface area was significantly larger in *A. brunoi* (HA 8.15 ± 0.98 cm^2; range 7.05–9.37 cm^2) than for *C.*

TABLE 1. Descriptive statistics of oxygen uptake ($\dot{V}O_2$, ml $O_2 \cdot h^{-1} \cdot kg^{-1}$) at 20 and 30°C and evaporative water loss (EWL) in *Aparasphenodon brunoi* and *Corythomantis greeningi*

	$\dot{V}O_2$		EWL			
	20°C	30°C	% EWL$_t$	% EWL$_{br}$	EWL$_h$	EWL$_{bs}$
A. *brunoi*	60.28 ± 14.83	104.46 ± 21.47	12.62 ± 3.63	1.81 ± 0.94	38.5 ± 19.67	73.02 ± 19.26
	(36.81–74.47)	(89.55–147.5)	(8.32–17.45)	(0.8–3.4)	(18.07–70.56)	(52.17–99.1)
C. *greeningi*	47.88 ± 7.6	74.15 ± 14.31	9.40 ± 4.66	0.97 ± 0.51	29.55 ± 14.56	47.66 ± 20.14
	(34.61–55.53)	(57.56–94.45)	(4.73–17.68)	(0.24–1.52)	(7.63–50.63)	(25.32–82.66)

EWL is presented as percentage of body mass per hour, for tree frogs with bodies exposed (%EWL$_t$) and sheltered inside bromeliads (%EWL$_{br}$). EWL$_h$ and EWL$_{bs}$ indicate the rate of EWL in area units (mg · cm^{-2} · hr^{-1}) through head and body surface, respectively. Values are mean ± SD, with range in parentheses. $n = 6$ in all cases.

greeningi (HA 4.97 ± 1.06 cm^2; range 3.84–6.97 cm^2) ($F_{1,10}$ = 29.4; P = 0.0003). The head area represented approximately 21.18% and 14.09% of A. *brunoi* and C. *greeningi* SA, respectively.

The mean values for oxygen uptake and EWL are given in Table 1. A. *brunoi* (t = 7.6; P = 0.0006) and C. *greeningi* (t = 4.43; P = 0.006) lost significantly less water (% of body mass) sheltered inside bromeliads than with bodies exposed to desiccation. Total EWL (% of body mass) in C. *greeningi* sheltered inside bromeliads ($F_{1,10}$ = 3.69) or exposed ($F_{1,10}$ = 1.79) were lower than A. *brunoi*, but such differences were not statistically significant ($P > 0.05$ in both cases). However, EWL per unit surface area through the body skin of C. *greeningi* was lower than that of A. *brunoi* ($F_{1,10}$ = 4.97; P = 0.05). On the other hand, the rate of water loss through head surface was not significantly different between species in absolute terms ($F_{1,10}$ = 3.69; P = 0.08) or per unit surface area ($F_{1,10}$ = 0.80; P = 0.39). A. *brunoi* (t = 3.62; P = 0.01) but not C. *greeningi* (t = 1.74; P = 0.14) lost less water per unit area through the head surface than the body skin.

Oxygen uptake was higher at 30°C than at 20°C for both species (t = 5.77, P = 0.002 for A. *brunoi* and t = 4.17, P = 0.008 for C. *greeningi*; see Table 1). The level of oxygen consumption did not differ between A. *brunoi* and C. *greeningi* at 20°C ($F_{1,10}$ = 3.32; P = 0.09). However, at 30°C, A. *brunoi* had a $\dot{V}O_2$ significantly higher than that of C. *greeningi* ($F_{1,10}$ = 8.28; P = 0.01). The temperature coefficient of A. *brunoi* (Q_{10} = 1.73) was higher than that of C. *greeningi* (Q_{10} = 1.55).

DISCUSSION

Living amphibians, despite their permeable integument, present behavioural, morphological and physiological modifications that enable them to live in almost all terrestrial environments (3,24). For the majority of species, control of EWL is primarily related to the use of moist micro-habitats and to reduction of the skin area exposed to desiccating environment (21). A. *brunoi* and C. *greeningi* subjected to desiccation without shelter assumed a water-conserving posture that decreases the surface area exposed to evapora-

tion. Such behaviour has been reported in many species as an efficient way to reduce EWL. For example, in *Eleutherodactylus portoricensis*, this posture was estimated to decrease water loss by about 50% (9). Under natural conditions, A. *brunoi* and C. *greeningi* may reduce skin area exposed to desiccation even more by their phragmotic habit (10,26). Accordingly, we found that phragmosis decreased total EWL by 86% and 90% in A. *brunoi* and C. *greeningi*, respectively.

However, phragmosis involves also a morphological specialisation, the cranial co-ossification, along with the reduction in surface area exposed. Moreover, cranial co-ossification has been suggested to decrease EWL through head surface (17,26). For A. *brunoi*, we found that the rate of EWL through head surface, per unit surface area, was significantly smaller than that through body skin. On the other hand, for C. *greeningi*, no significative difference was found between the rate of EWL, per unit surface area, through head and body skin. Such similarity is due the less permeable body skin of C. *greeningi* (see below) and could be indicative of a better general isolation of the integument of this specie from the external environment.

In the area where C. *greeningi* was collected, bromeliads are rare and the frogs shelter in tree trunk holes or rock crevices, often being exposed to the dry air in the "caatingas," particularly during activity (C. Jared, personal communication). On the other hand, A. *brunoi* occupies more moist habitats, especially with a higher relative air humidity due to the proximity with the Atlantic, remarkable by their richness in bromeliads [see (4,5,13) for geographic and climatic references]. Despite the discussion about the limitation of two-species comparative studies in inferring adaptation (6), we are tempted to suggest as plausible that the difference, or not, in EWL rates found by us could be related to differences in the species' habitats. Thus, we believe it possible that the better general skin isolation of C. *greeningi* indicates that phragmosis and cranial co-ossification itself could not be enough to support an adequate water balance in their dry inhospitable environments. Such problem may have been diminished, or even solved, by the smaller permeability of C. *greeningi* body skin. For A. *brunoi*, which live in relatively moist habitats, an adequate degree of water conservation could be yielded by phragmosis and cranial co-

ossification, even though their body skin is more permeable. A. *brunoi* has a larger head, with contours that more efficiently seals the bromeliad tube, than C. *greeningi* (personal observation), a feature that could also contribute to water conservation.

EWL was measured in two other species of casque-headed tree frogs: *Trachycephalus jordani* and *Pelobates syriacus* (17). In T. *jordani*, as for A. *brunoi*, cranial co-ossification was found to substantially decrease the rate of EWL. On the other hand, for P. *syriacus*, Seibert et al. (17) did not found a significative difference between the rate of EWL through body skin and head surface. This finding was related to a low EWL rate from the body skin of P. *syriacus*, an observation also applicable to C. *greeningi*. The relationship between cranial co-ossification and reduction of EWL are generally attributed to the poor vascularization and scant glands of the co-ossified tissues that imply in a reduction of their water content, resulting in low rates of EWL [see (17,24–26)]. However, in some species of casque-headed tree frogs, like P. *syriacus* (17) and C. *greeningi* (this study), the rate of EWL through body skin may not differ considerably from those of the co-ossified head surface. This finding indicates that the same degree of water conservation afforded by co-ossification could be reached by the improvement of the integument isolation barrier. Therefore, the co-ossification of the cranial region in anurans may not be directly related to a reduction in water loss rates. In this context, the study of alternative functions for this trait, such as its effectiveness in defence against predators, may provide new insights to the understanding the evolution and the functional significance of cranial co-ossification.

The reduction of skin permeability to water is known to limit its respiratory gas exchange capacity. Species with low skin permeability, especially those living in dry terrestrial environments, usually exchange gases almost entirely via lungs (21,23). Lung dependence to gas exchange associate with relatively impermeable skin are advantageous in dry habitats, because lungs are internalised and only intermittently exposed to the environment (21). Furthermore, lung respiration may be controlled faster and more efficiently than gas exchange through skin (2,27). The amount of oxygen uptake through skin and/or lungs were not separately evaluated for A. *brunoi* and C. *greeningi*. However, their low general skin permeability and phragmotic habits, which limits the skin area in free contact with air, probably limit the contribution of the skin in the respiration of A. *brunoi* and C. *greeningi*.

The metabolism of amphibians is thought to scale with body size and temperature. Our results on O_2 uptake in A. *brunoi* fit with the value predicted from the allometric equation derived by Gatten et al. (7) for anurans: the SMR measured for A. *brunoi* responding for 99.5% of the predicted value. On the other hand, SMR for C. *greeningi* was only 77.5% of the value predicted by the allometric equation [cf. (7)]. We found that oxygen uptake for A. *brunoi* and C.

greeningi increases with the increase of body temperature. However, the latter species showed a lower sensitivity to temperature. Although the Q_{10} value of A. *brunoi* is within the range commonly reported for anurans, the Q_{10} of C. *greeningi* is lower than that reported for other anurans (7,15).

The reduced metabolic rate of C. *greeningi* decreases the need of renovation of air into the lungs and consequently contributes to reduce EWL through lung surface. In the same way, the low Q_{10} of C. *greeningi* prevents a high metabolic increase after body temperature rise and could also contribute to a decrease in lung EWL. Such features of C. *greeningi* metabolism, and certainly of any organism, are not only related to water conservation, and many other traits might be involved, or even may be responsible for it. However, regardless the pressures acting on the metabolism of C. *greeningi*, their characteristics appear to be convenient to the occupation of dry habitats.

In summary, we believe it possible that in the areas where A. *brunoi* is found, an adequate degree of water conservation could be achieved primary by phragmosis associated with cranial coossification, whereas the more inhospitable environment of C. *greeningi* could have required a better general skin isolation and lower metabolism to face its dryness.

References

1. Bokermann, W.C.A. Una nueva especie de *Trachycephalus* de Bahia, Brasil. Neotropica 12:120–124;1966.

2. Boutilier, R.G. Characterization of ventilatory patterns in amphibians. In: Bicudo, J.E.P.W. (ed). The vertebrate gas transport cascade: Adaptations to environment and mode of life. Boca Raton, FL: CRC Press; 1993:72–86.

3. Duellman, W.; Trueb, L. Biology of Amphibians. New York: McGraw-Hill; 1986.

4. Ferreira, J.P. Enciclopédia dos municípios brasileiros, Vol. XXII. Espírito Santo e Rio de Janeiro. Rio de Janeiro: IBGE; 1959.

5. Ferreira, J.P. Enciclopédia dos municípios brasileiros, Vol. XVII. Paraíba e Rio Grande do Norte. Rio de Janeiro: IBGE; 1959.

6. Garland, T., Jr.; Adolph, S.C. Why not to do two-species comparative studies: Limitations on inferring adaptation. Physiol. Zool. 67:797–828;1994.

7. Gatten, R.E., Jr.; Miller, K.; Full, R.J. Energetics at rest and during locomotion. In: Feder, M.E.; Burggren, W.W. (eds). Environmental Physiology of the Amphibians. Chicago: The University of Chicago Press; 1992:314–377.

8. Heatwole, H. Ecological segregation of two species of tropical frogs of the genus *Eleutherodactylus*. Carib. J. Sci. 3:17–23; 1963.

9. Heatwole, H.; Torres, F.; Austin, S.B.; Heatwole, A. Studies of anuran water balance. I. Dynamics of evaporative water loss by the coqui, *Eleutherodactylus portoricensis*. Comp. Biochem. Physiol. 28:245–269;1969.

10. Jared, C.; Toledo, R.C.; Antoniazzi, M.M. A histological study

of the integument of *Corythomantis greeningi* (Amphibia, Anura, Hylidae). Rev. Brasil. Biol. 55:509–515;1995.

11. Lutz, B. Anfibios anuros do Distrito Federal. Mem. Inst. Oswaldo Cruz 52:155–238;1954.

12. Pough, F.H.; Taigen, T.L.; Stewart, M.M.; Brussard, P.F. Behavioral modification of evaporative water loss by a Puerto Rican frog. Ecology 64:244–252;1983.

13. RadamBrasil, Projeto. Folhas SF 23/24 Rio de Janeiro/Vitória; geologia, geomorfologia, vegetação e uso potencial da terra. Rio de Janeiro, RJ; Ministério das Minas e Energia/SG; 1983.

14. Reno, H.W.; Gehlbach, F.R.; Turner, R.A. Skin and aestivational cocoon of the aquatic amphibian, *Siren intermedia* Le Conte. Copeia 1972(4):625–631;1972.

15. Rome, L.C.; Stevens, E.D.; John-Alder, H.B. The influence of temperature and thermal acclimation on physiological function. In: Feder, M.E.; Burggren, W.W. (eds). Environmental Physiology of the Amphibians. Chicago: The University of Chicago Press; 1992:183–205.

16. Romer, A.S. The Vertebrate History, 4th Edition. Chicago: The University of Chicago Press; 1959.

17. Seibert, E.A.; Lillywhite, H.B.; Wasserburg, R.J. Cranial co-ossification in frogs: relationship to rate of evaporative water loss. Physiol. Zool. 47:261–265;1974.

18. Seymor, R.S.; Lee, A.K. Physiological adaptations of anuran amphibians to aridity: Australian prospects. Aust. Zool. 18:53–65;1974.

19. Shoemaker, V.H. Physiological ecology of amphibians in arid environments. J. Arid Environ. 14:145–153;1988.

20. Shoemaker, V.H.; Balding, D.; Ruibal, R.; McClanahan, L.L. Uricotelism and low evaporative water loss in a South American frog. Science 175:1018–1020;1972.

21. Shoemaker, V.H.; Hilmann, S.S.; Hillyard, S.D.; Jackson, D.C.; MacClanahan, L.L.; Withers, P.C.; Wygoda, M.L. Exchange of water, ions, and respiratory gases in terrestrial amphibians. In: Feder, M.E.; Burggren, W.W. (eds). Environmental Physiology of the Amphibians. Chicago: The University of Chicago Press; 1992:125–150.

22. Sokal, R.R.; Rolf, F.J. Biometry. San Francisco: Freeman Co.; 1986.

23. Stinner, J.N.; Shoemaker, V.H. Cutaneous gas exchange and low evaporative water loss in the frogs *Phyllomedusa sauvagei* and *Chiromantis xerampelina*. J. Comp. Physiol. 157B:423–427;1987.

24. Toledo, R.C.; Jared, C. Cutaneous adaptations to water balance in amphibians. Comp. Biochem. Physiol. 105A:593–608;1993.

25. Trueb, L. Morphology and development of the skull in the frog *Hyla septentrionalis*. Copeia 1966(3):562–573;1966.

26. Trueb, L. Evolutionary relationships of casque-headed tree frogs with coossified skulls (Family Hylidae). Univ. Kansas Publ. Mus. Natur. Hist. 18:547–716;1970.

27. Wang, T.; Abe, A.S. Oxygen uptake in snakes: Is there a reduction in fossorial species? Comp. Biochem. Physiol. 107A:483–485;1994.

28. West, N.H.; Van Vliet, B.N. Sensory mechanisms regulating the cardiovascular and respiratory systems. In: Feder, M.E.; Burggren, W.W. (eds). Environmental Physiology of the Amphibians. Chicago: The University of Chicago Press; 1992:151–182.

29. Wygoda, M.L. Low cutaneous evaporative water loss in arboreal frogs. Physiol. Zool. 57:329–337;1984.