

Short Notes

Seasonal water loss of the lizard *Lophognathus temporalis* in the wet-dry tropics of northern Australia

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Evaporative water loss (EWL) occurs principally across the skin (cutaneous water loss, CWL) of terrestrial reptiles (Shoemaker and Nagy, 1977; Mautz, 1982), but EWL also occurs across respiratory passages (respiratory water loss) and the eyes (ocular water loss) (Mautz, 1982). Over 70% of EWL in agamid lizards is from CWL (Eynan and Dmi'el, 1993). The factors determining the rate of CWL are skin resistance (Davis et al., 1980) and vapour density difference between the epidermis and the surrounding atmosphere (Zucker, 1980). Vapour density difference increases with increasing environmental temperature and wind speed and decreasing humidity and size of the animal (Davis et al., 1980). Respiratory water loss increases with aridity of habitat in agamid lizards (Leclaire, 1978). Ocular water loss may be a significant component of water loss where no significant barrier, such as transparent, spectacled eye-lids, exists (Mautz, 1982).

Reptiles inhabiting extremely desiccating environments, such as deserts, require exceptionally efficient water preserving mechanisms. CWL is usually relatively low in reptiles of such environments (Shoemaker and Nagy, 1977). Some lizards may have physiological adaptations to control CWL as they become dehydrated (Kobayashi et al., 1983; Dmi'el et al., 1997). Without such adaptations, EWL in lizards in a seasonal tropical environment is determined by the animal's activity (Heatwole and Vernon, 1977) and the environmental variables of temperature (Thorpe and Kontagiannis, 1977) and humidity (Hillman and Gorman, 1977). Although lizards of wet-dry tropical areas experience reasonably uniform temperatures throughout the year, they encounter a vast range of seasonal humidities and rainfall, and many species reduce activities during the driest times of the year as a response

to a decrease in food and/or water (Christian et al., 1995; Griffiths and Christian, 1996). The wet-dry tropical region of Australia experiences two distinct seasons based on rainfall: the wet season (December to March), when most of the yearly rainfall occurs, and the dry season (June to August) when rainfall virtually ceases. The months April to May and September to November are the wet-dry and dry-wet transitions respectively (Taylor and Tulloch, 1985).

In the wet-dry tropics of Australia, the agamid lizard *Lophognathus temporalis* is relatively inactive throughout the dry season, during which it significantly decreases body temperature (T_b) and metabolic rate (Christian et al., 1999). The aims of this study were to determine (1) the relative roles of cutaneous, respiratory and ocular water loss in this species and (2) whether EWL rates of *L. temporalis* change seasonally.

Water loss measurements were made on 11 lizards captured during the dry season, 10 captured during the dry-wet transition and 6 during the wet season. Lizards were immediately placed in cloth bags upon capture and taken to Northern Territory University for immediate analysis. A measurement of animal surface area was done in order to express CWL in terms of surface area. We obtained five preserved specimens, ranging from 10 to 41 g, from the Northern Territory Museum and Art Gallery. Masses were not corrected to account for possible differences between preserved and live animals. The specimens were weighed to the nearest 0.1 g on electronic scales, then the entire surface was wrapped in masking tape. The wrapped specimens were coloured with a permanent marker. The tape was removed and the darkened areas were cut and pieced together on paper to form a shape. The surface area of the shape was measured with an area meter (Delta T, Delta T Devices, Cambridge UK). The relationship between body mass and surface area of the museum specimens was used to construct a predictive equation (surface area = $16.74 + 4.36 \times \text{mass}$; $F_{1,3} = 70.6$, $P = 0.003$, $R^2 = 0.96$) from which we estimated the surface area of the live lizards after measuring their body mass.

Measurement of overall water loss first involved flowing air through a drying column, a flow meter (Sierra, Top Trak, Monterey CA) and then into an experimental chamber. Downstream from the experimental chamber the air passed over a humidity sensor (Vaisala HMP133Y, Helsinki). Airflow through the system was at a constant rate (0.1 l min^{-1}) as measured by the mass flow meter. The humidity and temperature were read directly from a volt meter connected to the humidity sensor. The system was first run with the experimental chamber empty to establish baseline water vapour density of excurrent air. Three experiments were performed on each lizard in order to compartmentalise EWL into cutaneous, respiratory and ocular components.

The first experiment involved placing a lizard in the experimental chamber and flowing the compressed air across it until excurrent humidity and temperature stabilised (approximately 3 hours). The difference between the experimental and baseline water vapour densities were due to EWL from the animal. Mass flow of water from the animal (M_w) was

determined by they equation:

$$M_w = V_e(\rho_e - \rho_i)$$

where V_e = experimental flow rate; ρ_e = experimental water vapour density and ρ_i = baseline water vapour density. M_w was divided by mass of the animal for mass-specific EWL and by surface area for surface area-specific EWL.

After total EWL was determined, baseline humidity was re-established and the second experiment was begun. This involved the lizard biting on a Y-shaped tube which was connected to two other tubes, one connected to the outside air and the other to a suction tap, allowing the animal to breath freely. The lizard's mouth and nostrils were covered with Impragum F (ESPE, Seefeld, Germany), a non-adhesive gum, which was applied as a paste and set, rubber-like in two to three minutes. Experiments were performed previously to ensure the Impragum F not only blocked EWL but did not dissipate water itself and provided an effective seal. The second experiment excluded respiratory water loss from the measurement.

Immediately following the second experiment, the lizard's eyes were covered with Parafilm M (American Can Co., Greenwich, CT), and water loss was measured as above for the third experiment. A baseline humidity was again established after the completion of the third experiment. Because ocular water loss was blocked and RWL was diverted, the third experiment was a direct measure of CWL.

At the completion of all experiments the results included a direct measure of total EWL (first experiment), CWL plus ocular (second experiment) and CWL alone (third experiment). Ocular and respiratory water loss were therefore determined by subtraction. Thus, the estimates of the various components of EWL are not independent measurements; however, the statistical comparisons that follow are not comparisons among components of EWL, but rather comparisons across seasons. Different experimental lizards were used in each season. We considered water loss from the cloaca (Mautz, 1982; Zucker, 1980) to be a component of CWL. Experiments were aborted if the lizards defecated or urinated. Analysis of covariance (ANCOVA) with mass as the covariant was used to test for seasonal difference in EWL after it was determined that the slopes were homogeneous at the 0.05 level. All data were log transformed prior to analysis.

The seasonal values of the measured and calculated variables are given in table 1 for each season. We found no significant difference in total EWL ($F_{1,18} = 0.6$, $P = 0.4$) between the dry season and the dry-wet transition. Therefore these data were combined for comparison with the wet season, and hereafter are referred to as the dry season data. The mean total EWL during the dry season (9.2 mg h^{-1}) was significantly less than the mean total EWL (23.1 mg h^{-1}) for the wet season ($F_{1,24} = 23.9$, $P < 0.0001$). There were no seasonal differences in RWL ($F_{1,24} = 0.03$, $P = 0.9$) or ocular water loss ($F_{1,24} = 3.2$, $P = 0.1$). CWL, on the other hand was significantly higher during the wet season ($F_{1,24} = 10.3$, $P = 0.004$).

Table 1. Means $\pm 1 s$ of the measured and calculated evaporative water loss (EWL) variables for *Lophognathus temporalis* during the dry and wet seasons.

	Dry season	Wet season
<i>n</i>	21	6
Mass (g)	30.5 (13.7)	34.8 (15.3)
Surface area (cm ²)	149.8 (59.8)	168.4 (66.5)
Total EWL (mg h ⁻¹)	9.2 (3.0)	23.1 (14.0)
Cutaneous water loss (mg h ⁻¹)	6.3 (2.7)	17.0 (13.8)
Respiratory water loss (mg h ⁻¹)	1.6 (1.5)	3.1 (2.7)
Ocular water loss (mg h ⁻¹)	1.3 (1.5)	3.0 (2.2)
Mass-specific EWL (mg g ⁻¹ h ⁻¹)	0.32 (0.10)	0.69 (0.31)
Area-specific EWL (mg cm ⁻² h ⁻¹)	0.06 (0.02)	0.14 (0.06)

Compared with values given by Mautz (1982) *L. temporalis* have EWL rates during the wet season that are within the range of values for tropical lizards of similar mass. However, EWL is substantially lower during the dry season, although not as low as would be expected for a desert lizard of similar mass. The EWL experiments were done under controlled conditions, with temperature, humidity and flow rate constant in all experiments. Activity was minimal within the experimental chambers, and animals of similar size were used in both seasons. Therefore, variability in EWL induced by temperature, wind velocity, activity and body size (Mautz, 1982) cannot explain the observed seasonal pattern. We found no significant difference in respiratory or ocular water loss rate between the seasons; however there was a significant seasonal change in cutaneous water loss rate. This suggests a change in the permeability of the skin in response to changing seasons, as documented for other lizards (Kobayashi et al., 1983; Kattan and Lillywhite, 1989).

The precise mechanism involved in this change is unknown, but it is likely that changes in the resistance of the outer layer of skin are involved (Mautz, 1982; Kattan and Lillywhite, 1989). Kattan and Lillywhite (1989) suggest that the changes in skin permeability in lizards are due to lipid deposition during acclimation to a drier environment, and that only by shedding the skin will the skin permeability return to previous levels. Further investigations, such as correlations with skin shedding cycles and examination of the seasonal skin structure are required to further understand the mechanisms behind the changes in cutaneous permeability in this species.

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References

- Christian, K.A., Bedford, G., Green, B., Griffiths, A., Newgrain, K., Schultz, T. (1999): Physiological ecology of a tropical dragon, *Lophognathus temporalis*. Aust. J. Ecol. 24: in press.
- Christian, K.A., Corbett, L.K., Green, B., Weavers, B.W. (1995): Seasonal activity and energetics of two species of varanid lizards in tropical Australia. Oecologia 103: 349-357.
- Davis, J.E., Spotila, J.R., Scheffler, W.C. (1980): Evaporative water loss from the American alligator *Alligator mississippiensis*: the relative importance of respiratory and cutaneous components and the regulatory role of the skin. Comp. Biochem. Physiol. 67A: 439-446.
- Dmi'el, R., Perry, G., Lazell, J. (1997): Evaporative water loss in nine insular populations of the lizard *Anolis cristallus* group in the British Virgin islands. Biotropica 29: 111-116.
- Eynan, M., Dmi'el, R. (1993): Skin resistance to water loss in agamid lizards. Oecologia 95: 290-294.
- Griffiths, A.D., Christian, K.A. (1996): Diet and habitat use of frillneck lizards in a seasonal tropical environment. Oecologia 106: 39-48.
- Heatwole, H.F., Vernon, J.E.N. (1977): Vital limit and evaporative water loss in lizards (Reptilia: Lacertilia): a critique and new data. J. Herpetol. 11: 341-348.
- Hillman, S.S., Gorman, G.C. (1977): Water loss, desiccation tolerance and survival under desiccating conditions in two species of Caribbean *Anolis*. Oecologia 29: 105-116.
- Kattan, G.H., Lillywhite, H.B. (1989): Humidity acclimation and skin permeability in the lizard *Anolis carolinensis*. Physiol. Zool. 62: 593-606.
- Kobayashi, D.W., Mautz, W.J., Nagy, K.A. (1983): Evaporative water loss: acclimation in *Anolis carolinensis* lizards. Copeia 1983: 701-704.
- Leclaire, R. (1978): Water loss in three sympatric species of lizards (Reptilia: Lacertilia) from Martinique, West Indies. J. Herpetol. 12: 177-182.
- Mautz, W.J. (1982): Patterns of evaporative water loss. In: Biology of the Reptilia, 12, Physiology C, p. 443-481. Gans, C., Pough, H.F., Eds., London, Academic Press.
- Shoemaker, V.H., Nagy, K.A. (1977): Osmoregulation in amphibians and reptiles. Ann. Rev. Physiol. 39: 449-471.
- Taylor, J.A., Tulloch, D. (1985): Rainfall in the wet-dry tropics: extreme events at Darwin and similarities between years during the period 1870-1983 inclusive. Aust. J. Ecol. 10: 281-295.
- Thorpe, G.S., Kontagiannis, J.E. (1977): Evaporative water loss in isolated populations of the coastal side blotched lizard, *Uta stansburiana hesperis*. Comp. Biochem. Physiol. 57A: 133-137.
- Zucker, A.H. (1980): Procedural and anatomical considerations of determining cutaneous water loss in squamate reptiles. Copeia 1980: 425-439.

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Advertisement calls of two Bolivian *Leptodactylus* (Amphibia: Anura: Leptodactylidae)

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Leptodactylus gracilis Duméril & Bibron, 1841 was recently recorded from Bolivian territory by Köhler et al. (1995). The authors identified *L. gracilis* based on morphological

characters only (see Scrocchi and Lavilla, 1986). However, the most reliable character to distinguish *L. gracilis* from its sibling species *L. geminus* is the advertisement call (Cei, 1980). During field-work in January 1998 in Bolivia we again found the species and recorded its call. *Leptodactylus rhodonotus* (Günther, 1869 "1868") is known to occur on the Andean slopes and the lowlands of the upper Amazon basin of Bolivia and Peru. The species inhabits lowland rainforests as well as montane rainforests between 200 and 2050 m above sea level (Reynolds and Foster, 1992; Rodríguez and Duellman, 1994). The advertisement call is unknown (Heyer, 1979). The purpose of this note is to verify the taxonomic status of *L. gracilis* in Bolivia based on bioacoustic data and to describe for the first time the advertisement call of *L. rhodonotus*.

Recording equipment included Sony WM-D6C and Aiwa HS-F150 tape recorders, respectively, a Sennheiser Me-80 directional microphone and TDK MA60 cassettes. Calls were analysed using a Medav Mosip-3000 sound spectrograph and Spektro 4.4 (version 1996) software. Frequency information was obtained through fast Fourier transformation (FFT; width 1024 points). Voucher specimens from recorded populations are deposited in the Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn (*L. gracilis* ZFMK 66841; *L. rhodonotus* ZFMK 66905).

Advertisement calls of *L. gracilis* (fig. 1) were recorded on 17 January 1998 at approximately 29 km southeast by road from Guadalupe ($18^{\circ}38'52''\text{S}/63^{\circ}58'33''\text{W}$), Provincia Vallegrande, Departamento Santa Cruz, Bolivia, 1650 m a.s.l. Males called from

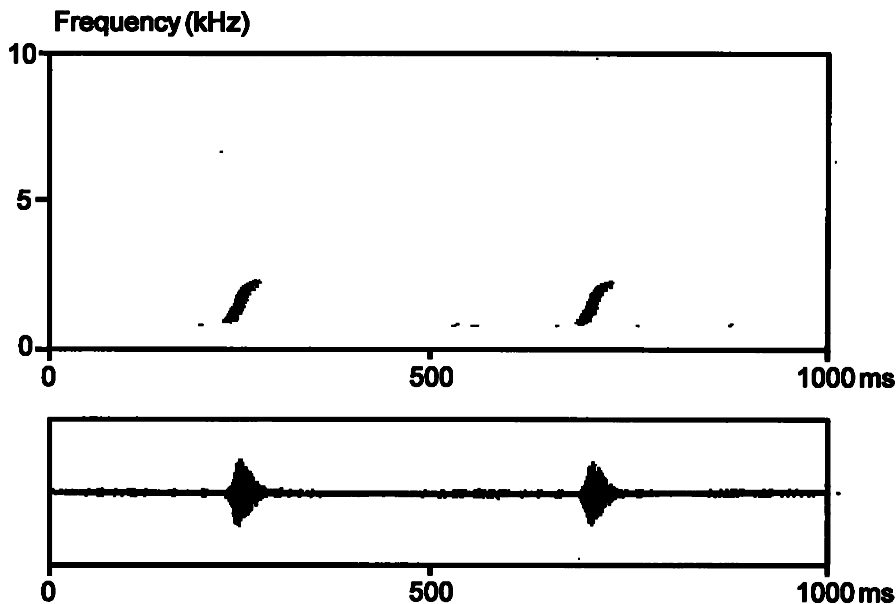


Figure 1. Spectrogram (above) and oscillogram (below) of advertisement call of *Leptodactylus gracilis* from 29 km SE of Guadalupe, Depto. Santa Cruz, Bolivia. Air temperature 18.0°C .

Table 1. Advertisement call characteristics of *Leptodactylus gracilis* and *L. rhodonotus* from Bolivia (mean followed by one standard deviation; range in parentheses).

	individuals analysed	calls analysed	air temp. (°C)	note duration (ms)	notes/minute	dominant frequency range (Hz)	maximum call energy (Hz)	calls pulsed
<i>L. gracilis</i>	1	23	18.0	74.9 ± 6.7 (67-87)	127.8 ± 19.2 (89-182)	830-2320	1970	+
<i>L. rhodonotus</i>	1	25	24.8	54.7 ± 4.9 (45-66)	173.2 ± 31.7 (106-214)	1680-2530	2160	+

the edge of an ephemeral pond beside the road hidden under fallen logs. The call consists of a single frequency modulated note repeated at regular intervals (127.8 notes/min). Mean note duration is 74.9 ms and frequency is distributed between 830 and 2320 Hz. Calls are indistinctly pulsed and lack harmonic structures. Comparison with call data of *L. gracilis* from Buenos Aires, Argentina, given by Heyer (1978) exhibits only little differences. In our recordings the note duration is slightly longer and the note repetition rate is a little bit lower. The calls we recorded differ considerably from those of *L. geminus* which have shorter note duration, much higher note repetition rate and a higher frequency range (2700-3100 versus 830-2320) (Barrio, 1973; Heyer, 1978). Based on similarities in morphology and vocalisation, Cardoso (1985) discussed a possible synonymy of *L. geminus* and *L. plaumanni*. Whether these two nominal species are conspecific or not, the advertisement call data provided for *L. plaumanni* by Cardoso (1985) differ from our recordings in the same manner as those published for *L. geminus*. Therefore, we conclude that the Bolivian populations are referable only to *L. gracilis* which as a result is not an exclusively lowland distributed species. Other anuran species as for example *Phrynohyas venulosa*, *Scinax fuscovarius*, *Odontophrynus americanus* and *Elachistocleis* cf. *ovalis* share this distribution pattern, since they are known to inhabit dry areas and semi-deciduous forests of eastern South America as well as the inter-Andean dry-valleys of Bolivia (Köhler et al., 1995). In addition to the above mentioned locality, we heard calls of *L. gracilis* at Guadalupe and at approximately 5 km south of Vallegrande, Provincia Vallegrande (ca. 2000 m a.s.l.). Juveniles were found in February 1998 under rocks at “El Fuerte”, 5 km east of Samaipata, Provincia Florida, at 1900 m a.s.l. (all localities within Departamento Santa Cruz, Bolivia).

Advertisement calls of *L. rhodonotus* (fig. 2) were recorded on 8 February 1998 at approximately 15.5 km south by road from Paracti to Palmar (17°06'06"S/65°30'36"W), Provincia Chapare, Departamento Cochabamba, Bolivia, 650 m a.s.l. Males called during heavy rain from a roadside ditch. The call consists of a single frequency modulated note repeated in regular intervals (173.2 notes/minute). Mean note duration is 54.7 ms and frequency is distributed between 1680 and 2530 Hz. The calls are distinctly pulsed. In some calls six to eight pulses are countable, in others the pulse structure appears to be

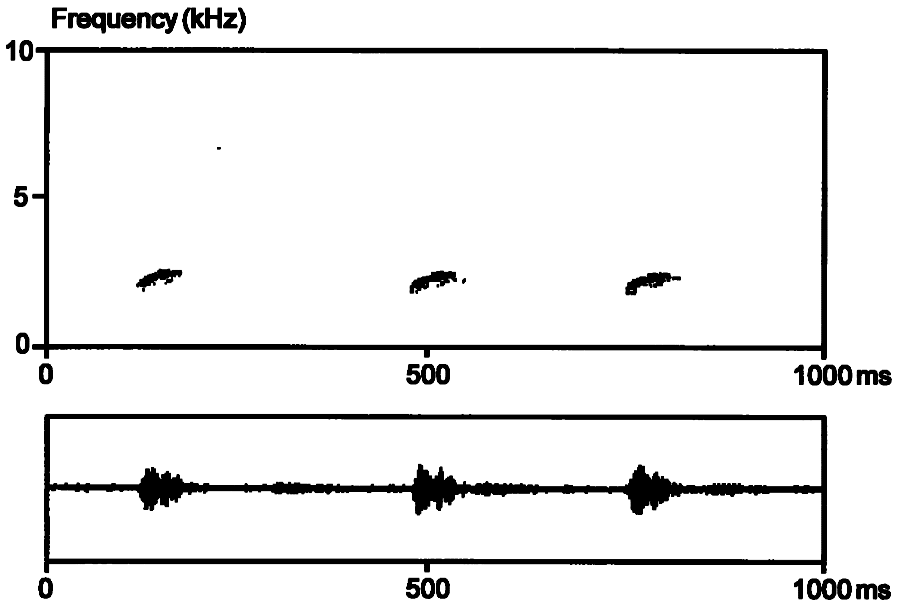


Figure 2. Spectrogram (above) and oscillogram (below) of advertisement call of *Leptodactylus rhodonotus* from a point 15.5 km S by road of Paracti, Depto. Cochabamba, Bolivia. Air temperature 24.8°C.

more complex. Calls exhibit harmonic structures. To the best of our knowledge no data are available for comparison.

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References

- Barrio, A. (1973): *Leptodactylus geminus*, una nueva especie del grupo *fuscus* (Anura, Leptodactylidae). *Physis* **32**: 199-206.
- Cardoso, A.J. (1985): Revalidation of *Leptodactylus plaumanni* (Amphibia: Leptodactylidae). *Pap. Avul. Zool. São Paulo* **36**: 87-90.
- Cei, J.M. (1980): Amphibians of Argentina. *Monogr. Monitore Zool. Ital.* **2**: 1-609.
- Heyer, W.R. (1978): Systematics of the *fuscus* species group of the frog genus *Leptodactylus* (Amphibia, Leptodactylidae). *Bull. Los Angeles Co. Mus. Nat. Hist.* **29**: 1-85.
- Heyer, W.R. (1979): Systematics of the *pentadactylus* species group of the frog genus *Leptodactylus* (Amphibia, Leptodactylidae). *Smiths. Contrib. Zool.* **301**: 1-43.
- Köhler, J., Ibisch, P.L., Dirksen, L., Böhme, W. (1995): Zur Herpetofauna der semihumiden Samaipata-Region, Bolivien. I. Amphibien. *Herpetofauna* **17**: 13-24.
- Reynolds, R.P., Foster, M.S. (1992): Four new species of frogs and one new species of snake from the Chapare region of Bolivia, with notes on other species. *Herpetol. Monogr.* **6**: 83-104.

- Rodríguez, L.O., Duellman, W.E. (1994): Guide to the frogs of the Iquitos region, Amazonian Peru. Nat. Hist. Mus. Univ. Kansas Special Publ. 22: 1-80.
- Scrocchi, G.J., Lavilla, E.O. (1986): Caracteres para la identificación de las especies crípticas *Leptodactylus gracilis* y *Leptodactylus geminus* (Anura: Leptodactylidae). Physis C 44: 93-95.

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Mass-rearing of plethodontid salamander eggs

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Pond-breeding anuran amphibians (e.g. *Rana*, *Bufo*, *Scaphiopus*, *Acris*, *Hyla*) have long served as model organisms in experimental ecological and genetic studies. These species freely spawn thousands of eggs that can be harvested easily and hatched rapidly with little special care making it possible to quickly set up large, statistically meaningful experiments (reviewed by Wilbur, 1997). For example, in a few days Wilbur and Fauth (1992) set up an experiment involving 32,000 *Bufo* and *Rana* tadpoles. In addition to the ease of studying anurans in experimental arenas, their external fertilization facilitates the use of quantitative genetic breeding designs to study anuran life history traits (e.g., Berven, 1982; Travis, 1980, 1981, 1983; Travis et al., 1987; Newman, 1988a, b, 1989).

Other common amphibians with different life histories have not been studied in large ecological experiments or using breeding designs. For example, lungless salamanders of the family Plethodontidae, the most diverse clade of salamanders (Dunn, 1926), are extremely common in eastern North American deciduous forests achieving biomasses that exceed those of birds or mammals (Burton and Likens, 1975). Plethodontid ecological diversity and life history variation are extraordinary, as are behavioral repertoires involved with species recognition and courtship (Tilley et al., 1990; Tilley and Bernardo, 1993; Arnold et al., 1993, 1996). Many aspects of plethodontid biology have been studied extensively for a century by systematists, behaviorists, developmentalists, and ecologists, but field-experimental and genetic analyses are rare.

Several attributes of plethodontids have hindered their use as model systems in genetical studies of ecological and behavioral problems. One difficulty is simply obtaining large numbers of eggs for experimental studies. Most plethodontids oviposit secretively, either terrestrially or subterraneously; many species' nests have seldom or never been observed in the field. Another problem is rearing eggs through to hatching. Unlike commonly studied anurans, plethodontids have internal fertilization and lay small clutches of large, yolky eggs with prolonged development (Collazo, 1996), and females brood the eggs. Females consume dead eggs, eliminating the danger of fungal infection of live eggs. Egg mortality

in the absence of brooding females is substantial due to desiccation and fungal infection (Forester, 1979). However, in the laboratory females tend to consume entire clutches when disturbed (Houck et al., 1985; personal observations). Thus, even if many clutches can be collected, it is difficult to rear large numbers of eggs to hatching.

Techniques have been developed for studying plethodontid eggs and hatchlings. Oviposition can be induced with hormones (Verrell, 1989; Collazo and Marks, 1994; Collazo, 1996) and small numbers of eggs can be carefully husbanded for developmental studies (Tilley, 1972; Collazo, 1996; Jockusch, 1996). Eggs have been obtained from more than 25 species (review: Jockusch, 1996). Thus, it is possible to obtain eggs of even secretive species by collecting gravid females in the field rather than by collecting eggs directly. Typically, eggs are maintained on moist paper toweling in closed plastic boxes, and are rinsed regularly. However, egg survival using these terrestrial husbandry methods is often low (Forester, 1979).

Houck et al. (1985) reared seven clutches of *Desmognathus ochrophaeus* (= *D. ocoee*) eggs using a terrestrial brooding technique. Eggs were housed in small perforated capsules maintained on moist filter paper in petri dishes which were housed in a larger box also lined with paper toweling. Eggs were rinsed daily for a month by submerging the capsule in water, and filter papers in the petri dishes were changed daily (Houck et al., 1985). This approach yielded 95% egg survival but was labor intensive (SJA, personal observation).

Vess and Harris (1997) used a similar approach for *Hemidactylium scutatum* to husband larger numbers of eggs. Eggs were housed on moist filter paper in covered plastic containers and they were cleaned with a series of steps that involved moving the eggs and rinsing them three times. Eggs were treated this way daily to every few days for most of embryonic development and then daily towards the end of development. Containers were rinsed, swabbed, ethanol sterilized, and received fresh filter paper daily during early development, but only weekly for near-term embryos. The reason for less frequent egg handling and container cleaning later in development was a concern that mechanical stimulation might cause premature hatching. Vess and Harris achieved high hatching success (467/570 eggs hatched, = 81.9%) with *H. scutatum*, and they report comparable success with *Desmognathus* eggs.

Despite its effectiveness, artificial terrestrial brooding of plethodontid salamander eggs is simply not practical for very large experiments. First, it is labor intensive and thus impractical for large-scale experiments. This large effort is required on a daily basis; lack of attention for a single day can allow progression of fungus to lethal levels (personal observations). Second, these methods require physical handling of eggs that can unintentionally damage embryos (e.g., puncturing eggs with forceps, dropping eggs, etc.). Last, it is possible to introduce heterogeneity in rearing conditions, particularly in large experimental designs requiring that several individuals handle eggs to maintain necessary daily vigilance. Here we describe a passive technique for rearing thousands of plethodontid eggs using equipment commonly used in fish aquaculture.

We have been conducting large controlled breeding experiments with mountain dusky salamanders (*Desmognathus ocoee*) involving hundreds of clutches. *Desmognathus ocoee* has an aquatic larval phase, but females oviposit terrestrially in stream banks under moss, or under moss that covers logs and rocks in the streambed. It is widely held that several species of *Desmognathus* only oviposit terrestrially, but we have observed inundated clutches of several of these species (*D. santeetlah*, *D. ocoee*, *D. carolinensis*, *D. aeneus*, *D. fuscus*) in the field, producing viable larvae (JB, pers. obs.). These observations suggested that submerged rearing might be a fruitful alternative to terrestrial brooding methods. Further detailed observations in the lab (JB) confirmed that *D. ocoee* eggs develop and hatch normally when submerged, without developing fungus as readily as when eggs are artificially brooded on paper towels (Houck et al., 1985). We reasoned that an egg washer that circulates water would be an efficient means of caring for eggs.

We used a device for rearing salmonid eggs: a Marisource (Tacoma, WA; 206-475-5772; available from Aquacenter, Leland, MS 38756; 1-800-748-8921) 4-Tray Vertical Incubation System with a screened lid (4.7×4.7 strand per cm mesh) coupled with a drip pan to catch the water from the bottom tray. The rearing system (egg washer hereafter) is a series of interchangeable molded plastic trays with removable screened lids. The design of the units forces water to upwell through the bottom rear of the tray and escape through the top screen into a drainage conduit and then into the tray below. Water was filtered and recirculated with a Marineland Magnum 350 Deluxe Canister filtration system with a water polisher cartridge (2,064 cm² filtration surface) and carbon filter. The inlet to the pump was placed in the optional drainage tray at the base of the egg washing unit, and the outlet was placed in the gutter of the upper tray. The egg wash unit and pump combined contained ~ 25 liters of water. We used city tap water both in Chicago, Illinois (1996) and in Highlands, North Carolina (1997) with no discernible negative effects on larvae. Water lost to evaporation was replaced as needed every few days with tap water. The pump circulated about 1400 liters / hour, so the water in the system recirculated more than 50 times per hour. Temperature was not controlled except that the entire apparatus was placed in a cool room (15-18°C) in which adult salamanders are regularly housed.

Recently laid eggs were introduced into the egg washer in small, perforated plastic dishes normally used for histological preparations (Houck et al., 1985). We used Fisherbrand (Fisher Scientific, Pittsburgh, PA, USA, 1-800-766-7000) hinged Histoprep polypropylene capsules (29 mm diameter \times 6 mm deep). Several sizes of capsules are available; larger capsules have larger perforations. Care must be exercised to use a fine enough perforation to prevent small eggs from escaping. For very small eggs, we lined the inside of the capsule with a piece of fiberglass mesh. Information can be written directly on the capsule.

Eggs were separated from each other using forceps and 2 to 12 eggs (depending on egg size) per clutch were placed in each of several capsules. We considered it important to split a single clutch among several capsules both to decrease overall egg density per capsule, and to avoid confounding a particular clutch with a particular microenvironment

in a single capsule or in a single tray of the egg washer. Unseparated egg clusters are more likely to die during early development than clusters that have been separated, presumably due to oxygen deficit within a cluster. Importantly, larger eggs require greater separation among capsules. We maintained at least 50% (but usually more) of the surface area inside each capsule open to water flow and gas exchange. Capsules were immersed in the egg washer taking care to put different capsules containing eggs of a single clutch in separate trays. The capsules float in the upwelling water so do not remain in a single position in the tray, but are kept in the tray by the screened lid.

We successfully used this system in two large breeding experiments. We attempted to rear 1,541 eggs from 102 females in 1996, and 1,056 eggs from 91 females in 1997. In our earliest trials we found that, when embryos were left in the egg washer until hatching, even seemingly large larvae inevitably escaped through the small (1×1 mm) perforations in the capsules. Thus, it is essential to remove near-term embryos from the egg washer. As embryos neared hatching (when embryos moved within the eggs), we transferred the contents of a single capsule (1-10 embryos) into small petri dishes with tight-fitting lids with 1-2 ml of water. Embryos were left undisturbed at room temperature after transfer to avoid causing premature hatching.

Because the eggs were being reared as part of another experiment, we did not attempt to evaluate egg survivorship systematically as a function of degree of physical separation in the capsules but we did make several repeated observations concerning egg survival. When an egg died in an unseparated egg cluster, adjacent eggs also died, but when eggs were separated and an egg died, nearby eggs often remain viable even if the dead egg deteriorated before it was removed. More crowded capsules tended to experience greater egg mortality. We seldom observed growth of fungal mycelia, although hyphae did occur on some dead eggs that were not removed immediately. However, live eggs were far less likely to become infected by fungal hyphae when submerged in the egg washer than during artificial terrestrial brooding trials. Overall there was 35.2% hatching success in 1996 and 54.2% in 1997. We attribute the lower overall hatching success in 1996 to the fact that we did not separate eggs as much as in subsequent clutches and those reared in 1997.

We observed minor capsule and tray effects on developmental rates. Because each clutch was split into at least two capsules, and because all capsules were checked for developmental progress on the same day, differences in developmental progress between capsules from the same clutch but occupying different trays were easy to detect. The differences were never dramatic, however (~ 1 day difference in hatching time through an average larval period of 35 days).

This egg-husbandry method may be widely applicable to rearing the eggs of other salamanders. The system should work well for aquatic breeders such as hemidactyliine plethodontids, as well as other stream breeding amphibians such as species of Ascaphidae, Proteidae, Cryptobranchidae, Rhyacotritonidae, Dicamptodontidae, and Salamandridae and various stream-breeding tropical frogs. Other aquatic organisms including invertebrates

might also be easily cultured using this system, with slight modifications of the capsules (use of fine screen to contain small eggs).

However, the egg washer may also function well for terrestrially-ovipositing, direct-developing species. A clutch of *Plethodon jordani* eggs was spontaneously laid in late July by a female collected a year earlier on Wayah Bald, Macon Co., NC. The clutch was discovered in the laboratory on August 1 and was immediately submerged in flowing water at 17°C. Most of the clutch developed and hatched normally. Thus, the eggs of *P. jordani*, a species with direct development, can be successfully reared underwater, suggesting that the eggs of directly-developing plethodontids can also be reared en masse using this egg washer.

Our approach is both efficient and relatively inexpensive (~ \$650 including filter), and eliminates a key constraint on future large ecological and genetic studies of early life history stages of plethodontids. Efficiency is mainly achieved because the device allows relatively passive care for many eggs. Our study used a fraction of the capacity of the 4-shelf incubator, and multiple units can be stacked to expand capacity. The incubator also obviates problems associated with mechanical handling of eggs, especially of near-term embryos that may be caused to hatch prematurely. There are no exposed egg surfaces upon which fungal hyphae proliferate, and the constant filtering maintains highly aerated and clean water. Heterogeneity due to handling differences between investigators is eliminated, and the slight position effects we observed can be eliminated by alternating tray position.

This method will allow expanded studies of the early life history of plethodontid and other stream-breeding amphibians, thereby allowing extensions of research programs that have heretofore focused on pond-breeding Anura. It will also allow new experimental and field studies of parental investment and early life history of plethodontids and other salamanders that have not been well-studied (Bernardo, 1996a, b).

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References

- Arnold, S.J., Reagan, N.L., Verrell, P.A. (1993): Reproductive isolation and speciation in plethodontid salamanders. *Herpetologica* **49**: 216-218.
- Arnold, S.J., Verrell, P.A., Tilley, S.G. (1996): The evolution of asymmetry in sexual isolation: a model and test case. *Evolution* **50**: 1024-1033.
- Bernardo, J. (1996a): Maternal effects in animal ecology. *Am. Zool.* **36**: 83-105.
- Bernardo, J. (1996b): The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. *Am. Zool.* **36**: 216-236.
- Berven, K.A. (1982): The genetic basis of altitudinal variation in the wood frog *Rana sylvatica* II. An experimental analysis of larval development. *Oecologia* **52**: 360-369.

- Burton, T.M., Likens, G.E. (1975): Salamander populations and biomass in the Hubbard Brook Experimental Forest, New Hampshire. *Copeia* **1975**: 541-546.
- Collazo, A. (1996): Evolutionary correlations between early development and life history in plethodontid salamanders and teleost fishes. *Am. Zool.* **36**: 116-131.
- Collazo, A., Marks, S.B. (1994): Development of *Gyrinophilus porphyriticus*: Identification of the ancestral developmental pattern in the salamander family Plethodontidae. *J. Exper. Zool.* **268**: 239-258.
- Dunn, E.R. (1926): The Salamanders of the family Plethodontidae. Smith College 50th Anniversary Publications, Smith College, Northampton, Mass.
- Forester, D.C. (1979): The adaptiveness of parental care in *Desmognathus ochrophaeus* (Urodela: Plethodontidae). *Copeia* **1979**: 332-341.
- Houck, L.D., Tilley, S.G., Arnold, S.J. (1985): Sperm competition in a plethodontid salamander: preliminary results. *J. Herpetol.* **19**: 420-423.
- Jockusch, E.L. (1996): Techniques for obtaining and raising plethodontid salamander eggs. *Int. J. Dev. Biol.* **40**: 911-912.
- Newman, R.A. (1988a): Genetic variation for larval anuran (*Scaphiopus couchii*) development time in an uncertain environment. *Evolution* **42**: 763-773.
- Newman, R.A. (1988b): Adaptive plasticity in development of *Scaphiopus couchii* tadpoles in desert ponds. *Evolution* **42**: 774-783.
- Newman, R.A. (1989): Developmental plasticity of *Scaphiopus couchii* tadpoles in an unpredictable environment. *Ecology* **70**: 1775-1787.
- Tilley, S.G. (1972): Aspects of parental care and embryonic development in *Desmognathus ochrophaeus*. *Copeia* **1972**: 532-540.
- Tilley, S.G., Bernardo, J. (1993): Life history evolution in plethodontid salamanders. *Herpetologica* **49**: 154-163.
- Tilley, S.G., Verrell, P.A., Arnold, S.J. (1990): Correspondence between sexual isolation and allozyme differentiation: A test in the salamander *Desmognathus ochrophaeus*. *Proc. Natl. Acad. Sci. USA* **87**: 2715-2719.
- Travis, J. (1980): Genetic variation for larval specific growth rate in the frog *Hyla gratiosa*. *Growth* **44**: 167-181.
- Travis, J. (1981): The control of larval growth variation in a population of *Pseudacris triseriata* (Anura: Hylidae). *Evolution* **35**: 423-432.
- Travis, J. (1983): Variation in growth and survival of *Hyla gratiosa* larvae in experimental enclosures. *Copeia* **1983**: 232-237.
- Travis, J., Emerson, S.B., Blouin, M. (1987): A quantitative-genetic analysis of larval life-history traits in *Hyla crucifer*. *Evolution* **41**: 145-156.
- Verrell, P.A. (1989): Hormonal induction of ovulation and oviposition in the salamander *Desmognathus ochrophaeus* (Plethodontidae). *Herpetol. Rev.* **20**: 42-43.
- Vess, T.J., Harris, R.N. (1997): Artificial brooding of salamander eggs. *Herpetol. Rev.* **28**: 80.
- Wilbur, H.M. (1997): Experimental ecology of food webs: complex systems in temporary ponds. *Ecology* **78**: 2279-2302.
- Wilbur, H.M., Fauth, J.E. (1990): Experimental aquatic food webs: interactions between two predators and two prey. *Am. Nat.* **135**: 176-204.

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