

## Adaptations of the reed frog *Hyperolius viridiflavus* (Amphibia, Anura, Hyperoliidae) to its arid environment

### II. Some aspects of the water economy of *Hyperolius viridiflavus nitidulus* under wet and dry season conditions

W. Geise and K.E. Linsenmair

Zoologisches Institut der Universität, Röntgenring 10, D-8700 Würzburg, Federal Republic of Germany

**Summary.** Adaptations to aridity of the reedfrog *Hyperolius viridiflavus nitidulus*, living in different parts of the seasonally very dry and hot West African savanna, are investigated.

1. During the dry season mainly juveniles (weighing 200–600 mg) were found in the field. A very low rate of evaporative water loss (EWL; about 1.2% of the body weight/day under laboratory dry season conditions) enables the frogs to estivate unshaded on dry plants. There they are exposed to temperatures occasionally reaching 45° C and are to sustain high radiation loads. The EWL of wet season frogs (WSF) was on average 30 times higher.

2. In dry season frogs (DSF) a thin layer of desiccated mucus seals the body surface reducing water loss and securing tight attachment to the substrate. The DSF are not in a state of torpor but are able to become active at any moment. The highest tolerable water loss of DSF amounts to 50% of their initial body weight. Since uptake of water or food often is impossible for more than two months, the small DSF have to survive these harsh conditions with very limited reserves of energy and water.

3. The low EWL of DSF does not engender any cooling effects. Only above a certain high temperature limit, defined as the critical thermal maximum (CTM; 43–44° C) we found a steep increase of EWL—probably indicating evaporative cooling. The CTM is affected by the temperature during acclimatization.

4. In contrast to WSF cutaneous respiration is not found in DSF. All CO<sub>2</sub> is delivered via the lungs by discontinuous ventilation. The simultaneous water loss via the respiratory tract makes up to 14.9 ± 8.9% of the entire water loss.

5. A very fast water uptake (69.3 ± 19.4%/h) via thin and vascular skin areas at the ventral flanks and the inner sides of the legs enables the frogs to use small quantities of water available for very short times only. This highly permeable skin is protected against desiccation by the typical squat resting position of the frogs.

6. DSF usually do neither urinate nor defecate; they are not proved to be uricotelic. Probably they store the nitrogenous wastes as urea in the body fluids and as purines in the iridophores and connective tissues. It is suggested that there is no selective advantage for uricotelism in the small *H. v. nitidulus*.

As we know for a long time some anurans successfully inhabit desert like areas. Their specific adaptations are summarized in a number of reviews (e.g. Chew 1961; Bentley 1966; Warburg 1972).

These species mostly avoid the extreme conditions of the dry season by burrowing into the ground. Their tolerance of high urea concentration allows the use of water stored in large quantities (up to 30% of body weight) in the urinary bladder (Ruibal 1962; McClanahan 1967; Canziani and Cannata 1980). In some of the burrowing species cocoon formation by multiple molting further reduces the evaporative loss of water (Lee and Mercer 1967; Seymour and Lee 1974; McClanahan et al. 1976; Loveridge and Crayé 1979).

Two arboreal frogs originating from different, only distantly related families – *Chiromantis xerampelina* (Rhacophoridae) in East Africa and *Phyllomedusa sawagei* (Hyllidae) in South America – have been found (Loveridge 1970; Shoemaker et al. 1972) estivating on plants in exposed positions where they have to withstand high temperatures and a high radiation load. They are “reptile-like” in showing uricotelism and low rates of evaporative water loss (EWL). Other arboreal frogs with similar adaptations have been investigated by Shoemaker & McClanahan (1975) and Drewes et al. (1977).

Several phyllomedusans cover their body surface with a thin lipid layer, secreted by skin glands and spread by a special wiping behavior (Blaylock et al. 1976), thus achieving an effective protection against uncontrolled EWL. The corresponding mechanisms in *Chiromantis* spec. are not well understood. In *Ch. petersi* a thick layer of iridophores in the skin is assumed to be mainly responsible for the control of EWL (Drewes et al. 1977). On the other hand Withers et al. (1984) clearly demonstrate a very low EWL in the rainforest species *Ch. rufescens*. In the skin of this species dense iridophore layers as in *Ch. petersi* are absent (Drewes et al. 1977). Withers et al. (1984) assume lipids associated with carbohydrates or proteins located in the intercellular spaces of the stratum corneum to function as a water diffusion barrier.

Low EWL but not uricotelism was observed in the reed frog *Hyperolius nasutus* (Loveridge 1976; Withers et al. 1982). The Australian frog *Litoria gracilentata* also has been found to be “waterproof” (Withers et al. 1984). Loveridge distinguished between “dry season frogs” (DSF) and “wet

season frogs" (WSF), the latter showing a much higher EWL. All estivating tree frogs are able to replace their water loss by very rapid water uptake via the skin (e.g. Drewes et al. 1977).

Most articles only discuss problems of water balance and nitrogen excretion. They are rarely concerned with the complexity of estivation mechanisms or the physiological processes which occur during the change from WSF to DSF and vice versa. Data about thermoregulation and metabolism under the desert like climatic conditions with open water being unavailable for at least many weeks and with air temperatures reaching more than 40° C are lacking.

During field studies in the Sahel (West Africa) we observed the reed frog *Hyperolius viridiflavus nitidulus* (Hyperoliidae; systematics see Schiøtz 1967, 1971) estivating on dry plants in the blazing sun. Mainly young frogs which obviously tolerated occasional maximum air temperatures up to 45° C were found.

As the frogs could be kept and bred we had the chance to study their physiology in the laboratory under conditions comparable to those of the rainy season on the one and the dry season on the other hand. Certainly the very unusual way of estivation in *H.v.nitidulus* is a result of the development of numerous specific adaptations concerning their morphology, behavior and many aspects of their physiology, e.g. water economy and energy metabolism as well as thermoregulation. Considering the unpredictable onset and duration of the dry season different strategies with regard to the storage and use of energy- and water reserves are to be expected in smaller versus bigger frogs.

Here we present results of those experiments concerned with some aspects of water balance, e.g. EWL, rehydration rates and tolerance against high temperatures.

#### Materials and methods

Our first field observations were made in March/April 1973 and 1975 during the dry season at South Mali, Southeast Uppervolta and North Benin. Detailed field studies were carried out in the Ivory Coast (Comoe National Parc) during the first months of the dry season from November to January 1981/82.

About 200 *H.v.nitidulus* - DSF (mainly juveniles) were collected to study their physiology under laboratory conditions. In the laboratory they were given water to replace their losses during the 4-7 weeks of transport and they were kept for about 3 months in plastic containers without water or food at 26-30° C and 30-50% RH. Frogs in an apparently bad condition got water earlier or were placed in a terrarium with water and food available (wet season conditions). At the end of the experiments all frogs were rehydrated. They were bred in terraria provided with plants and water at a temperature of 23-30° C and 50-100% RH and maintained on a diet of flies (*Drosophila*, *Musca*, *Phormia*, *Lucilia*, *Calliphora*). Besides DSF collected in the field, raised young frogs have been used to compare the physiological reactions of frogs living under wet or dry season conditions.

**Rates of water loss.** Each single frog was sitting in a small wire cage (non-water-absorbing steel). The cages were put in a climate chamber at a 12:12 h LD period (for temperatures and humidities see results). Minimum rates of EWL were determined over a period of 2-3 weeks every 2nd-3rd day and are given in % of the initial body weight per day.

In order to distinguish the percentage of the respiratory water loss from the total water loss, the frogs were permanently weighed on a Mettler micro-balance (ME 22) and the measurements were recorded up to 1 µg.

**Rehydration.** After determination of the water loss the rates of water uptake were measured by placing the frogs in tap water. The water just covered the pelvic region of the frogs. It never limited the rate of absorption within a rehydration period of 30 minutes. After this period excess water was removed with filter paper and the frogs were reweighed one hour later. In different experiments the time of availability of water for dehydrated frogs was reduced to 10 min. The aim of this procedure was to measure the initial absorption rate. All rates of uptake are given in %/h of the initial body weight.

**Respiration.** CO<sub>2</sub>-release and evaporation were measured using infrared gas analyzers (UNOR - 4N and Binos; details of these experiments and their results: Geise and Linsenmair, in prep.).

**Urate (uric acid).** The first feces pellets of the frogs excreted after rehydration were analyzed for their content of uric acid with a Boehringer Mannheim Diagnostic Kit.

All values are given as means +/- standard deviation.

#### Results

**Posture and mechanism of attachment in DSF; field data.** During the dry season mainly juvenile *H.v.nitidulus* (with a length of 1.0-1.65 cm weighing 200-600 mg) were found on dry plants (e.g. grasses) at a height of 1-1.5 m. At temperatures exceeding 39° C they were shining white but below this temperature their coloration often becomes darker and may be adjusted to some extent to that of the background. The frogs were not in a state of torpor, but were able to flee at any moment if endangered greatly.

Often the DSF are attached to the substratum with only a very small part of their ventral skin. The legs are tightened close to the body and the feet are hidden in skin folds (e.g. gular pouch) (Fig. 1a). In this typical posture the DSF often perch on thin vertical grass blades or - during the experiments in the laboratory - on a wire gauze. The close attachment to the substrate could be achieved by the ventral skin acting as a suction plate. But, since moistening of the ventral skin causes loss of hold and always induces the DSF to unfold their limbs and to expand toe pads it seems reasonable to assume another mechanism. Especially the examination of DSF sitting on a wire gauze yielded the result that the adhesion to the ground is achieved by dried skin gland secretions (Geise unpublished). If soaked with water this mucus loses its adhesive properties.

**Rates of water loss.** Under shortage of water *H.v.nitidulus* delivered neither urine nor feces. The rates of evaporative water loss (EWL) in estivating frogs sitting in their typical resting posture were extremely low for amphibians (Fig. 2). Experimental conditions were 36° C, 30-40% RH during the day (12 h) and 26° C, 50-60% RH in the night. After a few days of acclimatization water loss averaged 1.2%/day over a period of 16 days (mean value at the 12th day: 1.1 +/- 0.22%/day; n=15) under our experimental conditions including a rather intensive handling of the frogs for frequent weighings (see Discussion).

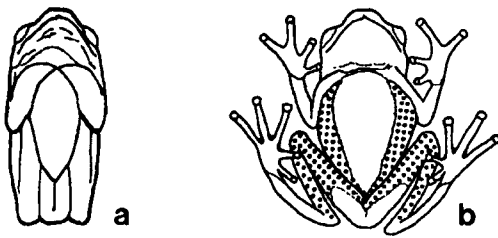


Fig. 1 a, b. Ventral view of a dry season frog (DSF). a While sitting in its typical resting posture the thin-skinned areas at the inner side of the limbs and at the ventral flanks - specialized for rapid water uptake - are hidden in skin folds and thus protected against desiccation. b During water uptake these vascular skin areas (dotted) are exposed

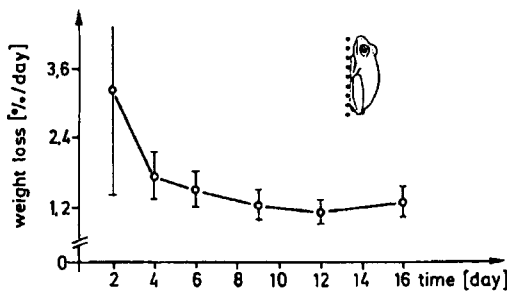


Fig. 2. Evaporative water loss (EWL) of DSF sitting on a wire gauze (weighing at 36° C/30% RH and 26° C/50% RH, at a light/dark period of 12/12 h; n=15)

Exposed to these conditions WSF would survive not more than two days. Two h after they had been taken out of the terrarium, WSF lost water at a rate of about 36%/day at comparatively mild climatic conditions (25° C and 40% RH). In spite of the lower temperature this value is 30 times higher as in DSF and is similar to the evaporation of a free water surface. In Fig. 3 single weighings of a WSF (a) and a DSF (b) are given just after they had been put into the wire cage. One out of several reasons for the high EWL of WSF is their high activity rate (movement and climbing in the wire cage) during the measurements (Fig. 3a, factor of increase in EWL, mainly caused by evaporation from the extended legs and arms and by mucus discharge:  $3.9 \pm 1.5$ ; n=34). When *H.v.nitidulus* - WSF were allowed to seal themselves to a cover glass instead of the wire gauze the frogs remained inactive in a DSF-like resting position. Their mean EWL then was reduced to 1/4 compared to the value of WSF sitting motionless on the wire gauze (Fig. 4; after 2 h from  $26.6 \pm 7.7\%$ /day to  $7.2 \pm 0.7\%$ /day). Under the same conditions no similar decrease in EWL was observed in DSF. Their normally exposed ventral skin seems to be protected against desiccation as well as the dorsal skin. They showed no increased activity when sitting on a permeable substrate (Fig. 3b).

The highest tolerable water loss is about 50% of the body weight which the frogs showed at the beginning of the dry season. Frogs having lost up to 45% always could be rehydrated successfully. Figure 5 shows the relation between body weight and EWL in DSF taken from the field and measured at 26-30° C, 40-50% RH in the laboratory. As indicated by the curve it has to be expected that only above a certain minimum body weight the DSF are able to survive a dry season lasting several months. In frogs

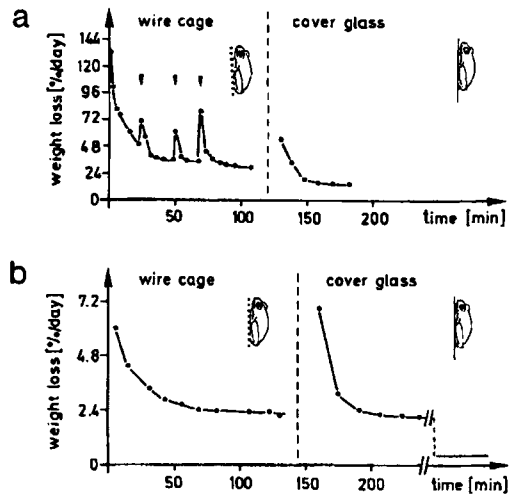


Fig. 3 a, b. Weighings of a single wet season frog (WSF) and a single dry season frog (DSF); comparison of EWL (at 25° C and about 40% RH; the actually measured EWL is computed as %/day) a The effect of activity on EWL in a WSF (904 mg). Except short periods of activity (▼) the WSF remained motionless in a DSF-like resting posture. While sitting on a cover glass activity in the measured frog could not be observed; b Weight loss of a DSF (461 mg). During the experiment the DSF remained inactive; the EWL did not change when the frog was sitting on a cover glass. After 1-2 days the EWL will have reached about 0.4%/day under these conditions

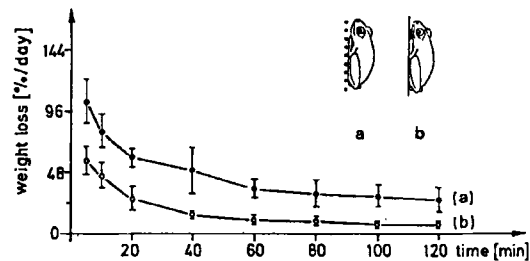


Fig. 4. Evaporative water loss of WSF (weighing at 25° C and about 40% RH; n=4; the actually measured EWL is computed as %/day). When sitting on a wire gauze, WSF loose great amounts of water via their pelvic region (curve a; movement induced water losses are not included). When frogs were allowed to seal themselves to a cover glass the losses are reduced to 1/4 (curve b)

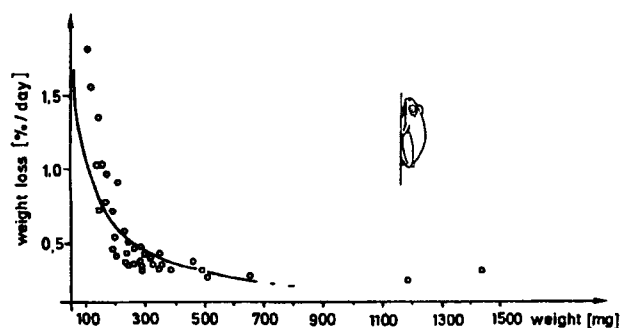


Fig. 5. Relationship between evaporative water loss and body mass in DSF (weighing at 26-30° C, 40-50% RH; n=38). It is assumed that only DSF with a minimum body weight of about 300 mg at the beginning of the dry season show an EWL which is low enough to assure the survival of a dry season lasting several months

not reaching this weight limit the EWL is so high that they usually will have lost all their expendable water before the beginning of the wet season. We suggest a minimum body weight of about 300 mg, with optimal reserves of water and stored energy at the beginning of the dry season, as an essential condition for a high probability of survival (see also Withers et al. 1982, Fig. 2).

**Respiratory water loss.** With decreasing EWL via the skin the inevitable water loss caused by ventilation of the respiratory system (lungs and mouth-cavity) becomes more significant. Figure 6 shows the original record of a continuous weighing. The small peaks in the steeper parts of the curve correspond to the breathing movements of the frog. Taking into account the overall time of gas exchange within one hour the portion of water loss via the respiratory tract was calculated as  $14.9 \pm 8.9\%$  ( $n=16$ ) of the average total water loss (at  $23^\circ\text{C}$ , 35% RH). Values between 5.6 and 32.4% were found (the latter is given in Fig. 6). This observation of interval-breathing in DSF was confirmed by measurements using gas analyzers. The irregular graph in Fig. 7 shows the  $\text{CO}_2$ -release of a WSF. As bimodale breathers WSF most probably deliver  $\text{CO}_2$  partly and more or less continuously via their moist skin and partly and discontinuously via their respiratory tract. For this reason the curve never reached the zero line. In contrast to the WSF the DSF solely show a discontinuous form of  $\text{CO}_2$ -release, as can easily be seen in Fig. 8. When no respiratory movements of the flanks and gular pouch were observed the  $\text{CO}_2$ -release decreased to zero. These results can be explained by assuming that there is no or only very little gas exchange via the skin of DSF. The minimum values of  $\text{CO}_2$ -release in the WSF could be interpreted to reflect the portion of skin respiration which is superimposed by  $\text{CO}_2$  expired from the lungs (Bentley and Shield 1973; Geise and Linsenmair, in prep.).

**Rehydration.** If the DSF are offered water they spread their legs so that the skin portions at the inner sides of the legs and ventral flanks are exposed (Fig. 1 b). These skin areas at the legs which are uncolored in the estivating juvenile turn to a deep red color in older frogs. They are supplied with a dense capillary network and show epidermal differentiations, known as "verrucae hydrophilicae" (Drewes et al. 1977; Kobelt and Linsenmair 1986). Obviously these regions have been evolved as special "organs" for water absorption. When tap water was available to DSF for 30 min the rate of water uptake averaged  $69.27 \pm 19.4\%/h$  of the body weight prior to rehydration ( $n=14$ ). The initial rates of uptake proved to be substantially higher (e.g. during the first 10 min; Table 1). In general the amount of water added was experimentally adjusted to cover just the pelvic region of the frogs. Partly submerging the frogs did not result in an increase of the rehydration rate but the animals showed escape behavior. If the DSF were forced to stay in contact with water for a longer time (e.g. 2 h) they become distended and – if not be taken out of the water – soon will die, of an osmotic shock most probably. Obviously the DSF are, certainly in contrast to the WSF, not able to balance their water uptake, except by behavioral means in "closing" the absorbing skin by assuming their typical squat position (currently under investigation).

**Evaporative cooling.** Despite the limited storage capacity for water DSF seem to use evaporative cooling under spe-

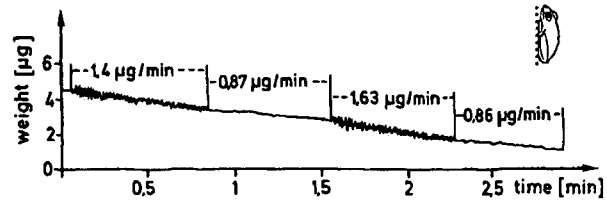


Fig. 6. Water loss of a DSF (377 mg) at  $23^\circ\text{C}$ , 35% RH; the serrated parts of the curve show the effect of respiration on water loss

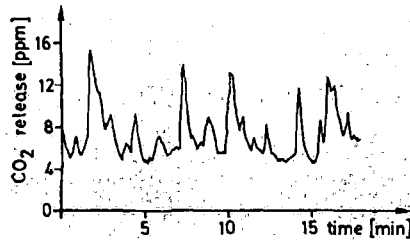


Fig. 7.  $\text{CO}_2$  release of a WSF (1,476 mg) at  $25^\circ\text{C}$  and about 90% RH

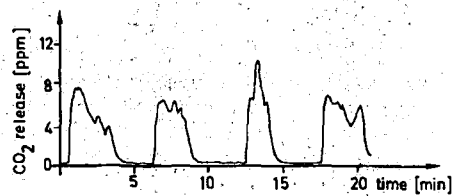


Fig. 8.  $\text{CO}_2$  release of a DSF (624 mg) at  $20^\circ\text{C}$  and 25% RH

Table 1. Rates of water uptake in dry season frogs

	Water available for 10 min				
Weight (mg)	368.4	374.5	404.9	572.8	673.2
Increase of weight (mg)	70	120	40	100	180
%/h	114	192.3	59.3	104.8	160.4
	After 2 h water available for 20 min				
Increase of weight (mg)	80	80	10	4.2	10
Total gain (%)	40.7	53.4	12.4	18.2	28.2

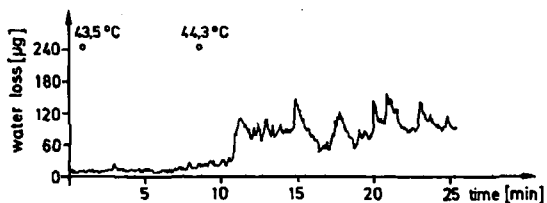


Fig. 9. Water loss of a DSF (359 mg) adapted to  $35^\circ\text{C}$  before and at the critical temperature maximum (CTM;  $44.3^\circ\text{C}$ )

cial conditions. Water loss increased abruptly and substantially at certain high temperatures. Steep increases in EWL as in Fig. 9 are supposed to be the onset of thermoregulation by evaporative cooling. The temperature at which evaporative cooling begins is defined as the critical thermal maximum (CTM). The CTM is influenced by the temperature of acclimatization (Table 2). A change of the acclimati-

**Table 2.** The effect of acclimatization temperature on the critical temperature maximum (CTM; determined by the onset of excessive water loss)

Temperature of acclimatization	Onset and end of excessive water loss (°C; means $\pm$ SD)	Diff. °C
27° C	Onset: $n=9$ ; $42.3 \pm 0.46$ End: $n=6$ ; $41.9 \pm 0.13$	0.4
35° C	Onset: $n=7$ ; $43.9 \pm 0.65$ End: $n=5$ ; $43.3 \pm 0.26$	0.6

zation temperature from 27 to 35° C caused an increase in CTM of about 1.6° C. The extremely high EWL ceased as soon as the temperature was lowered by only about 0.5° C (Table 2). The water loss during evaporative cooling increased nearly eight times ( $7.65 \pm 3.29$  times;  $n=7$ ) compared to the period before. Further increase in temperature under laboratory conditions always caused escape behavior, resulting in a water loss at least 30 times as high as in resting DSF.

**Urate (uric acid).** After water uptake for more than 15 min the DSF often defecated but urination could never be observed. Enzymatic analysis of these feces and of urine obtained by forced urination never gave any hint to the presence of uric acid.

## Discussion

**Rates of water loss.** Well adapted to aridity and high temperatures *H.v.nitidulus* showed a very low EWL of about 1.2%/day at an average temperature of about 30° C. This rate is comparable to that of *Chiromantis spec.*, *Phyllomedusa spec.* and some reptiles (Shoemaker and Nagy 1977; Mautz 1982). Minimum rates attained by frogs which were not frequently disturbed by experimental procedures could even reach much lower values under the described conditions (0.6%/day).

**Permeability barrier.** Since contrary to *Phyllomedusa species* (Blaylock et al. 1976) neither lipid glands nor a corresponding wiping behavior could be observed, a basically different method to reduce EWL is to be assumed for *H.v.nitidulus*. Location and mechanisms of the permeability barriers could not yet be determined with certainty.

In DSF dried mucus covers the whole body surface as a thin polish-like layer. This mucus could play an essential role in reducing water loss – perhaps in connection with the stratum corneum (Machin 1969). Most probably, it seals the gaps between the body and the tightened legs as well as the ducts of the skin glands. Mucus is secreted during movements and moltings. When the frogs resume their resting position the mucus dries again and forms a continuous layer on the frog's skin. WSF lose considerably more water than DSF even under comparatively mild experimental conditions. A great part of the entire water loss takes place via the pelvic skin. Possibly the WSF are not able to seal their skin and skin folds with mucus – perhaps because of a differing composition during wet season conditions. When the WSF were placed on a cover glass we consequently measured a very distinct decrease in EWL down to 1/4 compared to resting WSF with exposed ventral

side. The fact that in DSF sitting on a dense ground the EWL does not decrease substantially shows clearly the perfect sealing of their ventral skin parts (concerning the possible role of the iridophores and the ground substance as diffusion barriers: see Kobelt and Linsenmair, 1986). Dry mucus is proven to function as a diffusion barrier (Machin 1964, 1974) and as a mode of attachment in estivating snails. Reno et al. (1972) observed cocoon formation by gland secretions in the estivating urodele *Siren intermedia*.

**Evaporative cooling.** Measuring the water loss of DSF at very high temperatures (CTM) we observed a sudden and steep increase in EWL. Since simultaneous measurements of CO<sub>2</sub> release never yielded any evidence for greatly intensified gas exchange we conclude the increased EWL to be caused by mucus discharge which could serve for evaporative cooling at the CTM. At lower temperatures a discharge of mucus seems not to take place in resting frogs under conditions of water deficiency, except during movements and moltings. After mucus discharge the skin loses its parchment-like appearance. The importance of skin gland secretion for thermoregulation in amphibians living under much less severe environmental conditions was studied by Lillywhite (1971, 1975) in *Rana catesbeiana*. The mechanisms and the adaptive significance of mucus discharge he described may also be assigned to DSF of *H.v.nitidulus* but skin gland secretion seems to be restricted to a very narrow temperature range. The threshold value for the onset of evaporative cooling certainly is close to the lethal temperature. The CTM is altered by the temperature of acclimatization (Table 2) and evaporative cooling ceased when the temperature was lowered to 0.5° C below the CTM. These facts – onset of evaporative cooling only close to the lethal temperature, shifting of the CTM by acclimatization and the very sensible reaction to only small temperature changes – indicate that *H.v.nitidulus* uses its very limited water reserves in a highly economical way to cope with brief periods of otherwise most probably lethal temperatures.

**Rehydration.** High rehydration rates in DSF allow an effective utilization of very small quantities of water available after occasional dew and short rainfalls. Water uptake via a so called "pelvic patch" in anurans was described by several authors (McClanahan and Baldwin 1969; Fair 1970; Baldwin 1974; Drewes et al. 1977). In *H.v.nitidulus* this region seems to be much further specialized compared to e.g. *Bufo boreas* and *Bufo punctatus* (Fair 1970). In resting DSF these thin and vascular skin parts are covered by the tightened legs. The folds between legs and body are sealed with dried mucus. An uncomplete sealing of these highly permeable regions – e.g. caused by dirt between the skin folds – or exposure during activity results in extremely high EWL. Certainly the risk for high water losses is an important reason for DSF only to unfold their legs or to jump if endangered greatly. Those parts of the pelvic region which are normally exposed to the ambient air in the DSF show the usual white coloration caused by dense iridophore layers (Kobelt and Linsenmair 1986) and are protected against EWL as well as the dorsal skin.

**Respiratory water loss.** In general, the respiratory water loss in anurans amounts to not more than 1% of the total loss and therefore is negligible (Spotila and Berman 1976; Bent-

ley and Yorio 1979; Wygoda 1981). With an average of 14.9% of the total loss it becomes more important for the water balance of DSF of *H.v.nitidulus*. The highest respiratory water losses measured (about 30%) were similar to those found in reptiles (*Lepidophyma ssp.*; *Xantusia ssp.* see Mautz 1982; *Terrapene carolina*, *Anolis carolinensis* see Spotila and Berman 1976).

As bimodale breathers anurans usually deliver the bulk of CO<sub>2</sub> (60–90%) via their moist skin (Hutchison et al. 1968). The WSF of *H.v.nitidulus* do not deviate from this breathing pattern. In contrast to the WSF the diffusion of CO<sub>2</sub> through the dry skin of DSF is so greatly reduced that practically all CO<sub>2</sub> has to be emitted via the lungs. Since the respiratory water loss amounts to a considerable proportion of the total EWL, the evolution of specific adaptations has to be expected. The improvement of the efficiency of O<sub>2</sub> uptake from the inhaled air and the highest possible concentration of CO<sub>2</sub> in the exhaled air (Mautz 1982) would lead to an "economic" respiration and consequently to a more favorable water balance. The evolution of discontinuous ventilation in some estivating snails (Schmidt-Nielsen et al. 1971; Ghiretti and Ghiretti-Magaldi 1975; Herreid 1977) as well as in insects (summary in Miller 1974) is discussed as one possible mechanism of adaptation in this regard. Continuous weighings on a microbalance and measuring the gas exchange with infrared analyzers yielded the result that DSF obviously ventilate their lungs in a very similar manner. Discontinuous ventilation in anurans was described previously for *Bufo marinus* (Bentley and Shield 1973; MacIntyre and Toews 1976; Boutilier et al. 1979a, b). This toad showed distinctly separated "inflations" as reaction a) to desiccation of the skin surface (tolerated for 2 days) and b) to an increase of the external CO<sub>2</sub> concentration to 5–10% vol. In both cases adequate CO<sub>2</sub> release via the skin is impossible. It has to be delivered via the lungs by deep ventilations. The evolution of vascular lungs with a high internal compartmentalization together with a high developed buffering capacity for CO<sub>2</sub> in the body fluids and the control of respiration via CO<sub>2</sub> receptors can help to lower the respiratory water loss (Tenney and Tenney 1970; Boutilier et al. 1979a, b; Glass 1983).

*Weight dependent water loss and time of survival.* As it is commonly known smaller frogs show a higher relative weight gain by rehydration than bigger individuals – this for example was found by Fair (1970) comparing *B. boreas* and *B. punctatus*. We have evidence that it is also true for DSF of *H.v.nitidulus*. But despite of their higher rates of water uptake smaller DSF are handicapped because of their higher EWL and their higher metabolic rates in relation to bigger *H.v.nitidulus*. A fundamental precondition for DSF in order to survive a dry season lasting several months is supposed to be a minimum weight of about 300 mg at the beginning of the estivation period. Owing to the disadvantageous surface to volume ratio in smaller DSF a higher EWL follows so that even undisturbed frogs will not survive more than about 4 weeks without water supply.

Under experimental conditions (see above; including frequent manipulations) a 400 mg DSF loses water at a rate enabling it to survive a 30–40 day period without water and energy uptake. Then the highest tolerable water loss of about 50% is reached. It has to be expected – and we have already good evidence – that in the field EWL and

metabolic rate in experimentally undisturbed frogs are much lower, at least doubling the maximal survival period to about 60–80 days. Enforced movements as well as temperatures at the CTM causing high EWL drastically reduce the span of survival. First results indicate that a considerable amount of the total water loss has to be attributed to the frequent moltings which not only the WSF but also the DSF regularly perform; but this problem will be treated elsewhere. Acclimatization to high temperature is considered part of the process of induction of the dry season physiology and has to be understood as an adaptation which extends survival time of estivating frogs. Especially the examination of the CTM and the metabolic rates of DSF (Geise and Linsenmair, in prep.) yielded very obvious effects of acclimatization, leading to an economical consumption of water and energy reserves.

DSF which died after they had been taken from the field and kept under dry season conditions for additional 2–3 months had a water content of about 80% although they were kept without water for more than 30 days. This fact proves that they certainly did not die of water shortage. Furthermore the survival time in relatively small DSF could not be prolonged by providing them with water. These results indicate that especially for smaller DSF the amount of stored energy reserves is the decisive limiting factor for the maximal time of estivation. DSF could replenish their energy stores by feeding during the dry season. In numerous observations of DSF under water shortage we never – and even after water uptake we only very rarely – found prey capturing behavior. On the other hand feeding without additional water provided is described and discussed for the uricotelic anurans like *Chiromantis* and *Phyllomedusa* (Loveridge 1970; Shoemaker 1974). The DSF of *H.v.nitidulus* started regular feeding only after daily water supply of about 1/2 h during 3–4 successive days. Uricotelism allows the excretion of nitrogenous wastes without the loss of great amounts of water so that feeding is possible without the risk of a large solute load (e.g. urea). We assume that feeding during the dry season not only has to improve the energy situation but also must not impair the water balance. This would be the case if the gain of energy and water by feeding compensates the entire water loss – e.g. losses during capturing behavior and via excretion of nitrogenous wastes. Thus feeding is advantageous only if relatively big prey (in relation to the frog's body mass) without great expenditures of energy and water is captured. In contrast to estivating anurans like *Chiromantis* and *Phyllomedusa* this situation seems not to be realized in the distinctly smaller DSF of *H.v.nitidulus*. As preparations of the stomach of DSF during the transition period from the wet to the dry season showed they only feed on very small insects (e.g. little beetles) which hardly ever are available during the dry season. Furthermore the gains yielded by capturing small insects probably would not compensate the losses with regard to the energy and – more important – the water expenditures. Therefore uricotelism is likely not to have evolved in DSF of *H.v.nitidulus* as a possible mechanism of adaptation to aridity in spite of the fact that the necessary enzymatic machinery exists. Preliminary experiments showed that in DSF a great portion of nitrogenous wastes originating from the basal metabolism is stored in the body fluids as urea or as purine crystals in the iridophores (Kobelt and Linsenmair, 1986 and in prep.), the connective tissue and the outer layer of the liver. The latter is indicated

clearly by the increasing white coloration of the usually dark brown liver, beginning at the anterior part of this organ.

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