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WATER RELATIONS
OF THE
ENDEMIC NEW ZEALAND FROGS
LEIOPELMA ARCHEYI, *L. HAMILTONI*
AND *L. HOCHSTETTERI*

*A thesis
submitted in fulfilment
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of
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*by
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ABSTRACT

Aspects of habitat and water balance physiology were compared between the endemic and anatomically primitive New Zealand frogs *Leiopelma archeyi*, *L. hamiltoni* and *L. hochstetteri*. Field studies in an area of the Coromandel Range showed that the partially sympatric *L. archeyi* and *L. hochstetteri* had nearly disjunct distributions. *L. archeyi* occurred in terrestrial habitats on the forested ridgetops, whereas most *L. hochstetteri* were found in semiaquatic habitats along the edges of streams. *L. archeyi* also showed less preference for water than *L. hochstetteri* in the laboratory. Climatic conditions in the ridgetop habitat of *L. archeyi* were usually cool and humid, and resembled those previously reported for the allopatric and completely terrestrial *L. hamiltoni*.

In laboratory studies, all three species dehydrated rapidly in dry conditions. Resistance to evaporative water loss (EWL) was similar to that for a free water surface. *L. archeyi* and *L. hamiltoni* rehydrated rapidly (in 1-2 h) when returned to shallow water after dehydration to 9-15% weight loss. Rapid rehydration was due to increased water uptake through ventral skin and to reduced bladder urine accumulation (cutaneous and antidiuretic water balance responses). *L. hochstetteri* rehydrated very slowly (rehydration incomplete within 16 h) and showed only an antidiuretic response. *L. hochstetteri* also rehydrated more slowly from moist soil when compared with *L. archeyi*.

Treatment with arginine vasotocin (AVT, 7×10^{-11} mol g⁻¹) produced water balance responses similar to those seen during rehydration. *L. archeyi* and *L. hamiltoni* placed in shallow water showed rapid water retention, reaching weight gains of 31% and 29% respectively 4-5 h after treatment. *L. hochstetteri* gained weight at a slow and linear rate (9% over 9 h). Unlike *L. archeyi* and *L. hamiltoni*, *L. hochstetteri* showed no increase in cutaneous water uptake, and water retention was attributed solely to antidiuresis.

In vitro experiments confirmed that AVT (3×10^{-8} mol l⁻¹) caused increased osmotic water flow (OWF) through ventral pelvic skin of *L. archeyi* and *L. hamiltoni*. However, pelvic skin from *L. hochstetteri* and ventral pectoral and dorsal skin from all three species showed no hydroosmotic response. The bladders of the three

species showed small increases in OWF in response to AVT. *In vivo*, glomerular filtration rate fell following AVT treatment in all species, although the effect on *L. hochstetteri* was not statistically significant.

Pelvic skin of *L. archeyi* showed no obvious structural specialisation for rapid water uptake, when examined using light and scanning electron microscopy. Pelvic skin was smooth, structurally similar to non-glandular areas of pectoral or dorsal skin, and less vascularised than pelvic skin of *L. hochstetteri*.

Adults of all three species were ureotelic. In the terrestrial eggs and larvae of *L. archeyi*, intracapsular larvae were ammonotelic, but most larvae became ureotelic within 4 d after hatching. The eggs had no resistance to EWL, but absorbed water rapidly from moist surfaces. Male parental care (egg brooding, larval transport) led to reduced EWL from eggs and hatched larvae of *L. archeyi*.

This study demonstrates that the presence of a cutaneous hydroosmotic response varies between adults of the three species in an inverse manner with habitat water availability. It also supports previous studies indicating a closer ecological similarity and evolutionary relationship between *L. archeyi* and *L. hamiltoni*, than between either and *L. hochstetteri*.



FRONTISPIECE

Leiopelma archeyi emerged at night in the Tapu study area.

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ABBREVIATIONS

Commonly used SI units and abbreviations mentioned only occasionally in the text are not listed here.

asl = above sea level
AVP = arginine vasopressin
AVT = arginine vasotocin
C_{CR} = creatinine clearance
d = day
EWL = evaporative water loss
GFR = glomerular filtration rate
h = hour
id = internal diameter
kPa = kilopascal
L. = *Leiopelma* (wherever it occurs)
MC = substrate moisture content
min = minute
mo = month
n = sample size
N = nitrogen
NHP = neurohypophysial peptide
OT = oxytocin
OWF = osmotic water flow
P = statistical probability
RH = relative humidity
RH_a = ambient relative humidity
RH_r = retreat site relative humidity
r_s = Spearman rank correlation coefficient
s = second
SA = surface area
SE = standard error of the mean
SVL = snout to vent length
T = temperature
T_a = ambient temperature
T_r = retreat site temperature
UWL = urinary water loss
wk = week

Abbreviations (cont.)

W_0 = initial weight of frog with
bladder urine removed

12 h L : 12 h D = 12 h of light followed by 12 h of
darkness

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

GENERAL INTRODUCTION

The aim of this thesis is to determine whether there is a relationship between habitat and aspects of water balance physiology in the endemic New Zealand frogs *Leiopelma archeyi* Turbott 1942, *L. hamiltoni* (McCulloch 1919) and *L. hochstetteri* Fitzinger 1861. *L. archeyi* and *L. hamiltoni* are both terrestrial frogs which inhabit forested slopes, islands or mountain ridgetops lacking permanent free-standing water, whilst *L. hochstetteri* is semiaquatic, usually living along the edges of forested streams (Bell *et al.* 1985). The question addressed in this thesis is: do *L. archeyi* and *L. hamiltoni* have mechanisms for maintaining water balance which differ from those in the more aquatic species *L. hochstetteri*?

Leiopelma archeyi is a small and slender frog, often beautifully patterned with colours of brown, black, tan, pink and sometimes green (Plate i.1). *L. hamiltoni* is morphologically similar (Plate i.2), but lacks obvious green colouration and grows to a larger size (Bell 1978). Both species have a large dorso-lateral glandular ridge (parotoid ridge; Bell 1978) behind each eye, and essentially no toe webbing. In contrast, *L. hochstetteri* is drab in colour (adults are predominantly dark brown or dark olive-green), with a sturdy appearance, half-webbed toes and less pronounced glandular ridges than in either *L. archeyi* or *L. hamiltoni* (Plate i.3). Maximum sizes measured by Bell (1978) were 36, 49 and 44 mm snout-vent length (SVL) for *L. archeyi*, *L. hamiltoni* and *L. hochstetteri* respectively.

All three species are uncommon and inconspicuous. *L. hochstetteri* has the most widespread distribution, occurring in forested patches of the North Island from Northland to Pureora, and on Great Barrier Island (Bell *et al.* 1985; Fig. i.1). *L. archeyi* occurs on mountain ranges in or near the Coromandel Peninsula, in some cases overlapping in distribution with *L. hochstetteri*. *L. hamiltoni* has the most restricted distribution, being known only from an overgrown rock pile known as the "frog bank" on the summit of Stephens' Island, and from a forested slope on Maud Island. Both islands are in the Cook Strait - Malborough Sounds region (Fig. i.1).

Subfossil evidence indicates that *L. hamiltoni* and *L. hochstetteri* were once more widespread in New Zealand (Worthy 1986), and their

PLATE i.1 *Leiopelma archeyi* (mature female, 36 mm SVL). A = lateral view, B = dorsal view, C = ventral view (ovarian egg visible through body wall in C).

A



B



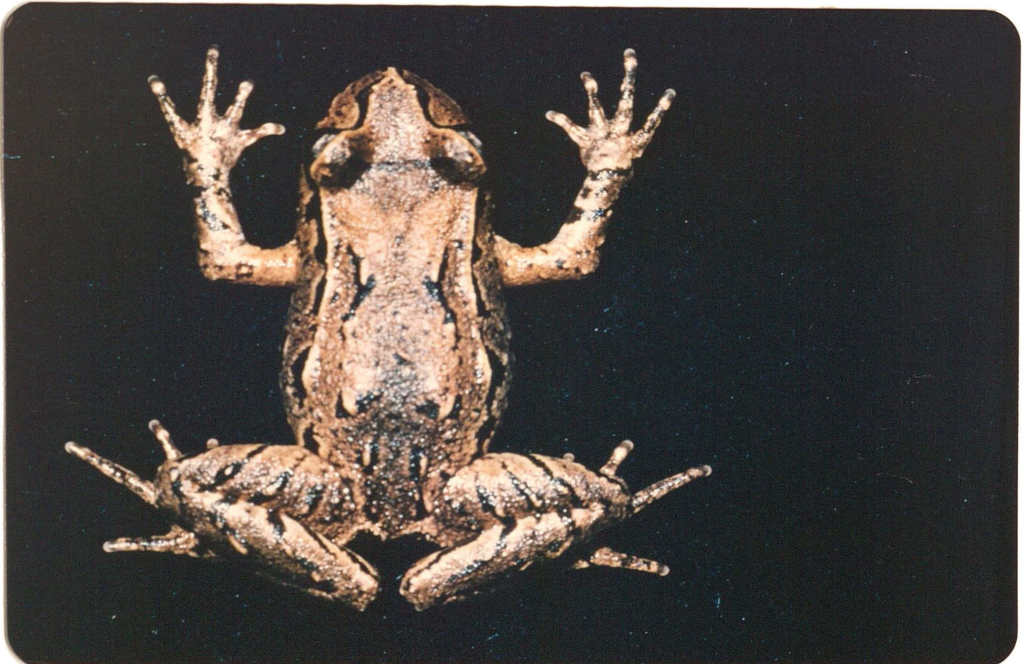
C



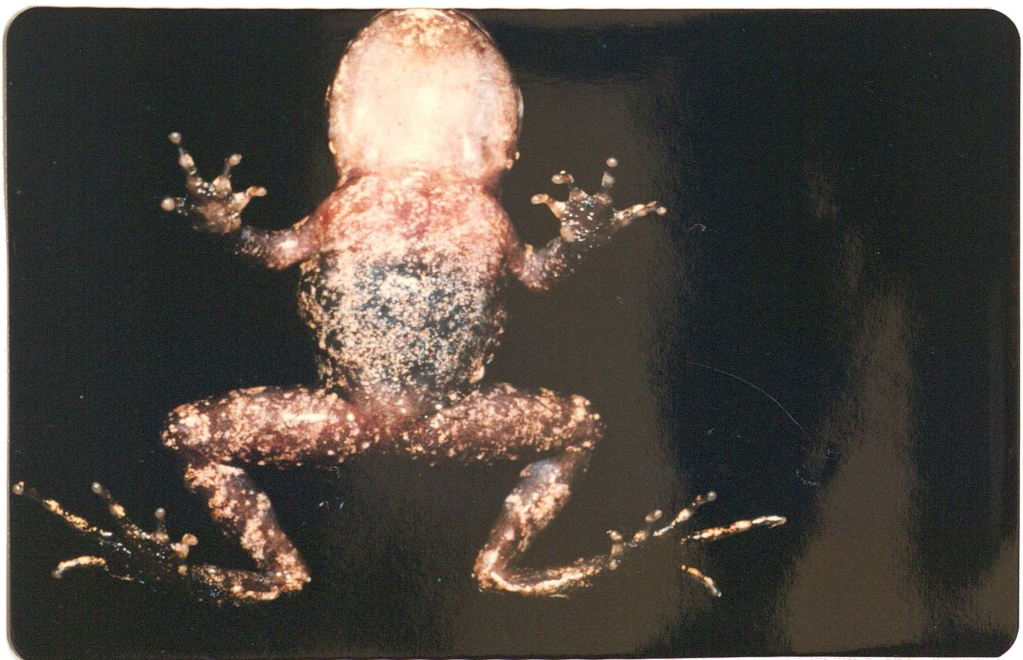
PLATE i.2 *L. hamiltoni*. A and B = lateral and dorsal views of a mature female (40 mm SVL). C = ventral view (mature male, 39 mm SVL).



A



B



C

PLATE i.3 *L. hochstetteri* (mature female, 41 mm SVL).
A = lateral view, B = dorsal view, C = ventral view.

A



B



C



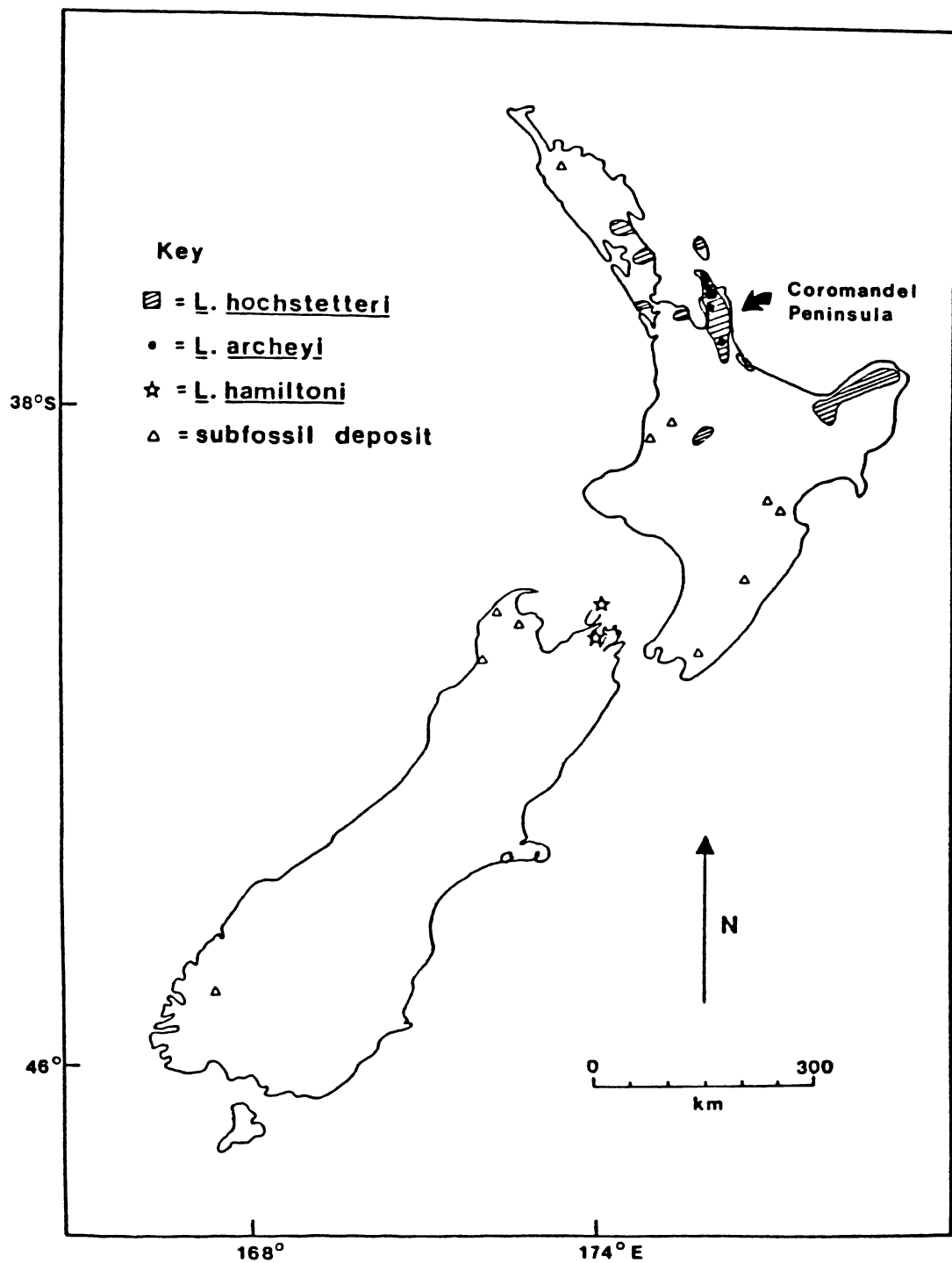


FIG. i.1 Distribution of extant species and subfossil deposits of *Leiopelma* in New Zealand (after Bell *et al.* 1985).

present restricted distributions are attributed partly to habitat destruction (Bell 1985a). The conservation status of all three species is described as "rare" in the Red Data Book of New Zealand (Williams and Given 1981), and the frogs are "absolutely protected" (from illegal disturbance or collection) by the New Zealand Wildlife Act 1953. Three additional and apparently extinct species of *Leiopelma* have recently been described from subfossil material (Worthy 1986; Fig. i.1).

L. archeyi, *L. hamiltoni* and *L. hochstetteri* are regarded as amongst the most anatomically primitive of living anurans (Griffiths 1963; Lynch 1973; Duellman 1975; Carroll 1977). Primitive features, some of which are shared with South American fossil anurans of Jurassic age (Estes and Reig 1973), are the presence of nine presacral vertebrae (most living frogs have eight or fewer), an amphicoelous vertebral structure, the presence of free ribs (unfused to the vertebrae) and the retention in the adult of the "tail-wagging" muscle *m. caudali(o)puboischiotibialis* (E.M. Stephenson 1961; Kluge and Farris 1969; Bell 1982a). The same features are present in the North American tailed frog *Ascaphus truei*, which is generally considered to belong to the genus most closely related amongst living frogs to *Leiopelma*. However, the relationship is not necessarily a close one, and recent studies place the two genera in separate families (Ascaphidae and Leiopelmatidae) (Savage 1973; Green *et al.* 1980), a distinction supported by Worthy (1986). Both genera are included in the suborder of "archaic frogs", the Archaeobatrachia (Duellman 1975).

Much of the previous work on *Leiopelma* has been reviewed by Bell (1982a, 1982b). Early studies by N.G. Stephenson and E.M. Stephenson (née Thomas) described aspects of development, skeletal structure and vascular systems in the genus, and reported on the distribution and habitats of *L. archeyi* and *L. hochstetteri* (E.M. Stephenson 1951, 1952, 1955, 1960, 1961; N.G. Stephenson 1951a, 1951b, 1955; Stephenson and Stephenson 1947, 1957; Stephenson and Thomas 1945). Later, the same authors and others examined karyotypes and oocyte cytology within the genus (Morescalchi 1968; Stephenson *et al.* 1972, 1974; Robinson *et al.* 1973; Green *et al.* 1984), and Moffat (1974) described development and structure of the vertebral column. Aspects of habitat and/or development were reported for *L. archeyi* by Archey (1922) (under the impression that he was examining *L. hochstetteri*), and for *L. hochstetteri* by Turbott (1942, 1949), McLennan (1985), Newman and

Towns (1985), and Whitaker and Hardy (1985). Crook *et al.* (1971), Newman (1977, 1982) and Newman *et al.* (1978) have described studies by the New Zealand Wildlife Service on the habits and habitats of the rarest species, *L. hamiltoni*, and Kane (1980) examined the diet of the same species. *L. hochstetteri* is the most widely studied of the three frogs, aspects of the vascular system having been described by Szarski (1951) and Czopek (1955), the structure of the carotid labyrinth by Carman (1967) and Ensor (1976), and the structure of gustatory papillae by MacDonald (1975). Olmo (1973) cited unpublished studies by Sexsmith on the nuclear DNA content of *L. archeyi*, and a popular account of the biology of the three species has been given by Robb (1980).

An extensive contribution to recent knowledge of the ecology, morphology, reproduction and development of the *Leiopelma* species has been made by Bell (1977, 1978, 1982a, 1982b, 1985a, 1985b; Bell *et al.* 1985). Bell's studies have emphasised a clear separation between *L. archeyi* and *L. hamiltoni* on the one hand, and *L. hochstetteri* on the other. *L. archeyi* and *L. hamiltoni* are morphologically similar, and have similar, although allopatric, habitats. Development in all three species is specialised and unusual amongst anurans, involving a relatively long period (about 6 wk) of intracapsular development within large, yolky eggs, followed by a period of similar length of non-feeding larval development. However, differences in detail exist between the two groups. *L. archeyi* and *L. hamiltoni* lay their egg clusters on land and these are brooded by male frogs. Following hatching, the tailed larvae complete their development on the back of the male. *L. hochstetteri* lays its eggs in moist seepages and the clusters are not brooded. Its hatched larvae are semiaquatic. Differences in parental care, larval morphology and behaviour between the two groups are attributed to the more aquatic breeding habitats of *L. hochstetteri* (Bell 1982a, 1985b).

Recent cytological and electrophoretic studies support the division of the three species into two groups. *L. hochstetteri* has 22 major chromosomes whereas *L. archeyi* and *L. hamiltoni* have 18 (Stephenson *et al.* 1972, 1974), and liver enzymes and blood proteins of *L. archeyi* and *L. hamiltoni* are more similar to those of each other than they are to those of *L. hochstetteri* (Daugherty *et al.* 1981, 1982). Assuming constant rates of genetic change, Daugherty *et al.* (1981, 1982) estimated that the divergence between the *L. archeyi/L. hamiltoni* and

L. hochstetteri lineages occurred 13-15 million years ago, and that between *L. archeyi* and *L. hamiltoni* about 3-5 million years ago. A recent study indicates that, other than in size, *L. archeyi* and *L. hamiltoni* are osteologically identical (Worthy 1986).

Current work on the *Leiopelma* species includes studies of leucocyte enzyme cytology in *L. hochstetteri* (Dr P.M.Hine, Fisheries Research Division, Wellington, pers. comm.) and of eye structure in all three species (Dr V.B.Meyer-Rochow, University of Waikato, pers. comm.). However, apart from a single unpublished study which investigated aspects of water and salt balance in *L. hochstetteri* (Cameron 1974), the environmental physiology of these interesting frogs remains unexamined. This thesis aims in part to help redress this imbalance, by examining the relationship between habitat and physiological aspects of water balance in the three species.

Before presenting the experimental results of this study, a review of current knowledge on water balance of amphibians is presented (Chapter 1). This review summarises differences which have been found between species from aquatic and more terrestrial habitats, and explains the rationale behind the choice of experiments carried out in this study. Following this, the habitats of the two species about which least information on habitat is available (*L. archeyi* and *L. hochstetteri*) are described, for an area of the Coromandel Peninsula where both occur (Chapter 2). Chapters 3-7 describe rates of dehydration and rehydration, responses to the neurohypophysial hormone arginine vasotocin, skin structure and nitrogen excretion in some or all of the three species. Reproductive and behavioural adaptations of the terrestrial frog *L. archeyi* are discussed in Chapters 8 and 9. The thesis concludes with a General Discussion (Chapter 10), in which the results from field studies of habitat and laboratory studies of water balance are integrated to help develop an overall view of water relations for the three species. In addition, the results are discussed in relation to the evolutionary relationships within the genus, and between the genus and other more advanced anurans.

CHAPTER 1

WATER BALANCE OF AMPHIBIANS: A REVIEW

Amphibians have attracted considerable interest over the last two hundred years in terms of their ability to maintain water balance. This interest reflects their position as members of the first class of tetrapods to evolve, the success of many modern forms in both aquatic and terrestrial habitats at various stages of the lifecycle, and their amenability to experimentation. Hydrated adult amphibians have a water content equivalent to about 79% of body weight and this must be regulated within certain limits in order to survive (Alvarado 1979). Most studies have focussed on the physiological mechanisms of adult amphibians (particularly adult anurans) which help to achieve this, and the following brief account reviews aspects of this work relevant to the present study. Although early efforts concentrated largely on three common laboratory genera of anurans (*Rana*, *Bufo* and *Xenopus*), increasing attention has been given over the last 20 years to less well-known anurans and to urodeles, and in many cases this has yielded valuable new information (see reviews by Bentley 1971a, 1982a, 1982b; Shoemaker and Nagy 1977; Alvarado 1979). As yet, features of water balance in the third order of living amphibians, the caecilians, remain essentially unknown.

Recently, interest has also been directed to the physiological problems facing terrestrial anuran eggs (e.g., Shoemaker and McClanahan 1973; Taigen *et al.* 1984) and to behavioural factors influencing water balance in free-roaming adult anurans (e.g., Pough *et al.* 1983); these aspects are discussed further in Chapters 7 and 8, and 9 respectively.

Evaporative water loss

Many studies have examined the ability of amphibians to resist dehydration. The results show that amphibians in dry surroundings can be placed into three broad groups according to their ability to control evaporative water loss (EWL) from the skin (Withers *et al.* 1982). The largest group includes those with a moist, permeable skin having little or no resistance to water loss (Bentley 1982a), and

includes both aquatic species, and terrestrial species from mesic environments. Evaporation from the skin of such species may occur at rapid surface area (SA)-specific rates depending on environmental conditions such as temperature, humidity and air speed (Tracy 1976), and water losses from the respiratory tract are insignificant by comparison (Spotila and Berman 1976; Bentley and Yorio 1979a; Wygoda 1981). In a widely cited study, Elkan (1976) claimed that "ground substance", a layer present in dorsal skin of terrestrial anurans within this group impeded water loss, but this claim has not been substantiated and attempts to do so suggest that its role is largely structural (Cameron 1980). Small adaptive differences in rates of weight-specific EWL between species in this group are probably due largely to differences in size and behaviour (Shoemaker and Nagy 1977), and many of the more terrestrial species become less active and adopt postures which reduce the area of exposed skin when placed in desiccating conditions (Heatwole *et al.* 1969; Gillis 1979; Pough *et al.* 1983). A recent report has suggested that SA-specific rates of EWL from arboreal anurans are characteristically about half those from non-arboreal anurans, independent of differences in posture and activity (Wygoda 1984), but the mechanism involved was not addressed.

The second group comprises those amphibians which are able to reduce EWL by enclosing themselves in cocoons whilst aestivating during droughts. These include the anurans *Leptopelis bocagei* (Loveridge and Crayé 1979), *Pyxicephalus adspersus* (Loveridge and Withers 1981) and *Lepidobatrachus llanensis* (McClanahan *et al.* 1983), which form cocoons from layers of shed integument. A similar process (McClanahan *et al.* 1976) or perhaps secretion from mucous-type skin glands (Reno *et al.* 1972) leads to cocoon formation in the urodele *Siren lacertina*.

The third group consists of several exceptional anurans in which the dorsal skin is itself virtually impermeable to water loss. This was reported first for the African rhacophorid *Chiromantis xerampelina* (Loveridge 1970) and has since been confirmed in several other anurans from xeric habitats, including South American phyllomedusine hylids (Shoemaker *et al.* 1972; Shoemaker and McClanahan 1975), *Chiromantis petersi* (Drewes *et al.* 1977), *C. rufescens* and Australian *Litoria gracilentata* (Withers *et al.* 1982, 1984) and African *Hyperolius* spp. (Withers *et al.* 1982, 1984; Geise and Linsenmair 1986). Rates of EWL from these species are similar to those from desert reptiles and about an order of magnitude lower than from "normal", non-cocooned anurans.

In *Phyllomedusa sauvagei* the so-called "waterproofing" is achieved by the secretion of waxy esters from dorsal skin glands (Blaylock *et al.* 1976; McClanahan *et al.* 1978), but the mechanism(s) for reducing EWL in the remaining species are uncertain (Withers *et al.* 1984; Geise and Linsenmair 1986).

Water uptake

In all amphibians, water uptake to replace that lost by evaporation is essential. Amphibians normally do not drink (Adolph 1927; Bentley and Yorio 1979b), and osmotic absorption across the skin is the most important route for water gain. This may occur not only from obvious sources of free water such as pools and streams, but also from moist soil (Walker and Whitford 1970; Tracy 1976). In anurans, water uptake tends to be faster in more terrestrial species, and this is attributed to both higher osmotic concentration of the body fluids and to greater skin permeability (Schmid 1965; Mullen and Alvarado 1976; Zamachowski 1977; Alvarado 1979).

Compared with other vertebrates, amphibians are very tolerant to desiccation and some arid-adapted anurans may survive water losses equivalent to 50% of initial bladder-empty body weight (Bentley 1966a). Dehydrated amphibians given access to water typically return to their original water content or body weight, a feature known as the "water balance response" (Shoemaker and McClanahan 1980). In many species this occurs very rapidly and involves an increase in the rate of cutaneous water uptake. For example, the hylid *Litoria ewingi* absorbs water through its ventral skin at a rate of $40 \text{ mg g}^{-1} \text{ h}^{-1}$ when hydrated, but after dehydration to 85% of initial bladder-empty body weight (W_0 , Brown and Brown 1980) this increases to $322 \text{ mg g}^{-1} \text{ h}^{-1}$ (Cree 1985a). In this species, a dehydration deficit which took 7 h to achieve in warm and moderately dry conditions (24°C , 60% relative humidity) was replaced within 30 min in shallow water. Rapid rehydration has been repeatedly observed in many semiaquatic and terrestrial amphibians, including bufonid, ranid, leptodactylid, rhacophorid and hyperoliid anurans (Bentley *et al.* 1958; Claussen 1969; Christensen 1974a; Shoemaker and McClanahan 1980; van Berkum *et al.* 1982; Geise and Linsenmair 1986), and plethodontid and salamandrid urodeles (Hillman 1974; Brown and Brown 1977, 1980; Brown *et al.* 1977). In these species, rapid rehydration is regarded as an

adaptation to life away from permanent sources of free water. Rehydration is comparatively slow in several aquatic amphibians, including the South African clawed toad *Xenopus laevis* (Ewer 1952), *Rana kuhli* (Shoemaker and McClanahan 1980) and the urodele *Desmognathus quadramaculatus* (Brown *et al.* 1977). In *X. laevis*, water uptake through the skin is unchanged by prior dehydration (Ewer 1952).

In many anurans which absorb water rapidly after dehydration, this ability may be restricted to a specific region of skin in the ventral pelvic area (McClanahan and Baldwin 1969; Baldwin 1974; Christensen 1974a; Jones 1978). This phenomenon was anticipated by Townson in 1799 (cited by van Berkum *et al.* 1982) and Overton (1904), who found that specimens of *Hyla arborea* with only their ventral surface or hind-quarters in water were able to rehydrate extremely rapidly. The highly permeable region may form a morphologically distinct area in bufonids (Baldwin 1974) and juvenile spadefoot toads (*Scaphiopus couchi*, Jones 1978), where it is known as the "pelvic patch". In several anurans it is densely vascularised (Roth 1973; Christensen 1974a; Cameron 1980; Kobelt and Linsenmair 1986), and blood flow to this region may increase during dehydration (Overton 1904; Christensen 1974a; Yokota and Hillman 1984). Such a morphologically distinct or well-vascularised region is not present in *X. laevis* (Roth 1973; Christensen 1974a) and has apparently yet to be reported for a urodele.

Nitrogen excretion

The major waste nitrogen products in the urine of many amphibians are ammonia and urea, the proportions of each varying with development and between species in accordance with habitat water availability (Cragg *et al.* 1961; Balinsky 1970). Ammonotelism is favoured under conditions of high water availability where it can be excreted in very dilute form, as in aquatic anuran tadpoles (Munro 1939, 1953; Cree 1985a). Species which are amphibious or terrestrial as adults become ureotelic at metamorphosis (Balinsky 1970), and anuran larvae which develop in terrestrial eggs may also be ureotelic (Shoemaker and McClanahan 1973). However, several aquatic anurans remain ammonotelic as adults (Cragg *et al.* 1961), although some, such as *X. laevis* (Balinsky and Baldwin 1961) and *Rana glandulosa* (Shoemaker and McClanahan 1980) have the capacity to become ureotelic if water

shortage occurs.

Considerable interest greeted the first report of uricotelism in an amphibian, *Chiromantis xerampelina* (Loveridge 1970), and excretion of uric acid as the predominant nitrogenous waste has since been confirmed in several other "waterproof" anurans (Shoemaker *et al.* 1972; Shoemaker and McClanahan 1975, 1982). The combination of the two reptilian features of uricotelism and a low rate of EWL is highly advantageous in arid habitats. *C. xerampelina* is reported to remain in exposed situations for several months during the African dry season (Loveridge 1970), and laboratory experiments suggest that *Phyllomedusa sauvagei* can remain in water balance without access to water, other than that in a diet of insects (Shoemaker and McClanahan 1975).

Urinary water loss

The urine produced by hydrated amphibians in water is normally copious and dilute, balancing in quantity the water absorbed through the skin (Bentley 1971a). When transferred to dry surroundings, *Rana pipiens* (Adolph 1927) and *R. clamitans* (Schmidt-Nielsen and Forster 1954) develop a marked antidiuresis within a few hours. In *R. clamitans* this results from a reduction of glomerular filtration rate (GFR) and increased reabsorption of water by the kidney tubules.

Under normal conditions, urine formed by the kidneys of amphibians is not voided directly to the outside but is stored for a time in the urinary bladder (Middler *et al.* 1968). This is a distensible sac which may be extremely capacious, holding fluid equivalent to 50% or more of W_0 in desert-dwelling anurans (Bentley 1966a, 1966b). More aquatic species typically have relatively small bladders; the lowest capacity reported is 1% for *X. laevis* (Bentley 1966a).

The possible adaptive significance of the anuran urinary bladder as a water reservoir was noted as early as 1799 by Townson (cited by Bentley 1966a). Overton (1904) reported that *Hyla arborea* survived desiccation much longer if the bladder was full at the start of dehydration. Later, Steen (1929) and Ewer (1952) showed that water is reabsorbed from the bladder during dehydration, and Ruibal (1962) demonstrated that this delays the inevitable rise in osmotic concentration of the body fluids experienced by a dehydrating toad.

Bladder water reabsorption during dehydration has also been reported for the urodele *Ambystoma tigrinum* (Alvarado 1972).

A further store of water in amphibians is that in the lymph sacs. This store may account for about 25% of body water in anurans (Bentley 1966a) and preferentially loses water during dehydration in comparison with vital organs such as the heart and brain (Smith and Jackson 1931).

Hormonal factors affecting water balance

Dehydration produces adaptive changes in skin permeability, bladder water reabsorption and urine production in many amphibians, leading to conservation of body water and enhanced water uptake. How are these changes effected? Why do some species, such as *Xenopus laevis*, fail to show all of these changes? The answer to these questions lies at least in part with the neurohypophysial hormone arginine vasotocin (AVT).

AVT is a small peptide found in representatives of all vertebrate classes (Fig. 1.1). Structurally, it is very similar to the usual mammalian neurohypophysial peptides (NHPs) oxytocin (OT) and arginine vasopressin (AVP), which are believed to have evolved from AVT by point mutation (Sawyer 1977). Indeed, the name arginine vasotocin comes from the fact that its structure combines the side chain of arginine vasopressin with the ring of oxytocin (Katsoyannis and du Vigneaud 1958).

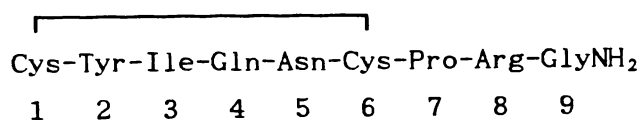


FIG. 1.1 Amino acid sequence for arginine vasotocin (AVT, 8-arg oxytocin). The amino acid sequence for arginine vasopressin (AVP) substitutes Phe for Ile in position 3. The sequence for oxytocin (OT) substitutes Leu for Arg in position 8. After Sawyer and Pang (1979).

The first hint of the role played by AVT in water balance of amphibians came from Brunn (1921), who observed that injection of mammalian neurohypophysial extracts into frogs sitting in water resulted in a weight gain due to water accumulation ("water retention" or "Brunn effect"). In 1941, Heller discovered that injection of the contents of the frog neurohypophysis had a similar but more potent effect. The identity of this amphibian "water balance principle" was later shown to be arginine vasotocin (Sawyer *et al.* 1959; Acher *et al.* 1960).

The extent of water retention produced by injected NHPs varies between amphibian species. Steggerda (1937) noted that the terrestrial toad *Bufo americanus* responded with a mean weight gain of 45% above W_0 , whereas the aquatic urodele *Necturus maculosus* (the mudpuppy) retained water equivalent to only 4% of W_0 . Responses of the semiaquatic frogs *Rana clamitans* and *R. pipiens* were intermediate (14% and 18% of W_0 respectively). *X. laevis* fails to respond at all (Ewer 1952; Heller and Bentley 1965). Subsequent studies have demonstrated that at least part of the explanation for these differences in response comes from differences in target tissue receptivity. AVT can affect water movements in amphibians at the skin, the kidneys and the urinary bladder, and not every species shows responses at all three sites.

The skin and the urinary bladder may respond with an increased rate of osmotic water transfer (Bentley 1971a, 1974). Since the body fluids are usually hyperosmotic to the external medium and to the urine, the net effect in the intact animal is an increased flow of water into the body fluids. NHPs can also produce antidiuresis at the kidney. In several anurans, including *R. catesbeiana* (Sawyer 1957a; Uranga and Sawyer 1960), *B. marinus* (Sawyer 1957b), and *R. esculenta* (Jard and Morel 1963; Jard 1966) this is produced by two separate effects: a decline in glomerular filtration rate (GFR) and a simultaneous increase in osmotic reabsorption of water across the renal tubule. However, tubular responses are not seen in all amphibians and may be absent from urodeles (Pang *et al.* 1982). The reduction in GFR is believed to result from constriction of the afferent blood vessels of the glomerulus (Pang 1977; Pang *et al.* 1980). Actions of AVT on water balance can therefore be summarised into two main types: "hydroosmotic" effects (increased osmotic water flow through epithelial membranes of skin, bladder and renal tubules) and a vasoconstrictor effect on the smooth muscle of glomerular arterioles

(Sawyer and Pang 1979).

In amphibians in which the effects of both dehydration and exogenous AVT or related NHPs have been examined, the responses are remarkably similar. This suggests that the water balance responses seen during rehydration are due to release of endogenous AVT. Anurans and urodeles which are able to regain water rapidly during rehydration show large amounts of water retention when treated with exogenous NHPs, and in these species all three target sites appear to be affected (e.g., *B. regularis* - Ewer 1952; *Taricha torosa* - Brown and Brown 1980). However, *X. laevis* (Ewer 1952) and *Desmognathus quadramaculatus* (Brown *et al.* 1977) show little or no increase in water uptake in response to dehydration, and no significant water retention when treated with NHPs (Heller and Bentley 1965; Brown *et al.* 1977). In these species there appears to be no hydroosmotic effect on the skin. The urodele *Salamandra maculosa* is apparently the only known amphibian in which AVT affects only the bladder (Bentley and Heller 1965).

Although many of the effects of exogenous AVT can be demonstrated with doses less than that stored in the amphibian neurohypophysis, additional evidence is required before one can conclude that the endogenous peptide functions as a hormone in the control of amphibian water balance. For instance, endogenous AVT should be released into the circulation in response to dehydration or other appropriate environmental stimuli, and removal of the source of endogenous AVT should result in physiological deficiencies which can be alleviated by treatment with exogenous NHPs (Bentley 1971a; Sawyer and Pang 1979).

Indirect evidence that AVT is released into the circulation of anurans came from observations that the peptide content of the neurohypophysis declines during dehydration (Levinsky and Sawyer 1953; Jørgensen *et al.* 1956), and that a substance appears in the plasma which causes water retention when injected into intact toads (Shoemaker 1965) and which leads to increased osmotic water flow through isolated toad bladders (Bentley 1969a). Direct measurement by radioimmunoassay has since confirmed that AVT is released during dehydration (Rosenbloom and Fisher 1974; Nouwen and Kühn 1983) and also during haemorrhage (Sawyer and Pang 1975). The most potent stimulus for release of AVT appears to be reduced blood volume or pressure; increased plasma osmolarity is of less importance (Sawyer and Pang 1975).

Early attempts to determine the effects of neurohypophysectomy in anurans yielded inconclusive results, apparently owing mainly to technical difficulties which are discussed by Bentley (1971a, 1974). Later experiments in which neurohypophysial function was impaired in *B. marinus* indicated significant effects on water balance. Toads with hypothalamic lesions showed reduced cutaneous and antidiuretic responses to dehydration (Shoemaker and Waring 1968; Bakker and Bradshaw 1977), but both responses occurred following treatment with toad pituitary extract (Shoemaker and Waring 1968). Totally hypophysectomised toads had reduced ability to increase bladder water reabsorption in response to a salt load, when compared with adeno-hypophysectomised controls (Middler *et al.* 1967).

These studies indicate that endogenous AVT does play a physiological role in the control of amphibian water balance, although other mechanisms are undoubtedly involved (Morel and Jard 1968; Bentley 1971a, 1974; Pang 1977; Sawyer and Pang 1979). The latter may include other endocrine substances such as the adeno-hypophysial polypeptide prolactin, which reduces osmotic water flow across the skin of several urodeles (Lodi *et al.* 1982; Brown *et al.* 1983) and the neurohypophysial peptide mesotocin, which produces glomerular diuresis in the urodeles and anurans examined (Galli-Gallardo *et al.* 1979; Pang *et al.* 1982). Recently, it has been suggested that glomerular and tubular antidiuresis in *R. catesbeiana* (Pang *et al.* 1982) and the cutaneous hydroosmotic response of some bufonid toads (Yokota and Hillman 1984; Segura *et al.* 1984) are additionally or even predominantly controlled by adrenergic neural mechanisms, and further studies are needed to examine this aspect in other amphibians (Pang 1983).

In addition to its effect on water uptake, AVT increases active transport of sodium across the skin and urinary bladder of many anurans ("natriferic" effect; Sawyer and Pang 1979). Although a small amount of water may passively accompany sodium transport, the natriferic and hydroosmotic responses to AVT are separate and independent processes (Sawyer and Pang 1979), probably mediated by separate receptors and separate pools of adenylate cyclase (Jard and Bockaert 1975). Evidence for the independence of the two responses comes from observations that: (i) large hydroosmotic effects are still seen in the absence of net sodium transport (Sawyer 1960; Bentley 1969b); (ii) various analogues of AVT do not have parallel

effects on the two processes (Bourguet and Maetz 1961); (iii) skin from certain regions or from certain species shows a natriferic response but not a hydroosmotic one (Bentley and Main 1972a; Marrero and Hillyard 1985). Since AVT is released in situations where an increase in sodium uptake does not make homeostatic sense, the physiological significance of the natriferic response remains obscure (Bentley 1971a, 1974, 1982b; Sawyer and Pang 1979).

Recent studies have also linked AVT with reproductive behaviour and oviducal contractions in amphibians (Diakow 1978; Guillette *et al.* 1985). Other non-osmoregulatory effects of exogenous AVT include the ability to raise blood pressure, to produce hyperglycaemia and to promote the release of corticotropin, but the significance of these is unclear (Bentley 1974, 1982b).

Responsiveness to NHPs: ecological versus phylogenetic hypotheses

It has been widely accepted since it was first suggested by Steggerda (1937), that the extent to which amphibians respond to NHPs correlates in an adaptive manner with habitat water availability (e.g., Bentley 1974; Alvarado 1979). Evidence for this "ecological" hypothesis is that species which lack a cutaneous response to NHPs or to dehydration are usually aquatic and therefore not likely to become dehydrated (Ewer 1952; Shoemaker and McClanahan 1980). Terrestrial species which rehydrate rapidly are able to take advantage of transient water sources and may also risk less predation (Bentley 1971a).

An alternative explanation for the absence of a well-developed cutaneous hydroosmotic response in some anurans is suggested by the results of Heller and Bentley (1965). Heller and Bentley noted an increasing rate of water retention in response to injected AVT in the anurans *X. laevis*, *Discoglossus pictus*, *Pelobates cultripes*, *B. bufo*, *R. esculenta* and *Hyla hyla*. Four hours after treatment, *X. laevis* had shown no gain in weight, *D. pictus* 12%, *P. cultripes* 13%, *B. bufo* 16% and *R. esculenta* 20%. *H. hyla* gained 53% in 1 h. Although these results seemed consistent with the "ecological hypothesis", Heller and Bentley (1965) suggested that they could also be viewed in a phylogenetic sense, since *Xenopus*, *Discoglossus* and *Pelobates* are amongst the more anatomically primitive or archaic frogs (all belong to the Archaeobatrachia of Duellman 1975). Thus, a possible

interpretation of the results is that a cutaneous response to AVT had not evolved or had evolved to only a poor extent in these less advanced anurans. This can be considered a "phylogenetic" hypothesis.

A similar conclusion was drawn in the only previous study to have examined water balance in any of the *Leiopelma* species (Cameron 1974). Cameron found that the rate of rehydration of immersed *L. hochstetteri* was much slower than that of the hylid *Litoria ewingi*. Furthermore, isolated ventral skin of *L. hochstetteri* showed no increase in osmotic water flow in the presence of mammalian NHPs, whereas that of *Litoria ewingi* did. Cameron considered that this lack of response was another example of the "primitive" nature of *L. hochstetteri*, and speculated that water exchange in the remaining species of *Leiopelma*, *L. archeyi* and *L. hamiltoni*, would be similar.

The present study

The aim of the present study is to demonstrate whether aspects of water balance vary between the three *Leiopelma* species in accordance with differences in habitat. This aim has been divided into several more specific objectives:

(i) to demonstrate that there is a difference in water availability between the habitats of the partially sympatric species *L. archeyi* and *L. hochstetteri*, and that "terrestrial" and "semiaquatic" are appropriate descriptions of their respective habitats;

(ii) to demonstrate whether physiological aspects of water balance in the three species (rates of dehydration and rehydration, responses to AVT, predominant mode of nitrogen excretion) vary in relation to habitat;

(iii) to test the ecological and phylogenetic hypotheses regarding cutaneous responsiveness to AVT amongst the *Leiopelma* species;

(iv) to determine some of the behavioural and reproductive adaptations which enable *L. archeyi* to survive in a terrestrial environment.

These are examined in the following chapters as follows. Chapter 2 describes the habitats of *L. archeyi* and *L. hochstetteri* in a selected

area of the Coromandel Peninsula. The habitats of these two species were examined for reasons of accessibility, and because of doubt over the extent of their overlap in distribution. Information on the habitat of *L. hamiltoni* has been previously published (Newman 1977; Newman *et al.* 1978). Chapters 3-5 describe laboratory studies of the water balance physiology of the three species. Rates of dehydration and rehydration are examined in Chapter 3. Chapter 4 shows the effects of AVT on water balance *in vivo*. The effects of AVT on specific tissues or organs - the skin, the kidneys and the bladder - are examined in Chapter 5. The relationship between the structure of the skin and its role in water uptake is examined in Chapter 6 for *L. archeyi* and *L. hochstetteri*, and compared with that for the locally available hylid *Litoria aurea*. Nitrogen excretion in adults of the three species of *Leiopelma* and at various stages of development of *L. archeyi* are examined in Chapter 7. Chapters 8 and 9 include a return to the field. Water relations of the terrestrial eggs and larvae of *L. archeyi* are discussed in Chapter 8, based on both field and laboratory studies. Chapter 9 examines the relationship between environmental conditions and activity of *L. archeyi* in the field, and compares in the laboratory the ability of *L. archeyi* and *L. hochstetteri* to absorb moisture from soil.

Finally, the conclusions from individual chapters are integrated in Chapter 10. In this chapter the evidence for the ecological and phylogenetic hypotheses is also discussed, using both information from the literature and the original results obtained in the present study.

CHAPTER 2

HABITATS OF *L. ARCHEYI* AND *L. HOCHSTETTERI*

INTRODUCTION

This chapter examines the habitats and microclimates of *Leiopelma archeyi* and *L. hochstetteri*. Adequate information on microhabitat is essential for assessing the ecological significance of results from comparative studies of anuran water balance, and its absence may have hindered interpretation in previous studies (Alvarado 1979). *L. archeyi* and *L. hochstetteri* have frequently been reported to inhabit cool and/or humid areas (e.g., Archey 1922; Turbott 1949; Stephenson and Stephenson 1957; Bell 1978, 1982a; Robb 1980; McLennan 1985), but virtually no quantitative information on ambient or microclimatic conditions in these habitats has yet been published. Furthermore, although recent studies refer to *L. archeyi* as terrestrial and *L. hochstetteri* as semiaquatic (Bell 1978, 1982a, 1982b, 1985a, 1985b), the extent of overlap in their distributions has been the subject of some dispute.

Turbott (1942) considered that the two species were completely allopatric, *L. archeyi* being restricted to mist-prone ridgetops and *L. hochstetteri* to lower altitudes close to streams. This was later proved incorrect by N.G. Stephenson and E.M. Stephenson (née Thomas), and by Turbott himself. Stephenson and Thomas (1945) reported that both may occur in either type of habitat, *L. hochstetteri* having been found on ridges away from surface water along with *L. archeyi*, and *L. archeyi* a few metres from the stream beds occupied by *L. hochstetteri*. Turbott (1949) conceded this, and added that he had found a specimen of *L. archeyi* partly immersed in stream water. Later, Stephenson and Stephenson (1957) referred to "close mingling of the two species" in regions of the Coromandel Peninsula, and E.M. Stephenson (1961) reported that in such regions it was a "common occurrence" to find members of both species under the same log or stone. However, recent and more quantitative information from Bell (1978) suggests that the extent of overlap is somewhat less than that implied by the Stephensons. Of 82 frogs found by Bell along the edges of forested creeks throughout the Coromandel Peninsula, 99% were *L. hochstetteri* and 1% *L. archeyi*; at Tapu Summit (the ridgetop of the

Coromandel Range between Tapu and Coroglen) the proportions were exactly reversed for 87 frogs found. Furthermore, there was a significant difference between the moistness of substrates on which the frogs were found: *L. hochstetteri* was only seen on "moist" (27% of frogs) or "wet" (73%) substrates, whereas *L. archeyi* was found on "dry" (3%), "moist" (85%) or "wet" (12%) ones (Bell 1978).

To interpret the ecological significance of results from comparative laboratory studies of water balance, I required more quantitative information on the distribution and habitats of *L. archeyi* and *L. hochstetteri*. Specifically, the questions I asked were:

- (i) are "terrestrial" and "semiaquatic" appropriate descriptions of the habitats of *L. archeyi* and *L. hochstetteri* respectively?
- (ii) to what degree do the two species overlap in distribution?
- (iii) what ambient and microclimatic conditions potentially affecting water loss from, and water uptake by, frogs prevail in the habitats of each species?

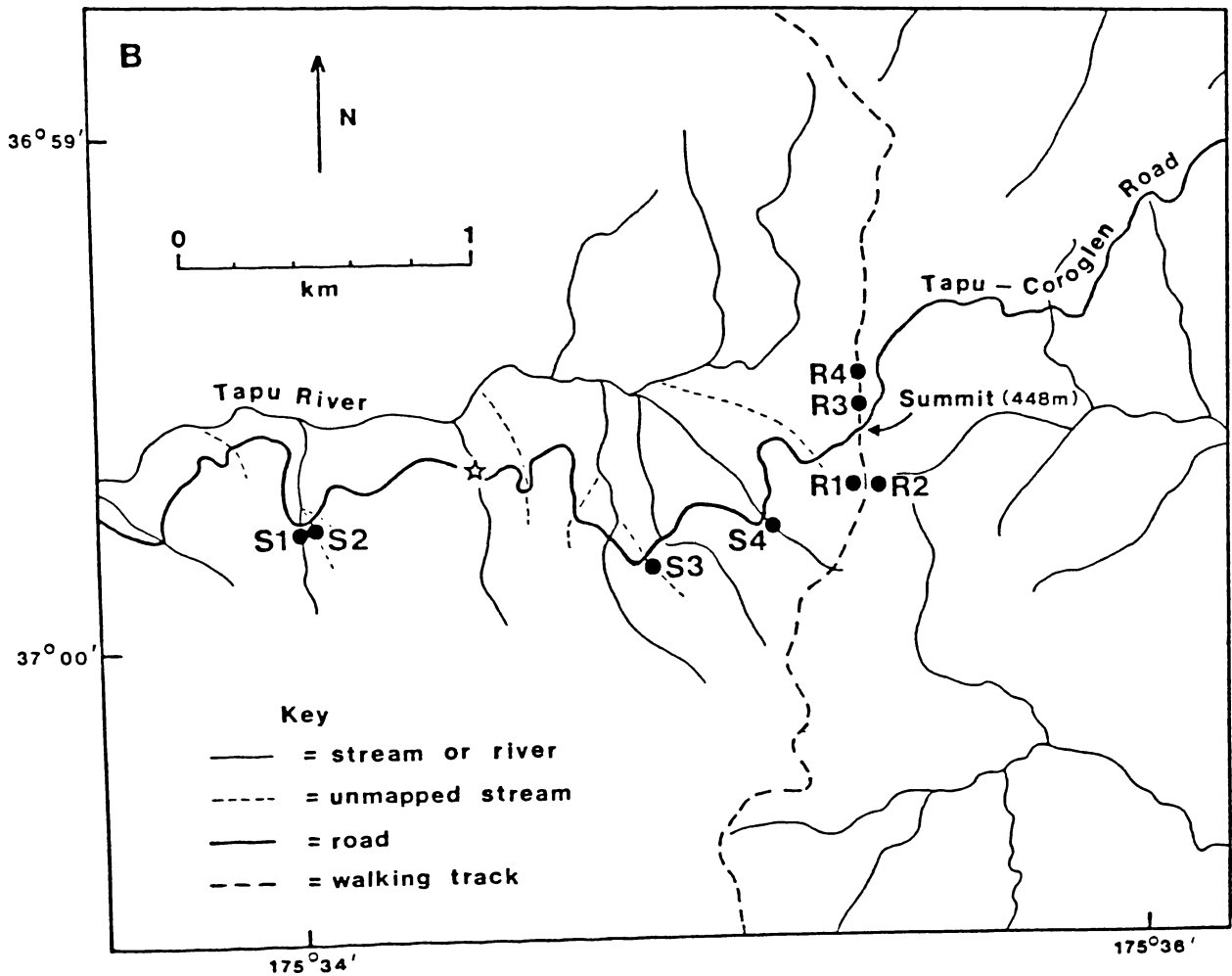
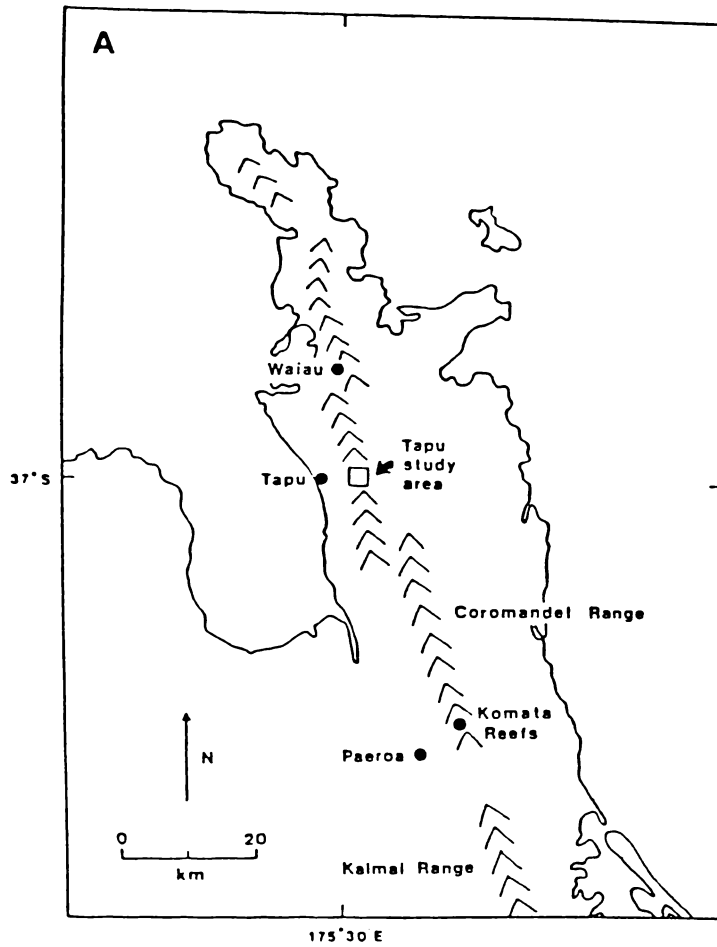
Several aspects of the habitat and microclimate of the third species of *Leiopelma*, *L. hamiltoni*, have previously been described (Crook *et al.* 1971; Newman 1977; Newman *et al.* 1978); and the results show that this species is completely terrestrial. Because of the inaccessibility of the islands on which *L. hamiltoni* is found, the habitats of this species were not examined further in the present study.

MATERIALS AND METHODS

Study area

Field studies were carried out on the ridgetop and western slopes of the Coromandel Range, above the settlement of Tapu on the Coromandel Peninsula and within Coromandel State Forest Park (Fig. 2.1A; Plate 2.1). The study area included streams and ridgetops forming part of the upper catchment of the Tapu River, and ranged in altitude from about 150-500 m above sea level (asl). It was covered by a lush canopy of warm-temperate mixed podocarp-broadleaf forest, dominated by rimu (*Dacrydium cupressinum*), rewa rewa (*Knightia excelsia*) and towhai (*Weinmannia sylvicola*), with emergent kauri (*Agathis australis*) and various tree ratas (*Metrosideros* spp.). Black tree ferns or mamaku

FIG. 2.1 The Tapu study area. A = location of the Tapu study area on the Coromandel Peninsula, and additional place names mentioned in the text. B = map of the Tapu study area (based on the published map NZMS 260 T11). In B, the approximate locations of additional unmapped streams or seepages have been included, and streams in the upper and lower left-hand corners have been omitted for clarity. The locations of the four streamside sites (S1 - S4) and the four ridgetop sites (R1 - R4) are shown by closed circles (for study site dimensions, see TABLE 2.1). The walking track passes along the ridgetop of the Coromandel Range, and an "★" marks the location from which PLATES 2.1A and B were photographed.





A



B

PLATE 2.1 View of the Tapu ridgetops in fine weather (A) and misty weather (B). These photographs were taken looking northeast from the spot marked "☆" on FIG. 2.1. The summit of the Tapu-Coroglen Road is hidden behind the trees on the extreme right of the photographs.

(*Sphaeropteris medullaris*) and nikau palms (*Rhopalostylis sapida*) dominated over stream gullies and were present in the subcanopy away from streams. Other common subcanopy trees and shrubs were mahoe (*Melicytus ramiflorus*), kohekohe (*Dysoxylum spectabile*), toropapa (*Alseuosmia macrophylla*), rangiora (*Brachyglottis repanda*), pate (*Schefflera digitata*) and kiekie (*Freycinetia baueriana banksii*). Ferns were common on the forest floor, and mosses and liverworts on rocks and tree trunks. The forest soil was a Komata clay-loam, typical of broadleaf forest soils derived from andesitic rock (the late Prof. H.S.Gibbs, previously of the University of Waikato, pers. comm.).

Within the study area, four streamside sites (S1-S4) and four ridgetop sites above the headwaters of streams (R1-R4) were chosen for intensive study (Fig. 2.1B). These sites were chosen because they included rocks shown by preliminary searches to be suitable for frogs to shelter under, and because they were easily accessible from the Tapu-Coroglen Road. The dimensions of these sites are given in Table 2.1. Briefly, the streamside sites included two trickling creeks with many small rocks alongside (S1 and S4; Plate 2.2A), a steeper, more swiftly flowing creek with fewer, larger rocks and some boulders alongside (S2; Plate 2.2B), and the rocky spray-zone and creek at the base of a waterfall (S3). Water depth in the four creeks was in most parts less than 20 cm. The ridgetop sites included a dark, shaded rock pile set in a slight depression (R1), and three more open sites with scattered or continuous rock piles (R2-R4; Plate 2.3). Because of its low canopy and exposure to prevailing westerly winds, R3 was the driest ridgetop site, whilst R1, with its taller, denser canopy and sheltered rock pile set in a depression was the wettest. Sites R1 and R2 were the closest of the four ridgetop sites to streams, the nearest headwater being about 50-70 m away. Sites R3 and R4 were about 250 m from the nearest permanent streams (Fig. 2.1B).

Distribution surveys

The distribution of *L. archeyi* and *L. hochstetteri* along streamsides and on ridgetops within the study area was examined during 13 monthly searches, from June 1983-June 1984 inclusive. Each month, daytime searches were made for frogs by lifting rocks, small logs and other debris on the forest floor and along stream edges. For the first 7 mo of the study 1-2 d per month were spent making searches, and the time

TABLE 2.1 Maximum dimensions of the eight study sites.

Study site	Length (m)	Width ¹ (m)	Plate
S1	60.0	1.5-2.0	2.2A
S2	39.0	1.3-1.7	2.2B
S3	15.0	2.5-3.0	
S4	30.0	2.0-3.5	
R1	13.0	6.0	
R2	15.0	5.0	
R3	11.0	17.0	2.3B
R4	17.0	4.0	2.3A

Note:

1. Width of sites S1-S4 includes width of stream (0.5-1.0 m); width of sites R3 and R4 includes width of walking track (0.5 m).

PLATE 2.2 Streamside site S1 (A), looking downstream toward the Tapu-Coroglen Road, and site S2 (B), looking upstream.



A



B



A



B

PLATE 2.3 Ridgetop site R4 (A), and close-up of forest floor of site R3 (B).

spent searching each day was divided approximately equally between streamside and ridgetop sites. Sites S1, R1 and R2 were always searched, but sites S2, S3, S4, R3 and R4 were only occasionally included. To increase the numbers of frogs found, searches during the final 6 mo were extended to 2-3 d per month and included all eight sites. Occasional searches were also made between the eight study sites to obtain additional information on distribution.

For each frog found, as many of the following details as possible were recorded:

- (i) distance to nearest visible free-standing water [recorded as 0 m (i.e., frog in water), >0 - 1 m, >1 - 10 m or > 10 m];
- (ii) substrate wetness ("wet" if the frog was in water or a film of surface water was visible, "moist" if a dry finger pressed to the substrate became moist, or "dry" if no substrate particles or moisture adhered to the finger);
- (iii) substrate moisture content: where frogs were found on soil, humus or mud, a sample of the substrate was sealed in a plastic container, returned to the laboratory, weighed, and dried at $\sim 105^{\circ}\text{C}$ to constant weight. The sample was sieved through a 0.85 mm mesh to remove twigs and stones, and then reweighed. Moisture content was calculated as the amount of water removed during drying as a percentage of dry substrate weight after sieving;
- (iv) substrate water potential: where frogs were on soil, humus, mud or silt of sufficient depth (> 4 cm), a "Quick Draw" soil moisture probe (Soilmoisture Equipment Corp., California, USA) was used to measure substrate water potential. This parameter indicates the force with which substrate moisture is bound to substrate particles: values are at or near 0 kPa for saturated substrates, but become increasingly more negative as the substrate dries. A useful reference value is the permanent plant wilting point, which occurs at about -1500 kPa (Nobel 1974). The instrument used here measured water potentials within the range 0 to -100 kPa (= 0 to -1 bar);
- (v) ambient and retreat site temperatures: on most occasions when frogs were found, paired ambient and retreat site temperatures were measured to the nearest 0.1°C using a thermistor probe (Model 8522 -10, Cole Parmer Instruments Co., Illinois, USA). Retreat site temperature (T_r) was measured by inserting the probe into a suspected (later confirmed) frog retreat site, or by inserting the probe into a confirmed retreat site as soon as a frog was discovered and then quickly replacing the covering rock (the latter method may slightly

overestimate true retreat site temperature, since the temperature gradient between ambient and retreat site air was disturbed by lifting the covering rock). Ambient air temperature (T_a) was then recorded just outside the retreat site;

(vi) relative humidity (RH): ambient relative humidity (RH_a) was measured about 0.5 m above ground level in a predetermined spot at each study site searched (as opposed to each retreat site), using a Sundo hair hygrometer (Stacker and Olms, Hamburg, West Germany). In addition, some paired estimates of ambient relative humidity and retreat site relative humidity (RH_r) were made for *L. archeyi*. RH_r was estimated in the same way as for T_r , using a quick-response hygrometer (Vaisala, Finland). After measuring RH_r , RH_a was recorded just outside the retreat site.

Environmental conditions at study sites

Evaporation rates at streamside and ridgetop habitats

An index of the dryness of the eight study sites was obtained by measuring rates of evaporation from matched Piché evaporimeters (Jane and Green 1983). These provide an estimate of the rate of evaporation resulting from a combination of factors, including air temperature, relative humidity, windspeed and radiation. Each evaporimeter was installed about 1 m above ground level, and evaporation rates were recorded over two 2 d periods of mainly fine, dry summer weather in February 1985.

Rainfall, temperature, leaf wetness and soil water potential

Further information on environmental conditions at two ridgetop sites was obtained using automatic data loggers. Three months after the monthly searches began, a TASMAN Mini Data Logger (Solid State Equipment Ltd, Lower Hutt, NZ) was installed at the R2 site. T_r under a rock on the edge of a nearby clearing (a known *L. archeyi* retreat site) was recorded using the supplied temperature sensor, and rainfall in the clearing was recorded with a tipping bucket rain gauge (Ota Keiki Seisakusho, Japan). Rainfall and T_r were recorded at 2 h intervals for 12 mo, ending in September 1984. Additional information on rainfall in the vicinity of the study area was obtained from a NZ Meteorological Service recording station at Waiau, 67 m asl and 19 km north of the Tapu study area (Fig. 2.1A).

In February 1984, a more sophisticated CR-21 micrologger (Campbell

Scientific Inc., USA) was installed at the driest ridgetop site (R3). Unfortunately, the risk of vandalism made installation on the drier, windier and more open western slope unwise (the equipment would have been visible from the walking track passing through the site), so instead it was placed in a shaded spot behind the cover of a fallen tree on the eastern slope of the ridgetop. Although well hidden from the track, this location was undoubtedly cooler and more humid than conditions in the rest of the study site. Retreat site temperature (T_r) in a known *L. archeyi* retreat site, and ambient temperature (T_a) above the retreat site were recorded with thermistor probes. The probes were matched to within 0.1°C , both when installed and when removed at the end of the study. Soil water potential was measured under an adjacent rock with a gypsum soil moisture block, and the occurrence of rain or mist was recorded with an artificial "leaf wetness sensor", placed on the forest floor. This sensor was calibrated to record "wet" when sprayed with a fine layer of mist, and "dry" when fully dry. All sensors and probes were from Campbell Scientific Inc., and recordings were made at 2 h intervals until October 1984.

Attempts were also made to record RH_r at both the R2 and R3 sites using a Jenway HP1 humidity probe (Jenway Ltd, Essex, England). However, these consistently failed, since as soon as RH reached 100% the sensor became inoperative. Evidently (and despite the supplier's assurance to the contrary) this probe is unsuitable for conditions where condensation frequently occurs.

RESULTS

Distribution and microhabitats of *L. archeyi* and *L. hochstetteri*

General

Over the 13 monthly searches, individual *L. archeyi* were observed on 215 occasions and *L. hochstetteri* on 204. The results showed convincingly that *L. archeyi* was almost entirely restricted to the ridgetops year-round (99% of observations) whilst *L. hochstetteri* was nearly always found in water or within 1 m of the edges of streams (94% of observations).

To aid interpretation, and because the numbers of frogs found during

monthly searches in the first 7 mo of the study were sometimes low, data from each month have been grouped into seasons. "Winter" includes data from June-August, "spring" from September-November, "summer" from December-February and "autumn" from March-May. Since there was usually little variation in frog distribution and in environmental conditions from one month to the next, and since similar numbers of each species of frog were found in each month, this does not bias the seasonal data towards different months for the two species.

Distance to water

In all seasons, almost all *L. archeyi* (94% or more) were found further than 10 m from surface water (Fig. 2.2). The exceptions occurred in spring (October 1983) and summer (December 1983) when heavy rainfall left puddles in depressions near some ridgetop sites, and in summer (February 1984) and autumn (May 1984) when solitary *L. archeyi* were found under rocks near the banks of streams.

In contrast, most *L. hochstetteri* (78% or more) were found within 1 m of stream edges, and a further 7% or more were found in stream water itself (Fig. 2.2). *L. hochstetteri* were never fully immersed in retreat sites, although adults often jumped into the stream when disturbed and sometimes stayed submerged for several minutes. In December 1983 following heavy rain, when higher than normal stream levels had submerged many normal retreat sites, *L. hochstetteri* were difficult to find and three of the four discovered were at ridgetop sites along with *L. archeyi*. Other instances when *L. hochstetteri* were found on the ridgetops included three in winter (June 1983 and June 1984), one in summer (January 1984) and one in autumn (April 1984). Of these eight *L. hochstetteri*, seven were found at sites R1 or R2, the two ridgetop sites closest to streams.

Substrate wetness

In all seasons, 70-90% of *L. archeyi* were found on moist substrates (Fig 2.3). The remainder were about equally distributed between wet and dry substrates (Plate 2.4) in summer and autumn, all on dry in winter, and all on wet in spring. All but three *L. archeyi* seen were under the cover of rocks or logs; the exceptions were emerged and active during wet daytime weather, either on the forest floor or in vegetation up to 1.5 m high.

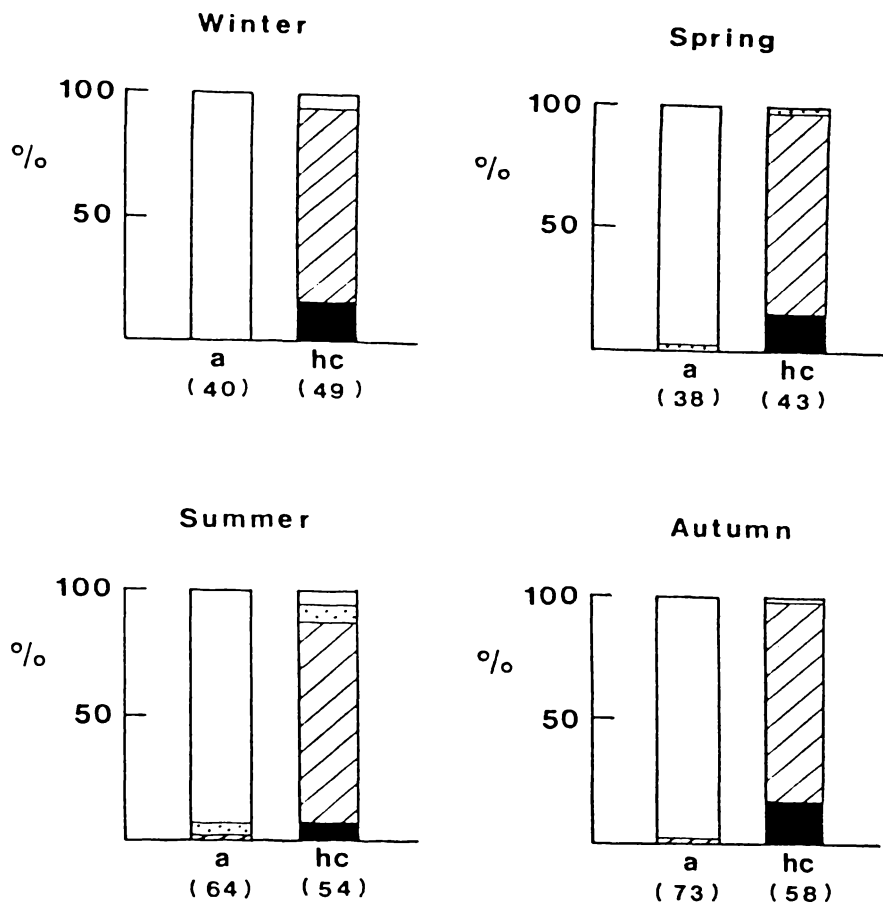


FIG. 2.2 Distance to free-standing water for *L. archeyi* (a) and *L. hochstetteri* (hc). Percentages of frogs found in each class are indicated as follows: solid colour = in water, hatched = >0 - 1 m, stipple = >1 - 10 m, open = >10 m. Sample sizes are given in parentheses.

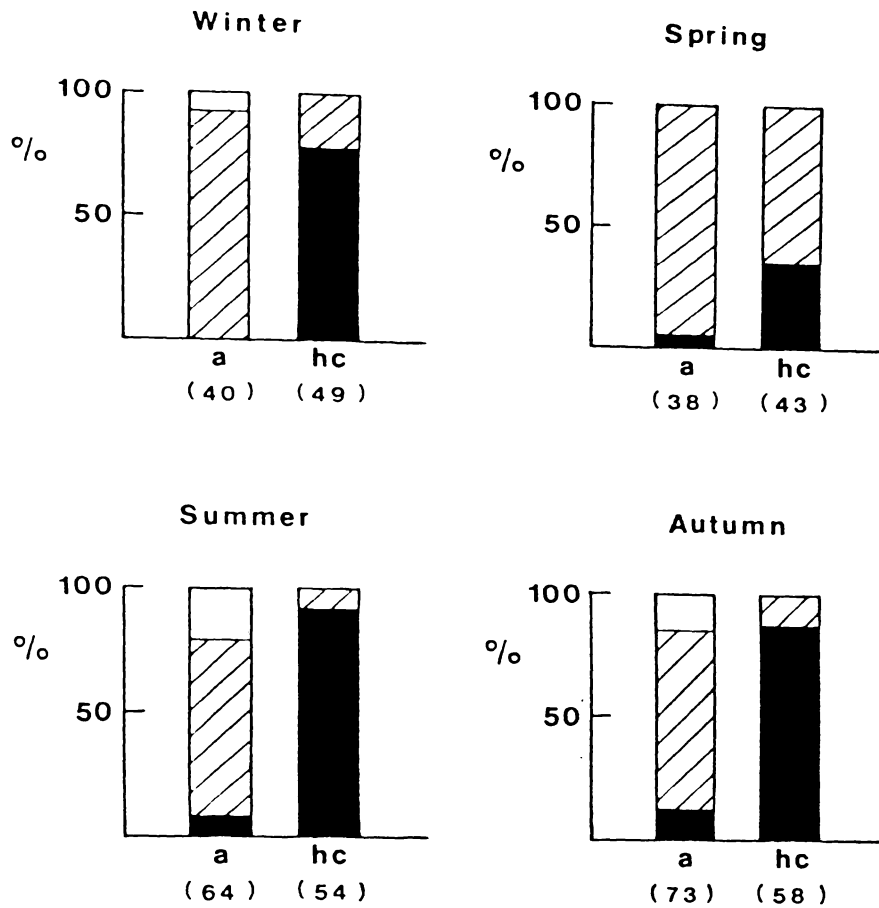


FIG. 2.3 Wetness of substrates on which *L. archeyi* (a) and *L. hochstetteri* (hc) were found. Percentages of frogs found in each class are indicated as follows: solid colour = wet, hatched = moist, open = dry. Sample sizes are given in parentheses.



PLATE 2.4 *L. archeyi* exposed on retreat site substrate of dry litter.

All *L. hochstetteri* were found in retreat sites by day, and all were on moist or wet substrates, never dry ones (Fig. 2.3; Plate 2.5). The percentage of frogs on wet substrates varied from 35% (in spring) to 91% (in summer).

Substrate moisture content and water potential

Most *L. archeyi* were found on forest substrates of rocks, soil or soil/humus, or fallen leaf litter. In contrast, most *L. hochstetteri* were on streamside substrates of mud, silt, gravel, pebbles or rocks. Twenty four percent of *L. archeyi* and 15% of *L. hochstetteri* were on substrates for which moisture content could be determined (i.e., soil, soil/humus or mud) (Fig. 2.4A). Moisture contents of substrates for both species were extremely variable within each season, but mean values were always high ($> 100\%$ MC). *L. hochstetteri* substrates varied to less high and low extremes of moisture content than those of *L. archeyi*. The lowest value recorded for *L. archeyi* was 32% MC in June 1984.

Soil moisture content does not provide any indication of the force with which water is held by substrate particles, and thus of its potential availability to a frog sitting on the surface (Tracy 1976). A more useful measure, which takes into account differences in substrate type and texture, is substrate water potential (here measured in -kPa; Fig. 2.4B). *L. hochstetteri* substrates were always saturated (> -6 kPa); those for *L. archeyi* were consistently lower in water potential (mean values -16 to -18 kPa), although still usually close to saturation. One autumn *L. archeyi* soil sample with a low moisture content (62%) had a water potential which was lower (more negative) than the range of the instrument used (-100 kPa). Unfortunately, samples of *L. archeyi* substrates with moisture contents lower than this (as low as 32% - Fig. 2.4A) were too shallow to measure water potential, but assuming a homogeneous soil texture and type, water potential would have been lower than -100 kPa for these samples also.

Soil water potential was recorded automatically at 2 h intervals in a cool, shaded area of the R3 site (*L. archeyi* habitat) from February to August 1984. Water potential remained close to saturation throughout this period (between -49 and -69 kPa).



A



B

PLATE 2.5 *L. hochstetteri* in streamside retreat sites. A = a typical view, showing two frogs (circled), concealed against the waterlogged substrate. B = a frog half-immersed in water (the maximum degree of immersion observed for undisturbed frogs).

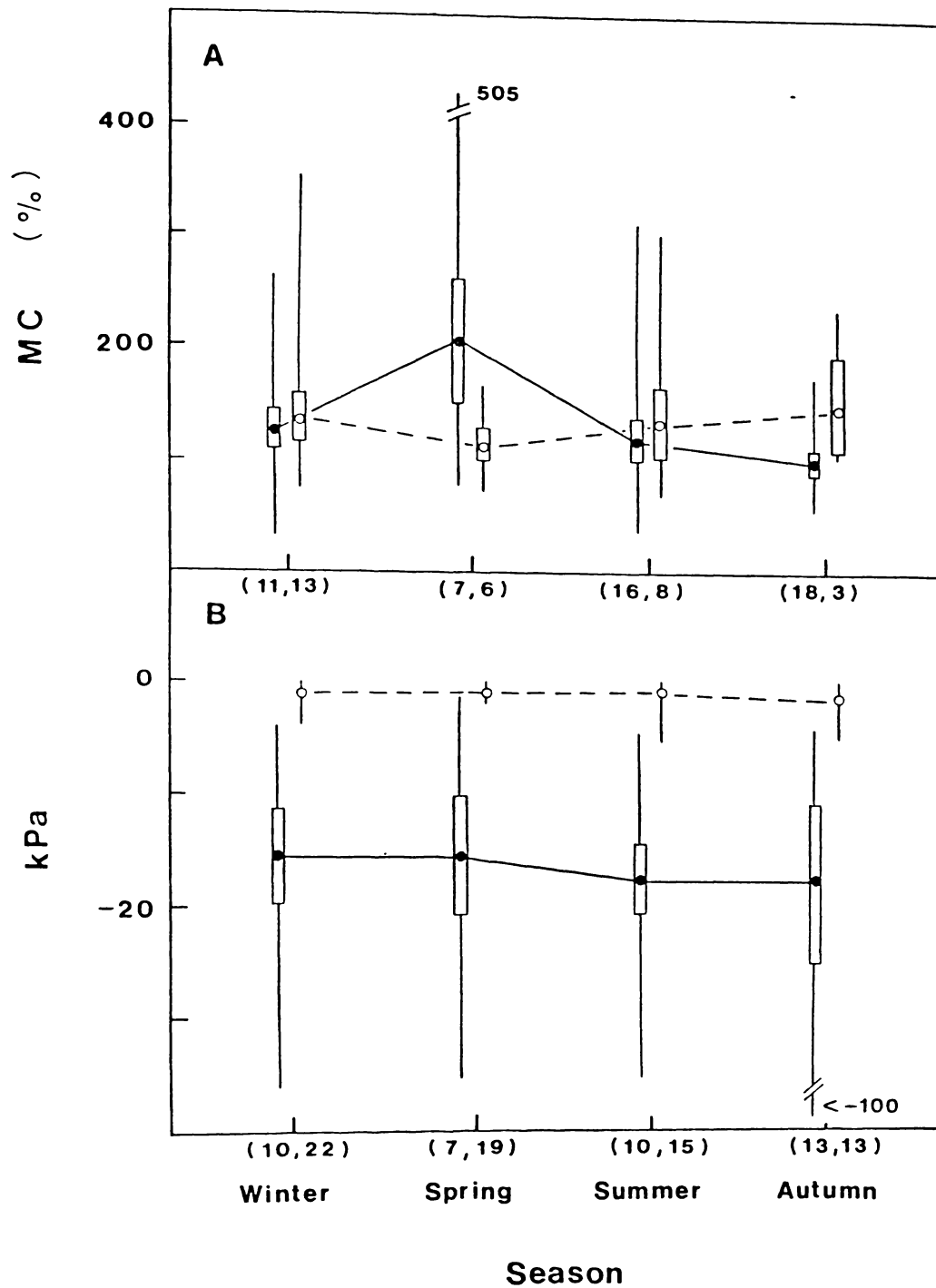


FIG. 2.4 Moisture content (A) and water potential (B) of water-containing substrates on which *L. archeyi* (●) and *L. hochstetteri* (○) were found ($\bar{x} \pm SE$ and range; SE deleted where necessary for clarity). The mean autumn water potential for *L. archeyi* substrates has been calculated assuming that the extreme value was in fact -100 kPa. Figures in parentheses give sample sizes for *L. archeyi*, then *L. hochstetteri*.

Ambient and retreat site temperatures were recorded for many frogs found during monthly searches. Ambient temperature records show two general features (Fig. 2.5A): (i) T_a remained cool throughout the year, the winter minimum being 6.3°C for both species, and the summer maximum 20.6°C for *L. archeyi* and 19.0°C for *L. hochstetteri*; (ii) mean values and ranges were similar for both species in all seasons.

Paired measurements of T_a and T_r in summer and winter confirm that, as expected, air in the retreat site is slightly cooler than ambient air by day (Table 2.2). For both species, the difference between mean T_a and mean T_r was similar in summer and winter (0.6 - 0.8°C), but as previously mentioned, these values may underestimate the temperature gradient between T_a and T_r because of the disturbance caused by lifting the cover of the retreat site. The greatest difference recorded between spot measurements of T_r and T_a was 3°C (T_r being the cooler), for a single *L. archeyi* retreat site in February 1984 (summer).

Measurements of T_r and T_a obtained during monthly searches were supplemented by automatic recordings at two ridgetop sites (Fig. 2.6). T_r under a rock exposed to morning sun at the R2 site fluctuated over a year between 6.5°C in July 1984 (winter) to 22.5°C in February and March 1984 (summer) (Fig. 2.6A). Under a permanently shaded rock at the R3 site, T_r recorded between February 1984 and September 1984 fluctuated between extremes of 4.6°C in July and 16.8°C in March (Fig. 2.6B). Both these retreat sites were occupied by *L. archeyi* on occasions during the recording period; the measurements therefore represent valid data for frog retreat sites for at least some times of the year.

T_a recorded at the R3 site fluctuated between greater extremes than T_r (Fig. 2.6B). Not only did T_r remain cooler than T_a by day (as previously shown for spot measurements - Table 2.2), but it usually remained warmer (by up to 4.4°C) at night. The extremes of T_a measured at this site were 18.4°C (in February) and 1.4°C (in July).

Relative humidity

Ambient humidity (RH_a) was recorded during monthly searches at study sites where *L. archeyi* and *L. hochstetteri* were found (Fig. 2.5B). Sites where *L. hochstetteri* was present were consistently high in RH_a ($> 82\%$), with mean values being greater than 90% even on dry sunny

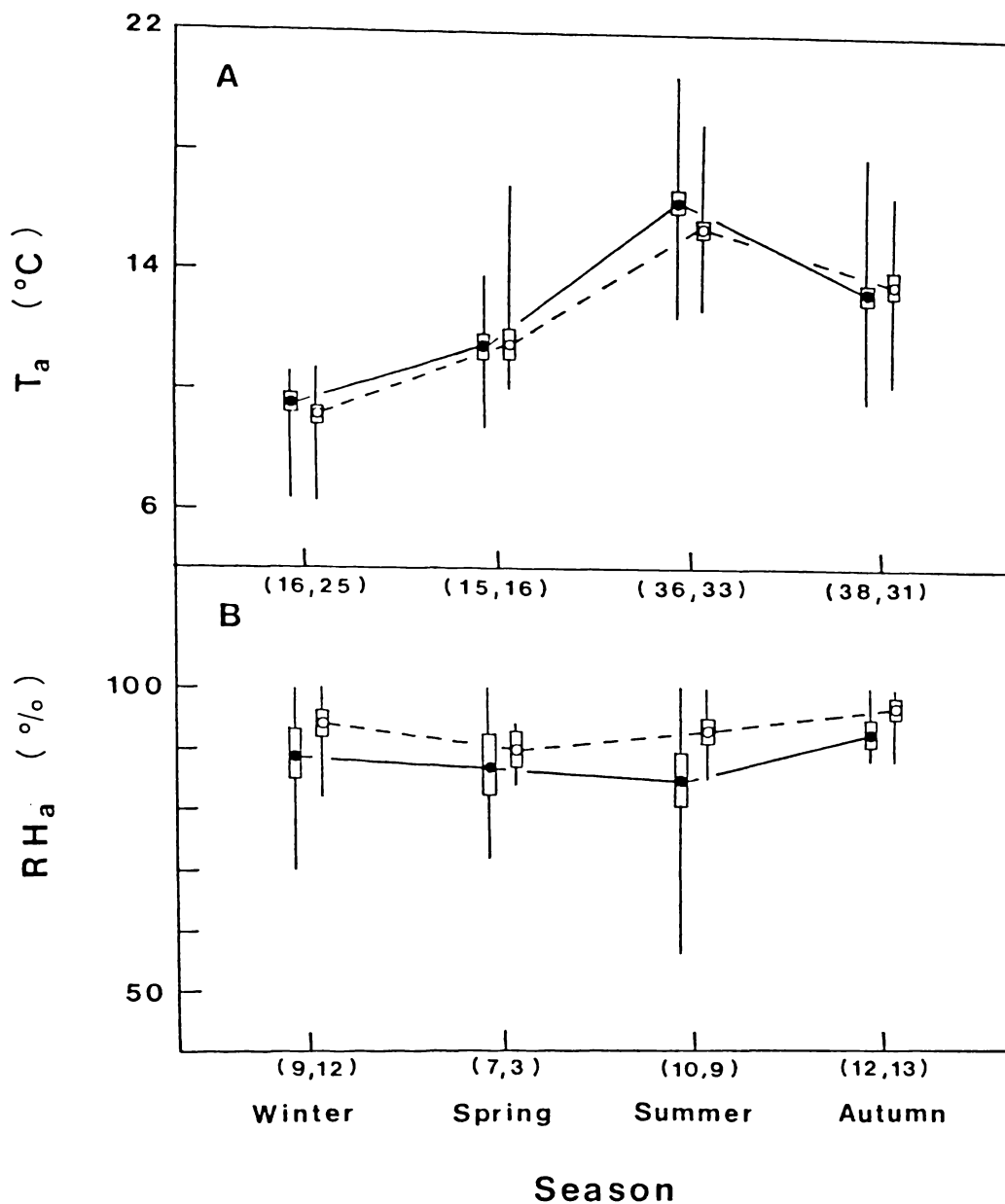


FIG. 2.5 Ambient air temperature (A) and relative humidity (B) measured during searches for *L. archeyi* (●) and *L. hochstetteri* (○) ($\bar{x} \pm SE$ and range). Ambient air temperature was measured outside individual frog retreat sites. Relative humidity was recorded once at each study site (no humidity measurements were made in December because of equipment failure, but values would have been within the range indicated for the remaining summer months). Figures in parentheses give sample sizes for *L. archeyi*, then *L. hochstetteri*.

TABLE 2.2 Paired measurements of ambient air temperature (T_a) and retreat site temperature (T_r) for *L. archeyi* and *L. hochstetteri* ($\bar{x} \pm SE$). Sample sizes give the number of paired measurements. Significance was tested using a paired Student's t-test (***) = $P < 0.001$).

Season	<i>L. archeyi</i>			<i>L. hochstetteri</i>		
	T_a (°C)	T_r (°C)	Significance	T_a (°C)	T_r (°C)	Significance
Summer	16.5 ± 0.4 n = 36	15.7 ± 0.3	t = 4.947 ***	15.5 ± 0.3 n = 33	14.9 ± 0.3	t = 7.519 ***
Winter	9.5 ± 0.3 n = 16	8.9 ± 0.3	t = 6.579 ***	9.1 ± 0.2 n = 25	8.4 ± 0.2	t = 5.185 ***

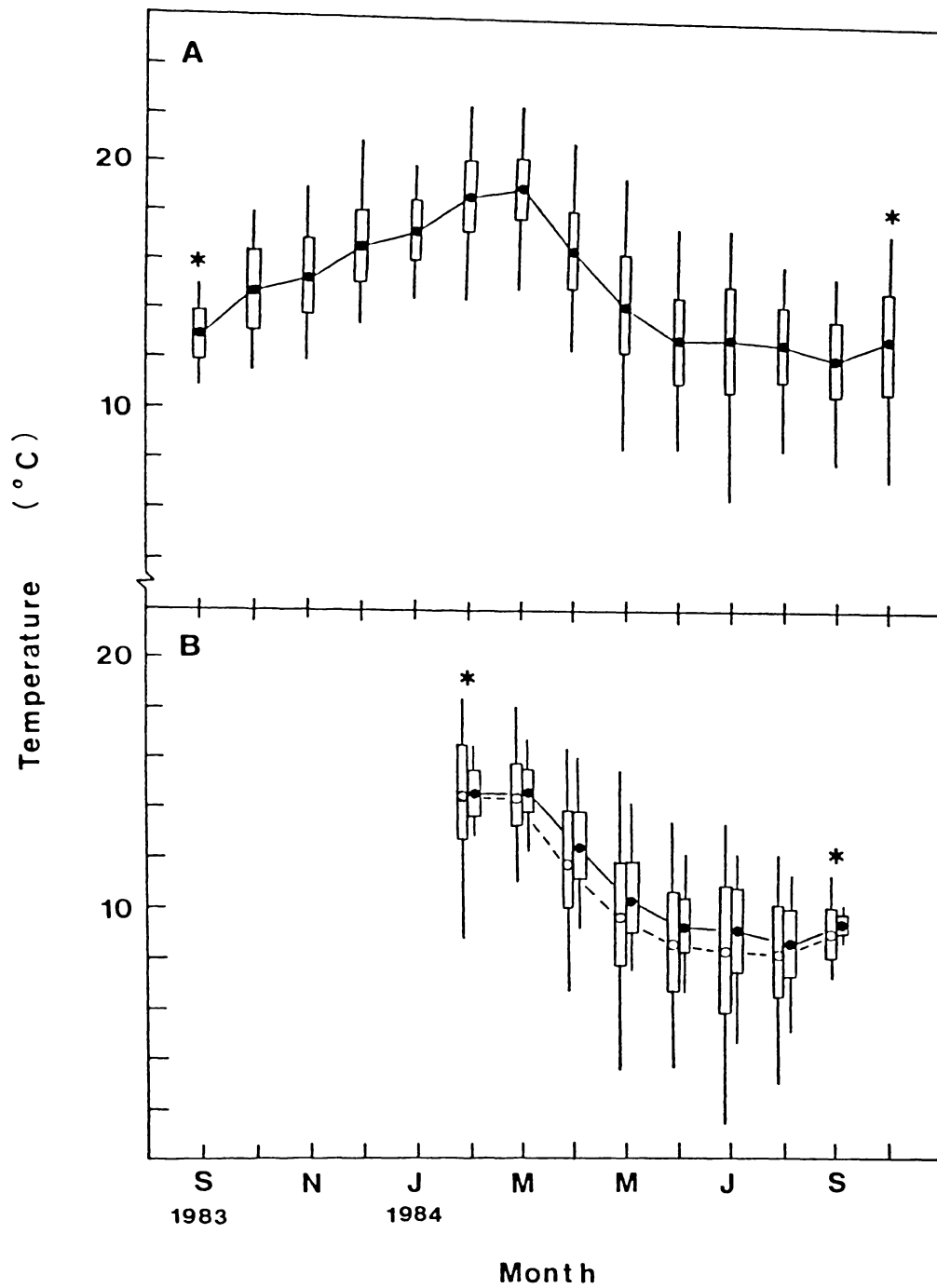


FIG. 2.6 Monthly temperatures at ridgetop sites R2 (A) and R3 (B) ($\bar{x} \pm$ standard deviation and range). Temperatures were recorded automatically at 2 h intervals. ● = retreat site temperature, ○ = ambient temperature, * = incomplete record.

days. RH_a at sites where *L. archeyi* was present was also frequently high (mean values > 85%) but varied more widely than for *L. hochstetteri*, particularly in summer. The lowest value observed for *L. archeyi* (56%) was recorded at the driest ridgetop site (R3) on a dry sunny day in January 1984. A similar value (59%) was recorded during a visit to the same site in February the following year.

During dry summer weather, conditions in the forest are cooler and more humid than in non-forested areas. For example, on a fine afternoon in February 1984, T_a at ridgetop sites ranged from 18-21°C and RH_a from 78-91%; on the road at the summit T_a was 29°C and RH_a 56%.

Paired measurements of RH_a and RH_r were made for 17 *L. archeyi* retreat sites in rainless weather. Retreat sites were significantly more humid ($88 \pm 1\%$, $\bar{x} \pm SE$) than ambient air ($83 \pm 2\%$) when compared by a paired Student's t-test ($t = 4.349$, $P < 0.001$); the mean value for RH_r undoubtedly underestimates true retreat site humidity because of the disturbance to retreat sites caused by inserting the probe.

Environmental conditions on the ridgetop

Rainfall

The Coromandel Range receives a high annual rainfall. Both the Tapu study area and the NZ Meteorological Service station at Waiau lie within an area of the range estimated to have a mean annual rainfall between 1775 and 2550 mm (Maunder 1974). At Waiau, records from 1950-1982 show that rainfall is generally well-distributed throughout the year, monthly means being lowest in January (119 mm) and highest in June (231 mm) (Fig. 2.7A). Over the 12 mo from June 1983 to May 1984 inclusive, 2294 mm of rain fell at Waiau; this compares favourably to the mean annual rainfall of 2125 mm, indicating that rainfall on the Coromandel Ranges over the year in which distribution surveys were made was not unusual.

On the Tapu ridgetop itself, rainfall was recorded from October 1983 to September 1984 inclusive (Fig. 2.7B). The annual rainfall of 2729 mm was slightly higher than at Waiau over the same period (2425 mm), largely because of a higher peak rainfall in October on the higher altitude Tapu ridgetop. Monthly totals in the study area ranged from a low of 83 mm in April 1984 to a high of 736 mm in October 1983. The

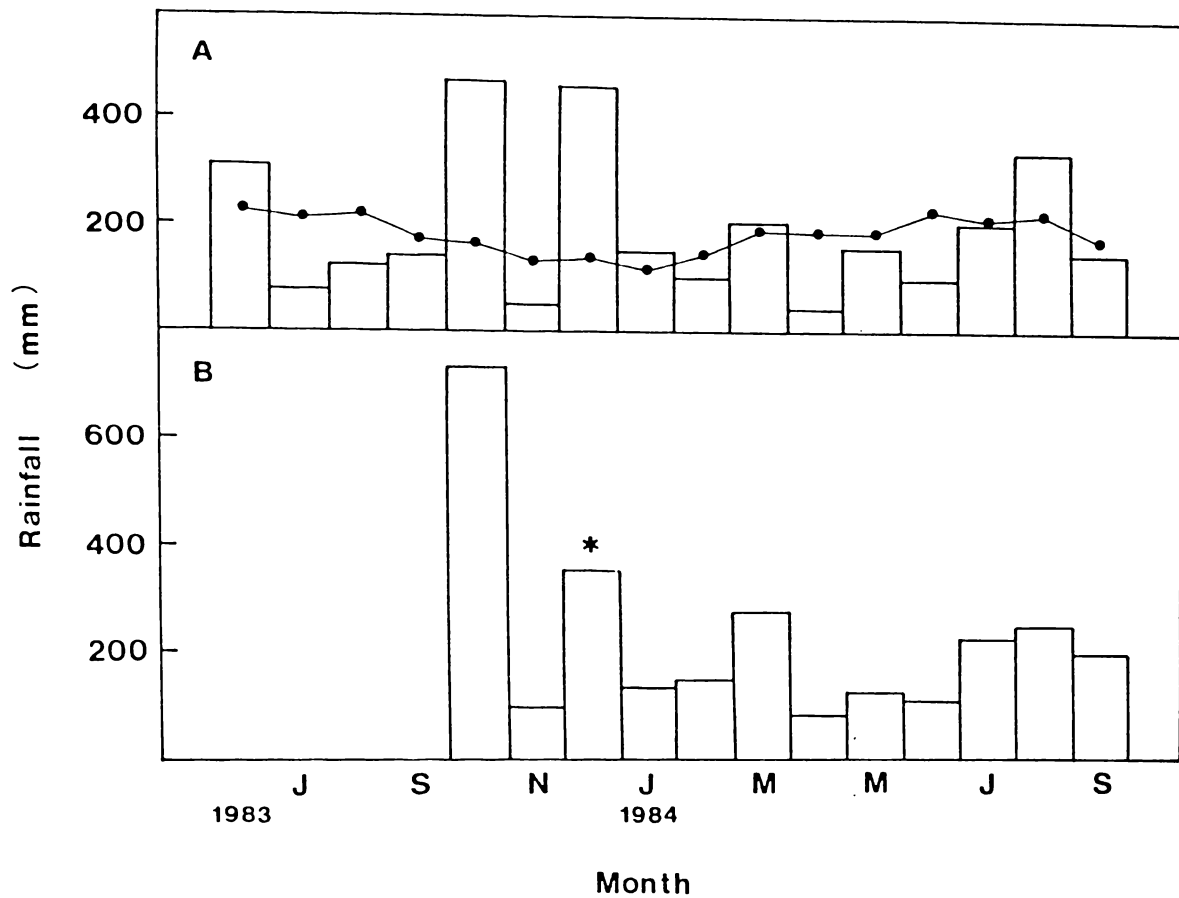


FIG. 2.7 Monthly rainfall at Waiiau (A) from June 1983, and at the Tapu study area (B) from October 1983. Mean monthly figures for Waiiau over the years 1950-1984 are also included (●) (all data for Waiiau courtesy of NZ Meteorological Service). * indicates incomplete record (raingauge became blocked with debris).

largest amount falling in 24 h was 163 mm (in October 1983) and the longest period without rain lasted 16 d (in April 1984). Periods of 7 d or longer without substantial rain (1 mm or less) occurred seven times throughout the year of recording.

Leaf wetness

Leaf wetness was recorded on the eastern slope of the R3 site from April-August 1984, a period of low-moderate rainfall (Fig. 2.7). Leaf wetness was recorded as either "wet" or "dry" at 2 h intervals, and the recordings for each day were summarised as either all wet, all dry or including both wet and dry readings. For each month, the percentage of days falling in each of these categories was calculated (Fig. 2.8). In April, the leaf wetness sensor remained dry for 70% of days in the month, but the proportion declined steadily until August, when on no days in the month was the sensor always dry. In general, leaf wetness showed a consistent relationship with rainfall, both by month (Fig. 2.8, cf. Fig. 2.7) and by day. If no rain was recorded on a particular day, then the leaf wetness sensor usually remained dry for all readings on that day. This indicates that the occurrence of mist which wets the vegetation but fails to be registered as rainfall is rare.

Evaporation rates at streamside and ridgetop sites

Evaporation rates at the eight study sites were compared during fine, dry summer weather when differences were expected to be most marked (Table 2.3). Mean rates of evaporation from the four streamside sites (*L. hochstetteri* habitat) were consistently low ($\leq 3.9 \text{ mm d}^{-1}$). The ridgetop sites (*L. archeyi* habitat) had mean evaporation rates 1.5-5.0x as high. Of the four ridgetop sites, evaporation occurred least rapidly from the cool, dark, sheltered R1 site and most rapidly from the sunnier and windier R3 site.

Summary of environmental conditions in habitats of *L. archeyi* and *L. hochstetteri*

Data collected during the 13 monthly distribution surveys have been grouped for statistical analysis (Fig. 2.9). The grouped data show the following features.

(1) Distance to water: 97% of *L. archeyi* were found more than 10 m from the nearest puddle or stream, whilst 94% of *L. hochstetteri* were either partly immersed in, or within 1 m of water. The difference in

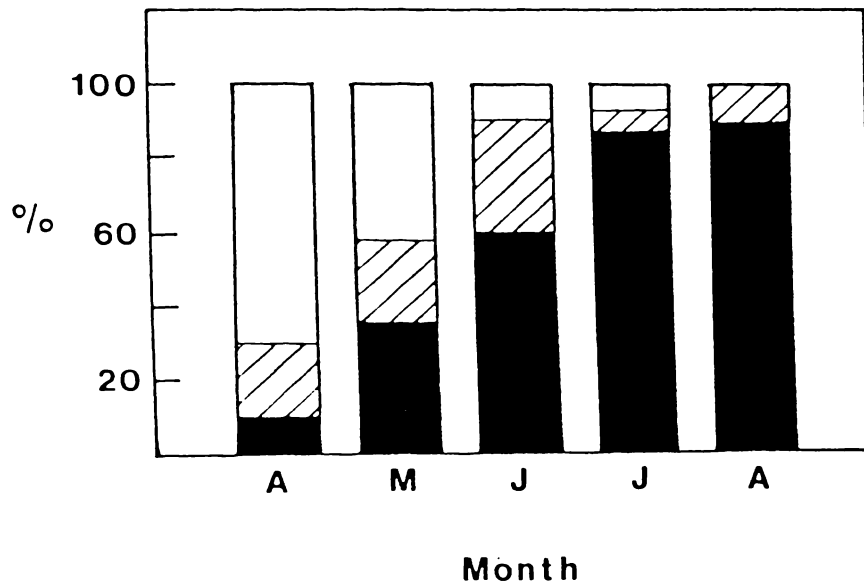


FIG. 2.8 Percentage of days per month on which the leaf wetness sensor at the R3 site was always wet (solid), sometimes wet (hatched), or always dry (open). Recordings were made at 2 h intervals between April and August 1984.

TABLE 2.3 Evaporation rates from streamside and ridgetop study sites during two 2 d periods of fine, dry summer weather in February 1985 (\bar{x} , with actual values given in parentheses).

Evaporation rate (mm d^{-1})							
Streamside sites				Ridgetop sites			
S1	S2	S3	S4	R1	R2	R3	R4
3.9 (2.3,5.4)	3.9 (2.3,5.4)	3.7 (2.8,4.5)	1.1 (1.7,0.5)	5.9 (5.1,6.7)	9.4 (7.4,11.4)	19.4 (15.5,23.3)	8.9 (7.2,10.5)

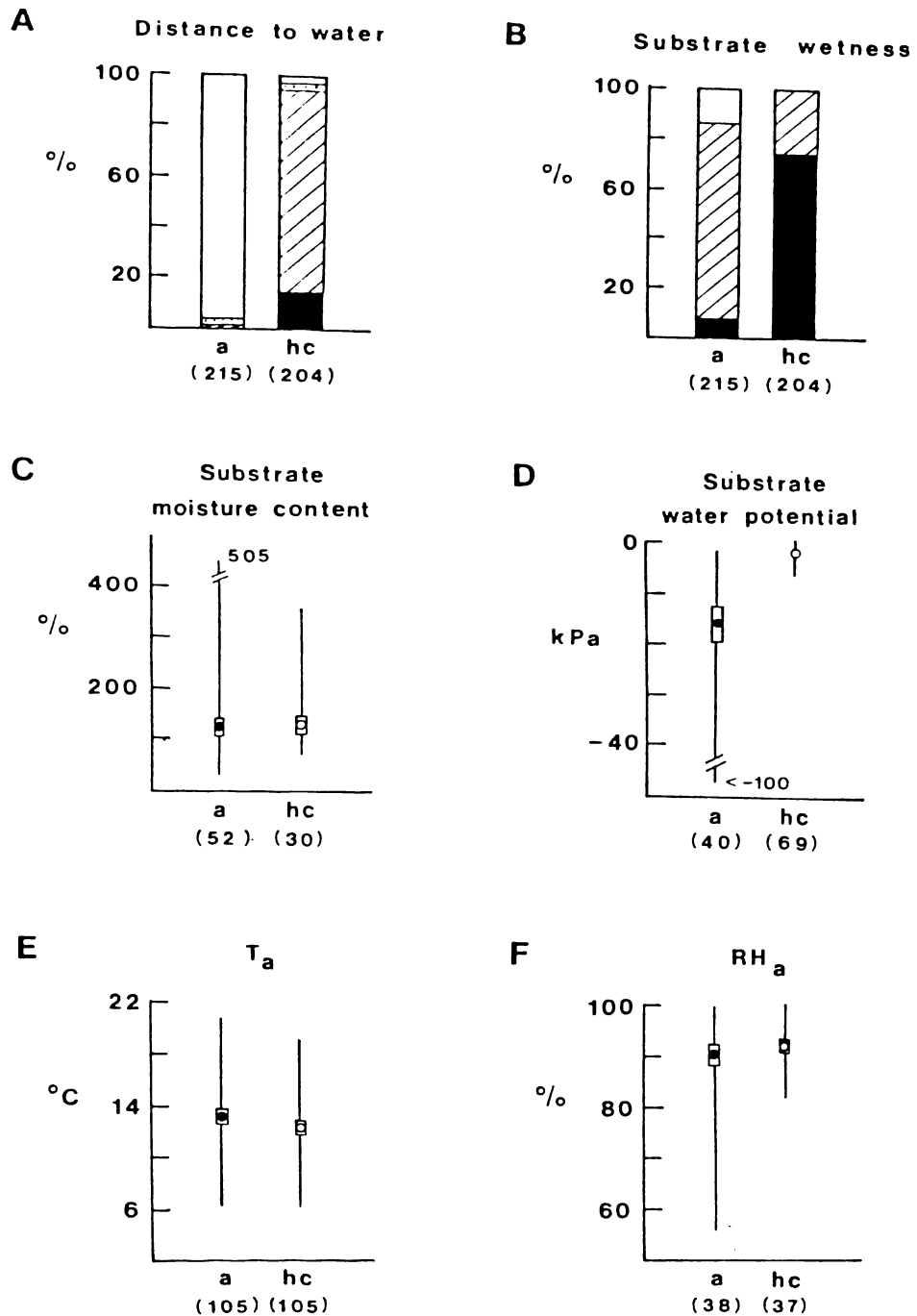


FIG. 2.9 Summary of environmental conditions recorded in the habitats of *L. archeyi* (a) and *L. hochstetteri* (hc) during 13 monthly searches (June 1983 - June 1984). For keys to A and B see FIGS 2.2 and 2.3 respectively. Data for C, D, E and F are $\bar{x} \pm SE$ and range (SE deleted where necessary for clarity). Sample sizes are given in parentheses.

frequency distribution between the two species is highly significant when compared by chi-square analysis ($\chi^2 = 5772$, $P < 0.001$). The results for *L. hochstetteri* are broadly comparable with those recently reported for the same species in the Motu River catchment on East Cape by McLennan (1985), who found this species in or up to 4 m from water.

(2) Substrate wetness: 79% of *L. archeyi* were found on moist substrates, 14% on dry and 7% on wet. No *L. hochstetteri* was found on dry substrates and 75% were on wet ones. These frequency distributions also differ significantly between the two species ($\chi^2 = 1343$, $P < 0.001$), and resemble those previously reported by Bell (1978) (see Introduction).

(3) Substrate moisture content: 24% or fewer of each species were on substrates for which moisture content could be determined (soil, soil/humus or mud). These substrates were almost always very high in moisture content (mean values for both species about 130% MC). However, values as low as 32% were recorded for *L. archeyi* substrates during dry periods.

(4) Substrate water potential: for each species, about 25% of frogs found were on permeable substrates for which water potential could be measured. All *L. hochstetteri* in this category were on waterlogged substrates (water potential > -6 kPa). Most *L. archeyi* were also on substrates close to saturation (> -38 kPa), but several were on drier soil with moisture contents of 62% or lower, which in one case at least had a water potential of < -100 kPa.

(5) Ambient temperature: spot measurements of T_a remained cool year-round, and the ranges recorded were similar for both species.

(6) Ambient relative humidity: mean values were high and similar for both species (90-92% RH), but individual values varied to lower extremes for *L. archeyi* (56% RH) than for *L. hochstetteri* (82% RH) during dry summer weather. The difference in variance between species is significant ($F = 4.456$, $P < 0.05$).

Effect of flooding on streams occupied by *L. hochstetteri*

On 16 February 1985, 8 mo after the completion of the monthly searches, torrential rain led to major flooding in low-lying areas of the Coromandel Peninsula. At Waiiau, 277 mm of rain (nearly twice the mean monthly rainfall for this station) fell in 24 h. Three weeks later, the eight study sites were revisited to examine the effect of flooding on the habitats of the two frog species. Although the ridgetop sites and the two highest altitude streams (S3 and S4) were

little affected, the stream lowest in altitude (S1) was totally devastated. What had previously been a cool, shaded, clear-flowing, rocky forest stream about 1 m wide (Plate 2.2A) was transformed into an open mud plain about 10 m wide, strewn with fallen trees and other debris at the road's edge and traversed by an innocuous and meandering muddy trickle. The site appeared completely destroyed as suitable *L. hochstetteri* habitat. However, 9 mo later in November 1985 a stream channel had reformed, the water had cleared and rocks suitable as frog retreat sites had been deposited on the banks of the upper zone shown in Plate 2.6. Three *L. hochstetteri* were found in this region. Later in January 1986, 11 mo after the flood, three different *L. hochstetteri* were discovered about 3 m further downstream. Since these frogs would have been unable to survive the peak flood levels of 1-2 m above the normal level, they must have colonised the stream after flood waters subsided.

DISCUSSION

The known distributions of *L. archeyi* and *L. hochstetteri* within the Tapu study area have been summarised in Fig. 2.10. This distribution map, which is based on observations made during the 13 monthly surveys plus additional observations between January 1983 and January 1986, indicates a large degree of ecological separation between the two species. Clearly, there is little overlap in distribution. *L. archeyi* is almost exclusively restricted to the forested ridgetops or to the spurs separating adjacent stream gullies, with only two specimens being found under rocks on the banks of streams. In contrast, almost all *L. hochstetteri* were found on waterlogged substrates along stream edges. Individuals of this species were rarely found in the same ridgetop sites as *L. archeyi*, in only two instances sharing the same retreat site as *L. archeyi*. These results thus confirm and extend the observations made by Bell (1978), but fail to support the "close mingling" of the two species reported by Stephenson and Stephenson (1957).

Apart from the availability of surface water, the range of ambient humidities experienced, and in some study sites the exposure to wind, there are no major physical differences between ridgetop and streamside habitats. Both are covered by forest of similar composition, both contain retreat sites suitable for frogs to shelter



PLATE 2.6 Streamside site S1 in November 1985, 9 mo after a major flood. Three *L. hochstetteri* were found in the region arrowed.

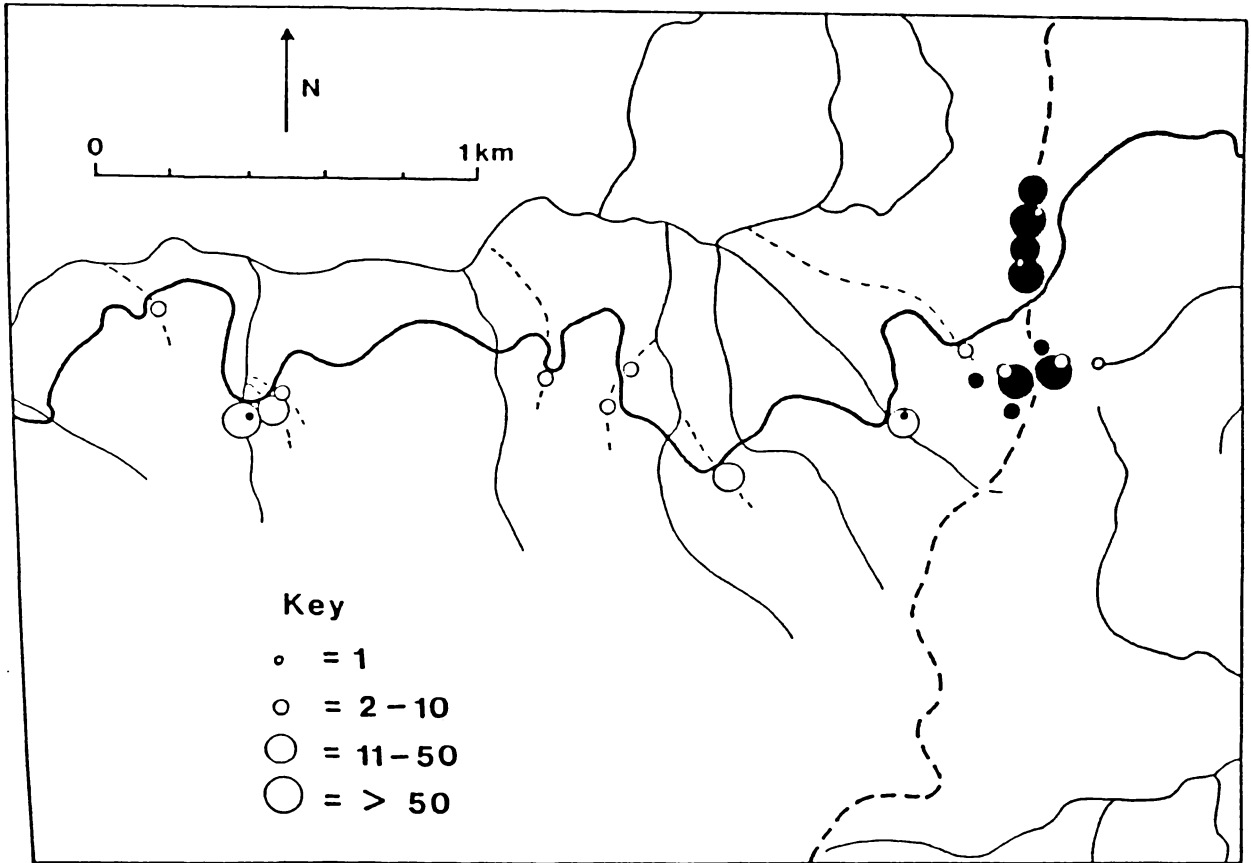


FIG. 2.10 Known distributions of *L. archeyi* (●) and *L. hochstetteri* (○) within the Tapu study area, based on observations between January 1983 and January 1986. The number of frogs observed is indicated by the size of the circles (see Key). For further details of the study area, see FIG. 2.1B.

in, and both have similar temperature regimes. These similarities suggest that a difference in substrate moisture preference may be the major factor determining the difference in distribution of the two species. Laboratory studies confirm that *L. hochstetteri* has a greater preference for water than does *L. archeyi* (Appendix I).

Although *L. archeyi* and *L. hochstetteri* have nearly disjunct distributions in the Tapu study area, in terms of spatial distance their separation may not be great. For instance, ridgetop sites R1 and R2, which were usually populated solely by *L. archeyi*, were only about 50-70 m distant from a stream headwater which contained only *L. hochstetteri*. These two ridgetop sites were also the ones to contain *L. hochstetteri* most frequently. Although the simultaneous presence of *L. archeyi* and *L. hochstetteri* in the same retreat sites was never the "common occurrence" reported by E.M. Stephenson (1961) (accounting for less than 1% of observations of each species), 4% of *L. hochstetteri* were found on ridgetops and this situation requires some consideration.

A likely explanation for the occasional presence of *L. hochstetteri* on the ridgetops lies in the close proximity of some ridgetop sites to stream headwaters. Following heavy rain, when higher than normal stream levels submerge many normal streamside retreat sites, *L. hochstetteri* are difficult to find close to streams. They may, however, be present on ridgetops. This suggests that heavy rain, possibly in conjunction with rising stream levels, stimulates the movement of *L. hochstetteri* into the forest, with some individuals reaching the ridgetop sites normally inhabited only by *L. archeyi*. Frogs which failed to move away during floods would be drowned and/or swept downstream. As conditions dry out, *L. hochstetteri* on the ridgetops presumably return to their regular streamside habitat. *L. hochstetteri* are clearly able to recolonise previously flooded streams when water levels subside and flows return to normal, provided suitable retreat sites are present. Furthermore, since eggs of this species have only been found in seepages close to streams (Robb 1980; Bell 1985b, McLennan 1985), *L. hochstetteri* are unlikely to remain on the ridgetops indefinitely.

Clearly, records of the movements of individual frogs are required to support this hypothesis. However, since the object of this study was to describe the habitats of the two species, not of individual frogs,

no detailed mark-recapture study of the movements of particular individuals was undertaken. The only movement records come from an attempt to estimate the frequency of recapture of individual *L. hochstetteri* from one monthly search to the next, for which 11 individuals were toe-clipped at the S1 study site in March 1984. None of these frogs was recaptured in two searches of this site following the 1985 flood, and only two were recaptured during five searches prior to flooding (of a total of 58 frogs found). One was recaptured once and one twice; both were recaptured within about 1 m of the spot where released following toe-clipping.

These recapture records, plus casual observation of frog sizes and colour patterns, suggest that the frequency of recapture from one month to the next was not high for either species. Certainly, it was the exception rather than the rule to find the same retreat site occupied by the same frog in successive months. Information from other sources also suggests that only a small proportion of the resident population of *L. archeyi* was found during daytime searches on the ridgetop. For instance, during nighttime searches of the R3 site in wet weather up to 33 *L. archeyi* were seen (Chapter 9), yet the greatest number found during daytime searches was 11. Frogs not found by day presumably occupy inaccessible retreat sites under tree roots or immovable objects. Dr. B.D. Bell (Victoria University of Wellington, pers. comm.) is presently carrying out a mark-recapture study of *L. archeyi* on the Tapu ridgetop between sites R3 and R4 and estimates the population size in an area of 100 m² to be about 100 frogs, with only about 25-33% being found in a single daytime search.

In several respects the habitat and habits of *L. hochstetteri* resemble those of the North American tailed frog *Ascaphus truei*. Like *L. hochstetteri*, *A. truei* is found by day in the vicinity of cool mountain streams or in spray-drenched cliff fissures by waterfalls (Metter 1964). However, whereas *L. hochstetteri* is most often found alongside rather than in streams, *A. truei* may be present under rocks in the stream water itself. Streams inhabited by *A. truei* are subject to flash floods, and adults can be found on the banks of previously flood-damaged streams (Metter 1968). As in the Tapu study area, summer air temperatures in the habitat of *A. truei* are cool (seldom reaching 23°C), but winter temperatures are colder, with snow and frost being common. On dry summer nights adult *A. truei* forage along streams, and in wet weather they may roam up to 33 m distant (Metter

1964, 1967). Daugherty and Sheldon (1982) speculated that high rainfall in the western portion of the range of *A. truei* may permit more extensive movement away from streams and between drainages than in the Rocky Mountains, where suitable streams are isolated from each other by dry precipitous mountain ridges.

Unlike the amphibious *L. hochstetteri* and *A. truei*, *L. archeyi* is a completely terrestrial frog. During dry summer weather this species remains on the ridgetops: there is no evidence that movement to moister habitats occurs during dry conditions. Although the air is cooler and more humid in the forest than on the nearby roadside, summer conditions on the ridgetops would be taxing to a permeable-skinned frog without the presence of suitable retreat sites. During rainless periods, retreat sites are cooler and more humid by day than ambient air. The cover of the retreat minimises drying of the substrate beneath so that soil water potential usually remains close to saturation, and frogs in retreat sites are protected from the desiccating effects of wind. If the soil in the retreat site is saturated and the covering rock is nearly sealed to the substrate, RH, probably approaches the interstitial humidity for saturated soil (99% or higher; Griffin 1963). High humidities, cool temperatures and low wind speed all reduce evaporation rates from permeable-skinned frogs (Tracy 1976) and water uptake may be possible from saturated soils (Walker and Whitford 1970). Although temporary puddles are present on the ridgetops following heavy rain, *L. archeyi* were never seen even partly immersed in water, contrary to the report of Turbott (1949). Individuals may, however, emerge from their retreat sites during wet daytime weather.

No measurements of environmental conditions in the habitat of *L. hamiltoni* were made in the present study. However, published data indicate that ambient temperatures and relative humidities for this third species of *Leiopelma* are remarkably similar to those reported here for *L. archeyi* (Newman 1977, Newman *et al.* 1978; Table 2.4), despite the fact that Stephens' Island and Maud Island lie 4° of latitude south of the Tapu study area. The similarity in conditions presumably reflects the insular geography of the Coromandel Peninsula, and the higher altitude of the Tapu study area (Table 2.4).

Similar combinations of cool temperature, high humidity, high rainfall and/or frequent mist to those in the Tapu study area must be present

TABLE 2.4 Comparison of the habitats of *L. archeyi* and *L. hamiltoni*. Temperature and relative humidity values for *L. archeyi* are means of spot measurements made during monthly searches in the present study. Those for *L. hamiltoni* are means of three-hourly daytime measurements made between September 1974 and August 1975 inclusive. Rainfall was measured over one year in the study areas, except for Maud Island where the values given indicate the isohyet range within which mean annual rainfall lies (all data for *L. hamiltoni* from Newman *et al.* 1978).

Factor	<i>L. archeyi</i> Tapu study area	<i>L. hamiltoni</i>	
		Maud Island forest floor	Stephens' Island "frog bank"
Altitude (m asl)	400 - 500	90	290
Annual rainfall (mm)	2729	1400 - 1600	914
Temperature (°C)			
spring (Sept-Nov)	11.4	12.9	13.4
summer (Dec-Feb)	16.5	18.9	20.1
autumn (Mar-May)	13.3	14.7	14.8
winter (Jun-Aug)	9.5	8.9	9.1
Relative humidity (%)			
spring	93	89	88
summer	90	83	79
autumn	97	91	87
winter	89	92	86

throughout many regions of mainland New Zealand; this raises the possibility that further, presently unknown populations of these secretive and inconspicuous frogs exist. A chance report to me from Mr R.Pearce (University of Waikato, pers. comm.) of the presence of "small brown frogs" in the Komata Reefs region of the southern Coromandel Range, about 9 km northeast of the township of Paeroa (Fig. 2.1A) illustrates this potential. Following this report I visited the Komata Reefs region in January 1984, and confirmed the presence of *L. archeyi* here (grid reference NZMS 260 T13 548258). The two frogs found were at an altitude of about 600 m asl, in forested habitat very similar to that in the Tapu study area. This finding has potential conservation importance, since it raises the number of known localities for *L. archeyi* from three (Tapu Summit, Tokatea Ridge, Mt Moehau) to four. Similar conditions of high rainfall (> 2500 mm per annum), frequent fog, low evaporation rates, waterlogged soils and cool air temperatures are also found in the nearby Kaimai Range (Fig 2.1A) (Green and Jane 1983; Jane and Green 1983). Assuming that suitable retreat sites are available, there seems no reason why *L. archeyi* should not be present here also.

In summary, the results of this field study show that there is a difference in availability of surface water between the habitats of *L. archeyi* and *L. hochstetteri* in the Tapu study area. Like *L. hamiltoni*, *L. archeyi* can justifiably be considered a terrestrial frog, whilst *L. hochstetteri* is semiaquatic or amphibious. In the following five chapters I examine how differences in water balance physiology relate to the observed differences in habitat between the three species. In the final two chapters of this thesis, some of the reproductive and behavioural adaptations which enable *L. archeyi* to survive and breed in a terrestrial environment are investigated.

CHAPTER 3

DEHYDRATION AND REHYDRATION

INTRODUCTION

Anurans fall into three broad classes with respect to evaporative water loss (EWL): (i) those that form cocoons to reduce EWL during aestivation, (ii) those xeric species in which the dorsal skin is virtually impermeable to water loss because of waxy secretions or other unknown mechanisms, and (iii) the majority, in which the skin has little or no resistance to EWL and which therefore dehydrate rapidly (Withers *et al.* 1982). Within the latter group there may be small differences between arboreal and non-arboreal species, the former losing water less rapidly (Wygoda 1984). Many anurans, however, are remarkably tolerant to dehydration and are able to rehydrate rapidly when given access to water. In several species this is due to an increase in osmotic water uptake through the ventral skin (McClanahan and Baldwin 1969; Christensen 1974a) and in some bufonid toads which have extensively sculptured skin, water may be transported from the ventral surface over almost the entire body in surface grooves, presumably by capillary action (Christensen 1974a; Lillywhite and Licht 1974). However, the aquatic species *Xenopus laevis* (Ewer 1952) and several ranids (Shoemaker and McClanahan 1980) fail to rehydrate rapidly, and in these anurans it appears that cutaneous water uptake is not increased by dehydration.

The objectives of this chapter are to demonstrate: (i) whether there are any differences in rates of EWL between *L. archeyi*, *L. hamiltoni* and *L. hochstetteri* in accordance with their differences in habitat, (ii) whether any species has mechanisms for reducing EWL below the high rates typical of most anurans, and (iii) whether the three species show any differences in rates of rehydration.

MATERIALS AND METHODS

1. Collection and maintenance of experimental animals

All *Leiopelma* species are protected by law, and only limited numbers

were available for laboratory experimentation. Most techniques were therefore non-terminal and several were practised first with more commonly available anurans; in some cases this yielded valuable comparative information (Chapters 5 and 6; Appendix II).

Laboratory experiments with *L. archeyi* (1.5-5.3 g) and *L. hochstetteri* (1.6-8.1 g) were performed with a stock of 20 animals of each species, including immature females and mature adults of both sexes. The first ten of each were collected from the Tapu study area in April and November 1983, and the remaining ten in October 1984 and February 1985. *L. archeyi* were collected from the ridgetop south of the R1 site and north of the R4 site. *L. hochstetteri* were collected from streams crossing the Tapu-Coroglen road on the western side of the summit. Some were taken from the S1 and S2 study sites in 1983 prior to beginning the field study described in Chapter 2, but the remainder were taken from areas outside study sites, in most cases separate streams.

In the laboratory, *L. archeyi* were housed in a 700 x 400 x 400 mm vivarium containing damp forest soil, leaf litter, rocks and logs. *L. hochstetteri* were kept in a vivarium of the same size containing rocks and stones and with stream water trickling through. Stream water originated from the Waitakararu Stream, Waikato and circulated in a closed system. The vivaria were kept in a cool room at 10-19°C on a 12 h L: 12 h D photoperiod, and *in vivo* experiments were performed in the same room. Frogs were fed a varied diet of houseflies, blowflies, nymphal locusts and mealworms; feeding was discontinued 4 d before experiments to minimise the frequency of defaecation during experiments. Animals were rested a minimum of 4 and usually at least 7 d between experiments.

Experiments with the rarer *L. hamiltoni* (2.2-8.4 g) were performed in two situations. Those described in this chapter and in Experiment 1 of Chapter 4 were carried out on Maud Island in May 1984, using 15 freshly caught unsexed animals held overnight on damp paper towels. These experiments were performed indoors under natural lighting (about 11 h L: 13 h D) and temperature (10-18°C) conditions. The frogs were returned to nature when experiments were complete, as required by the collection permit.

Later experiments with *L. hamiltoni* (Chapters 4, 5 and 7) were

performed using five mature females and three mature males collected from Maud Island by Dr B.D.Bell in May 1985. These were housed in the laboratory at the University of Waikato in a vivarium already containing three *L. archeyi*.

Most frogs remained apparently healthy for periods up to 23 mo in captivity. Many gained weight, and most females developed large ovarian eggs (visible through the abdominal skin). Exceptions were one *L. hochstetteri* which died after becoming excessively oedematous, perhaps indicating a lymph heart failure, and one *L. archeyi* which was noticeably ailing within 2-3 wk of collection (lethargic when prodded, remaining in the open by day) and which was killed for examination of skin structure (Chapter 6).

2. Dehydration, rehydration and hydrated water uptake experiments

Animals

Dehydration, rehydration and hydrated water uptake experiments were performed with *L. archeyi* (1.5-5.3 g) and *L. hochstetteri* (1.6-8.1 g) during June-November 1983, using stock animals held in captivity 2-7 mo. Experiments with *L. hamiltoni* (2.2-8.1 g) were carried out on Maud Island in May 1984 using animals collected the previous night.

Dehydration

Dehydration rates were determined by measuring weight loss from inactive animals in dry surroundings. Conditions of temperature, relative humidity (RH) and air flow were chosen to approximate those potentially experienced by frogs in their natural habitats. The effect of RH on water loss from *L. archeyi* and *L. hochstetteri* was examined by dehydrating frogs at $93 \pm 1\%$, $80 \pm 3\%$ and $59 \pm 4\%$ RH, at $14 \pm 1^\circ\text{C}$ in still air. Because of time and facility constraints on Maud Island, *L. hamiltoni* were dehydrated at $64 \pm 2\%$ RH and $15 \pm 1^\circ\text{C}$ only. Mean weights of frogs used in dehydration experiments were similar for the three species: 4.2 ± 0.1 g ($\bar{x} \pm \text{SE}$) for *L. archeyi*, 5.7 ± 0.3 g for *L. hochstetteri* and 4.2 ± 0.3 g for *L. hamiltoni*.

Prior to dehydration, bladder urine was removed using the usual methods for anurans (gentle pressure on the abdomen, insertion of a cannula into the cloaca - e.g., Heatwole *et al.* 1969; Shoemaker and McClanahan 1980), although occasionally these may not have guaranteed complete emptying of the bladder, particularly for the more muscular

L. hochstetteri. Frogs were then rinsed, blotted dry with paper towels, and their initial weights (W_0) were determined to the nearest mg. They were then placed in pre-weighed 35 ml dishes with mesh lids, just large enough to accommodate each frog without allowing excessive movement. Dehydration was carried out inside an internally supported 0.08 m³ black plastic bag which could be closed as necessary to maintain the desired humidity. Animals usually remained inactive in these dark conditions. During experiments at 93% RH trays of water were kept inside the bag, but other humidities were determined by outside weather conditions. Relative humidity inside the bag was monitored to 1% RH with a hair hygrometer and temperature to 0.5°C with a mercury thermometer. The humidity of the boundary layer 5 mm above inactive *L. archeyi* and *L. hochstetteri* dehydrating at 59% RH was measured using a quick-response humidity sensor (Vaisala HMP-11, Finland).

Rates of weight loss were measured over 4-5 h. A surprising feature of all *Leiopelma* species was that spontaneous urination continued to occur during dehydration; this meant that water loss could be divided into two components, evaporative water loss (EWL) and urinary water loss (UWL) (the latter included a small amount of mucous secretion). Each frog in its dish was weighed hourly and if urine was observed, frogs and dishes were wiped dry and reweighed to determine the amount. Weight losses due to defaecation were excluded from results.

Except for experiments at 93% RH, dehydration was then continued under sometimes more widely varying humidities until 9-15% of W_0 had been lost, to provide frogs for use in later rehydration experiments. Humidity remained relatively stable (within 75-82% RH) during dehydration of *L. archeyi* and *L. hochstetteri* at 80% RH; regular measurements of EWL and UWL were therefore continued throughout this experiment. The rate of EWL from a free water surface (a 35 ml dish containing stream water) was also measured during this experiment.

Hydrated water uptake and urine accumulation

Rates of water uptake and urine accumulation were first measured in hydrated frogs placed in water. These served as controls for later experiments in which frogs were initially dehydrated. The method followed was essentially that used by Brown and Brown (1977, 1980) for urodeles. Weighed animals with their bladders emptied were placed in water for 15 min, then removed, blotted and reweighed. Accumulated

urine was removed from the bladder, and the animals were weighed once more. The weight gained whilst in water gives the rate of water uptake, and the decline in weight observed when urine is removed provides an estimate of urine output. Clearly, this assumes that urination into the bathing water does not occur. In *Leiopelma* it proved necessary to reduce the time in water from the 1-2 h used by Brown and Brown (1977, 1980), since frogs left 30 min or longer sometimes urinated. Initially, attempts were made to ligate the cloaca or to insert cloacal cannulae connected to urine collection bags, but these were usually unsuccessful and in some cases seemed to interfere with spontaneous urine production. Because these procedures also risked damaging the animals or their skin, both of which were required for later experiments, they were not pursued further. However, if undisturbed frogs were left in water for only 15 min they rarely urinated (confirmed by analysing bathing water for urea plus ammonia nitrogen, using a modified urease-Berthelot reaction - Sigma Diagnostic Kit No. 640). A 15 min interval was therefore used, and the rare instances of urination which were detected by a drop in weight or by excretion of nitrogenous wastes were excluded from the results.

Details of the procedure were as follows. Frogs were weighed after removal of urine and then placed in 35 ml dishes containing 3 ml stream water, sufficient to cover just the ventral surface to about 3 mm depth. Rates of water uptake and urine accumulation were determined for four successive 15 min intervals and added to give hourly rates. Weights of individual frogs were reproducible to within 8 mg after rewetting and blotting. Values of urine accumulation are only estimates of urine output, since complete emptying of the bladder could not be guaranteed.

The role of the ventral skin in water uptake, relative to the entire skin surface, was determined for *L. archeyi* and *L. hochstetteri*. Experiments similar to those described above for ventral uptake were performed, except that this time frogs were immersed in water up to their heads (referred to as immersed uptake).

Rehydration

Frogs were first dehydrated by 9-15% of W_0 and then placed in shallow water to rehydrate. Rates of ventral water uptake and urine accumulation were measured every 15 min for 1 h and thereafter at

longer intervals. Frogs were closely observed during the first hour to see if drinking occurred, and in some cases a few drops of carmine suspension was added to the water to show whether water was transported onto the frogs' dorsal surfaces. *L. archeyi* and *L. hochstetteri* were also allowed to rehydrate when immersed; in this experiment weights were recorded every 30 min for 2 h. Nitrogen content of water bathing rehydrating animals was not measured.

Measurement of surface area

Physical processes such as EWL and cutaneous water uptake are clearly dependent on amount of exposed surface area (SA), and thus on shape and total body size. Measurements of EWL and immersed uptake per unit weight (W) in frogs have often been converted to unit SA using general formulae for tetrapods such as that of Benedict (1932), where $SA \text{ (cm}^2\text{)} = 10 \times W \text{ (g)}^{0.67}$ (e.g., Bentley and Yorio 1979a). However, these fail to take account of skin surfaces which are pressed together or in contact with the substrate and thus not participating in EWL; they also may not be relevant to species of different shapes.

In the EWL experiments described here, amount of exposed SA was estimated at a later date by smoothing an unstretched sheet of plastic food wrap over each frog in its resting position and then tracing the frog's outline, a feasible method for these inactive frogs. For immersed water uptake experiments plastic wrap was smoothed over the frog with its limbs outstretched. Its outline (excluding the head) was traced and doubled to give the approximate immersed area of both the upper and lower surfaces. During ventral uptake experiments the area of ventral skin in contact with the base of the container was traced. All tracings were transferred to stiff paper and the areas of these were measured using a Portable Area Meter (Li-Cor 3000, Nebraska, USA). Values for immersed area proved to be 77-98% of those calculated using Benedict's (1932) formula.

Analysis

Results were compared using Student's t-test (modified for paired samples where appropriate), or by one-way analysis of variance (ANOVA).

RESULTS

Dehydration

Weight loss due to both EWL and UWL occurred from all species at all humidities. Results are shown in detail for *L. archeyi* and *L. hochstetteri* at 80% RH (Fig. 3.1). In both species, the mean rate of EWL remained constant throughout whilst the rate of UWL declined. The same trends (although not rates) were observed in all species at other humidities where dehydration continued beyond 5 h. Animals were usually inactive during dehydration and adopted crouched postures similar to those seen in resting animals in the wild. The ventral surface was usually in contact with the base or wall of the container, the head was close to the substrate, eyes were half-closed, and limbs were held close to the body although not tucked in tightly against or underneath it.

Rate of EWL varied in an inverse linear relationship with RH for both *L. archeyi* and *L. hochstetteri*, whether expressed in terms of body weight or exposed SA (Fig 3.2A and B). No significant differences in EWL on a weight or SA basis were observed between these two species at any humidity ($t \leq 2.245$, $P > 0.05$). *L. hamiltoni* were dehydrated at a slightly higher temperature (15°C, 64% RH) and showed a slightly higher rate of EWL than for *L. archeyi* and *L. hochstetteri* dehydrated at 14°C and 59% RH. At 80% RH, EWL from *L. archeyi* ($0.88 \pm 0.04 \text{ mg cm}^{-2} \text{ h}^{-1}$; $\bar{x} \pm \text{SE}$) and *L. hochstetteri* ($0.86 \pm 0.08 \text{ mg cm}^{-2} \text{ h}^{-1}$) was similar to that from a free water surface ($0.77 \text{ mg cm}^{-2} \text{ h}^{-1}$) (Fig. 3.2B). The humidity of the boundary layer 5 mm above inactive *L. archeyi* and *L. hochstetteri* dehydrating at 59% RH was 78-80% RH.

Rates of UWL measured over 4-5 h showed no obvious relationship with RH, except that rates for *L. archeyi* at 93% RH were more variable and generally higher than at lower humidities (Fig. 3.2C). Variation in UWL between the three humidities was significant by one-way ANOVA for *L. archeyi* ($F = 6.27$, $P < 0.05$), but not for *L. hochstetteri* ($F = 2.80$, $P > 0.05$). In all experiments where dehydration continued to 9-15% weight loss, urinary losses declined during dehydration. The mean percentage of total weight loss accounted for by UWL at the end of dehydration varied between 19 and 43% (Table 3.1), and there was often considerable variation between animals in the same experiment. At both 59% and 80% RH, the mean percentage for *L. hochstetteri* was higher than that for *L. archeyi* at the same humidity or for

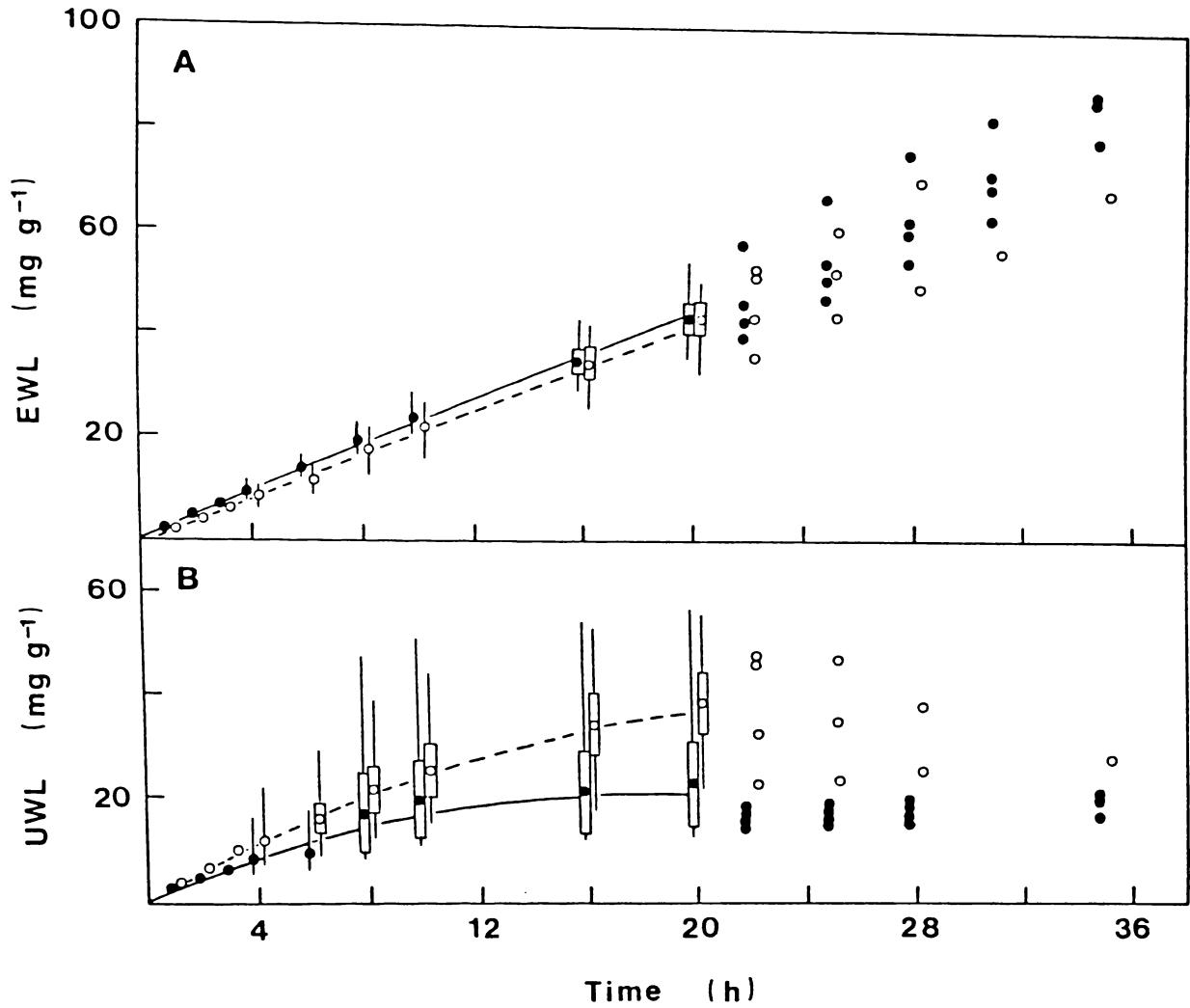


FIG. 3.1 Evaporative water loss (EWL) (A) and urinary water loss (UWL) (B) from *L. archeyi* (●) and *L. hochstetteri* (○) at 80% RH and 14°C ($\bar{x} \pm$ SE and range for $n = 5$ for first 20 h, and individual values for remaining animals thereafter; range and SE deleted where necessary for clarity; lines fitted by least squares regression in A and by eye in B).

Regression equations for A are

$$\begin{aligned} \text{EWL (mg g}^{-1}\text{)} &= 1.11 + 2.12 \text{ h (} L. \text{ archeyi)} \\ r &= 0.97 \end{aligned}$$

$$\begin{aligned} \text{EWL (mg g}^{-1}\text{)} &= -0.27 + 2.15 \text{ h (} L. \text{ hochstetteri)} \\ r &= 0.97, \end{aligned}$$

where h is time in hours.

FIG. 3.2 Effect of relative humidity on water loss over 4-5 h from *L. archeyi* (●) and *L. hochstetteri* (o) at 14°C, and from *L. hamiltoni* (Δ) at 15°C. A = evaporative water loss (EWL) from inactive animals, expressed per unit body weight. B = EWL from inactive animals expressed per unit surface area of exposed skin. C = urinary water loss (UWL) ($\bar{x} \pm SE$ and range for $n = 5$ at each humidity; SE deleted where necessary for clarity; * = free water surface at 14°C; lines fitted by least squares regression).

Regression equations for weight-specific EWL in A are:

$$\begin{aligned} \text{EWL (mg g}^{-1} \text{ h}^{-1}) &= 11.8 - 0.12 \text{ RH (} L. \text{ archeyi)} \\ r &= 0.94 \end{aligned}$$

$$\begin{aligned} \text{EWL (mg g}^{-1} \text{ h}^{-1}) &= 10.2 - 0.10 \text{ RH (} L. \text{ hochstetteri)} \\ r &= 0.94, \end{aligned}$$

where RH is relative humidity as a percentage.

Regression equations for area-specific EWL in B are:

$$\begin{aligned} \text{EWL (mg cm}^{-2} \text{ h}^{-1}) &= 4.64 - 0.045 \text{ RH (} L. \text{ archeyi)} \\ r &= 0.94 \end{aligned}$$

$$\begin{aligned} \text{EWL (mg cm}^{-2} \text{ h}^{-1}) &= 4.21 - 0.041 \text{ RH (} L. \text{ hochstetteri)} \\ r &= 0.97 \end{aligned}$$

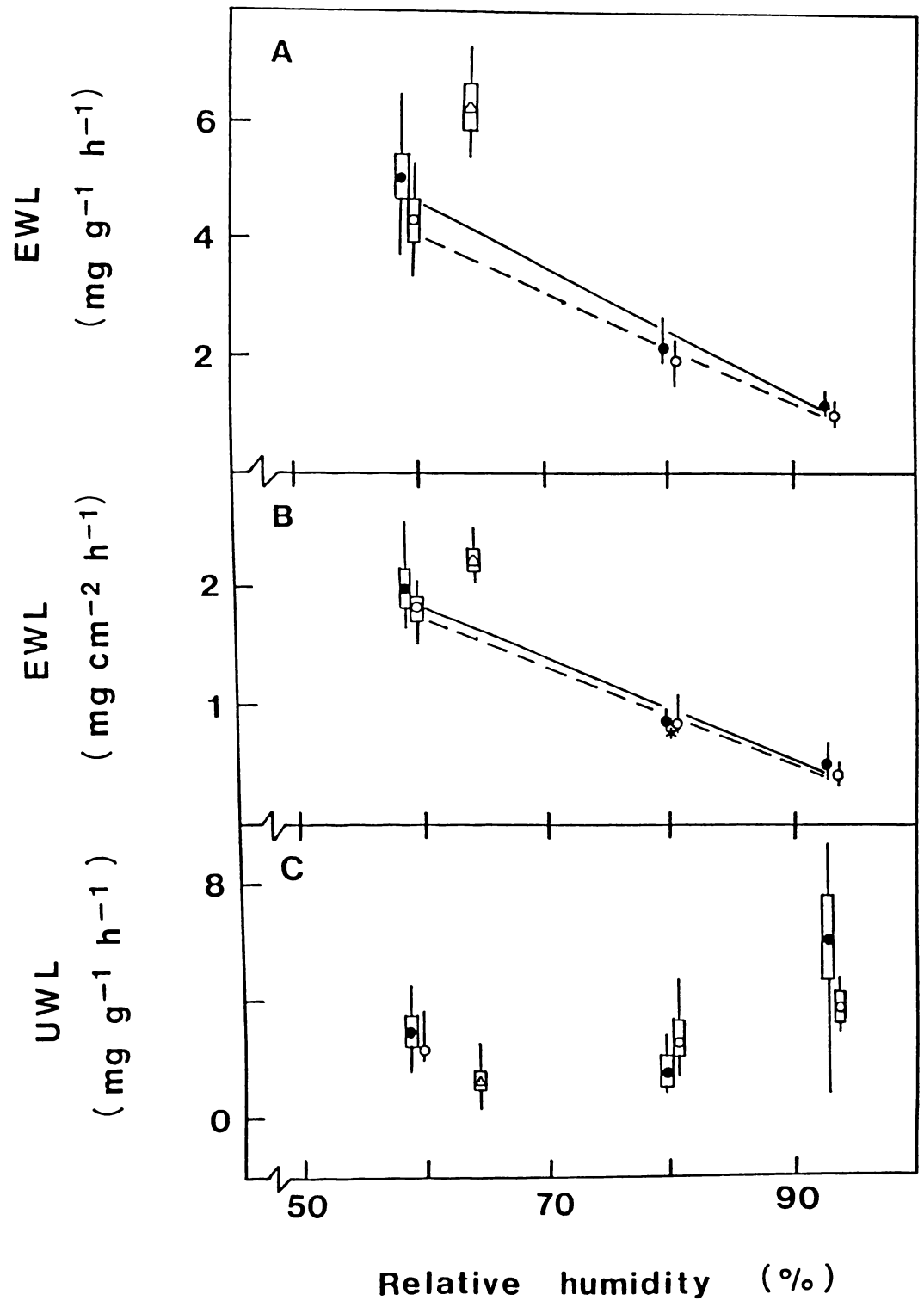


TABLE 3.1 Urinary water loss as a percentage of total water loss (UWL + EWL) during dehydration of *Leiopelma* species by 9-15% of W_0 ($\bar{x} \pm SE$). The humidity value at the top of each column gives the humidity during the first 4-5 h of the experiment. The values in parentheses give the humidity range that occurred throughout dehydration to 9-15% weight loss (* = $P < 0.05$).

Species	UWL as a percentage of total water loss at humidities of:		
	59% RH (56-78)	64% RH (62-83)	80% RH (75-82)
<i>L. archeyi</i> n = 5	18.9 ± 2.2	-	25.7 ± 7.5
<i>L. hamiltoni</i> n = 5	-	18.9 ± 3.7	-
<i>L. hochstetteri</i> n = 5	28.4 ± 3.4	-	42.5 ± 4.8
Significance of comparison between <i>L. archeyi</i> and <i>L. hochstetteri</i>	t = 2.318 *	-	t = 1.891 P > 0.05

L. hamiltoni at 64% RH. However, the difference between *L. hochstetteri* and *L. archeyi* was statistically significant at 59% RH only ($P < 0.05$; Table 3.1).

Hydrated water uptake, urine accumulation and rehydration

Hydrated *L. archeyi* and *L. hamiltoni* with ventral surfaces in water had rates of water uptake similar to those of urine accumulation. Water uptake of *L. archeyi* at 15°C averaged $24 \pm 4 \text{ mg g}^{-1} \text{ h}^{-1}$ and urine accumulation $28 \pm 2 \text{ mg g}^{-1} \text{ h}^{-1}$ ($t = 0.711$, $P > 0.5$); the corresponding values for *L. hamiltoni* at 12°C were 13 ± 2 and $17 \pm 1 \text{ mg g}^{-1} \text{ h}^{-1}$ respectively ($t = 1.780$, $P > 0.1$).

Dehydrated *L. archeyi* and *L. hamiltoni* placed in shallow water rapidly restored their initial body weight (within 1-2 h; Figs 3.3A and 3.4A). Weight gain during rehydration was due to water absorption through the ventral skin, since neither mouths nor vents were immersed, drinking was not observed and carmine in the water did not move onto the frogs' dorsal surfaces. Rates of water uptake during the first 15 min averaged $123 \pm 21 \text{ mg g}^{-1} \text{ h}^{-1}$ for *L. archeyi* and $109 \pm 8 \text{ mg g}^{-1} \text{ h}^{-1}$ for *L. hamiltoni* (Figs 3.3B and 3.4B). These do not differ significantly ($t = 0.630$, $P > 0.05$). In both species, rates of water uptake during the first 2 h were significantly greater than for hydrated frogs ($t \geq 2.874$, $P < 0.05$). Urine accumulation was significantly lower than the hydrated value during the first hour of rehydration for *L. archeyi* ($t \geq 6.850$, $P < 0.001$), and for the first 15 min for *L. hamiltoni* ($t = 6.058$, $P < 0.001$). Several *L. archeyi* declined in weight between 2 and 4 h after beginning rehydration, indicating that spontaneous urination into the bathing water had occurred.

Hydrated *L. hochstetteri* in shallow water had a low rate of water uptake ($9 \pm 2 \text{ mg g}^{-1} \text{ h}^{-1}$) which was lower than that of urine accumulation ($23 \pm 1 \text{ mg g}^{-1} \text{ h}^{-1}$; $t = 7.281$, $P < 0.01$). The imbalance possibly indicates that bladder urine had not been fully removed at the start of the experiment, causing an overestimate of urine accumulation. Rehydration of *L. hochstetteri* proceeded at a very slow rate, and even after 16 h in water W_0 had not been restored (Fig. 3.5A). Water uptake during the first 15 min of rehydration was $14 \pm 2 \text{ mg g}^{-1} \text{ h}^{-1}$; similar rates were recorded throughout the first 4 h of rehydration and these did not differ significantly from the hydrated level ($t \leq 1.862$, $P > 0.05$). Although smaller amounts of urine were removed from the bladders of rehydrating than hydrated frogs ($t \geq$

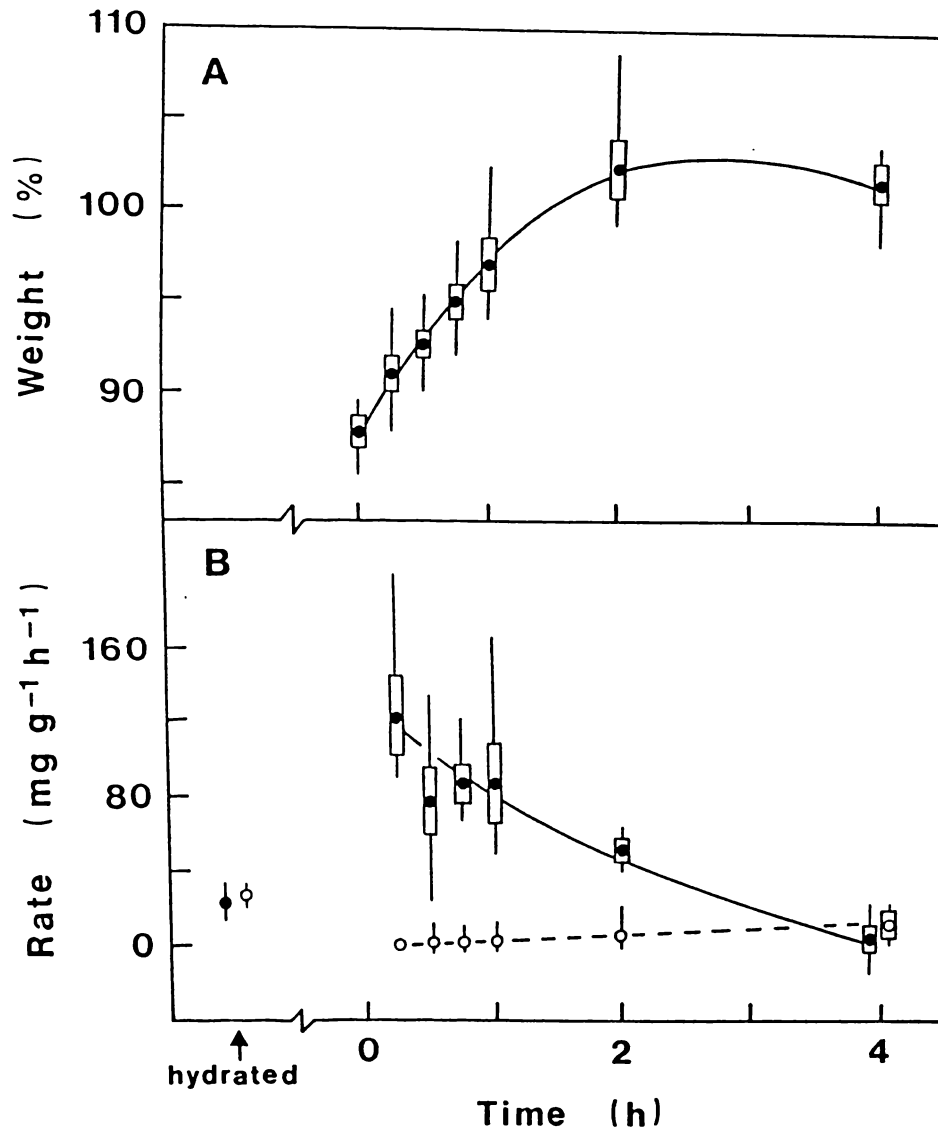


FIG. 3.3 Rehydration of *L. archeyi* in shallow water. A = weight prior to urine removal as a percentage of W_0 . B = rate of water uptake (●) and urine accumulation (○). Values for hydrated frogs are included for comparison in B ($\bar{x} \pm SE$ and range for $n = 5$; SE deleted where necessary for clarity; $T = 15^\circ\text{C}$).

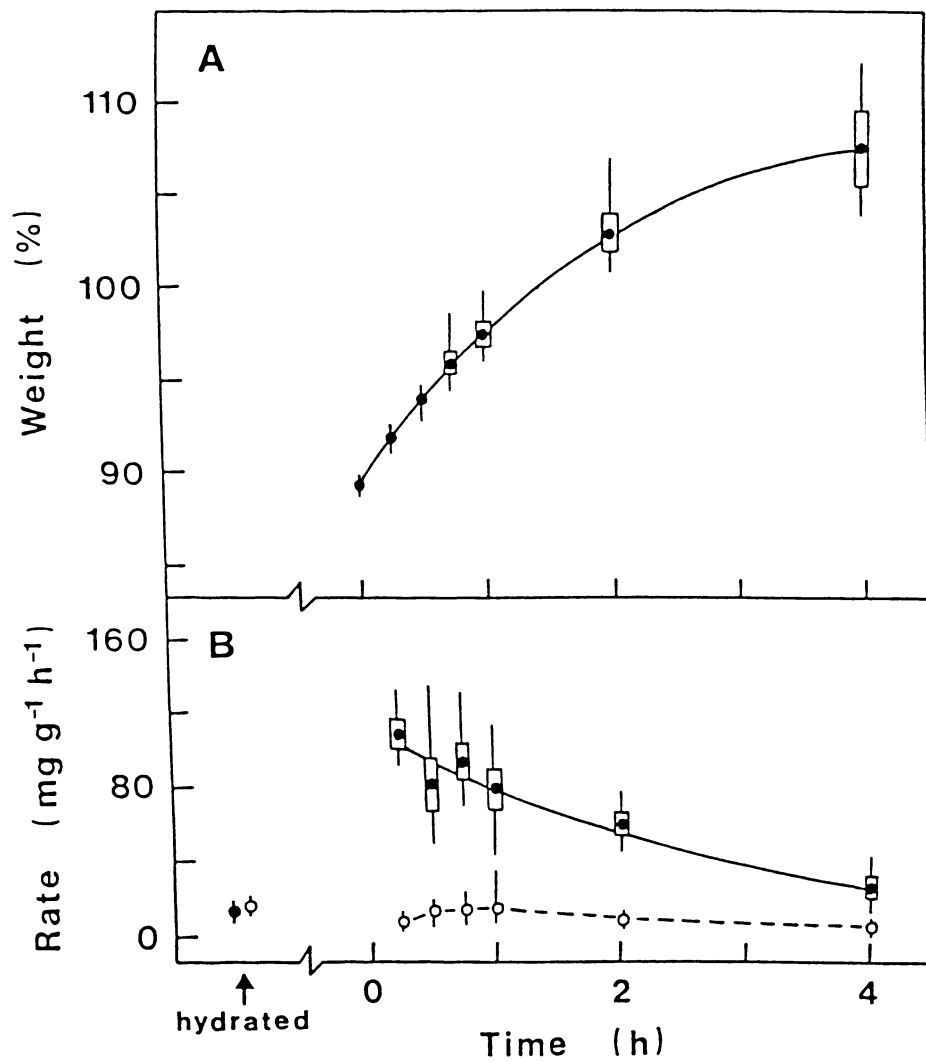


FIG. 3.4 Rehydration of *L. hamiltoni* in shallow water (T = 12°C; other details as in FIG. 3.3).

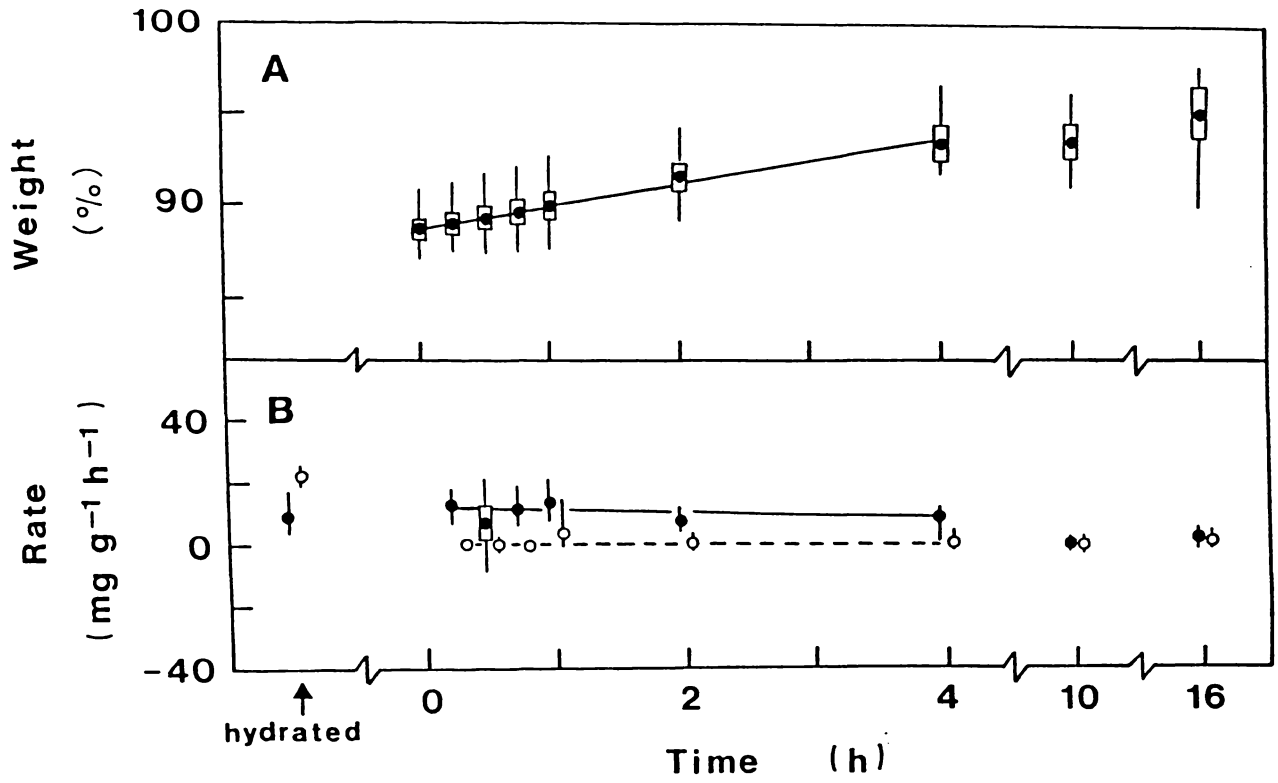


FIG.3.5 Rehydration of *L. hochstetteri* in shallow water (details as in FIG. 3.3).

6.030, $P < 0.001$), urination did not stop entirely during rehydration since some animals declined in weight whilst in water (Fig. 3.5), or urinated when being blotted dry.

L. archeyi and *L. hochstetteri* were also allowed to rehydrate when immersed (Fig. 3.6). Water uptake of *L. archeyi* during the first 15 min averaged $153 \pm 19 \text{ mg g}^{-1} \text{ h}^{-1}$, and all frogs had regained their original weight within 1 h. *L. hochstetteri* declined in weight during the first 30 min of rehydration, indicating spontaneous urination; following this the rate of water uptake averaged $33 \pm 8 \text{ mg g}^{-1} \text{ h}^{-1}$.

Rates of water uptake on a SA basis were calculated for hydrated and dehydrated *L. hamiltoni*, *L. archeyi* and *L. hochstetteri*, in the latter two species for both immersed frogs and frogs in shallow water (Fig. 3.7). In *L. archeyi*, water absorption occurred much more rapidly through the ventral skin of frogs in shallow water (per square centimetre of skin in water) than through the average skin surface of immersed frogs; this was true both for hydrated frogs and during rehydration ($t \geq 2.905$, $P < 0.05$). The rate of water uptake through ventral skin of *L. hamiltoni* did not differ significantly from that of *L. archeyi* during rehydration ($t = 0.644$, $P > 0.5$), although when hydrated it was lower than that of *L. archeyi* ($t = 3.064$, $P < 0.05$). Ventral skin of *L. hochstetteri* absorbed water at a similar rate to the average skin surface of immersed animals, both when hydrated and during rehydration ($t \leq 2.729$, $P > 0.05$).

Differences in rates of water uptake between the three species were reflected by differences in behaviour. Hydrated or dehydrated *L. hochstetteri* placed in water settled rapidly, and occasional escape movements consisted of trying to swim through the container walls. In contrast, hydrated *L. archeyi* or those rehydrated for more than 1 h struggled vigorously when immersed and frequently attempted to climb out. One immersed *L. archeyi* became comatose after 2 h rehydration, although it later recovered in air. Possibly, cutaneous respiration in this normally terrestrial species is impaired by immersion. When rehydrating in shallow water, *L. archeyi* and *L. hamiltoni* normally sat with little movement until their original weight was restored.

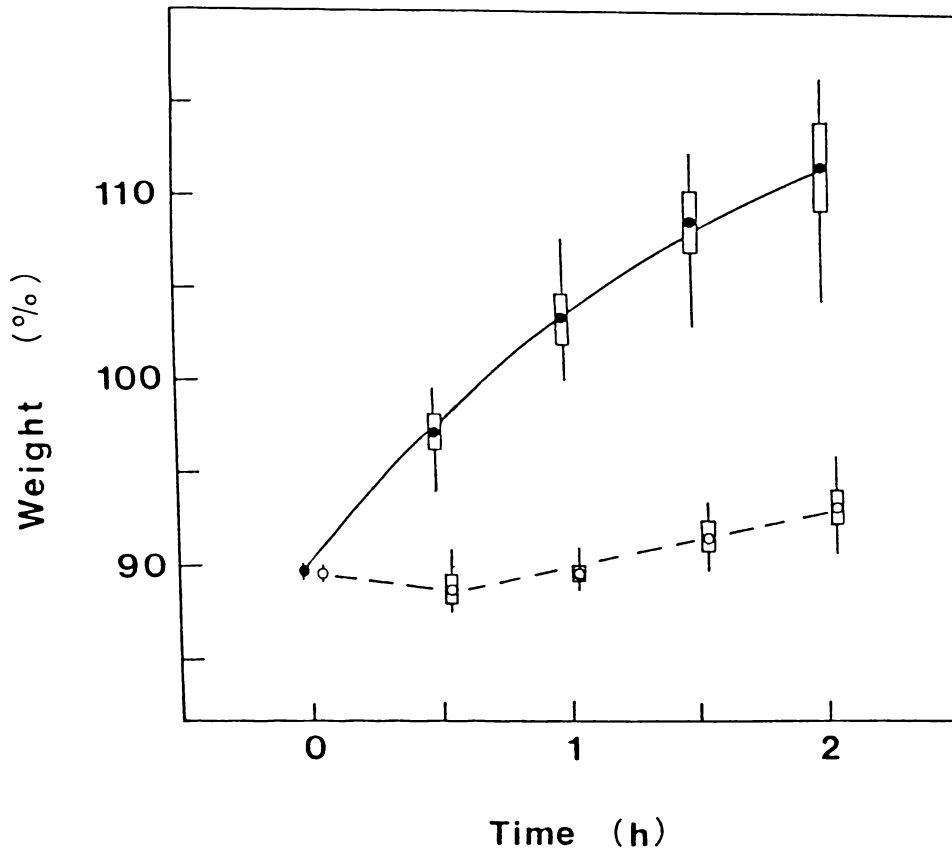


FIG. 3.6 Rehydration of *L. archeyi* (●) and *L. hochstetteri* (○) when immersed ($\bar{x} \pm SE$ and range for $n = 5$; SE deleted where necessary for clarity; $T = 14^{\circ}\text{C}$).

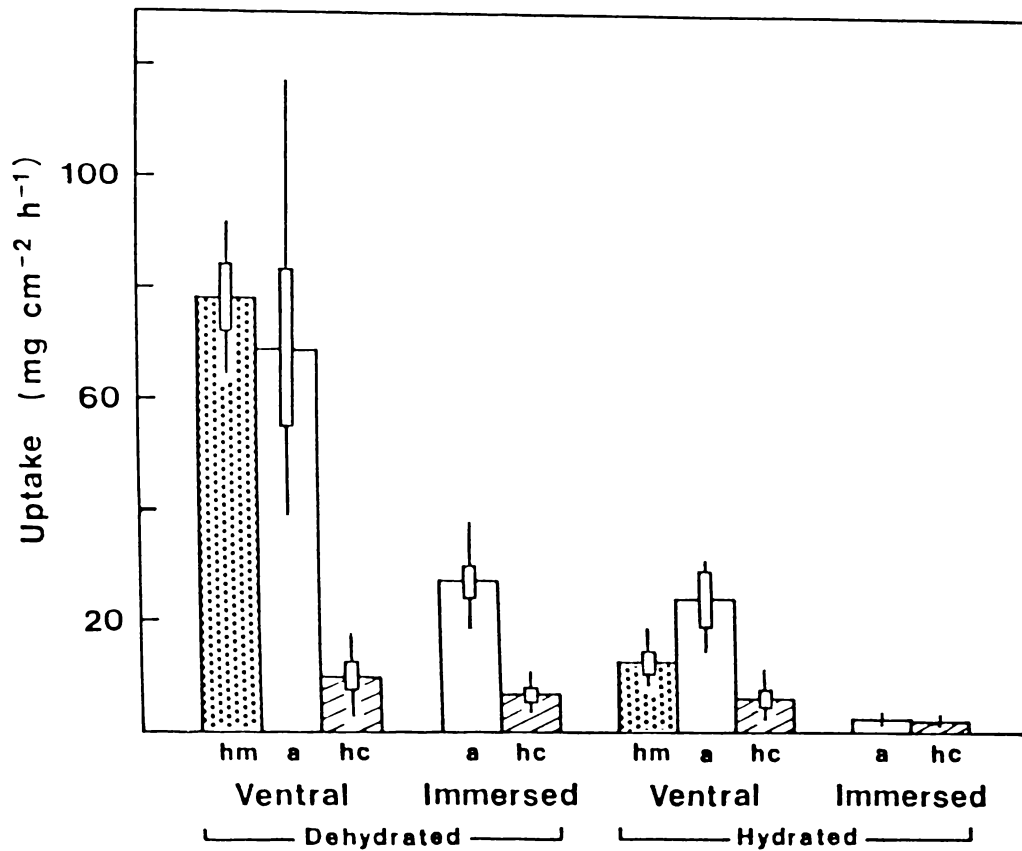


FIG. 3.7 Water uptake through the skin of hydrated and dehydrated *Leiolopma* species in shallow water (= ventral uptake) and when immersed. Rates are expressed per unit surface area of skin in water. Rates for dehydrated *L. archeyi* and *L. hamiltoni* were for the first hour of rehydration. Some *L. hochstetteri* urinated during the first 0.5 h of rehydration, and rates for this species were therefore calculated for the first hour of rehydration in which weight consistently increased (usually between 0.5 and 1.5 h) ($\bar{x} \pm SE$ and range for $n = 5$; a = *L. archeyi*, hm = *L. hamiltoni*, hc = *L. hochstetteri*; other details as in FIGS 3.3-3.6).

DISCUSSION

Dehydration

All three *Leiopelma* species dehydrate rapidly in dry conditions. Weight loss is due to constant rates of EWL and declining rates of UWL. At 80% RH, EWL from *L. archeyi* and *L. hochstetteri* occurred at a rate similar to that from a free water surface, as in *Rana pipiens* (Adolph 1933) and *Hyla regilla* (Withers *et al.* 1982). This indicates that the skin of these frogs poses little or no barrier to EWL.

Comparison of actual rates of EWL measured in this study with rates published for other anurans is difficult because both the physical conditions under which EWL has been measured (RH, temperature, wind speed) and the ways in which results have been expressed (weight-specific, total SA-specific or exposed SA-specific) have varied considerably (e.g., Loveridge 1970; Warburg 1971a; Shoemaker and McClanahan 1975; Tracy 1976; Bentley and Yorio 1979a; Degani and Warburg 1984). One way to compare rates of EWL between frogs of different sizes dehydrated at different humidities and/or temperatures is to calculate total resistance to water loss (R) according to the formula used by Withers *et al.* (1982) (after Nobel 1974):

$$R = \frac{\Delta C_{wv}}{EWL},$$

where ΔC_{wv} is the water vapour concentration deficit between the skin and ambient air (in mg cm^{-3}) and EWL is exposed SA-specific EWL (in $\text{mg cm}^{-2} \text{s}^{-1}$). Respiratory water losses are assumed to be insignificant (Spotila and Berman 1976; Bentley and Yorio 1979a; Wygoda 1981).

Total resistance was calculated in this way for the three *Leiopelma* species. Temperature at the skin surface was assumed to be equal to that of ambient air (Withers *et al.* 1982), and water vapour concentrations were taken from Campbell (1977). Resistance was low (between 5 and 10 s cm^{-1}) for all species at all humidities examined, and was similar to that for a free water surface at 80% RH (12 s cm^{-1}). These values are comparable to those for other "normal" anurans and free water surfaces in still air (7-16 s cm^{-1}), and much lower than those for cocooned or "waterproof" frogs (190-990 s cm^{-1} ; Table 3.2). Total resistance calculated in this way gives the sum of resistance provided by the boundary layer of humid air around the

TABLE 3.2 Resistance of representative anurans to EWL [R = total resistance; * indicates that resistance values were calculated by Withers *et al.* (1984) from the reference cited].

Species	R (s cm ⁻¹)		Reference
	Still air	Flowing air	
Free water surface	16	2	Withers <i>et al.</i> (1982)
Free water surface	12	-	Present study
"Normal" anurans:			
<i>Xenopus laevis</i>	7	-	Loveridge (1970)*
<i>Rana angolensis</i>	12	-	Loveridge (1970)*
<i>Bufo regularis</i>	9	-	Loveridge (1970)*
<i>Hyla regilla</i>	15	2	Withers <i>et al.</i> (1982)
<i>Osteopilus septentrionalis</i>	-	1	Withers <i>et al.</i> (1984)
<i>Bufo cognatus</i>	-	2	Withers <i>et al.</i> (1984)
<i>Rana pipiens</i>	-	2	Withers <i>et al.</i> (1984)
<i>Litoria caerulea</i>	-	2	Withers <i>et al.</i> (1984)
<i>Leiopelma archeyi</i>	6-10	-	Present study
<i>Leiopelma hamiltoni</i>	5	-	Present study
<i>Leiopelma hochstetteri</i>	8-10	-	Present study
Cocooned anurans:			
<i>Pyxicephalus adspersus</i>	190	190	Loveridge and Withers (1981)*
<i>Leptopelis bocagei</i>	-	40	Loveridge and Crayé (1979)
<i>Pterohyla fodiens</i>	-	457	Ruibal and Hillman (1981)*
<i>Lepidobatrachus llanensis</i>	-	128	McClanahan <i>et al.</i> (1983)
"Waterproof" anurans:			
<i>Hyperolius</i> spp.	257	25-96	Withers <i>et al.</i> (1982, 1984)
<i>Chiromantis</i> spp.	990	404	Loveridge (1970)*; Drewes <i>et al.</i> (1977)*; Withers <i>et al.</i> (1984)
<i>Litoria gracilentia</i>	-	118	Withers <i>et al.</i> (1984)
<i>Phyllomedusa azurea</i>	-	242	Withers <i>et al.</i> (1984)

inactive frog and that provided by the skin itself (McClanahan *et al.* 1983). When the boundary layer is disturbed by air movements then resistance drops (e.g., to 2 s cm^{-1} for the "normal" frog *Hyla regilla*; Withers *et al.* 1982, 1984). In contrast, cocooned or "waterproof" frogs have total resistances of $25\text{-}457 \text{ s cm}^{-1}$ in similar conditions (Table 3.2).

Although the skin of many anurans presents little or no physiological barrier to EWL per unit SA, the amount of skin exposed and thus participating in EWL can be reduced by postural adjustments. The semi-compact posture of the *Leiopelma* species is probably not as effective in this respect as the tightly hunched "water-conserving posture" seen in the arboreal frogs *Eleutherodactylus portoricensis* (Heatwole *et al.* 1969), *E. coqui* (Pough *et al.* 1983) and *Litoria ewingi* (Cree 1985a). This may reflect the less desiccating conditions which leiopelmatid frogs experience in their natural habitat. During dry conditions these frogs typically remain on moist or wet substrates in or near humid crevices (Chapter 2), whereas *E. coqui* (Pough *et al.* 1983) and *Litoria ewingi* (Cree 1985a) can be found in relatively exposed arboreal sites. *E. portoricensis* which relinquish the water-conserving posture show increases in weight-specific EWL of up to 130% (Heatwole *et al.* 1969).

EWL of *L. archeyi* and *L. hochstetteri* varied inversely and approximately linearly with RH over the range examined, as in *Rana pipiens* (Adolph 1932) and *Bufo valliceps* (Campbell and Davis 1971). The regression line given in Fig. 3.2A can be extrapolated to various humidities observed in the field for *L. archeyi* to estimate the value of the retreat site in reducing EWL. At 14°C and 56% RH (the lowest ambient humidity observed in the field, Chapter 2) calculated EWL is $5.4 \text{ mg g}^{-1} \text{ h}^{-1}$, whereas in a retreat above saturated soil at an estimated maximum humidity of 99% RH it would be $0.4 \text{ mg g}^{-1} \text{ h}^{-1}$. Assuming a maximum tolerance to dehydration equal to 37% of W_0 as in *R. pipiens* (Hillman 1980), a lethal water loss would be reached in just under 3 d if exposed at 56% RH, and at 37 d in the retreat site. Thus, EWL is potentially reduced about 13-fold by cryptozoic behaviour. In fact, the lethal loss would be reached sooner in the exposed situation than calculated here, since daytime summer ambient temperatures are higher and wind speeds greater than in laboratory dehydration experiments.

In all three *Leiopelma* species, urinary water losses declined gradually as dehydration proceeded, suggesting only a gradual antidiuretic response to dry conditions. Urination during dehydration was initially unexpected since several other frogs, including *R. pipiens* (Adolph 1927) and *Litoria ewingi* and *Litoria raniformis* (Cree 1985a), all of which periodically return to water, cease urinating when placed in dry conditions. However, since both *L. archeyi* and *L. hamiltoni* remain on land for their entire life cycle, such a response in these species would be inappropriate. Urinary water losses during dehydration to 9-15% of W_0 tended to be greater for *L. hochstetteri* than for the two terrestrial species, but this trend needs confirmation. One factor which may have contributed to UWL in these experiments is micturition of residual bladder urine. If a frog's bladder was not completely emptied at the start of the experiment, an early decline in urine production could have been obscured by the voiding of urine retained in the bladder. However, it seems unlikely that this could account for the prolonged period over which UWL was observed (e.g., 8 h or more in the case of *L. archeyi* and *L. hochstetteri* in Fig. 3.1), so that continued urine production during the early stages of dehydration is probable.

Rehydration

Hydrated *L. archeyi* and *L. hamiltoni* with ventral surfaces in water have rates of water uptake and urine accumulation essentially balanced at about $15-25 \text{ mg g}^{-1} \text{ h}^{-1}$ (the rates being lower for *L. hamiltoni*, which was examined at a cooler temperature). However, after relatively mild dehydration the rate of cutaneous water uptake is markedly increased and urine accumulation reduced, leading to a rapid restoration of original body weight. Increased cutaneous uptake is clearly the major factor in restoring original weight, since complete cessation of urine release would account for only 16-28% of the observed rates of rehydration. As W_0 is approached, the rate of cutaneous uptake declines towards the rate measured for hydrated animals, and spontaneous urination may occur.

Responses of *L. hochstetteri* to dehydration show a marked contrast to those of the other two species. Rehydration from shallow water occurs at a very slow and linear rate ($\sim 1\%$ of W_0 per hour), and cutaneous water uptake is not increased. The rate of dehydration can be entirely explained by a reduction in urine output, which in hydrated

animals amounted to $23 \text{ mg g}^{-1} \text{ h}^{-1}$ or 2.3% of W_0 per hour. However, urination does not cease entirely since several animals spontaneously urinated during rehydration, both in shallow water and when immersed.

The cutaneous and urinary responses to dehydration seen in rehydrating *L. archeyi* and *L. hamiltoni* constitute the typical anuran water balance response (Shoemaker and McClanahan 1980). Rapid rehydration has been reported for many anurans, although differences in the extent of previous dehydration, the time period over which rehydration rates were measured and in the way results are expressed makes exact comparisons of rehydration rates difficult. In terms of body weight, Katz and Graham (1980) found that dehydrated *Rana ridibunda* and *Bufo viridis* increased in weight by 90 and 110 $\text{mg g}^{-1} \text{ h}^{-1}$ respectively when immersed, lower than observed here for immersed *L. archeyi* ($153 \text{ mg g}^{-1} \text{ h}^{-1}$ during the first 30 min). However, several studies report higher rates of immersed uptake. During the first 15 min of rehydration, Claussen (1969) observed values for six species of anurans ranging from 100 $\text{mg g}^{-1} \text{ h}^{-1}$ in *B. debilis* to 380 $\text{mg g}^{-1} \text{ h}^{-1}$ in *B. boreas*, and Shoemaker and McClanahan (1980) reported rates of 229-251 $\text{mg g}^{-1} \text{ h}^{-1}$ for three arboreal or wide-ranging ranids, 320-407 $\text{mg g}^{-1} \text{ h}^{-1}$ for two bufonids, and 310-820 $\text{mg g}^{-1} \text{ h}^{-1}$ for three arboreal rhacophorids, the latter value being amongst the highest known for an anuran. However, differences in body size between species make comparisons on the basis of SA more meaningful. In terms of SA, species of the burrowing genera *Heleioporus* and *Neobatrachus* gained water when immersed at 44-60 and 33-99 $\text{mg cm}^{-2} \text{ h}^{-1}$ respectively (Bentley *et al.* 1958), *B. punctatus* 62 $\text{mg cm}^{-2} \text{ h}^{-1}$ (McClanahan and Baldwin 1969), and juvenile *Scaphiopus couchi* 36 $\text{mg cm}^{-2} \text{ h}^{-1}$ (Jones 1978). The comparable value for *L. archeyi* over the first hour of rehydration was 27 $\text{mg cm}^{-2} \text{ h}^{-1}$.

Published values for anurans rehydrating in shallow water or on wet surfaces are also higher than found here for *L. archeyi* (123 $\text{mg g}^{-1} \text{ h}^{-1}$ during the first 15 min) and *L. hamiltoni* (109 $\text{mg g}^{-1} \text{ h}^{-1}$). *B. punctatus* gained water at a rate of 192 $\text{mg g}^{-1} \text{ h}^{-1}$ (McClanahan and Baldwin 1969) and the hylids *Litoria ewingi* and *Litoria raniformis* 320 and 240 $\text{mg g}^{-1} \text{ h}^{-1}$ respectively (Cree 1985a). However, in all the water uptake studies cited above for immersed anurans and those in shallow water, the extent of prior dehydration was greater (15-30% weight loss) and temperature, where stated, was higher (20-30°C) than used here (9-15% dehydration deficit, 12-15°C). Therefore, higher

rates of water uptake than in *L. archeyi* and *L. hamiltoni* are not unexpected.

In additional studies, rehydration rates were examined in *Ascaphus truei*, *Xenopus laevis* and the locally available hylid *Litoria aurea* (Appendix II), under similar conditions to those used here for the *Leiopelma* species. Juvenile *Litoria aurea* (which are of a similar size to adult *Leiopelma*) also show cutaneous and urinary responses to dehydration, but the cutaneous component of the response is more marked than in either *L. archeyi* or *L. hamiltoni*. For example, water uptake during the first 15 min of rehydration in shallow water averaged $292 \pm 63 \text{ mg g}^{-1} \text{ h}^{-1}$ for *Litoria aurea*, more than twice the rate measured for *L. archeyi* ($123 \pm 21 \text{ mg g}^{-1} \text{ h}^{-1}$; $t = 2.561$, $P < 0.05$) or for *L. hamiltoni* ($109 \pm 8 \text{ mg g}^{-1} \text{ h}^{-1}$; $t = 2.890$, $P < 0.05$). In terms of SA, *Litoria aurea* gained $153 \pm 21 \text{ mg cm}^{-2} \text{ h}^{-1}$ during the first hour of rehydration, whilst *L. archeyi* absorbed $69 \pm 13 \text{ mg cm}^{-2} \text{ h}^{-1}$ ($t = 3.386$, $P < 0.01$) and *L. hamiltoni* $78 \pm 6 \text{ mg cm}^{-2} \text{ h}^{-1}$ ($t = 3.417$, $P < 0.01$). Differences between rates of water uptake for rehydrating *Litoria aurea* and those for *L. archeyi* are correlated with a difference in structure of the ventral skin (Chapter 6).

The slow and constant rate of weight gain seen in rehydrating *L. hochstetteri* is typical of amphibians which are believed to lack the cutaneous component of the water balance response (Shoemaker and McClanahan 1980). Shoemaker and McClanahan recorded nearly constant rates of water uptake every 15 min for 1 h during rehydration of the ranids *R. kuhli* ($40\text{--}57 \text{ mg g}^{-1} \text{ h}^{-1}$), *R. blythi* ($57\text{--}73 \text{ mg g}^{-1} \text{ h}^{-1}$) and *R. glandulosa* ($85\text{--}94 \text{ mg g}^{-1} \text{ h}^{-1}$) at 25°C ; the comparable rate for immersed *L. hochstetteri* at 15°C was $33 \text{ mg g}^{-1} \text{ h}^{-1}$ during periods when weight consistently increased. Other amphibians which rehydrate at a slow and fairly constant rate include *X. laevis* ($19 \text{ mg g}^{-1} \text{ h}^{-1}$ in Ewer 1952; $5 \text{ mg g}^{-1} \text{ h}^{-1}$ in Appendix II of the present study), *Ascaphus truei* ($8 \text{ mg g}^{-1} \text{ h}^{-1}$; Appendix II), the horned toad *Ceratophrys ornata* from South Chaco (about $25 \text{ mg g}^{-1} \text{ h}^{-1}$ from Fig. 2 of Canziani and Cannata 1980) and the urodele *Desmognathus quadramaculatus* ($22 \text{ mg g}^{-1} \text{ h}^{-1}$; Brown *et al.* 1977). In terms of SA, rates of water uptake in shallow water for rehydrating *L. hochstetteri* ($10 \text{ mg cm}^{-2} \text{ h}^{-1}$) and a single *A. truei* ($11 \text{ mg cm}^{-2} \text{ h}^{-1}$; Appendix II) were virtually identical.

Water uptake on a SA basis was similar for rehydrating *L. hochstetteri*

whether animals were immersed ($7 \text{ mg cm}^{-2} \text{ h}^{-1}$) or in shallow water ($10 \text{ mg cm}^{-2} \text{ h}^{-1}$). This suggests that osmotic permeability of all regions of skin is unchanged by dehydration in this species. However, water uptake of rehydrating *L. archeyi* was much faster through ventral skin ($69 \text{ mg cm}^{-2} \text{ h}^{-1}$) than through the average skin surface of immersed frogs ($27 \text{ mg cm}^{-2} \text{ h}^{-1}$). Uptake through ventral skin of rehydrating *L. hamiltoni* was also high ($78 \text{ mg cm}^{-2} \text{ h}^{-1}$). Rapid absorption of water through the ventral skin has previously been reported for dehydrated *Hyla arborea* (Overton 1904), *B. punctatus* (McClanahan and Baldwin 1969), *Scaphiopus couchi* toadlets (Jones 1978) and *Litoria ewingi* and *Litoria raniformis* (Cree 1985a). Unlike the skin of *B. bufo* (Christensen 1974a) the skin of *L. archeyi* and *L. hamiltoni* is not extensively sculptured and water transport onto the dorsal surface does not noticeably occur.

The ability to absorb water rapidly through the ventral skin is of obvious advantage to these terrestrial frogs. Standing water for immersion is rare or absent in their natural habitats, but ventral skin often comes into contact with moist or wet surfaces including soil, rocks and vegetation (Chapter 2). In contrast, *L. hochstetteri* is usually found in streamside retreats where dehydration is unlikely to occur and water is constantly available for absorption. A slow rate of rehydration is therefore probably of little or no disadvantage to this species and indeed, high ventral skin permeability could well be a liability.

CHAPTER 4

EFFECTS OF AVT ON WATER RETENTION *IN VIVO*

INTRODUCTION

The water balance responses of dehydrated amphibians are believed to be caused, at least in part, by release of the neurohypophysial hormone AVT (Bentley 1971a, 1974). Treatment of hydrated anurans or urodeles with exogenous AVT or similar neurohypophysial peptides produces water balance responses similar to those resulting from dehydration: species which rehydrate rapidly show large weight gains due to water retention, whereas those such as *Xenopus laevis* which rehydrate slowly demonstrate little or none (Ewer 1952; Brown *et al.* 1977). The neurohypophysis of *X. laevis* contains similar amounts of presumptive AVT to those of anurans which rehydrate rapidly (Bentley 1969b), and AVT has also been detected in its blood (Nouwen and Kühn 1983) at a concentration which appears similar to that in other dehydrated anurans (Bentley 1969a). Thus, it seems that the slow rate of water uptake in dehydrated *X. laevis* is due not to an absence of secreted AVT but to a difference in tissue responsiveness (Bentley 1971a).

Experiments described in the previous chapter showed that *L. archeyi* and *L. hamiltoni* have a water balance response to dehydration which differs from that of *L. hochstetteri*: the two terrestrial species rehydrate rapidly from a 9-15% dehydration deficit, whilst *L. hochstetteri* rehydrates very slowly. Assuming that the three species all release similar amounts of AVT when dehydrated, the results suggest that their different rehydration rates are caused by differences in response to AVT. This hypothesis is examined in the present chapter by comparing the effect of exogenous AVT on water balance of the three species *in vivo*.

MATERIALS AND METHODS

Animals

Experiments with the highest dose of AVT (7×10^{-11} mol g^{-1}) were

carried out in February-March 1984 with stock *L. archeyi* and *L. hochstetteri*, and on Maud Island in May 1984 with freshly collected *L. hamiltoni* (see Materials and Methods section of Chapter 3: 1. Collection and maintenance of experimental animals). Experiments with lower doses were performed during June-September 1985 using stock *L. archeyi* and *L. hochstetteri*, and *L. hamiltoni* which had been collected from Maud Island in May 1985. Size ranges were 2.6-4.9 g (*L. archeyi*), 2.2-8.1 g (*L. hamiltoni*) and 3.3-7.6 g (*L. hochstetteri*).

Experiment 1. Effect of 7×10^{-11} mol g⁻¹ AVT

During the rehydration experiments described in Chapter 3 I observed that *L. archeyi* and *L. hamiltoni* were able to rehydrate rapidly even when sitting in shallow water, suggesting that AVT increases water uptake through the ventral skin of these species. This hypothesis was tested in the following experiments by immersing only the frogs' ventral surfaces (to a depth of about 3 mm) following treatment with AVT. The same procedure was also used for *L. hochstetteri*.

Initial experiments were carried out using a dose of 7×10^{-11} mol AVT g⁻¹ W₀ to enable comparison with similar studies (Heller and Bentley 1965; Brown and Brown 1980). Hydrated frogs with bladder urine removed were weighed and then placed in shallow stream water (3 ml) for 1 h to ensure complete hydration. Bladder urine was removed, the frogs were reweighed, and synthetic AVT (7×10^{-11} mol g⁻¹ W₀ = 20 mU g⁻¹) was injected under the abdominal skin. The stock solution of hormone was prepared by dissolving 1 mg AVT in 2.8 ml of 0.25% glacial acetic acid (Baldwin 1974) containing 0.5% chlorobutanol as a preservative (Alvarado and Johnson 1965). Prior to injection, this refrigerated stock was diluted with 0.6% NaCl to give a strength of 1.75×10^{-11} mol μl⁻¹. The injection volume was 4 μl g⁻¹ W₀. An identical buffer-preservative-salt solution without hormone was injected into control animals. Five *L. archeyi* and five *L. hochstetteri* which did not receive injections were included as additional controls.

Weights of animals before and after urine removal were then measured hourly for up to 11 h. Bathing water was replaced at each weighing, and the surface area (SA) of ventral skin in water was measured as described in Chapter 3. During experiments with *L. archeyi* and

L. hochstetteri, samples of bathing water from each of the first 5 h after injection were frozen and later analysed for urea plus ammonia nitrogen to determine whether urination had occurred. Lack of facilities on Maud Island precluded these analyses for *L. hamiltoni*.

Rates of water uptake and urine accumulation were calculated from the hourly weighings. W_0 was taken to be the bladder-empty weight at the time of injection (0 h) rather than when put into water 1 h previously as in a previous report (Cree 1985b), since the former method shows more clearly the gain in weight attributable solely to AVT treatment.

Experiment 2. Dose-response studies

Although the dose of AVT used in Experiment 1 is similar to that of other studies, its effects may be pharmacological since it represents about 7-70x the amount of AVT stored in the neurohypophyses of adult anurans (10^{-11} - 10^{-12} mol g^{-1} according to Bentley 1971a). Further experiments were therefore carried out to demonstrate whether similar results could be obtained with doses up to 10 000x as low as the original. These experiments were performed in the same way as above, except that the hormone stock was prepared by dissolving 1 mg AVT in 2.8 ml deionised water and freezing it at $-18^{\circ}C$ until use, following the advice of Dr L.J.Guillette Jr., University of Florida, USA. Hormone doses used in Experiments 1 and 2 and their estimated physiological significance (proportion of estimated neurohypophysial content) are listed in Table 4.1 (note that not all doses were given to all species).

Hormone

Synthetic AVT was obtained from Sigma Chemical Company at an activity of 200 U mg^{-1} (details of assay not available, but presumably rat pressor bioassay). The stock used in Experiment 1 was from Lot 92F-0637 and no details of purity or peptide content were supplied. Experiment 2 stock was from Lot 83F-0690 and had a purity of ~94%. The peptide content was ~86% with the balance believed to be water and salts.

TABLE 4.1 Doses of AVT administered to *Leiopelma* species *in vivo*, and their estimated physiological significance. The proportion of neurohypophysial peptide (NHP) content has been estimated assuming that anuran neurohypophyses contain 10^{-12} - 10^{-11} mol g⁻¹ AVT (Bentley 1971a) (a = *L. archeyi*, hm = *L. hamiltoni*, hc = *L. hochstetteri*).

Dose of AVT (mol g ⁻¹)	Species treated	Estimated proportion of NHP content
7×10^{-11}	a, hm, hc	7 - 70 x
7×10^{-12}	hm	0.7 - 7 x
7×10^{-13}	a, hm, hc	0.07 - 0.7 x
7×10^{-15}	a, hm	0.0007 - 0.007 x

RESULTS

Experiment 1. Effect of 7×10^{-11} mol g^{-1} AVT

L. archeyi and *L. hamiltoni* treated with 7×10^{-11} mol g^{-1} AVT rapidly increased in weight (Figs 4.1A and 4.2A). Maximum mean weights were reached 4-5 h after injection and averaged $31 \pm 4\%$ ($\bar{x} \pm SE$) above W_0 for *L. archeyi* and $29 \pm 3\%$ above W_0 for *L. hamiltoni*. Fluid retention in the femoral and particularly the ventral lymph sacs of both species was obvious at this time. There was some variation in the time at which individual frogs reached their maximum weights (these occurred 3-8 h after injection), but the averages of these individual maxima were similar to those for the maximum mean weights at 4-5 h ($32 \pm 4\%$ and $30 \pm 2\%$ above W_0 for *L. archeyi* and *L. hamiltoni* respectively). Control animals (Figs 4.1A and 4.2A) and five non-injected *L. archeyi* (not shown) remained within $\pm 10\%$ of W_0 .

The dramatic weight gain seen in AVT-treated *L. archeyi* and *L. hamiltoni* reflected a large increase in water uptake (Figs 4.1B and 4.2B). Rates of water uptake peaked 2 h after injection at 127 ± 15 mg g^{-1} h^{-1} for *L. archeyi* and 99 ± 15 mg g^{-1} h^{-1} for *L. hamiltoni*. In terms of SA of ventral skin in water, these rates are equivalent to 86 ± 17 and 69 ± 3 mg cm^{-2} h^{-1} respectively. Rates of urine accumulation in AVT-treated *L. archeyi* and *L. hamiltoni* were low for the first hour after treatment (Figs 4.1C and 4.2C), and traces only of urea plus ammonia nitrogen were detected in bathing water of AVT-treated *L. archeyi* between 1 and 5 h after injection (Table 4.2), suggesting that little spontaneous urination occurred during this time. However, large amounts of urine were collected from the bladders of both species from 3-4 h after injection onwards (Figs 4.1C and 4.2C) and spontaneous urination into the bathing water was apparent by the sixth hour (indicated by the negative rates of water uptake in Figs 4.1B and 4.2B; note that controls also showed evidence of spontaneous urination between hourly weighings). Initial body weights of AVT-treated animals were restored or approached at 23 h after injection.

L. hochstetteri showed a rather different response to the same dose of AVT. Mean weight increased at a slow and linear rate, reaching 9% above W_0 9 h after injection (Fig. 4.3A). Water uptake averaged 13 ± 2 mg g^{-1} h^{-1} 1 h after injection (Fig 4.3B) (equivalent to 14 ± 1 mg

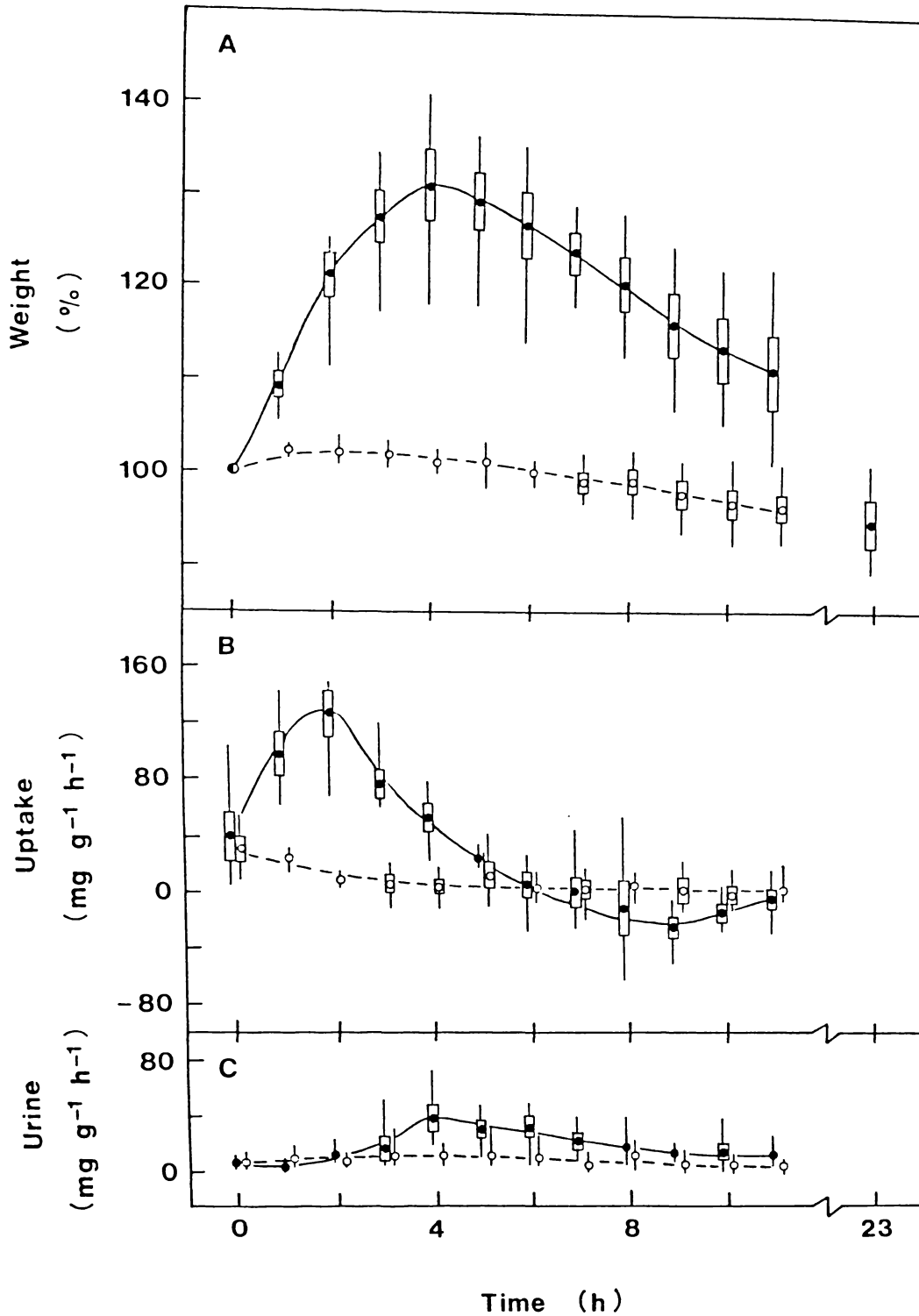


FIG. 4.1 Effect of AVT (7×10^{-11} mol g⁻¹) on water balance of *L. archeyi*. A = weight prior to urine removal as a percentage of W_0 , except at 0 and 23 h where weights are after urine removal. B = rate of water uptake. C = rate of urine accumulation. AVT was injected into frogs at 0 h after they had spent the previous hour in shallow water; animals were returned to their holding cages at 11 h ($\bar{x} \pm$ SE and range; ● = AVT-treated, n = 5; ○ = controls, n = 5; T = 18°C).

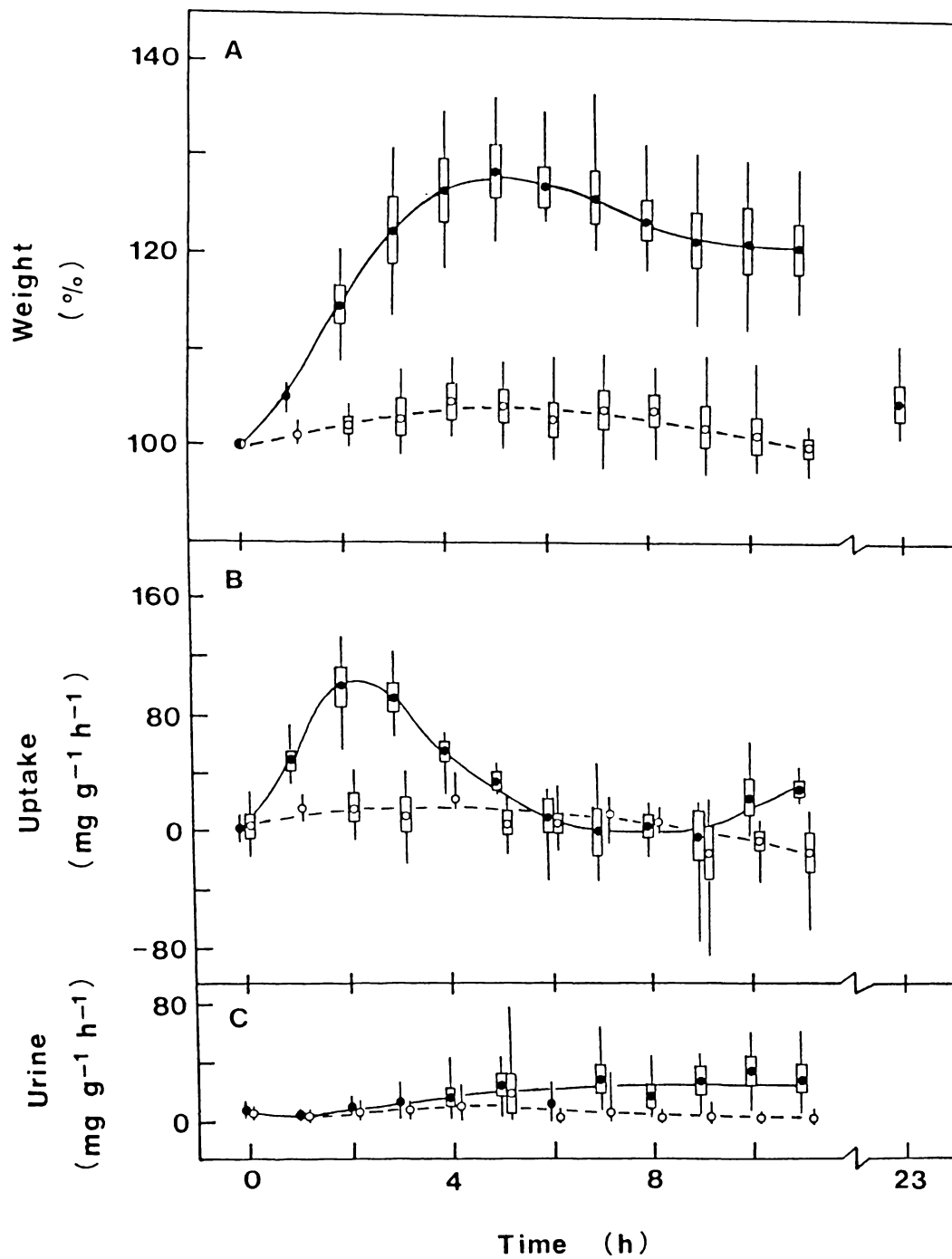


FIG. 4.2 Effect of AVT (7×10^{-11} mol g⁻¹) on water balance of *L. hamiltoni* ($T = 15^{\circ}\text{C}$; other details as in FIG. 4.1).

TABLE 4.2 Urea plus ammonia nitrogen content of bathing water from experiments in which *L. archeyi* and *L. hochstetteri* were treated with 7×10^{-11} mol g^{-1} AVT. Values are $\mu g N g^{-1} h^{-1}$ ($\bar{x} \pm SE$) for the first 5 h after injection. Levels $\leq \sim 1.0 \mu g g^{-1} h^{-1}$ are within the range of error of the method (* indicates that five *L. archeyi* were treated with AVT but bathing water was kept and analysed for only four of these).

Species and treatment		0-1 h	1-2 h	2-3 h	3-4 h	4-5 h
<i>L. archeyi</i>	AVT n = 4*	4.5 \pm 1.7	1.3 \pm 1.3	1.0 \pm 0.7	0.5 \pm 0.3	1.0 \pm 0.3
	Control n = 5	4.4 \pm 1.6	3.8 \pm 1.6	3.6 \pm 1.8	4.0 \pm 1.4	1.0 \pm 0.7
<i>L. hochstetteri</i>	AVT n = 5	3.1 \pm 1.3	0	0	0.2 \pm 0.1	0.7 \pm 0.3
	Control n = 5	4.2 \pm 3.2	5.0 \pm 2.8	1.2 \pm 0.6	0.6 \pm 0.5	0.5 \pm 0.3

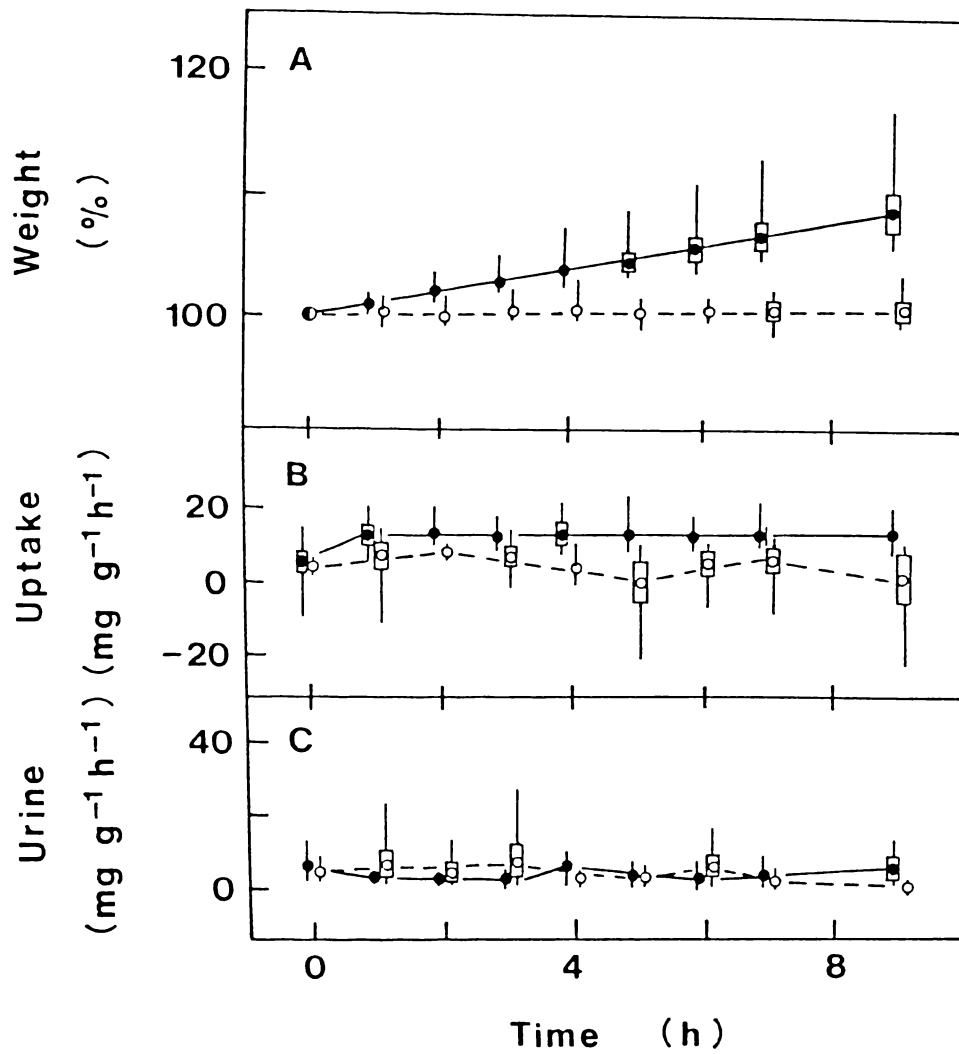


FIG. 4.3 Effect of AVT (7×10^{-11} mol g⁻¹) on water balance of *L. hochstetteri* (n = 7 for AVT-treated, n = 5 for controls; other details as in FIG. 4.1).

$\text{cm}^{-2} \text{ h}^{-1}$) and this rate remained essentially constant for the remainder of the experiment. Controls (Fig 4.3A) and five non-injected animals (not shown) remained within $\pm 4\%$ of W_0 , and the low and often negative rates of water uptake recorded for control animals (Fig. 4.3B) indicate that spontaneous urination between hourly weighings frequently occurred. Thus, rates of urine accumulation shown for control animals in Fig. 4.3C underestimate true urine output.

In contrast, there was little evidence that AVT-treated *L. hochstetteri* spontaneously urinated between weighings. Rates of water uptake were always greater than zero (Fig. 4.3B), and negligible amounts of urea plus ammonia nitrogen were detected in bathing water collected between 1 and 5 h after injection (Table 4.2). Rates of bladder urine accumulation were also generally lower and less variable for the first 3 h after AVT treatment when compared with the (underestimated) rates for control animals (Fig. 4.3C), suggesting that the weight gain seen in AVT-treated *L. hochstetteri* may have been caused by a reduction of urine output.

Experiment 2. Dose-response studies

Responses to the above and lower doses of AVT were compared by calculating mean values for the maximal weight gain (water retention) observed within 8 h of hormone administration (9 h in the case of *L. hochstetteri*). These were compared with control values using Student's t-test. All doses produced significant water retention in *L. archeyi*, although the effect of the lowest dose ($7 \times 10^{-15} \text{ mol g}^{-1}$) was minimal (only 6% weight gain, Table 4.3). *L. hamiltoni* showed significant water retention when treated with 7×10^{-11} , 7×10^{-12} and $7 \times 10^{-13} \text{ mol g}^{-1}$ AVT, but the small weight gain observed following treatment with $7 \times 10^{-15} \text{ mol g}^{-1}$ was not significant (Table 4.4). *L. hochstetteri* treated with $7 \times 10^{-11} \text{ mol g}^{-1}$ showed significant water retention, but $7 \times 10^{-13} \text{ mol g}^{-1}$ was without effect (Table 4.5).

DISCUSSION

Hydrated *L. archeyi* and *L. hamiltoni* treated with $7 \times 10^{-11} \text{ mol g}^{-1}$ AVT show a large and temporary increase in body weight due to water retention (Brunn effect). This can be attributed to both increased

TABLE 4.3 Effect of dose of AVT on maximal water retention of *L. archeyi* *in vivo* ($\bar{x} \pm \text{SE}$). Water retention is calculated as the maximum weight gain occurring for each individual between 0 and 8 h after treatment, expressed as a percentage of W_0 (** = $P < 0.01$; *** = $P < 0.001$).

Dose of AVT (mol g ⁻¹)	Water retention (%)	Significance compared with control
Control n = 5	3.0 ± 0.3	
7 × 10 ⁻¹¹ n = 5	31.5 ± 3.5	t = 7.992 ***
7 × 10 ⁻¹³ n = 3	23.6 ± 5.3	t = 3.876 **
7 × 10 ⁻¹⁵ n = 3	6.1 ± 0.7	t = 4.008 **

TABLE 4.4 Effect of dose of AVT on maximal water retention of *L. hamiltoni in vivo* ($\bar{x} \pm \text{SE}$). Δ = maximal water retention over 5 h; all other doses = maximum over 8 h (see TABLE 4.3 for details).

Dose of AVT (mol g ⁻¹)	Water retention (%)	Significance compared with control
<u>Maud Island 1984:</u>		
Control n = 5	6.5 ± 2.0	
7 × 10 ⁻¹¹ n = 5	30.1 ± 2.4	t = 7.490 ***
<u>Lab. 1985:</u>		
Control n = 4	2.0 ± 0.3	
7 × 10 ⁻¹² n = 4	37.6 ± 5.3 ^Δ	t = 6.689 ***
7 × 10 ⁻¹³ n = 3	25.8 ± 4.1	t = 5.725 **
7 × 10 ⁻¹⁵ n = 3	5.2 ± 1.7	t = 1.888 P > 0.1

TABLE 4.5 Effect of dose of AVT on maximal water retention of *L. hochstetteri* *in vivo* over 9 h ($\bar{x} \pm \text{SE}$; see TABLE 4.3 for details).

Dose of AVT (mol g ⁻¹)	Water retention (%)	Significance compared with control
Control n = 5	2.2 ± 0.5	
7 × 10 ⁻¹¹ n = 7	9.1 ± 1.5	t = 4.416 **
7 × 10 ⁻¹³ n = 4	2.2 ± 0.6	t = 0.070 P > 0.1

cutaneous water uptake and decreased urine output. A reduction in urine output is suggested by the initially low rates of urine accumulation in the bladder and the presence of only traces of nitrogenous wastes in the bathing water of *L. archeyi* between 1-5 h following hormone treatment. However, cessation of urine release cannot account for weight gains greater than about 1.7-2.8% of W_0 per hour (normal rates of urine accumulation recorded in Chapter 3 were 17 and 28 $\text{mg g}^{-1} \text{h}^{-1}$ for *L. hamiltoni* and *L. archeyi* respectively), and therefore an increase in cutaneous water uptake must be the major factor in producing the large amounts of water retention observed.

In contrast, there is no evidence that $7 \times 10^{-11} \text{ mol g}^{-1}$ AVT increased cutaneous water uptake in *L. hochstetteri*. Water retention of this species occurred at a slow and linear rate ($\sim 1\%$ of W_0 per hour) and the rate of water uptake over the first hour following AVT treatment ($13 \pm 2 \text{ mg g}^{-1} \text{h}^{-1}$) does not differ significantly from that recorded for hydrated animals in Chapter 3 ($9 \pm 2 \text{ mg g}^{-1} \text{h}^{-1}$; $t = 1.241$, $P > 0.2$). The water retention observed must therefore be due to a reduction in urine output. The mean rate of urine accumulation recorded in Chapter 3 for hydrated animals was $23 \text{ mg g}^{-1} \text{h}^{-1}$ (2.3% of W_0 per hour), which is more than sufficient to account for the rate of water retention observed here. Weights of AVT-treated *L. hochstetteri* continued to increase for at least 9 h after treatment, suggesting a more sustained response than in either *L. archeyi* or *L. hamiltoni*.

In several anurans, AVT reduces urine output by affecting both the kidney (reducing glomerular filtration rate and/or increasing tubular reabsorption of water) and the bladder (increasing bladder water reabsorption) (Bentley 1971a; Sawyer and Pang 1979). A renal antidiuretic effect seems likely for all three species examined here. This is based on the assumption that urine production of anurans normally keeps pace with the extent of water loading, as shown for *Rana pipiens* (Sawyer 1951a) and *R. clamitans* (Schmidt-Nielsen and Forster 1954). The fact that AVT-treated frogs continue to increase in weight for several hours after hormone administration therefore implies that renal function has been depressed (Heller 1950). However, an increase in bladder water reabsorption cannot be ruled out, and in *Bufo marinus* this may account for up to 50% of the total water retention observed following injection of neurohypophysial peptides (Bentley and Ferguson 1967).

The water balance responses of the three *Leiopelma* species to exogenous AVT are remarkably similar to those produced by dehydration. In both AVT-treated and rehydrating *L. archeyi* and *L. hamiltoni*, cutaneous water uptake through the ventral skin is increased and urine output apparently reduced, whereas *L. hochstetteri* shows no evidence for increased cutaneous uptake in either situation. Maximal rates of water uptake calculated on a SA basis for AVT-treated animals do not differ significantly from the rates of water uptake observed during shallow rehydration in Chapter 3 (Fig. 3.7) [in $\text{mg cm}^{-2} \text{h}^{-1}$, *L. archeyi* AVT-treated = 86 ± 17 , rehydrating = 69 ± 13 ($t = 0.830$, $P > 0.4$); *L. hamiltoni* AVT-treated = 69 ± 3 , rehydrating = 78 ± 6 ($t = 1.413$, $P > 0.1$); *L. hochstetteri* AVT-treated = 14 ± 1 , rehydrating = 10 ± 2 ($t = 0.108$, $P > 0.9$)]. These similarities support the hypothesis that the water balance responses seen during rehydration are due to release of endogenous AVT.

The effects of similar doses of NHPs on water retention in anuran and urodele amphibians have been summarised in Tables 4.6 and 4.7. Although the values listed in these tables are not strictly comparable because of differences in season, temperature, hormone treatment, the time interval over which weight gains were measured, and whether or not bladder urine was removed prior to weighing, a general trend nevertheless exists between responsiveness to NHPs and habitat. This trend applies both to the maximum extent of water retention and to the rate at which water retention occurs.

For instance, the aquatic anurans *Xenopus laevis* and *Calyptocephalella gayi* show little or no water retention at all (see Tables 4.6 and 4.7 for references). The aquatic urodeles *Necturus maculosus*, *Amphiuma means*, *Siren lacertina* and *Desmognathus quadramaculatus* show small maximum weight gains ($\leq 6\%$ of W_0) and water retention occurs very slowly ($\leq 1\%$ of W_0 per hour). In at least the first three of these urodeles the effect is believed to be solely antidiuretic (Bentley and Heller 1964; Bentley 1973). The terrestrial urodeles *Aneides lugubris* and *Taricha torosa* and many terrestrial anurans show maximum weight gains of more than 20%, and these occur relatively rapidly ($\sim 5\%$ per hour or faster). This is assumed to be evidence of a cutaneous response (Hillman 1974; Brown and Brown 1980). The greatest rate of water retention occurred in the tree frog *Hyla arborea*, which gained 53% of its original weight in 1 h (Heller and Bentley 1965).

TABLE 4.6 Magnitude and rate of water retention in adult anurans treated with neurohypophysial peptides.

Species	Habitat ¹	Hormone ²	Hormone dose ² (AVT dose in mol g ⁻¹)	Maximum water retention ³ (%)	Rate of water retention ⁴ (% h ⁻¹)	Reference
<i>Xenopus laevis</i>	A	AVT	2 × 10 ⁻¹⁰	0	0	Heller and Bentley (1965)
<i>Calyptocephalella gayi</i>	A	AVT	10 ⁻⁹	1	-	Garcia <i>et al.</i> (1970)
<i>Discoglossus pictus</i>	A/SA	AVT	3 × 10 ⁻¹⁰	19*	3.2	Heller and Bentley (1965)
<i>Rana clamitans</i>	A/SA	Pituitrin	10 IU 100g ⁻¹	14	4.7	Steggerda (1937)
<i>R. catesbeiana</i>	SA	AVT	4 × 10 ⁻¹²	19*	4.8	Alvarado and Johnson (1966)
		AVT	1 × 10 ⁻¹¹	13	1.9	Bentley (1973)
<i>R. ridibunda</i>	SA	AVT	4 × 10 ⁻¹⁰	14*	3.5	Degani (1985)
<i>R. pipiens</i>	SA	Pituitrin	10 IU 100g ⁻¹	18	6.0	Steggerda (1937)
		Pituitrin	10 IU 100g ⁻¹	10-26	-	Boyd and Brown (1938)
		Pituitrin	5 IU 100g ⁻¹	12*	4.0	Sawyer (1951b)
<i>R. septentrionalis</i>	SA	Pituitrin	10 IU 100g ⁻¹	said not to differ significantly from <i>R. pipiens</i>	-	Boyd and Brown (1938)
<i>R. esculenta</i>	SA	AVT	8 × 10 ⁻¹¹	22	-	Heller (1965)
		AVT	4 × 10 ⁻¹¹	20	6.7	Heller (1941)
<i>R. temporaria</i>	SA	Frog pituitary extract	-	13	3.7	Heller (1941)
<i>Pelobates syriacus</i>	F	AVT	-	4*	4.0	Warburg (1971a)
<i>P. cultripes</i>	F	AVT	-	13	3.3	Heller and Bentley (1965)
<i>Crinia georgiana</i>	T	AVT	10 ⁻¹²	13	2.7	Bentley and Main (1972b)
<i>Bufo marinus</i>	T	AVT	10 ⁻¹²	10	-	Bentley (1969b)
		OT	5 U 100g ⁻¹	22*	5.5	Bentley and Ferguson (1967)
<i>B. viridis</i>	T	AVT	4 × 10 ⁻¹⁰	16*	-	Degani (1985)

TABLE 4.6 (cont.)

Species	Habitat	Hormone	Hormone dose (AVT dose in mol g ⁻¹)	Maximum water retention (%)	Rate of water retention (% h ⁻¹)	Reference
<i>B. bufo</i>	T	AVT	2 × 10 ⁻¹²	20	-	Heller (1965)
		Mammalian neurohypophysial extract	-	(40)	-	Howes (1940)
<i>B. carens</i>	T	Pitressin	1 IU 100g ⁻¹	22	-	Ewer (1952)
<i>B. regularis</i>	T	Pitressin	5-10 IU 100g ⁻¹	22	7.3	Ewer (1951)
<i>B. americanus</i>	T	Pituitrin	10 IU 100g ⁻¹	45	6.0	Steggerda (1937)
<i>Hyla arborea</i>	ARB	AVT	4 × 10 ⁻¹⁰	53*	53.0	Heller and Bentley (1965)
<i>Leiopelma hochstetteri</i>	SA	AVT	7 × 10 ⁻¹¹	9* (18)	1.0	Present study
<i>Leiopelma archeyi</i>	T	AVT	7 × 10 ⁻¹¹	31 (41)	7.8	Present study
<i>Leiopelma hamiltoni</i>	T	AVT	7 × 10 ⁻¹¹	29 (37)	5.8	Present study
		AVT	7 × 10 ⁻¹²	38* (52)	7.6	Present study
<i>Ascaphus truei</i>	A/SA	AVT	7 × 10 ⁻¹¹	1	1.0	Present study (Appendix II)
<i>Litoria aurea</i>	SA	AVT	7 × 10 ⁻¹¹	27	6.8	Present study (Appendix II)

- Notes:
1. Predominant habitat: A = aquatic, SA = semiaquatic, F = fossorial, T = terrestrial, ARB = arboreal [information from original references, Bentley (1971a), Cogger (1975) or Arnold and Burton (1978)].
 2. Hormone and dose: as far as possible, data were collected for AVT doses of 10⁻¹¹ - 10⁻¹⁰ mol g⁻¹. Where necessary, doses have been estimated from the authors' original units of concentration by assuming an activity of 200 U mg⁻¹. Pituitrin and Pitressin are mammalian neurohypophysial extracts (IU = international units; - indicates no data).
 3. Maximum water retention (Brunn effect): values give maximum weight gain as a percentage of W₀ (* indicates cases where weight may not have been at a maximum). Values without parentheses are presumed or stated to be mean values. Values inside parentheses are highest individual values.
 4. Rate of water retention: average rate of weight gain over the time taken to reach maximum weight (- indicates insufficient data to calculate).

TABLE 4.7 Magnitude and rate of water retention in urodeles treated with neurohypophysial peptides (all adult unless otherwise stated; see TABLE 4.6 for details).

Species	Habitat	Hormone	Hormone dose (AVT dose in mol g ⁻¹)	Maximum water retention (%)	Rate of water retention (% h ⁻¹)	Reference
<i>Necturus maculosus</i>	A	Pituitrin	10 IU 100g ⁻¹	4	0.7	Steggerda (1937)
		AVT	1 × 10 ⁻¹⁰	3	0.5	Bentley and Heller (1964)
<i>Amphiuma means</i>	A	AVT	1 × 10 ⁻¹⁰	4*	0.4	Bentley (1973)
<i>Siren lacertina</i>	A	AVT	1 × 10 ⁻¹⁰	5*	0.6	Bentley (1973)
<i>Desmognathus quadramaculatus</i>	A	AVP	1 × 10 ⁻¹⁰	6	1.0	Brown <i>et al.</i> (1977)
<i>Ambystoma tigrinum</i> larva	A	AVT	1 × 10 ⁻¹⁰	4	0.5	Bentley and Heller (1964)
<i>Ambystoma tigrinum</i> adult	T/F	AVT	4 × 10 ⁻¹¹	8*	1.0	Alvarado and Johnson (1965)
<i>Plethodon cinereus</i>	T	AVP	1 × 10 ⁻¹⁰	2	0.5	Brown <i>et al.</i> (1977)
<i>P. glutinosus</i>	T	AVP	1 × 10 ⁻¹⁰	7	1.6	Brown <i>et al.</i> (1977)
<i>P. hoffmani</i>	T	AVP	1 × 10 ⁻¹⁰	8*	1.3	Brown <i>et al.</i> (1977)
	(more so than <i>P. cinereus</i> or <i>P. glutinosus</i>)					
<i>P. punctatus</i>	"	AVP	1 × 10 ⁻¹⁰	17	4.3	Brown <i>et al.</i> (1977)
<i>Salamandra maculosa</i>	T	AVT	4 × 10 ⁻¹¹	8(14)	2.0	Bentley and Heller (1965)
<i>S. salamandra</i>	T	AVT	3 × 10 ⁻¹⁰	8*	8.3	Warburg (1971b)
<i>Triturus alpestris</i>	T	AVT	4 × 10 ⁻¹¹	15	2.5	Bentley and Heller (1964)
<i>T. vittatus</i> aquatic phase	A	AVT	3 × 10 ⁻¹⁰	8*	7.8	Warburg (1971b)
terrestrial phase	T	AVT	3 × 10 ⁻¹⁰	17*	16.7	Warburg (1971b)
<i>Notophthalmus viridescens</i> aquatic phase	A	AVP	40 mU g ⁻¹	24*	12.0	Brown and Brown (1977)
terrestrial phase	T	AVP	40 mU g ⁻¹	15	7.5	Brown and Brown (1977)
<i>Aneides lugubris</i>	T	AVP	4 × 10 ⁻⁸	21*	5.3	Hillman (1974)
<i>Taricha torosa</i> terrestrial phase	T	AVT	7 × 10 ⁻¹¹	21	5.3	Brown and Brown (1980)

The results of the present study support these general trends. *L. archeyi* and *L. hamiltoni* treated with 7×10^{-11} mol g⁻¹ AVT show mean weight gains of 29-31%, which are similar to those of other terrestrial or arboreal anurans in which a cutaneous response has been inferred. In contrast, the small weight gain seen in *L. hochstetteri* is comparable to that of aquatic urodeles which are also believed to have only an antidiuretic response. The rate of water retention observed for *L. hochstetteri* (1% per hour) is identical to that for *D. quadramaculatus* (Table 4.7), which was noted in Chapter 3 as having a similar rate of rehydration to *L. hochstetteri*.

In additional experiments, responses of *Litoria aurea* and *Ascaphus truei* to 7×10^{-11} mol g⁻¹ AVT were examined (Appendix II; Table 4.6). The single *A. truei* examined showed a maximum weight gain of 1% over 8 h, indicating, as in *L. hochstetteri*, an absence of a cutaneous response to AVT. However, *Litoria aurea* demonstrated a water balance response similar to that of *L. archeyi* and *L. hamiltoni* (maximum mean water retention 27% of W_0). The latter result is consistent with the broadly similar water balance responses to dehydration in the three latter species.

Experiments with lower doses of AVT show that significant water retention was also evident in *L. archeyi* and *L. hamiltoni* treated with 7×10^{-13} mol g⁻¹ (equivalent to about 0.07-0.7x presumed neurohypophysial content). *L. archeyi* treated with one-hundredth this dose also showed a small but still significant amount of water retention. The actual concentration that these doses of exogenous AVT produced in the body fluids is unknown, but can be estimated by assuming that the injected peptide is distributed throughout an extracellular fluid volume equivalent to 25% of W_0 (Christensen and Jørgensen 1972). Based on this assumption, an injected dose of 7×10^{-13} mol g⁻¹ produces an estimated concentration of $\sim 3 \times 10^{-9}$ mol l⁻¹. Given that endogenous AVT circulates in the plasma or serum of dehydrated anurans at concentrations between 10^{-11} and 10^{-9} mol l⁻¹ [calculated from bioassay data of Bentley (1969a) and RIA data of Rosenbloom and Fisher (1974), Sawyer and Pang (1975) and Nouwen and Kühn (1983)], and that AVT injected subcutaneously may not diffuse completely throughout the extracellular fluids before it begins to be inactivated, a dose of 7×10^{-13} mol g⁻¹ seems consistent with the highest potential endogenous levels.

Responses of *L. archeyi* and *L. hamiltoni* treated with the same dose of AVT were extremely similar: 7×10^{-11} mol g⁻¹ produced mean values for maximal water retention of 30-32% W₀, 7×10^{-13} mol g⁻¹ produced maximum weight gains of 24-26% and 7×10^{-15} mol g⁻¹ 5-6%. However, the large weight gain seen in *L. hamiltoni* treated with 7×10^{-12} mol g⁻¹ (38%; Table 4.4) was surprising. This experiment was terminated after only 5 h because of the alarmingly high weight gain (52%) seen in one individual, which had become extremely bloated and had also developed an apparent haemorrhage under the pelvic skin. Fortunately, several days after return to its vivarium the engorgement had disappeared and the frog had returned to its original weight. This dose was the first to be tested on laboratory *L. hamiltoni* (which had been collected from Maud Island 12 d previously), and it seems that these animals were more responsive to AVT than those treated with a higher dose on Maud Island in May 1984. Large responses of the ventral skin to AVT *in vitro* were later noted in the same animals (see Chapter 5). Field conditions were relatively dry on Maud Island in May 1985 when these animals were collected (Dr B.D.Bell, pers. comm.), and this may have increased their responsiveness to AVT. Seasonal differences in responsiveness to injected NHPs have been observed in North American amphibians, with summer animals showing greater responses than winter ones (Brown *et al.* 1977; Parsons *et al.* 1978), although the separate effects of dry conditions and temperature were not addressed.

Comparison of the dose-response results obtained here for *L. archeyi* and *L. hamiltoni* with those for other anuran species is difficult, in that most published studies have only examined the effects of doses of $\sim 10^{-13}$ mol g⁻¹ and higher. The lowest dose used to my knowledge is 6.6×10^{-14} mol g⁻¹, which had no effect on water retention in *Rana catesbeiana* (Alvarado and Johnson 1966). However, 3.5×10^{-13} mol g⁻¹ did produce significant water retention (11%). Heller (1965) found that *Bufo bufo* treated with $\sim 3 \times 10^{-13}$ mol g⁻¹ AVT increased in weight by 9%, and by 16% when treated with $\sim 8 \times 10^{-13}$ mol g⁻¹. Christensen and Jørgensen (1972) reported that a dose of 0.25 µg AVT 100g⁻¹ ($\sim 3 \times 10^{-12}$ mol g⁻¹) produced maximal water retention in *B. bufo*. From these and other studies (Bentley and Heller 1965; Elliott 1968; Bentley 1969a) it is evident that anurans generally show increasing water retention over a dose range of $\sim 10^{-13}$ - 10^{-11} mol g⁻¹ AVT. *L. archeyi* and *L. hamiltoni* are therefore at least as sensitive and possibly more sensitive to low doses of AVT than are other anurans.

The situation with *L. hochstetteri* is more equivocal. Significant water retention was seen at the possibly pharmacological dose of 7×10^{-11} mol g⁻¹ AVT but 7×10^{-13} mol g⁻¹ had no effect. This seems reminiscent of the situation in the urodeles *Siren lacertina* and *Amphiuma means*, where significant water retention (4-5% of W_0) occurred with a dose of 1.2×10^{-10} mol g⁻¹, but 1.2×10^{-11} mol g⁻¹ caused no response (Bentley 1973). Since the former dose represents about 10-100× the AVT content of their neurohypophyses, the physiological significance of the effect is regarded as questionable (Bentley 1973). However, given that in *L. hochstetteri* actual neurohypophysial content and circulating levels of AVT remain unknown and that small and transient changes in urine output would not be detected by the weighing method used here, a firm conclusion as to the significance of the results from dose-response studies cannot be made. What is clear, however, is that water retention in *L. hochstetteri* is not evident at the lower doses of AVT to which *L. archeyi* and *L. hamiltoni* are sensitive.

In summary, the results presented in this chapter have shown that exogenous AVT produces effects on water balance similar to those seen during rehydration in the three *Leiopelma* species. In *L. archeyi* and *L. hamiltoni* a dose of 7×10^{-11} mol g⁻¹ increases cutaneous water uptake through the ventral skin and probably reduces urine output. These effects cause a large increase in body weight which is demonstrable at presumably physiological doses. In *L. hochstetteri* a dose of 7×10^{-11} mol g⁻¹ does not affect cutaneous water uptake but does produce a small weight gain, and this is attributed to reduced urine output. Whether urine output of this species is affected by AVT at lower doses is uncertain.

CHAPTER 5

EFFECTS OF AVT ON THE SKIN, THE KIDNEYS AND THE BLADDER

INTRODUCTION

Experiments described in the previous chapter showed that exogenous AVT caused increased water uptake *in vivo* through ventral skin of *L. archeyi* and *L. hamiltoni*, but not of *L. hochstetteri*. AVT also appeared to reduce the urine output of all three species, but whether this resulted from renal antidiuresis, increased bladder reabsorption or both was not known.

The objectives of this chapter are to determine for each species the effects of AVT on water exchange at the three possible receptor sites: the skin, the kidneys and the bladder. Effects on the skin were examined by measuring osmotic water flow *in vitro* through three regions (ventral pelvic, ventral pectoral and dorsal skin) in the absence and presence of AVT. This technique was practised first with the hylid *Litoria aurea* and the results for this species are included. The effect on glomerular filtration rate at the kidney was determined for the three *Leiopelma* species by measuring creatinine clearance before and after AVT treatment *in vivo* (tubular reabsorption was not examined). Bladder responses were examined by measuring osmotic water flow through isolated bladder lobes in the absence and presence of AVT. Together with additional data on bladder capacities, the results provide an indication of the relative contributions of the skin, the kidneys and the bladder to the total water balance response of each species.

MATERIALS AND METHODS

Animals

L. archeyi and *L. hochstetteri* used in Experiment 1 (Effect of AVT on cutaneous osmotic water flow) were stock animals held in captivity 12-23 mo before use in February-March 1985. *L. hamiltoni* were collected in May 1985 and used within 8 wk of capture. All animals had been used in previous experiments examining dose-response effects

of AVT *in vivo* (Experiment 2 of Chapter 4), and all except three *L. hamiltoni* were female. Blood samples were also collected from several of these animals and used for measurements of plasma osmotic concentration (Appendix III).

Frogs used in Experiment 2 (Effect of AVT on glomerular filtration rate) were collected in 1985 during February (*L. archeyi* and *L. hochstetteri*) and May (*L. hamiltoni*). This experiment involved blood sampling and it was therefore carried out as promptly as possible (within 4 d - 6 wk of capture) to minimise the reduction in blood volume which is sometimes observed in captive anurans (Dr C.H. Daugherty, Victoria University of Wellington, pers. comm.). Only *L. hamiltoni* had been used in prior experiments (Experiment 2 of Chapter 4).

Experiment 3 (Effect of AVT on osmotic water flow through the bladder) used bladder lobes from animals used in Experiments 1 and 2. Initial trials with *L. archeyi* and *L. hochstetteri* were carried out with lobes from Experiment 1 animals, but most final results came from animals used in Experiment 2. All except one (an *L. hochstetteri* lobe) were from females. All *L. hamiltoni* lobes came from females used in Experiment 1.

Weights of animals used in the three experiments were 3.0-5.0 g (*L. archeyi*), 4.4-8.4 g (*L. hamiltoni*) and 3.2-9.4 g (*L. hochstetteri*).

Male *Litoria aurea* (11.3-14.5 g) used in Experiment 1 were collected in December 1984 from breeding ponds near Raglan, Waikato. They were kept on sand with access to water at $17 \pm 2^\circ\text{C}$ on a 12 h L: 12 h D photoperiod for 1 d - 6 wk before use, and were fed mealworms.

Experiment 1. Effect of AVT on cutaneous osmotic water flow

The effect of AVT on osmotic water flow (OWF) through isolated skin preparations was determined at $22 \pm 1.5^\circ\text{C}$ following the gravimetric procedure of Bentley and Main (1972a). Skin from the lower abdomen ("pelvic"), from the ventral surface of the pectoral girdle ("pectoral") and from the mid-lower back ("dorsal") of double-pithed animals (Fig. 5.1) was removed and rested for ~20 min in aerated amphibian Ringer's solution (Ringer) of the following composition (g

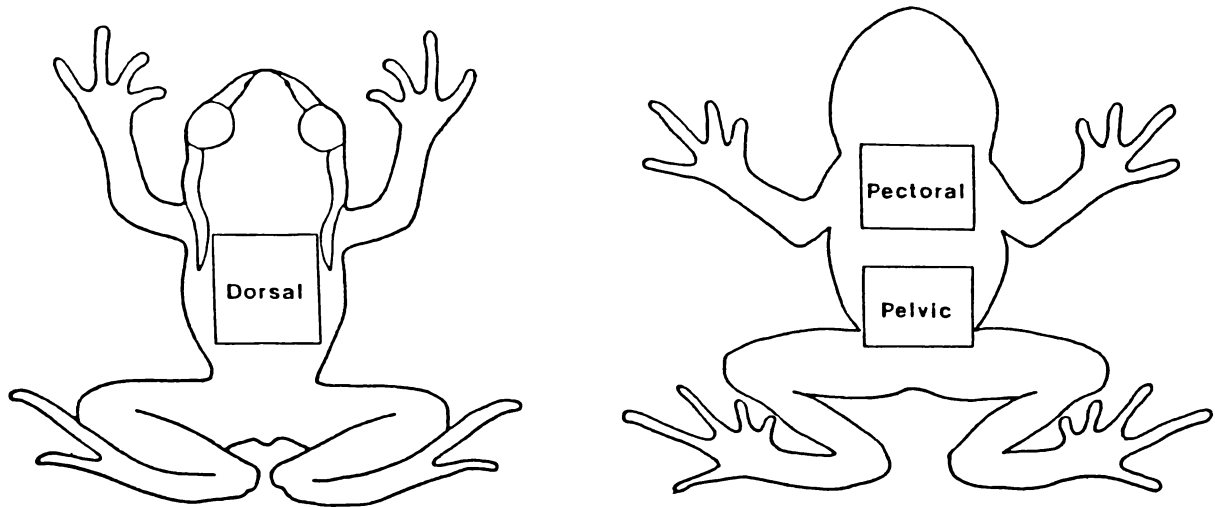


FIG. 5.1 Regions of skin used for measurement of osmotic water flow.

l^{-1}): NaCl 6.5, KCl 0.14, $CaCl_2$ 0.12, $NaHCO_3$ 0.10, Na_2HPO_4 0.01, glucose 2.0. Each skin piece was then tied, epidermis inside, onto the end of a hollow glass tube (id 4.4 mm). Tubes were filled with 10% Ringer and immersed in full-strength aerated Ringer so that fluid levels inside and outside the tube were equal (Plate 5.1). The osmotic gradient between inside and outside solutions was 192 mOsm kg^{-1} (osmotic concentrations determined using a Wescor Model 5100-C vapour pressure osmometer, Utah, USA). Weights of the preparations were reproducible to within 3.5 mg (maximum difference between any two consecutive of ten successive weighings). Preparations were rested for ~30 min, and then weight loss to the nearest 0.1 mg was measured over 2-3 ~50 min periods until stable resting values were obtained (preparations of isolated frog skin are known to show large and highly variable OWF when first mounted; Franz and van Bruggen 1967). The tubes were then refilled with fresh 10% Ringer before weight loss in full-strength Ringer containing AVT was measured. Hormone concentrations were 3×10^{-8} , 3×10^{-7} and $3 \times 10^{-6} \text{ mol l}^{-1}$ in successive periods (equivalent to 8 mU ml^{-1} , 80 mU ml^{-1} and 800 mU ml^{-1} respectively). Some *L. hamiltoni* pelvic preparations, including four from animals used in Experiment 2, were exposed to alternative doses of AVT. Preparations were then rinsed three times in Ringer, refilled and placed in full-strength Ringer without hormone for a further two periods.

Experiment 2. Effect of AVT on glomerular filtration rate

The effect of AVT on glomerular filtration rate (GFR) was determined at $16 \pm 1^\circ\text{C}$ by measuring creatinine clearance of frogs before and after treatment with AVT. Creatinine is a valid marker for determining GFR in *Rana catesbeiana* (Forster 1938; Sawyer 1957a) and *Bufo marinus* (Sawyer 1957b), and this was assumed to be true for *Leiopelma* also. Creatinine is not reabsorbed by the urinary bladder of *R. catesbeiana* (Sawyer 1960).

The procedure for loading animals with creatinine was similar to that of Schmidt-Nielsen and Lee (1962). A solution of 5% creatinine in 0.6% NaCl was injected under the abdominal skin (injection volume $10 \mu\text{l g}^{-1}$) and animals were held overnight with access to water to allow distribution of the marker in body fluids. The following morning, bladder urine was removed and only frogs which had apparently voided their entire bladder contents were used. GFR was then assessed by

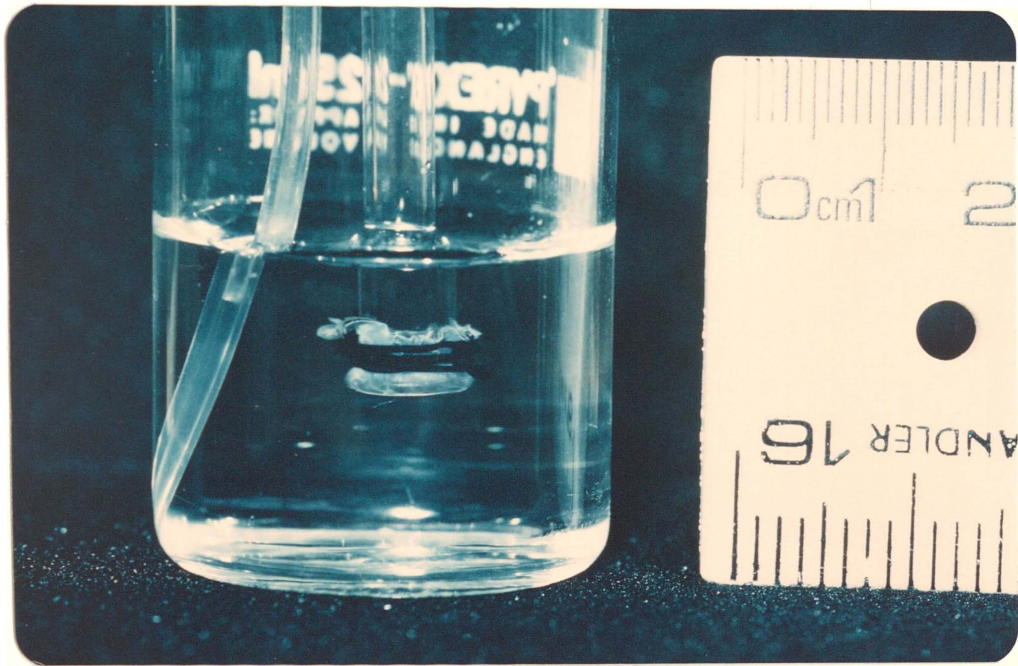


PLATE 5.1 Preparation of *L. hochstetteri* pelvic skin used for measurement of osmotic water flow. The glass tube contains dilute Ringer (one-tenth original concentration), which bathes the outer surface of the skin. The inner surface is bathed by full-strength Ringer (in beaker). Air is supplied in a polythene tube. Ruler shows scale in millimetre divisions.

determining clearance of the marker into the surrounding water during a 1 h control period, and then during a 1 h experimental period following treatment with AVT (Brown and Brown 1980). Frogs were weighed, treated with a control solution of 0.6% NaCl (4 $\mu\text{l g}^{-1}$ injected subcutaneously) and placed in 3 ml distilled water for 1 h. At the end of this period, bladder urine was removed and added to the bathing medium. Frogs were then reweighed and treated with 7×10^{-11} mol g^{-1} AVT dissolved in 0.6% NaCl (4 $\mu\text{l g}^{-1}$ injected subcutaneously). After a further hour in a fresh sample of bathing water, unvoided urine was expelled into the bathing medium (later dissection confirmed that bladders had been fully emptied). Frogs were weighed once more and then double-pithed.

A blood sample was quickly collected from the transected ventral aorta in a heparinised capillary tube, and centrifuged at 2000 rpm (max) for 3 min. Plasma and bathing water samples were frozen at -18°C . Creatinine content was later determined using a Creatinine Analyzer 2 (Beckman Instruments, California, USA) operated by Mr D.Mikkelson, Waikato Public Hospital. Plasma samples were diluted 1:2 or 1:3 with deionised water to obtain the necessary volume for analysis (40 μl).

Creatinine clearance (C_{CR}) was calculated in $\mu\text{l g}^{-1} \text{h}^{-1}$ as:

$$C_{\text{CR}} = \frac{Q}{P_{\text{CR}} \times W_0 \times t}$$

where Q = the quantity of creatinine in the bathing fluid (in mmol), P_{CR} = the calculated plasma creatinine concentration at the mid-point of each experimental period (in mmol μl^{-1}), W_0 = initial body weight with bladder urine removed (in g) and t = time (in h). Ideally, plasma concentrations should be measured at the mid-point of both control and AVT periods; however, this presents a problem in small amphibians where removal of sufficient blood for analysis may in itself have profound effects on renal function (Stiffler and Alvarado 1974; Stiffler *et al.* 1984). Moreover, in the *Leiopelma* species it was sometimes difficult to obtain sufficient blood for analysis even with the terminal method of aortic transection: the blood of these frogs seems to have peculiar agglutination properties and on occasions stopped flowing after volumes as little as 15 μl had been collected. This difficulty has been noted by others working with the same species (Dr C.H.Daugherty, pers. comm.).

Terminal plasma samples were therefore obtained and corrected for falling creatinine levels as follows. Plasma creatinine concentration at the beginning of the AVT period was assumed to be equal to final measured plasma creatinine concentration plus the amount of creatinine excreted into the bathing water during this period, distributed in an extracellular creatinine space equal to 25% of W_0 . Using this figure, plasma creatinine concentration at the beginning of the control period could be calculated in the same way. Mid-point plasma creatinine concentrations for each period were assumed to be the means of initial and final plasma concentrations. This method is similar to that used by Stiffler and Alvarado (1974) and Stiffler *et al.* (1984) for calculating mid-point inulin clearance in small urodeles.

A potential source of error in these calculations is that the control plasma creatinine concentrations may be underestimated for *L. archeyi* and *L. hamiltoni*, since these species show large water gains following AVT treatment which would tend to dilute final plasma creatinine concentration below the level present during the control period. This would produce an overestimate of control C_{CR} . The extent of this is unknown but is assumed to be insignificant, since most absorbed water probably accumulates initially in the lymph spaces rather than in the plasma. In the case of *L. hochstetteri* the problem does not exist since little water retention occurs in response to AVT.

Experiment 3. Effect of AVT on osmotic water flow through the bladder

This experiment was carried out at $22 \pm 1.5^\circ\text{C}$ following essentially the classic gravimetric method of Bentley (1958). Each bladder lobe was removed and tied, mucosa innermost, onto the end of a hollow glass tube (id 2.5 mm). Intact preparations were filled with 10% Ringer and immersed in full-strength Ringer.

Preparations were then rested for ~90 min to allow any residual effects of AVT treatment prior to death to wear off. This was necessary because most *L. archeyi* and all *L. hochstetteri* lobes used in final experiments came from animals which had been treated with AVT 1 h prior to death (the same animals as used in Experiment 2). Bladder lobes of *L. hamiltoni* were from animals used in Experiment 1 (not AVT-treated) but were rested for the same length of time.

Weight losses over ~50 min control and experimental periods were then

measured to the nearest 0.1 mg. The concentration of AVT in the external medium was 3×10^{-8} mol l⁻¹ (8 mU ml⁻¹) during the experimental period. Some *L. archeyi* and *L. hamiltoni* preparations which were larger and remained intact for many hours were exposed to further treatments. Weights of preparations were reproducible to within 1.5 mg (maximum difference between any two consecutive of ten successive weighings).

During these experiments, care was taken to maintain a fluid head of 10 mm inside each tube and to refill preparations to the same initial weight (± 1.5 mg) prior to each treatment; this was essential to maintain constant distension of bladder lobes. In an earlier series of experiments in which the latter precaution was not taken (with bladder lobes from *L. archeyi* and *L. hochstetteri* used in Experiment 1) it was found that some bladder lobes tended to become increasingly distensible during the course of an experiment. These lobes therefore tended to be refilled to a larger volume before they were exposed to AVT. Only later did I realise that the high rates of OWF measured during exposure to AVT were to an unknown extent an artifact of increased bladder surface area (SA). Results from the AVT treatment period were therefore discarded.

The experiment was then repeated using lobes from *L. archeyi* and *L. hochstetteri* used in Experiment 2. To check that any residual effects of the prior AVT treatment *in vivo* had worn off 90 min after mounting, control rates of OWF through lobes of animals used in Experiment 2 were compared with control rates for lobes from Experiment 1 animals. In both species, mean OWF did not differ significantly between the two groups ($t \leq 1.615$, $P \geq 0.2$). Therefore, the effects of the *in vivo* AVT treatment can be discounted.

At the end of an experiment, the volume of each preparation was measured by fluid displacement to the nearest 0.05 ml. The SA of exposed bladder tissue was estimated by assuming preparations were spherical and correcting for the area inside the tube not covered by bladder tissue.

Experiment 4. Bladder capacity

Bladder capacity of the three species was estimated in three ways: (i) by measurement of the fluid volume displaced by mounted bladder lobes *in vitro*, as described above; (ii) in a small number of cases, by stretching isolated lobes over a glass rod of known cross-sectional area prior to mounting, measuring the length enclosed by each lobe to the nearest mm, and calculating the volume of the enclosed cylinder; (iii) by measuring voluntary bladder volume (the amount of urine voluntarily stored in the bladder) in stock animals on a large number of occasions. Animals were removed from their holding terraria, weighed, emptied of bladder urine and reweighed. Several of the larger urine samples obtained were used for measurement of urea nitrogen and ammonia nitrogen concentrations (Chapter 7) and/or urine osmotic concentration (Appendix III).

Hormone

Stock solutions of AVT (Sigma Lots 73F-0299 and 83F-0690) were prepared as described in Experiment 2 of Chapter 4. The purity of these lots was ~94-96% and the peptide content ~75-86%.

RESULTS

Experiment 1. Effect of AVT on cutaneous osmotic water flow

L. archeyi and *L. hamiltoni*

Skin from these two species showed generally similar responses to AVT (Figs 5.2 and 5.3). Mean OWF during the second control period (hereafter referred to as the resting value) was 41 mg cm⁻² h⁻¹ or less for all skin regions. In *L. archeyi*, resting OWF tended to be higher and more variable for pelvic skin than for pectoral or dorsal skin; however, one-way analysis of variance (ANOVA) tests indicated no significant regional differences in resting OWF for either species (Table 5.1). When exposed to 3×10^{-8} mol l⁻¹ AVT, pelvic OWF increased to 2.4× the resting value for *L. archeyi* (from 41 ± 17 to 97 ± 17 mg cm⁻² h⁻¹; $\bar{x} \pm$ SE) and to 7.8× the resting value for *L. hamiltoni* (from 11 ± 7 to 86 ± 4 mg cm⁻² h⁻¹). Thereafter, the rate of increase in OWF declined for pelvic skin of *L. archeyi* exposed to higher concentrations of AVT; the highest mean rate recorded was

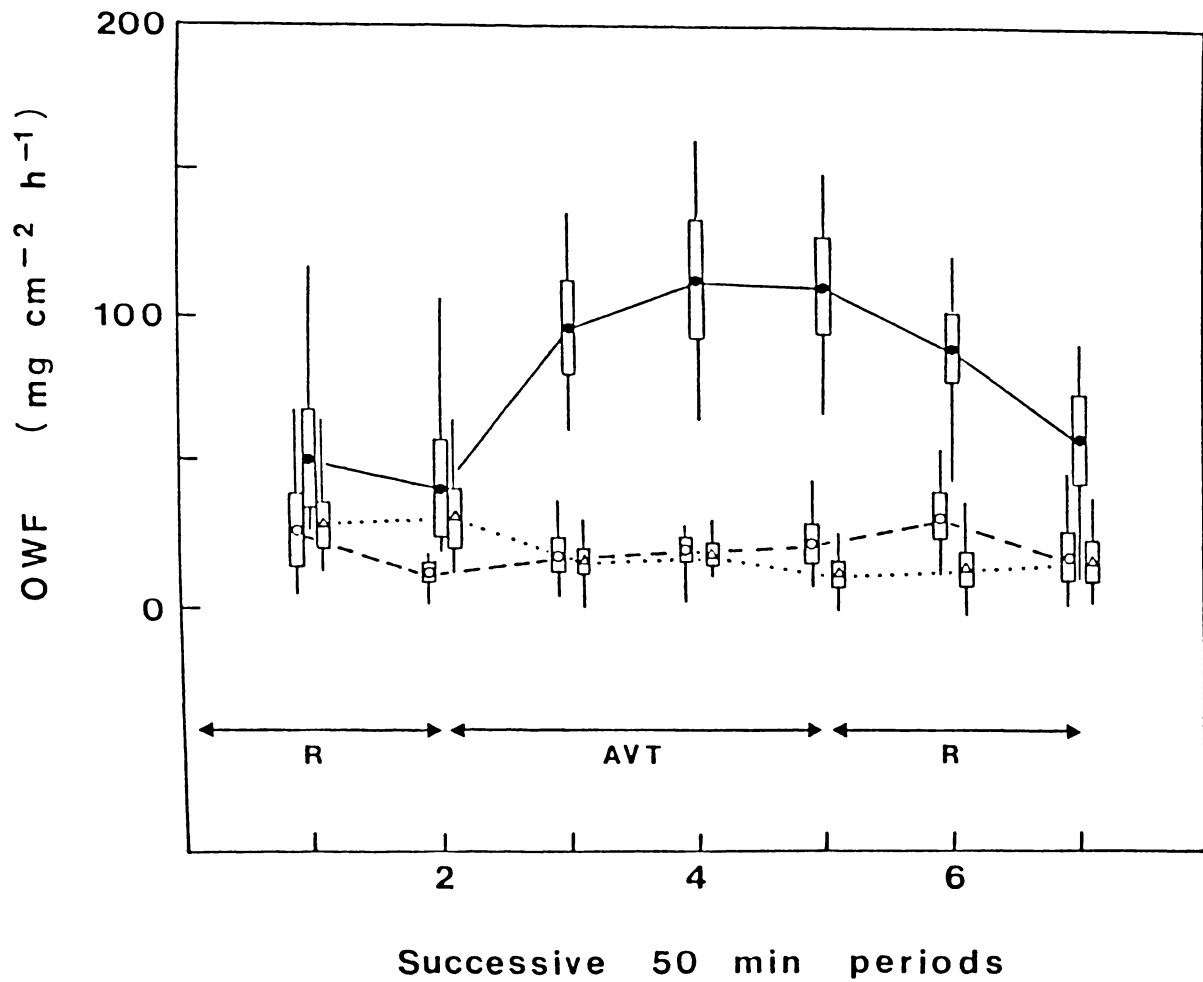


FIG. 5.2 Effect of AVT on osmotic water flow (OWF) through skin of female *L. archeyi* ($\bar{x} \pm \text{SE}$ and range). AVT concentration was $3 \times 10^{-8} \text{ mol l}^{-1}$ during the third period, $3 \times 10^{-7} \text{ mol l}^{-1}$ during the fourth and $3 \times 10^{-6} \text{ mol l}^{-1}$ during the fifth (R = Ringer only; ● = pelvic skin, $n = 5$; ○ = pectoral skin, $n = 5$; Δ = dorsal skin, $n = 5$).

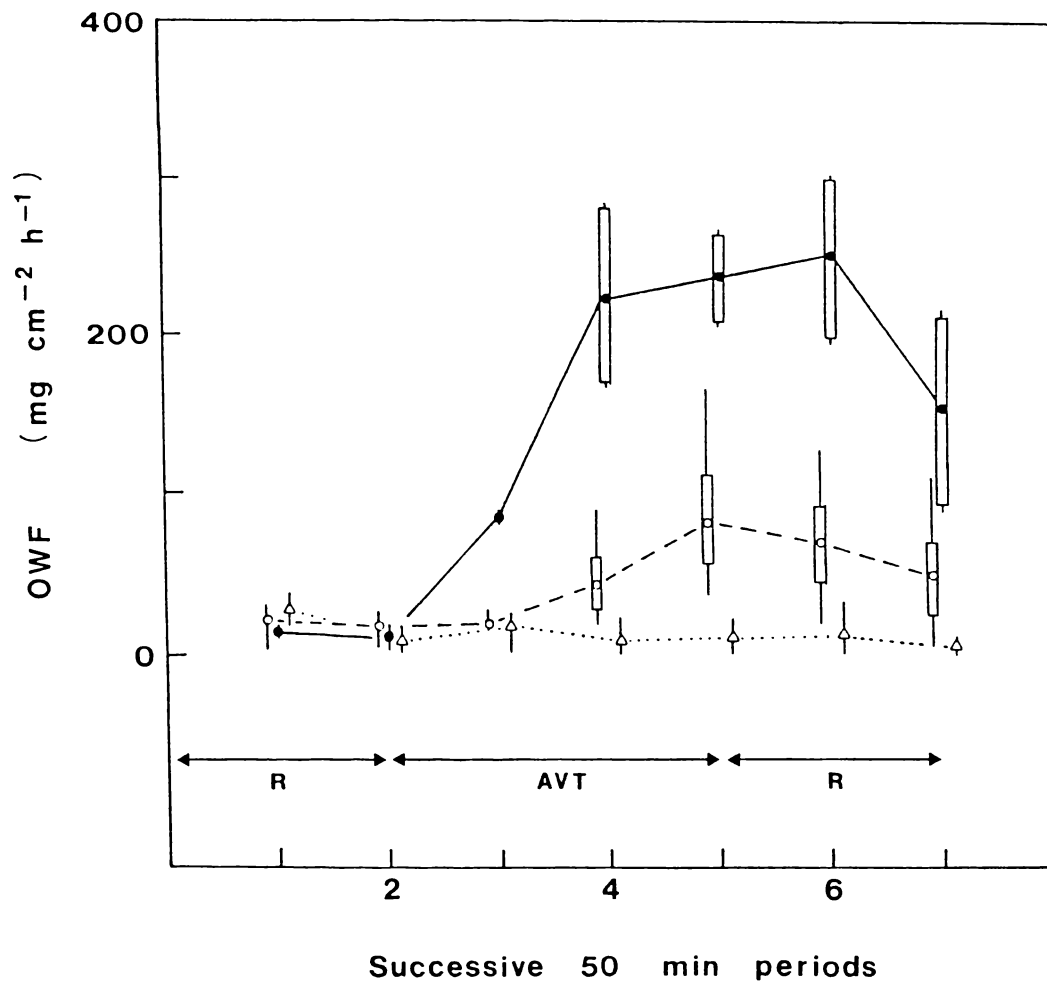


FIG. 5.3 Effect of AVT on osmotic water flow through skin of female *L. hamiltoni* (for pelvic skin $n = 2$, pectoral $n = 4$, dorsal $n = 4$; other details as in FIG. 5.2).

TABLE 5.1 F-values for comparisons of cutaneous osmotic water flow (OWF) in *Leiopelma* species and *Litoria aurea* [degrees of freedom in parentheses; * = $P < 0.05$, ** = $P < 0.01$; NSD = no significant difference ($P > 0.05$)].

Comparison between	Species			
	<i>L. archeyi</i>	<i>L. hamiltoni</i>	<i>L. hochstetteri</i>	<i>Litoria aurea</i>
Resting OWF (pelvic vs pectoral vs dorsal) ¹	1.74 (2,12) NSD	0.81 (2,7) NSD	0.55 (2,12) NSD	13.44 (2,13) **
Pelvic OWF (resting vs AVT) ²	3.60 (3,16) *	10.86 (3,4) *	1.95 (3,16) NSD	13.10 (2,18) **
Pectoral OWF (resting vs AVT) ²	0.79 (3,16) NSD	2.83 (3,12) NSD	0.68 (3,16) NSD	0.03 (2,9) NSD
Dorsal OWF (resting vs AVT) ²	1.44 (3,16) NSD	0.60 (3,12) NSD	2.08 (3,16) NSD	1.79 (2,12) NSD

Notes:

1. To compare resting OWF through different skin regions, a one-way analysis of variance was made between resting values recorded in period 2 (see text).
2. To assess the effect of AVT on OWF through each region of skin, a one-way analysis of variance was made between values recorded in periods 2-5 inclusive for *Leiopelma* species (FIGS 5.1, 5.2 and 5.4) and between periods 2-4 inclusive for *Litoria aurea* (FIG 5.5).

114 \pm 21 mg cm⁻² h⁻¹ in 3 \times 10⁻⁷ mol l⁻¹. Water flux through pelvic skin of *L. hamiltoni* continued to increase dramatically in the presence of AVT, and the highest mean rate recorded was 236 \pm 31 mg cm⁻² h⁻¹ (in 3 \times 10⁻⁶ mol l⁻¹). In both species, the effect of AVT on pelvic OWF was significant by one-way ANOVA ($P < 0.05$; Table 5.1). When preparations of pelvic skin were rinsed and returned to Ringer without AVT, OWF eventually fell, indicating that the effect of the hormone was reversible (Figs. 5.2 and 5.3).

Similar increases in OWF were seen in pelvic skin of *L. hamiltoni* exposed to different concentrations of AVT (Fig. 5.4). Preparations which were exposed to 3 \times 10⁻¹¹ mol l⁻¹ AVT showed little response during the first period of exposure, but OWF increased to values as high as 188 mg cm⁻² h⁻¹ during the following period. Another preparation, which was to have been part of the series depicted in Fig. 5.3 but which was inadvertently transferred direct from hormone-free Ringer into 3 \times 10⁻⁷ mol l⁻¹ and then into 3 \times 10⁻⁶ mol l⁻¹ AVT, showed increases in OWF in these concentrations virtually identical to those seen in preparations placed into 3 \times 10⁻⁸ mol l⁻¹ and then into 3 \times 10⁻⁷ mol l⁻¹ (Fig. 5.4, cf. Fig. 5.3). In this preparation, the maximum stimulated OWF was 240 mg cm⁻² h⁻¹ (in 3 \times 10⁻⁶ mol l⁻¹ AVT).

Pectoral skin of *L. archeyi* and dorsal skin of both species showed no response to any concentration of AVT (Figs 5.2 and 5.3, Table 5.1). Pectoral skin of *L. hamiltoni* did not respond to 3 \times 10⁻⁸ mol l⁻¹ AVT, but some preparations showed moderate increases in OWF during subsequent treatments (Fig. 5.3). Although this trend was not statistically significant according to one-way ANOVA ($P > 0.05$, Table 5.1) the highest individual value recorded (166 mg cm⁻² h⁻¹ in 3 \times 10⁻⁶ mol l⁻¹) is far greater than can be explained by weighing errors alone.

L. hochstetteri

Mean OWF through all regions of skin dropped during the second control period to resting values of 17-23 mg cm⁻² h⁻¹ (Fig. 5.5). No regional differences were apparent ($P > 0.05$, Table 5.1). Rates of OWF did not increase during the remainder of the experiment and no responses to any concentration of AVT were observed (Table 5.1).

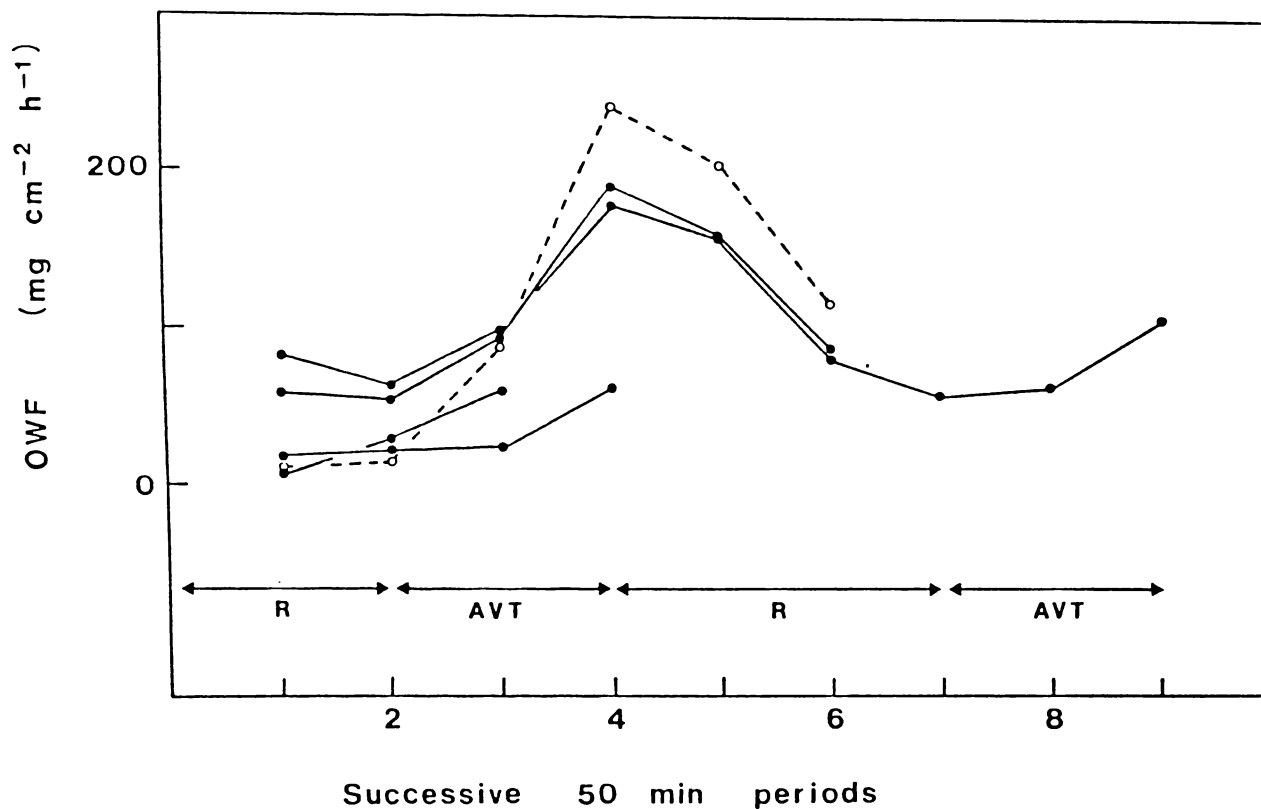


FIG. 5.4 Effect of various concentrations of AVT on osmotic water flow through pelvic skin of *L. hamiltoni*. o = one preparation which was exposed to $3 \times 10^{-7} \text{ mol l}^{-1}$ AVT during the third period and $3 \times 10^{-6} \text{ mol l}^{-1}$ during the fourth. This skin was from a female used in Experiment 1 (see text). ● = four preparations which were exposed to $3 \times 10^{-11} \text{ mol l}^{-1}$ AVT during periods 3 and 4 and in one case again during periods 8 and 9. These four preparations were from three males and one female used in Experiment 2.

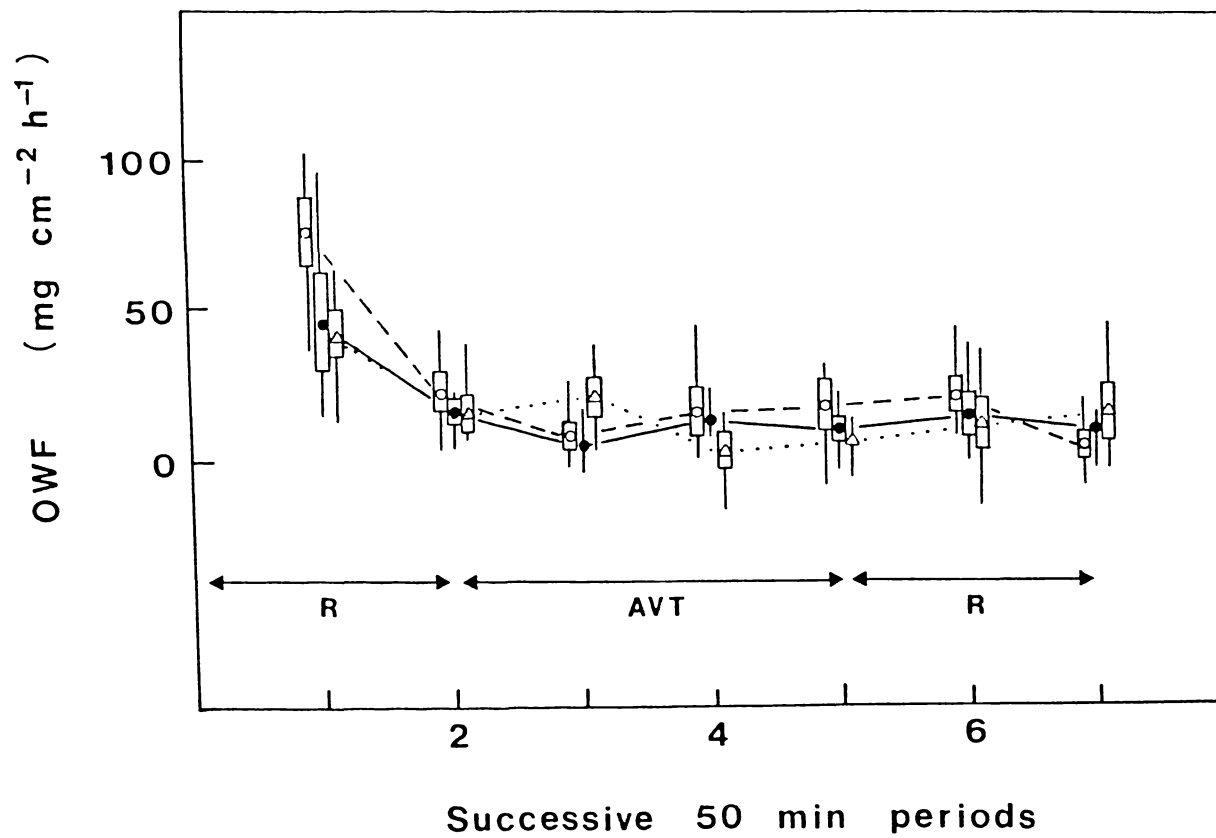


FIG. 5.5 Effect of AVT on osmotic water flow through skin of female *L. hochstetteri* (details as in FIG. 5.2).

Litoria aurea

OWF through all skin regions was initially high, but dropped during the second control period to resting values of $96 \pm 11 \text{ mg cm}^{-2} \text{ h}^{-1}$ for pelvic skin, $30 \pm 17 \text{ mg cm}^{-2} \text{ h}^{-1}$ for pectoral skin and $21 \pm 7 \text{ mg cm}^{-2} \text{ h}^{-1}$ for dorsal skin (Fig. 5.6). These regional differences in resting OWF were significant by one way ANOVA ($P < 0.01$, Table 5.1). When pelvic skin was placed in $3 \times 10^{-8} \text{ mol l}^{-1}$ AVT, OWF increased dramatically to $332 \pm 45 \text{ mg cm}^{-2} \text{ h}^{-1}$; this declined slightly to $299 \pm 40 \text{ mg cm}^{-2} \text{ h}^{-1}$ when the preparations were exposed to $3 \times 10^{-7} \text{ mol l}^{-1}$. OWF returned to near the resting value after two periods in Ringer without hormone. The effect of AVT on OWF through pelvic skin was highly significant ($P < 0.01$) but pectoral and dorsal skin showed no significant response ($P > 0.05$, Table 5.1).

Experiment 2. Effect of AVT on glomerular filtration rate

Control values for C_{CR} were similar for all species ($21\text{-}25 \mu\text{l g}^{-1} \text{ h}^{-1}$; Table 5.2). Following AVT treatment, C_{CR} dropped significantly to 0 and $1 \mu\text{l g}^{-1} \text{ h}^{-1}$ for *L. archeyi* and *L. hamiltoni* respectively ($P \leq 0.05$ by paired Student's t-test). Although mean C_{CR} also dropped for six AVT-treated *L. hochstetteri* (by 55%), there were wide individual variances and the effect was not statistically significant ($P > 0.2$). Four *L. hochstetteri* did show a considerable reduction in mean C_{CR} (from 31 ± 11 to $5 \pm 1 \mu\text{l g}^{-1} \text{ h}^{-1}$, but the remaining two animals showed increases in clearance (from 5 ± 2 to $19 \pm 10 \mu\text{l g}^{-1} \text{ h}^{-1}$).

Weight increases resulting from water uptake during this experiment are given in Table 5.3. All species showed significant increases in weight following AVT treatment, and as expected, the extent of water retention was much greater in *L. archeyi* and *L. hamiltoni* than in *L. hochstetteri*.

Experiment 3. Effect of AVT on osmotic water flow through the bladder

Isolated bladder lobes of all three species are small and difficult to mount. However, the intact preparations which were obtained showed small and significant increases in OWF (in mg h^{-1}) when exposed to AVT (Table 5.4), and the relative magnitude of the increase was similar for all three species (stimulated value = $1.5\times$ control value for *L. hamiltoni*, $1.6\times$ for *L. archeyi* and $2.1\times$ for *L. hochstetteri*). Longer experiments with two *L. archeyi* and seven *L. hamiltoni* lobes

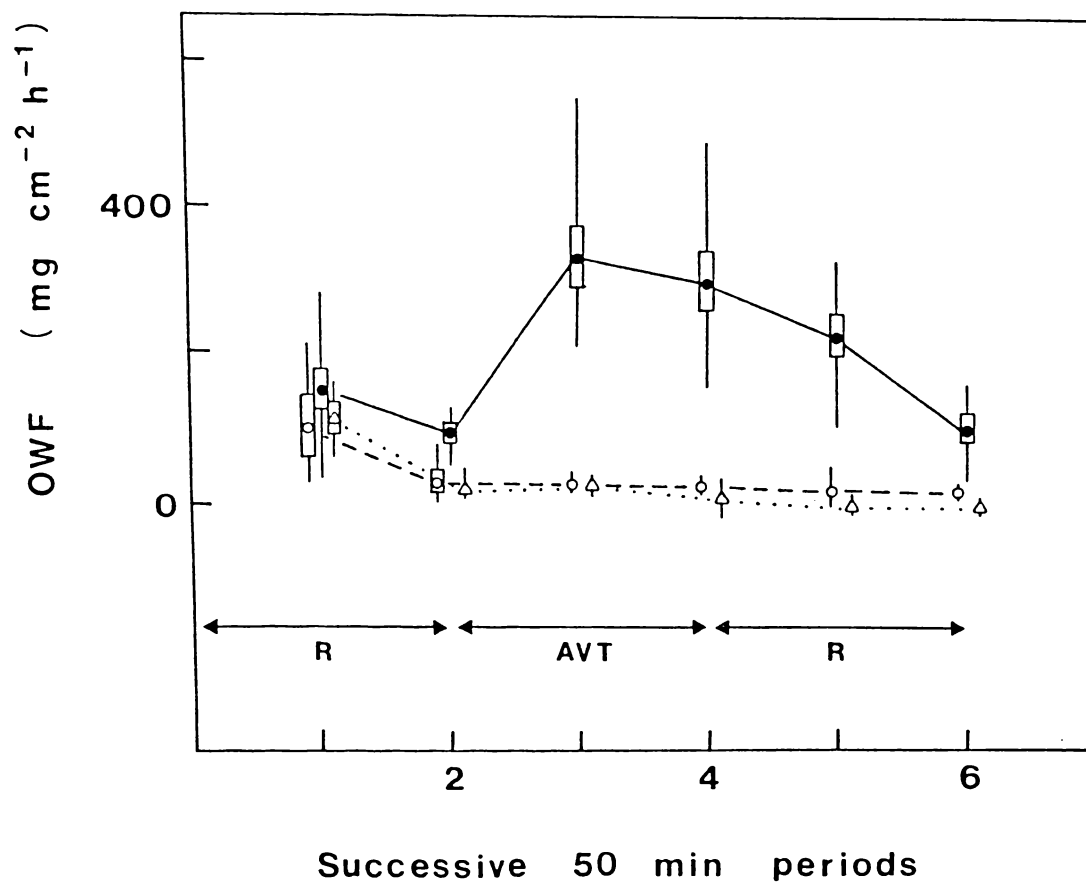


FIG. 5.6 Effect of AVT on osmotic water flow through skin of male *Litoria aurea*. AVT concentration was $3 \times 10^{-8} \text{ mol l}^{-1}$ during the third period and $3 \times 10^{-7} \text{ mol l}^{-1}$ during the fourth (for pelvic skin $n = 7$, pectoral $n = 4$, dorsal $n = 5$; other details as in FIG. 5.2).

TABLE 5.2 Effect of AVT on creatinine clearance of *Leiopelma* species
 ($\bar{x} \pm SE$; * = $P < 0.05$, ** = $P < 0.01$).

Species	Clearance ($\mu\text{l g}^{-1} \text{h}^{-1}$)		Significance
	Control	AVT ($7 \times 10^{-11} \text{ mol g}^{-1}$)	
<i>L. archeyi</i> n = 6	25 \pm 8	0	t = 3.014 *
<i>L. hamiltoni</i> n = 4	21 \pm 3	1 \pm 1	t = 6.366 **
<i>L. hochstetteri</i> n = 6	22 \pm 9	10 \pm 4	t = 1.102 0.4 > P > 0.2

TABLE 5.3 Weight increases of *Leiopelma* species during experiment examining the effect of AVT on creatinine clearance (see TABLE 5.2). Weights at end of control and AVT periods are given as a percentage of W_0 ($\bar{x} \pm SE$; ** = $P < 0.01$, *** = $P < 0.001$).

Species	W_0 (g)	Weight at end of control period (%)	Weight at end of AVT period (%)	Significance of increase following AVT treatment
<i>L. archeyi</i> n = 6	3.2 ± 0.1	100.5 ± 0.7	108.6 ± 1.5	t = 7.116 ***
<i>L. hamiltoni</i> n = 4	5.3 ± 0.3	100.7 ± 0.4	107.7 ± 1.2	t = 6.377 **
<i>L. hochstetteri</i> n = 6	4.8 ± 0.3	101.3 ± 0.2	102.6 ± 0.3	t = 4.496 **

TABLE 5.4 Effect of AVT on osmotic water flow (OWF) through isolated bladder lobes of *Leiopelma* species ($\bar{x} \pm SE$). *L. archeyi* and *L. hochstetteri* lobes came from animals used in Experiment 2 and *L. hamiltoni* lobes came from animals used in Experiment 1 (see text) (* = $P < 0.05$, ** = $P < 0.01$).

Species	Weight of animals ($\bar{x} \pm SE$)	Volume of isolated lobes (range in ml)	OWF (mg h^{-1})		Significance
			Control	AVT ($3 \times 10^{-8} \text{ mol l}^{-1}$)	
<i>L. archeyi</i>	3.3 ± 0.2 n = 3	0.10 - 0.15 n = 5	11.8 ± 3.9	18.4 ± 4.8	t = 4.634 **
<i>L. hamiltoni</i>	7.6 ± 0.4 n = 4	0.10 - 0.25 n = 7	22.4 ± 5.7	34.6 ± 9.6	t = 2.692 *
<i>L. hochstetteri</i>	4.8 ± 0.5 n = 6	0.05 - 0.10 n = 6	5.9 ± 1.8	12.5 ± 1.9	t = 4.556 **

demonstrated that the effect of AVT was both reversible and repeatable (Fig. 5.7). Absolute values for OWF (in mg h^{-1}) shown in Table 5.4 were smallest for *L. hochstetteri*, intermediate for *L. archeyi* and largest for *L. hamiltoni*; this is to some extent attributable to a direct relationship with bladder lobe volume (Table 5.4). In an attempt to compensate for this, OWF was recalculated on a SA basis (Table 5.5). Again, values for *L. hochstetteri* are lowest, perhaps a reflection of the apparently thicker, whiter and less distensible nature of the bladder tissue than in *L. archeyi* and *L. hamiltoni*. Values for *L. hamiltoni* may be exaggerated because SA was probably underestimated for these larger bladders, which tended to become sac-like rather than spherical when distended.

Experiment 4. Bladder capacity

Data collected from several sources indicate that relative to body weight, *L. hochstetteri* has the smallest bladder of the three species. For instance, when stretched over a glass rod prior to mounting, the isolated lobes from a 3.2 g, 31.6 mm SVL *L. hochstetteri* enclosed a bladder volume which, if both lobes of an intact bi-lobed bladder were filled with water, would be equivalent to 3% of W_0 . This was half that estimated for a similar-sized *L. archeyi* (6% for a frog of 3.3 g and 32.6 mm SVL). The maximum value calculated from four *L. hamiltoni* lobes was also 6%. Second, the volume of fluid displaced by mounted bladder lobes was smallest for *L. hochstetteri* despite coming from frogs of intermediate size (Table 5.4). Relative to body weight, the maximum volumes of mounted isolated bladder lobes (Table 5.4) are equivalent to a total bi-lobed bladder volume of 4% of W_0 for *L. hochstetteri*, 6% for *L. hamiltoni* and 8% for *L. archeyi*.

Both the above methods may, however, underestimate true bladder capacity, since some bladder tissue is inevitably left behind when lobes are removed and the degree to which intact bladders can be distended may be greater than that estimated *in vitro*. Measurements of voluntary bladder volume tend to support these reservations. In captivity, *L. archeyi* retained urine volumes of up to 12% of W_0 ($n = 74$), *L. hamiltoni* 8% ($n = 19$) and *L. hochstetteri* 4% ($n = 84$).

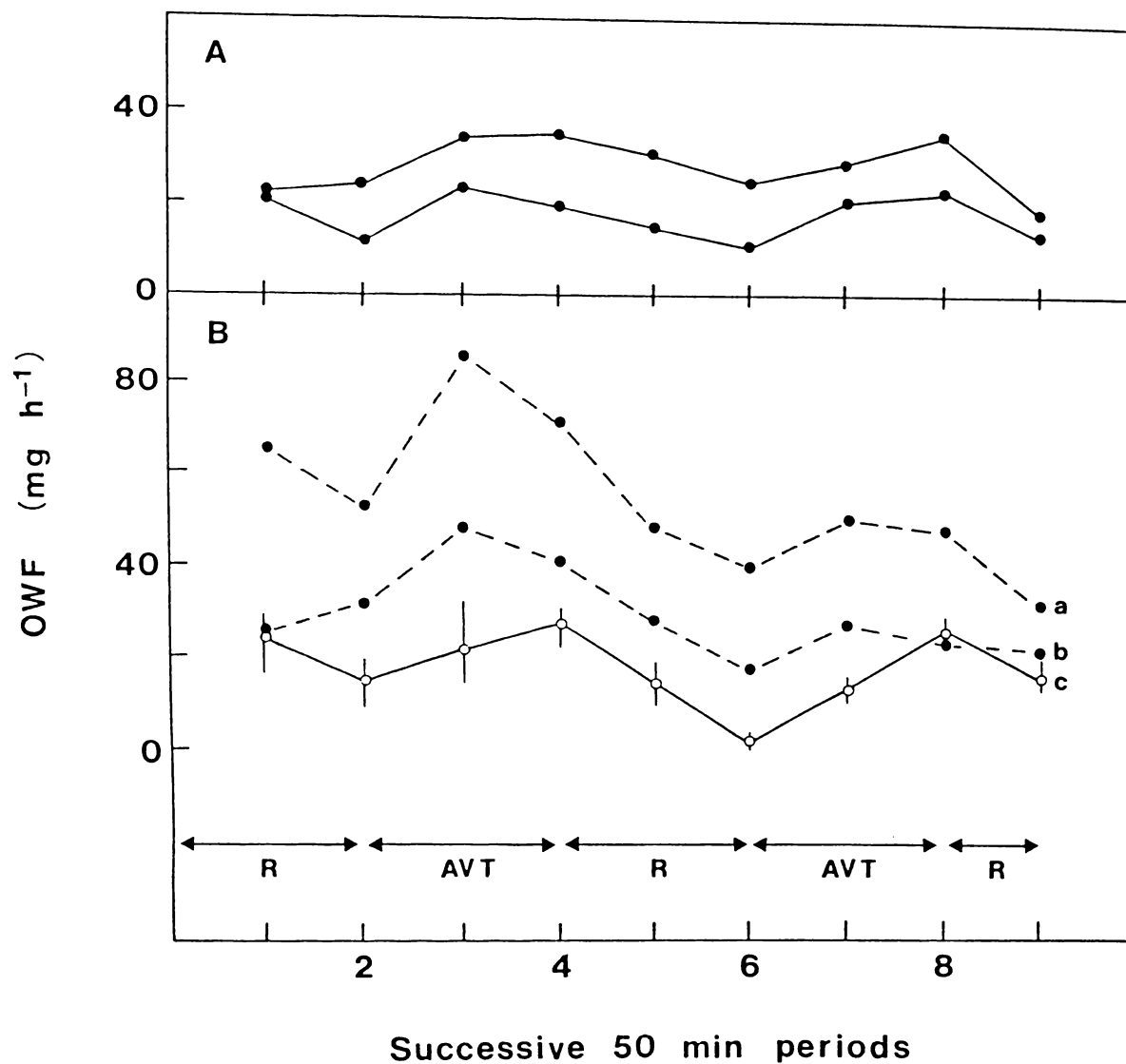


FIG. 5.7 Long-run effects of AVT on osmotic water flow through isolated bladder lobes of *L. archeyi* and *L. hamiltoni*. All lobes came from animals used in Experiment 1 (see text). A = individual lobes from a single female *L. archeyi* (lobe volumes 0.10 ml). B = lobes from female *L. hamiltoni* grouped according to volume: a and b = individual lobes of 0.25 and 0.20 ml respectively; c = five lobes of 0.10 - 0.15 ml (\bar{x} and range). AVT concentration was $3 \times 10^{-8} \text{ mol l}^{-1}$.

TABLE 5.5 Effect of AVT on osmotic water flow (OWF) per unit surface area through isolated bladder lobes of *Leiopelma* species. Values are calculated from data in TABLE 5.4, assuming that each preparation is spherical and correcting for the amount of surface area enclosed by the inside of the tube.

Species	OWF (mg cm ⁻² h ⁻¹)		Significance
	Control	AVT (3 × 10 ⁻⁸ mol l ⁻¹)	
<i>L. archeyi</i> n = 5	10.0 ± 2.7	16.0 ± 2.9	t = 6.253 **
<i>L. hamiltoni</i> n = 7	15.6 ± 2.6	23.6 ± 4.3	t = 3.015 *
<i>L. hochstetteri</i> n = 6	6.4 ± 2.1	14.4 ± 1.3	t = 3.750 **

DISCUSSION

Effect of AVT on cutaneous osmotic water flow

The results from Experiment 1 demonstrate that AVT increases OWF through ventral skin of *L. archeyi*, *L. hamiltoni* and *Litoria aurea*, but not of *L. hochstetteri*. The effect was localised to the ventral pelvic region of *L. archeyi* and *Litoria aurea*, but pectoral skin of *L. hamiltoni* also sometimes responded. Dorsal skin of all four species showed no response to AVT in these experiments.

Detailed comparisons of the results for *L. archeyi* and *L. hamiltoni* show that skin from the latter species was more responsive to AVT (compare Figs 5.2 and 5.3). First, although mean OWF through pelvic skin exposed to 3×10^{-8} mol l⁻¹ AVT was similar for both species (86 and 97 mg cm⁻² h⁻¹ for *L. hamiltoni* and *L. archeyi* respectively), the extent of the increase above the mean resting level was much greater for *L. hamiltoni* (7.8x) than for *L. archeyi* (2.4x). Second, pelvic skin of *L. hamiltoni* continued to increase dramatically in OWF when exposed to subsequent concentrations of AVT, whereas that of *L. archeyi* did not. Finally, some preparations of pectoral skin from *L. hamiltoni* also showed increased OWF when exposed to AVT whereas those from *L. archeyi* did not.

Differences in acclimation conditions mean that these differences in response may not be species-specific. *L. hamiltoni* were collected during a relatively dry period on Maud Island and were used within 6 wk of capture. *In vivo* experiments with the same animals suggested that they were highly responsive to AVT (Experiment 2 of Chapter 4). *L. archeyi*, however, had been collected 1-2 y previously, and mesic conditions in captivity may have reduced their responsiveness to AVT. Season (Parsons *et al.* 1978), length of time in captivity (Baldwin 1974) and wetness of conditions in captivity (Christensen 1975) are all known to affect cutaneous responses of anurans to AVT *in vitro*.

As mentioned above, pelvic skin of *L. hamiltoni* exposed to 3×10^{-8} mol l⁻¹ AVT continued to increase in permeability when exposed to successively higher concentrations of AVT (Fig. 5.3). Trials with other concentrations of AVT suggest that this phenomenon was time-related rather than dose-related (Fig. 5.4). For example, when exposed first to 3×10^{-7} mol l⁻¹ and then to 3×10^{-6} mol l⁻¹ AVT, a

single preparation showed successive increases in OWF virtually identical to those seen in skins which were exposed first to 3×10^{-8} mol l⁻¹ and then 3×10^{-7} mol l⁻¹ (Fig. 5.3). Furthermore, preparations which were exposed to a lower concentration (3×10^{-11} mol l⁻¹) in both periods also showed an increase in OWF during the second period (Fig. 5.4).

The observation that 3×10^{-11} mol l⁻¹ AVT led to increased OWF through pelvic skin of *L. hamiltoni* is significant, since this concentration is of the same order of magnitude as levels of endogenous AVT measured by RIA in serum of *Rana ridibunda* (Nouwen and Kühn 1983). The response is also notable when compared with *in vitro* dose-response studies for other anurans. Johnsen and Nielsen (1984) presented data showing that 7.8×10^{-11} mol l⁻¹ AVT had no effect on water flux through a single skin preparation from *Rana esculenta*, and that not until the concentration was increased to 1.3×10^{-9} mol l⁻¹ did OWF increase. Christensen (1974b) reported that the threshold for stimulation of OWF in isolated unperfused pelvic skin of *Bufo bufo* was between 10^{-10} and 10^{-9} mol l⁻¹ AVT. These comparisons suggest that the threshold for stimulation of OWF through pelvic skin of *L. hamiltoni* is lower than that for either *R. esculenta* or *B. bufo* and approaches that of the bladder of *B. marinus* (10^{-12} - 10^{-11} mol l⁻¹ AVT - Eggena *et al.* 1968; Bentley 1969a), a membrane traditionally regarded as more sensitive than anuran skin (Bentley 1966b).

One explanation for the apparent difference in sensitivity between the skin of *L. hamiltoni* and that of *R. esculenta* or *B. bufo* may lie in the freshly caught condition of *L. hamiltoni*. An additional reason may involve the time required for development of AVT-induced increased OWF. In the present study, pelvic skin of *L. hamiltoni* exposed to 3×10^{-11} mol l⁻¹ AVT did not increase markedly in OWF until the second 50 min period in hormone. However, in both *R. esculenta* (Johnsen and Nielsen 1984) and *B. bufo* (Christensen 1974b), OWF was measured over a period of about 30-60 min after addition of AVT to the medium, and it is possible that this interval was simply too short to detect responses at very low doses. The period which elapses between exposure of skin preparations to neurohypophysial peptides (NHPs) and increased OWF presumably reflects the time required for peptide in the medium to diffuse through the dermis before reaching the epithelial receptors. When isolated epithelium from the skin of *R. esculenta* (i.e., with dermis removed) is exposed to oxytocin (OT), the time-lag

to increased OWF is reduced (Rajerison *et al.* 1972).

The failure to demonstrate an increase in OWF through the skin of *L. hochstetteri*, even after long periods in AVT and at pharmacological concentrations, confirms results from Chapter 4 indicating that the skin of this species is unresponsive to the hydroosmotic effects of the hormone. Cameron (1974) also failed to demonstrate an increase in OWF through isolated ventral skin of *L. hochstetteri* in the presence of AVP or OT. Although seasonal refractoriness to the cutaneous hydroosmotic effect of AVT has been reported for at least one anuran (*Scaphiopus couchi*; Hillyard 1976) this is unlikely to be the explanation for the absence of response in *L. hochstetteri*. *S. couchi* is a fossorial desert species and significant cutaneous responses to AVT were found by Hillyard only on the day following emergence from the burrow (however, a more recent study of rehydration rates suggests a response is also evident at other times; Jones 1978). Since emergence is triggered by ephemeral rain, the response observed by Hillyard (1976) was considered appropriate to the availability of water. In contrast, *L. hochstetteri* is usually found year-round in a streamside habitat where there are no major seasonal variations in water availability (Chapter 2). There is therefore no reason to suppose that cutaneous responses to AVT would be apparent at any other time of year.

Resting cutaneous OWF and responses to NHPs have been examined in many anurans. Representative values are listed in Tables 5.6 (for pelvic, ventral or abdominal skin), 5.7 (pectoral skin) and 5.8 (dorsal skin). Four generalisations can be drawn from these data: (i) resting OWF through pelvic skin tends to be lower for aquatic or semiaquatic species ($\leq 28 \text{ mg cm}^{-2} \text{ h}^{-1}$) than for terrestrial or arboreal species (up to $78 \text{ mg cm}^{-2} \text{ h}^{-1}$) (Table 5.6); (ii) NHP-stimulated OWF through pelvic skin of terrestrial or arboreal species is higher than that for semiaquatic or aquatic species, which may show little or no response (Table 5.6); (iii) resting OWF through pectoral skin is similar to or lower than that for pelvic skin of the same species and usually shows little or no hydroosmotic response (Table 5.7); (iv) dorsal skin has low resting OWF and usually shows no hydroosmotic response (Table 5.8).

The results for the three *Leiopelma* species are consistent with these generalisations. Pelvic skin of *L. hochstetteri* has low resting OWF

TABLE 5.6 Resting and stimulated osmotic water flow through isolated pelvic (Pv), ventral (V) or abdominal (Abd) skin of anurans. Stimulated values are for exposure to AVT or other NHPs. Osmotic gradients were 180-236 mOsm l⁻¹ and temperatures, where stated, were 15-25°C (~ indicates value read from graph; - = no data; habitat codes as in TABLE 4.6).

Species	Habitat	Hormone	Hormone concentration (AVT conc. in mol l ⁻¹)	Skin region	Osmotic water flow (mg cm ⁻² h ⁻¹)		Reference
					resting	stimulated	
<i>Xenopus laevis</i>	A	-	-	Pv	4	-	Bentley and Main (1972a)
		-	-	Pv	~3	-	Christensen (1974a)
<i>Leiopelma hochstetteri</i>	SA	AVT	4 × 10 ⁻⁸	-	8	9	Bentley (1969b)
		AVP	10 mU ml ⁻¹	V	3	4	Cameron (1974)
<i>Rana catesbeiana</i>	SA	OT	10 mU ml ⁻¹	V	3	4	Cameron (1974)
		AVT	4 × 10 ⁻⁸	-	13	61	Bentley (1969b)
<i>R. esculenta</i>	SA	AVT	4 × 10 ⁻⁸	-	10	43	Johnsen and Nielsen (1984)
		OT	4 mU ml ⁻¹	-	9	26	Maetz (1963)
<i>R. pipiens</i>	SA	-	-	V	28	-	Brown and Brown (1973)
		AVT	4 × 10 ⁻⁸	Pv	7	29	Bentley and Main (1972a)
		AVP	500 mU ml ⁻¹	Abd	5	17	Parsons <i>et al.</i> (1978)
<i>Litoria raniformis</i> ¹	SA	AVP	500 mU ml ⁻¹	Abd	5	6	Parsons <i>et al.</i> (1978)
		OT	10 mU ml ⁻¹	V	7	50	Cameron (1974)
<i>Neobatrachus pelobatoides</i>	F	AVT	4 × 10 ⁻⁸	Pv	9	23	Bentley and Main (1972a)
<i>Crinia georgiana</i>	T	AVT	4 × 10 ⁻⁸	V	3	14	Bentley and Main (1972b)
<i>Bufo boreas</i>	T	AVT	(1 × 10 ⁻⁶) ²	Pv	27	114	Kent and McClanahan (1980)
		AVP	101 mU ml ⁻¹	Pv	62	171	Baldwin (1974)
<i>B. bufo</i> kept in water	T	AVT	5 × 10 ⁻⁸	Pv	18	46	Christensen (1975)
		AVT	5 × 10 ⁻⁸	Pv	55	113	Christensen (1975)
kept on dry filter paper with access to water							

TABLE 5.6 (cont.)

Species	Habitat	Hormone	Hormone concentration (AVT conc. in mol l ⁻¹)	Skin region	Osmotic water flow (mg cm ⁻² h ⁻¹)		Reference	
					resting	stimulated		
<i>B. marinus</i>	T	AVT	4 × 10 ⁻⁸	-	28	85	Bentley (1969b)	
		AVT	4 × 10 ⁻⁸	Pv	37	109	Bentley and Main (1972a)	
		AVP	100 mU ml ⁻¹	Pv	34	124	Cameron (1980)	
		"low responders"	AVP	100 mU ml ⁻¹	Abd	31	42	De Sousa and Grosso (1982)
		"high responders"	AVP	100 mU ml ⁻¹	Abd	23	109	De Sousa and Grosso (1982)
		<i>B. punctatus</i>	T	AVP	101 mU ml ⁻¹	Pv	15	208
<i>B. woodhousei</i>	T	AVP	36 mU ml ⁻¹	Pv	~40	~70	Marrero and Hillyard (1985)	
<i>Litoria ewingi</i>	T/ARB	OT	10 mU ml ⁻¹	V	21	135	Cameron (1974)	
<i>Litoria moorei</i>	ARB	AVT	4 × 10 ⁻⁸	Pv	26	50	Bentley and Main (1972a)	
<i>Hyla arenicolor</i>	ARB	AVT	4 × 10 ⁻⁸	V	18	164	Yorio and Bentley (1977)	
<i>H. squirella</i>	ARB	AVT	4 × 10 ⁻⁸	V	35	110	Yorio and Bentley (1977)	
<i>H. femoralis</i>	ARB	AVT	4 × 10 ⁻⁸	V	20	254	Yorio and Bentley (1977)	
<i>Agalychnis dacnicolor</i>	ARB	AVT	4 × 10 ⁻⁸	V	78	182	Yorio and Bentley (1977)	
<i>Leiopelma hochstetteri</i>	SA	AVT	3 × 10 ⁻⁸	Pv	16	5	Present study	
<i>Leiopelma archeyi</i>	T	AVT	3 × 10 ⁻⁸	Pv	41	97	Present study	
<i>Leiopelma hamiltoni</i>	T	AVT	3 × 10 ⁻⁸	Pv	11	86	Present study	
<i>Litoria aurea</i>	SA	AVT	3 × 10 ⁻⁸	Pv	96	332	Present study	

Notes:

1. Described as *Litoria aurea* by Cameron (1974), but now known as the closely related *L. raniformis* (Dr M.C. Cameron, pers. comm.).
2. Concentration in mol l⁻¹ estimated from the stated concentration of 2.5 µg ml⁻¹ and activity of 85 U mg⁻¹.

TABLE 5.7 Resting and stimulated osmotic water flow through isolated pectoral skin of anurans (details as in TABLE 5.6).

Species	Habitat	Hormone	Hormone concentration (AVT conc. in mol l ⁻¹)	Osmotic water flow (mg cm ⁻² h ⁻¹)		Reference
				resting	stimulated	
<i>Xenopus laevis</i>	A	-	-	4	-	Bentley and Main (1972a)
		-	-	~3	-	Christensen (1974a)
<i>Rana esculenta</i>	SA	-	-	~6	-	Christensen (1974a)
<i>R. pipiens</i>	SA	AVT	4 × 10 ⁻⁸	6	7	Bentley and Main (1972a)
<i>R. temporaria</i>	SA/T	-	-	~6	-	Christensen (1974a)
<i>R. arvalis</i>	SA/T	-	-	~6	-	Christensen (1974a)
<i>Bufo boreas</i>	T	AVP	101 mU ml ⁻¹	41	34	Baldwin (1974)
<i>B. bufo</i> kept in water	T	AVT	5 × 10 ⁻⁸	10	26	Christensen (1975)
<i>B. bufo</i> kept with access to water	T	AVT	5 × 10 ⁻⁸	18	25	Christensen (1975)
<i>B. marinus</i>	T	AVT	4 × 10 ⁻⁸	24	59	Bentley and Main (1972a)
		AVP	100 mU ml ⁻¹	35	68	Cameron (1980)
<i>B. punctatus</i>	T	AVP	101 mU ml ⁻¹	10	7	Baldwin (1974)
<i>B. woodhousei</i>	T	AVP	36 mU ml ⁻¹	~28	~30	Marrero and Hillyard (1985)
<i>Litoria moorei</i>	ARB	AVT	4 × 10 ⁻⁸	11	13	Bentley and Main (1972a)
<i>Leiopelma hochstetteri</i>	SA	AVT	3 × 10 ⁻⁸	23	8	Present study
<i>Leiopelma archeyi</i>	T	AVT	3 × 10 ⁻⁸	18	21	Present study
<i>Leiopelma hamiltoni</i>	T	AVT	3 × 10 ⁻⁸	17	20	Present study
<i>Litoria aurea</i>	SA	AVT	3 × 10 ⁻⁸	30	30	Present study

TABLE 5.8 Resting and stimulated osmotic water flow through isolated dorsal skin of anurans (temperatures, where stated, were 15-22°C; other details as in TABLE 5.6).

Species	Habitat	Hormone	Hormone concentration (AVT conc. in mol l ⁻¹)	Osmotic water flow (mg cm ⁻² h ⁻¹)		Reference
				resting	stimulated	
<i>Xenopus laevis</i>	A	-	-	3	-	Bentley and Main (1972a)
<i>Leiopelma hochstetteri</i>	SA	AVP	10 mU ml ⁻¹	3	3	Cameron (1974)
		OT	10 mU ml ⁻¹	3	4	Cameron (1974)
<i>Rana pipiens</i>	SA	AVT	4 × 10 ⁻⁸	5	11	Bentley and Main (1972a)
<i>Litoria raniformis</i> ¹	SA	OT	10 mU ml ⁻¹	3	3	Cameron (1974)
<i>Neobatrachus pelobatoides</i>	F	AVT	4 × 10 ⁻⁸	6	30	Bentley and Main (1972a)
<i>Bufo marinus</i>	T	AVT	4 × 10 ⁻⁸	21	28	Bentley and Main (1972a)
		AVP	100 mU ml ⁻¹	16	21	Cameron (1980)
<i>Litoria ewingi</i>	T/ARB	OT	10 mU ml ⁻¹	4	4	Cameron (1974)
<i>Litoria moorei</i>	ARB	AVT	4 × 10 ⁻⁸	7	3	Bentley and Main (1972a)
<i>Hyla arenicolor</i>	ARB	AVT	4 × 10 ⁻⁸	0	1	Yorio and Bentley (1977)
<i>H. squirrella</i>	ARB	AVT	4 × 10 ⁻⁸	6	4	Yorio and Bentley (1977)
<i>H. femoralis</i>	ARB	AVT	4 × 10 ⁻⁸	1	3	Yorio and Bentley (1977)
<i>Agalychnis dacnicolor</i>	ARB	AVT	4 × 10 ⁻⁸	3	2	Yorio and Bentley (1977)
<i>Leiopelma hochstetteri</i>	SA	AVT	3 × 10 ⁻⁸	17	20	Present study
<i>Leiopelma archeyi</i>	T	AVT	3 × 10 ⁻⁸	31	17	Present study
<i>Leiopelma hamiltoni</i>	T	AVT	3 × 10 ⁻⁸	9	17	Present study
<i>Litoria aurea</i>	SA	AVT	3 × 10 ⁻⁸	21	26	Present study

[16 mg cm⁻² h⁻¹ in the present study; 3 mg cm⁻² h⁻¹ in Cameron (1974) where larger pieces of skin with correspondingly better reproducibility in weighing were used], and shows no hydroosmotic response to AVT. Pelvic skin from *L. archeyi* and *L. hamiltoni* increases markedly in OWF when exposed to AVT, and stimulated values in 3 × 10⁻⁸ mol l⁻¹ AVT are within the ranges reported for other terrestrial or arboreal species (from 14 mg cm⁻² h⁻¹ for *Crinia georgiana*, to 254 mg cm⁻² h⁻¹ for *Hyla femoralis*; see Table 5.6 for references).

Pectoral skin of all three *Leiopelma* species did not increase significantly in OWF when exposed to 3 × 10⁻⁸ mol l⁻¹ AVT for 50 min (Table 5.7), although some preparations from *L. hamiltoni* responded after a longer interval and an increase in concentration. Small increases in OWF have been observed in pectoral skin of *Bufo marinus* exposed to AVT (Table 5.7).

Dorsal skin of all three *Leiopelma* species and most other anurans fails to respond to AVT (Table 5.8). This is clearly appropriate for terrestrial or arboreal species, since only the ventral and particularly the ventral pelvic skin would regularly come into contact with moist surfaces from which water could be absorbed. However, dorsal skin of *Neobatrachus pelobatoides* does show a significant increase in OWF in the presence of AVT (Bentley and Main 1972a). *N. pelobatoides* is a fossorial species where both dorsal and ventral skin can potentially come in contact with moist soil in the burrow.

Results obtained here for the hylid frog *Litoria aurea* initially appear inconsistent with its description as a semiaquatic species. Both resting and AVT-stimulated rates of OWF through pelvic skin are higher than reported for other anurans, including terrestrial and arboreal species (Table 5.6). Possible explanations for this are that: (i) *Litoria aurea* is unusually aquatic for a hylid and the strong hydroosmotic response may be a reflection of a previously more arboreal existence; (ii) *Litoria aurea* is a basking species (Robb 1980; Bell 1982a) and as such may be subject to high rates of EWL. Anurans which bask must continually replenish the dorsal integument with water to prevent it desiccating (Lillywhite 1971); in many species, including *Litoria aurea*, this is achieved by the secretion of copious mucus (Lillywhite and Licht 1975). The ability to increase water uptake through the ventral skin to compensate for mucus

production may be an important feature of water balance in basking species such as *Litoria aurea*.

Effect of AVT on glomerular filtration rate

Control values for GFR are similar for the three *Leiopelma* species (21-25 $\mu\text{l g}^{-1} \text{h}^{-1}$) and within the ranges reported for other amphibians (Table 5.9). As indicated in Table 5.9, GFR may vary widely, both between seasons and between species. For instance, GFR in the salamandrid urodeles *Notophthalmus viridescens* and *Taricha torosa* is characteristically as much as an order of magnitude higher than in many amphibians (Brown and Brown 1977, 1980). Shoemaker and Nagy (1977) considered GFRs of 20-50 $\mu\text{l g}^{-1} \text{h}^{-1}$ to be typical of most amphibians in water, and the values obtained here for the three *Leiopelma* species are clearly consistent with this.

Treatment with AVT reduced C_{CR} to 0 and 1 $\mu\text{l g}^{-1} \text{h}^{-1}$ in *L. archeyi* and *L. hamiltoni* respectively, implying a near or complete shutdown of urine production. It is possible that more sensitive methods of measuring GFR (e.g., using radioactive inulin as a marker) might detect very low levels of filtration following AVT treatment which were not detected here. Neurohypophysial peptides have been shown to reduce GFR in many amphibians, including *R. pipiens* (Sawyer 1951a), *R. catesbeiana* (Sawyer 1957a), *B. marinus* (Sawyer 1957b), *R. esculenta* (Jard and Morel 1963), *Necturus maculosus* (Garland *et al.* 1975), *Notophthalmus viridescens* (Brown and Brown 1977) and *T. torosa* (Brown and Brown 1980). Species which show similar responses to those seen here for *L. archeyi* and *L. hamiltoni* include *B. marinus icterus*, where GFR fell from 14 to 3 $\mu\text{l g}^{-1} \text{h}^{-1}$ following treatment with AVP (calculated from the data of Cirne *et al.* 1981), and larval *Ambystoma tigrinum*, where the reduction was from 25 to 4 $\mu\text{l g}^{-1} \text{h}^{-1}$ (Alvarado and Johnson 1965).

The effect of AVT on GFR of *L. hochstetteri* was less clear than for *L. archeyi* and *L. hamiltoni*. Although mean C_{CR} dropped in four animals following AVT treatment (by 84%), the remaining two animals showed an increase in clearance. McBean and Goldstein (1970) also failed to demonstrate a consistent effect on GFR following injection of AVT into *Xenopus laevis* (which, like *L. hochstetteri*, lacks a cutaneous response), but in later studies where AVT was infused intravenously, a significant reduction in GFR was obtained (Henderson

TABLE 5.9 Glomerular filtration rates (GFR) of hydrated amphibians in tap water or distilled water (~ indicates value read from graph; - = no data).

Species	T (°C)	GFR ($\mu\text{l g}^{-1} \text{h}^{-1}$)	Notes	Reference
Anurans:				
<i>Xenopus laevis</i>	24-26	30	cloaca cannulated	McBean and Goldstein (1970)
<i>Rana clamitans</i>	20-25	34		Schmidt-Nielsen and Forster (1954)
<i>R. catesbeiana</i>	-	8-36		Forster (1938)
	-	~20	ureters cannulated	Sawyer (1957a); Uranga and Sawyer (1960)
<i>R. esculenta</i>	-	47-53	ureters cannulated	Jard and Morel (1963), Jard (1966)
<i>R. cancrivora</i>	27-31	~60	cloaca cannulated	Schmidt-Nielsen and Lee (1962)
<i>Bufo arenarum</i>	-	135	summer, cloaca cannulated	Uranga (1958)
	18-22	24	winter, cloaca cannulated	Uranga (1958)
<i>B. boreas</i>	30	63		Shoemaker and Bickler (1979)
<i>B. marinus</i>	-	~60	ureters cannulated	Sawyer (1957b)
	-	90		Middler <i>et al.</i> (1968)
	-	78	ureters cannulated	Jackson and Henderson (1976)
<i>Phyllomedusa sauvagei</i>	30	92		Shoemaker and Bickler (1979)
<i>Leiopelma hochstetteri</i>	16	22		Present study
<i>Leiopelma archeyi</i>	16	25		Present study
<i>Leiopelma hamiltoni</i>	16	21		Present study
Urodeles:				
<i>Dicamptodon ensatus</i>	15	17	spring-autumn	Stiffler and Alvarado (1974)
<i>Ambystoma gracile</i> larvae	15	11	spring-autumn	Stiffler and Alvarado (1974)
	15	6	winter	Stiffler and Alvarado (1974)
<i>A. tigrinum</i> larvae	15-17	25		Alvarado and Johnson (1965)
	23	13		Stiffler <i>et al.</i> (1982)
	23	34	summer	Stiffler <i>et al.</i> (1984)
	23	11	autumn	Stiffler <i>et al.</i> (1984)
<i>Notophthalmus viridescens</i> aquatic phase	20-22	155		Brown and Brown (1977)
<i>Taricha torosa</i> terrestrial phase	18	340		Brown and Brown (1980)

et al. 1972). In *L. hochstetteri*, it seems likely that AVT does reduce GFR, but the effect may have been masked in two individuals in the experiment described here because of incomplete drainage of bladder urine at the end of the control period. This would lead to an underestimate of control GFR and an overestimate of GFR following AVT treatment. Knowing whether or not all bladder urine has been removed is probably the major limitation of the "free-flow" method (Stiffler and Alvarado 1974) used here for determining GFR, and additional experiments are therefore required to confirm the antidiuretic effects of AVT in *L. hochstetteri*. An alternative procedure would be to cannulate the ureters as others have done for *R. catesbeiana* and *B. marinus* (see Table 5.9); however, this would be a technically difficult task in such a small frog as *L. hochstetteri*, and according to Bakker and Bradshaw (1977) the procedure may in itself produce a disturbance of water balance.

Effect of AVT on osmotic water flow through the bladder

Resting OWF through bladder tissue is fairly similar for the three *Leiopelma* species (6-15 mg cm⁻² h⁻¹) and comparable to that of the toad *B. marinus* (7-11 mg cm⁻² h⁻¹; Bentley 1966b). Bladder lobes of all three *Leiopelma* species show increased OWF in the presence of AVT but the magnitude of the effect is small (an increase of 1.5x for *L. hamiltoni* to 2.1x for *L. hochstetteri*; Table 5.4). In contrast, increases in OWF of 16-100x have repeatedly been demonstrated for bladder lobes of *B. marinus* (e.g., Bentley 1958, 1971b; Eggena *et al.* 1968; Eggena 1972), and similar increases (from 10-100x) occur in bladders of *B. bufo* and *R. esculenta* (Maetz 1963; Bentley 1964; Takamitsu *et al.* 1980). In terms of SA, OWF through the bladder of *B. marinus* increased from a resting value of essentially zero to about 240 mg cm⁻² h⁻¹ in the presence of AVP (De Sousa and Grosso 1982) and in *R. catesbeiana* from 4 to 96 mg cm⁻² h⁻¹ in the presence of Pituitrin (Sawyer 1960). It is unlikely that the small responses of *Leiopelma* bladders were caused by an insufficiently high concentration of AVT, since the bladder of *B. marinus* responds at a concentration more than 1000x as dilute as that used here (i.e., 10⁻¹²-10⁻¹¹ mol l⁻¹; Eggena *et al.* 1968). In additional experiments, three lobes of *L. archeyi* were exposed to a higher AVT concentration (3 × 10⁻⁷ mol l⁻¹), but no increase in OWF greater than 2.7x was detected.

The small bladder responses of the *Leiopelma* species are comparable to

those observed for several urodeles. Stimulated values for OWF in the presence of NHPs were 1.4× the resting value for *Taricha torosa* (Brown and Brown 1980), 2.3× for *Notophthalmus viridescens* (Brown and Brown 1977), 3.7× for *Aneides lugubris* (Hillman 1974) and 4.7× for *Salamandra maculosa* (Bentley and Heller 1965); all these responses were considered statistically significant. However, the bladder of *X. laevis* apparently lacks a hydroosmotic response (Bentley 1974) as do those of the urodeles *Ambystoma tigrinum* (larvae and adults) and *Triturus* species (either *T. alpestris* or *T. cristatus*; Bentley and Heller 1964), *Necturus maculosus* (Bentley 1971b), and *Siren lacertina* and *Amphiuma means* (Bentley 1973).

The small bladder capacities recorded for the three *Leiopelma* species (4-12% of W_0) are consistent with their small hydroosmotic responses. Bladder capacities reported for other amphibians range from 1% of W_0 in *X. laevis* to 50% or more in arid-adapted anurans, with values of 21-51% being common for terrestrial or arboreal anurans and terrestrial urodeles (Table 5.10). The maximum value of 12% recorded for *L. archeyi* is similar to that of the aquatic frog *Rana clamitans* (Schmid 1969). The lower value of 8% for *L. hamiltoni* probably reflects the relatively small number (19) of voluntary bladder volumes measured for this species. *L. hochstetteri* has the smallest bladder capacity, and the maximum value of 4% recorded here is comparable to those for aquatic and semiaquatic urodeles.

Relative contributions of the skin, the kidneys and the bladder to the water balance response

L. archeyi and *L. hamiltoni* showed similar responses to AVT in all aspects examined here and can be considered together in the following discussion. In both these species, AVT has potent water-conserving effects on the skin and the kidney, increasing OWF through pelvic skin at least 2.4× and essentially halting the formation of primary urine at the kidney. However, the bladder is relatively small and shows only a small hydroosmotic response. These observations suggest that the bladder plays a relatively unimportant role in the water balance responses of these two frogs.

This conclusion is strengthened when the effects of AVT on GFR and on cutaneous OWF *in vitro* seen here are compared with the rate of water retention observed following AVT treatment *in vivo* (Chapter 4) and the

TABLE 5.10 Maximum bladder capacities of amphibians as a percentage of W_0 (* indicates that values were means of the five largest capacities observed; habitat codes as in TABLE 4.6).

Species	Habitat	Bladder capacity (%)	Reference
Anurans:			
<i>Xenopus laevis</i>	A	1	Bentley (1966a)
<i>Rana clamitans</i>	A	15	Claussen (1974)
<i>Bufo fowleri</i>	T	21	Claussen (1974)
<i>B. americanus</i>	T	22	Schmid (1969)
<i>Bufo marinus</i>	T	25	Shoemaker (1964)
<i>Litoria leseuri</i>	ARB	30	Main and Bentley (1964)
<i>Litoria moorei</i>	ARB	30	Main and Bentley (1964)
<i>Pyxicephalus adspersus</i>	F	32	Loveridge and Withers (1981)
<i>B. cognatus</i>	T/F	45	Ruibal (1962)
<i>Notaden nichollsi</i>	F	50	Main and Bentley (1964)
<i>Neobatrachus wilsmorei</i>	F	50	Main and Bentley (1964)
<i>Cyclorana platycephalus</i>	F	50	Main and Bentley (1964)
<i>Leiopelma hochstetteri</i>	SA	4	Present study
<i>Leiopelma hamiltoni</i>	T	8	Present study
<i>Leiopelma archeyi</i>	T	12	Present study
Urodeles:			
<i>Necturus maculosus</i>	A	5	Bentley and Heller (1964)
<i>Desmognathus monticolus</i>	SA	2*	Spight (1967a)
<i>D. fuscus</i>	SA	2*	Spight (1967a)
<i>Triturus cristatus</i>	SA	2	Bentley and Heller (1964)
<i>Notophthalmus viridescens</i>	aquatic phase newts	20	Brown and Brown (1977)
<i>Plethodon jordani</i>	T	6*	Spight (1967a)
<i>Ambystoma opacum</i>	T	12*	Spight (1967a)
<i>A. tigrinum</i>	T	20	Bentley and Heller (1964)
<i>Salamandra maculosa</i>	T	34	Bentley and Heller (1965)
<i>Aneides lugubris</i>	T	50	Hillman (1974)
<i>Taricha torosa</i>	T	51	Brown and Brown (1980)

rate of rehydration from shallow water (Chapter 3). *In vitro*, the maximum OWF through "ventral" skin in response to AVT treatment averaged $67 \text{ mg cm}^{-2} \text{ h}^{-1}$ for *L. archeyi* and $159 \text{ mg cm}^{-2} \text{ h}^{-1}$ for *L. hamiltoni* (calculated as the average of the mean pelvic and pectoral responses to $3 \times 10^{-6} \text{ mol l}^{-1}$ AVT). These are similar to or higher than maximum rates of water uptake through ventral skin after AVT treatment *in vivo* ($86 \text{ mg cm}^{-2} \text{ h}^{-1}$ for *L. archeyi* and $69 \text{ mg cm}^{-2} \text{ h}^{-1}$ for *L. hamiltoni*) and during rehydration ($68 \text{ mg cm}^{-2} \text{ h}^{-1}$ for *L. archeyi* and $78 \text{ mg cm}^{-2} \text{ h}^{-1}$ for *L. hamiltoni*). Thus, the effect of AVT on OWF through the skin *in vitro* is adequate to account for the SA-specific rate of water uptake observed during the water balance response *in vivo* (either following AVT treatment or during rehydration).

Calculations made on a weight-specific basis provide further support for the apparent unimportance of the bladder in the water balance response. In *in vivo* AVT experiments, the bladder was drained prior to treatment and urine production subsequently declined, so that bladders are unlikely to have been full. Nevertheless, one can estimate the amount of water retention that reabsorption from full bladders would contribute, using the *in vitro* values for stimulated OWF obtained in the present chapter. In *L. archeyi*, the mean stimulated OWF was 18.4 mg h^{-1} for isolated lobes from frogs of mean weight 3.3 g (Table 5.4), equivalent to a reabsorption rate of $11 \text{ mg g}^{-1} \text{ h}^{-1}$ for a frog with an intact bi-lobed bladder. The calculated value for *L. hamiltoni* is similar ($9 \text{ mg g}^{-1} \text{ h}^{-1}$). This is slightly less than half that contributed by the reduction in GFR occurring over the first hour following AVT treatment ($25 \mu\text{l g}^{-1} \text{ h}^{-1} \equiv 25 \text{ mg g}^{-1} \text{ h}^{-1}$ for *L. archeyi*, 20 for *L. hamiltoni*), and considerably less than the stimulated rate of cutaneous water uptake observed *in vivo* ($93 \text{ mg g}^{-1} \text{ h}^{-1}$ from Experiment 1 of Chapter 4 for *L. archeyi*, $52 \text{ mg g}^{-1} \text{ h}^{-1}$ for *L. hamiltoni*).

Similar calculations can be made for *L. hochstetteri*. In this species, cutaneous OWF *in vitro* is unchanged by AVT treatment and averages $14 \text{ mg cm}^{-2} \text{ h}^{-1}$ for "ventral" skin in $3 \times 10^{-6} \text{ mol l}^{-1}$ AVT. This compares favourably to the rate of water uptake observed following AVT treatment *in vivo* ($14 \text{ mg cm}^{-2} \text{ h}^{-1}$, Chapter 4) and with that occurring during rehydration ($10 \text{ mg cm}^{-2} \text{ h}^{-1}$, Chapter 3). What then accounts for the net weight gain of about $13 \text{ mg g}^{-1} \text{ h}^{-1}$ which is observed during rehydration and following AVT treatment *in vivo*?

Results from this chapter indicate that the reduction in mean GFR produced by AVT treatment is sufficient to account for this, despite the fact that the reduction was not statistically significant. Treatment with AVT reduced mean GFR of the six animals treated from 22 to $10 \mu\text{l g}^{-1} \text{h}^{-1}$, a saving of $12 \mu\text{l g}^{-1} \text{h}^{-1}$. Assuming no change in the amount of water reabsorbed by the renal tubules following AVT treatment, this is equivalent to a weight gain of $12 \text{ mg g}^{-1} \text{h}^{-1}$. Bladder water reabsorption may also contribute to the water balance response of this species, but the small hydroosmotic response seen *in vitro* (Table 5.4) suggests that this is unlikely to be the sole effect involved. If both bladder lobes were full, the *in vitro* rate of reabsorption following AVT treatment could account for an increase in body weight of about $5 \text{ mg g}^{-1} \text{h}^{-1}$ (calculated from an AVT-stimulated reabsorption rate of 12.5 mg h^{-1} per lobe for a frog of 4.8 g; Table 5.4). This is less than half the rate of water retention observed. Thus, it seems that in this species a reduction (although not cessation) of urine production may be the major contributor to the water balance response.

No attempt was made in this study to measure tubular reabsorption. AVT has been shown to increase tubular reabsorption of water in *B. marinus* (Sawyer 1957b) and *R. catesbeiana* (Uranga and Sawyer 1960), but not in the urodele *N. maculosus* (Sawyer and Pang 1979; Pang and Sawyer 1982). Circumstantial evidence suggests that increased tubular reabsorption may also contribute little to the water balance responses of the three *Leiopelma* species. In AVT-treated *L. archeyi* and *L. hamiltoni*, the reduction in GFR is so pronounced that there would be little or no primary urine from which water could be reabsorbed. In AVT-treated *L. hochstetteri* filtration apparently continues, but the amount of urine excreted during the first hour after treatment *in vivo* ($3 \text{ mg g}^{-1} \text{h}^{-1}$ in Experiment 1 of Chapter 4) plus that potentially reabsorbed by the bladder ($5 \text{ mg g}^{-1} \text{h}^{-1}$ as calculated above) accounts for within $2 \text{ mg g}^{-1} \text{h}^{-1}$ of the amount filtered ($10 \text{ mg g}^{-1} \text{h}^{-1}$), suggesting that tubular reabsorption may be relatively unimportant. Brown and Brown (1977) came to a similar conclusion in their study of *Notophthalmus viridescens*.

The apparent unimportance of bladder water reabsorption in the water balance responses of *L. archeyi* and *L. hamiltoni* contrasts with the situation in *B. marinus*. This toad has a capacious bladder with a fluid volume equivalent to at least 25% of W_0 (Shoemaker 1964), and

increased bladder water reabsorption accounts for about 50% of the water retention observed following AVT treatment *in vivo* (Bentley and Ferguson 1967). When one considers the size of *B. marinus* relative to that of *L. archeyi* and *L. hamiltoni*, this difference in the relative role of the bladder appears to make physiological sense. *B. marinus* is a large anuran reaching up to 230 mm SVL (Tyler 1976) with consequently a relatively small SA:volume ratio. *L. archeyi* and *L. hamiltoni* are minute in comparison and therefore have a relatively large SA:volume ratio. Thus, per unit body weight, the area of skin available for reabsorption is larger in *L. archeyi* and *L. hamiltoni*, whereas the volume available to accommodate a bladder is larger in *B. marinus*. *B. marinus* also shows a much larger bladder hydroosmotic response. In this context it is interesting to note that urodeles in which bladder water reabsorption appears to be of minor importance in the water balance response are also small [*Plethodon glutinosus*, ~3 g (Brown *et al.* 1977) and *N. viridescens*, 1.7-3.5 g (Brown and Brown 1977)], whereas two in which it plays a significant role are larger [*Aneides lugubris*, 4-13 g (Hillman 1974) and *Salamandra maculosa*, 11-47 g (Bentley and Heller 1965)]. These comparisons suggest that in general, small amphibians with a large SA:volume ratio rely more on cutaneous water uptake whilst larger species place relatively greater emphasis on bladder water reabsorption in the water balance response.

CHAPTER 6

SKIN STRUCTURE

INTRODUCTION

The skin of anurans has many diverse functions. Apart from absorbing water and salts, the skin plays a role in colouration and colour change, exchange of respiratory gases, mechanical protection, chemical defence, protection against parasites, odoriferous communication, sensation of mechanical and chemical stimuli, and the development of specialised mechanical devices such as claws (Lindemann and Voûte 1976). Additionally, in some arid-adapted species the skin may contain structures or substances restricting the evaporative loss of water (Blaylock *et al.* 1976; Kobelt and Linsenmair 1986).

The characteristic structure of anuran skin has been reviewed by Elkan (1976) and Cameron (1980). Briefly, it consists of four layers, which are organised from the outer to the inner surface as follows:

- (i) stratum corneum - a thin layer of keratinised epithelium, which is continuously or periodically discarded and replaced during moulting;
- (ii) epidermis - from 2-10 layers of epithelial cells;
- (iii) dermis (corium) - this can be divided into two regions: an outer stratum spongiosum and an inner stratum compactum. The stratum spongiosum consists of a network of collagen and elastic fibres, smooth muscle fibres, nerves and capillaries. Mucous glands and granular glands (the latter are also known as poison, serous or venom glands) lie in the stratum spongiosum, and ducts from these penetrate the epidermis to reach the exterior. The stratum compactum comprises layers of collagen fibres;
- (iv) tela subcutanea - a thin sheet forming the outer lining of the subcutaneous lymph sacs. The tela subcutanea contains blood vessels and nerves, and fibres may run vertically from the tela subcutanea through the dermis.

Although anuran skin has the general features described above, differences in structure between different regions exist and these are interpreted as evidence of specialisation for particular functions (Noble 1925; Elias and Shapiro 1957; Bagnara 1976, Bentley 1982a; Bani *et al.* 1985). In particular, pelvic skin of many terrestrial and

arboreal species has two main structural features which are believed to enhance rapid water uptake. First, pelvic skin often has surface irregularities ("sculpturing"; Lillywhite and Licht 1974), with raised areas (warts, verrucae, tubercles, mounds) being separated by channels or infoldings (Noble 1925; Cameron 1974; Blaylock *et al.* 1976). Second, pelvic skin is frequently hypervascularised when compared with other areas of skin (Roth 1973; Christensen 1974a, Drewes *et al.* 1977; Cameron 1980).

The aim of this chapter is to determine whether there is a relationship between the structure of the skin and its role in rapid water uptake for *L. archeyi* and *L. hochstetteri*. Is pelvic skin of *L. archeyi* hypervascularised or more extensively "sculptured" when compared with skin from other body regions, and are these features absent from pelvic skin of *L. hochstetteri*, which shows no hydroosmotic response? The techniques employed were practised first with the hylid *Litoria aurea*, and the results for this species are included for comparison.

MATERIALS AND METHODS

Material

Skin from pelvic, pectoral and dorsal regions of double-pithed frogs was removed and examined using light microscopy and scanning electron microscopy. In *Bufo marinus*, double pithing causes the pelvic blood vessels to become engorged with blood, thus improving their detection (Cameron 1980). The regions examined in the present study were comparable to those used for measurement of osmotic water flow *in vitro* (see Fig. 5.1 of Chapter 5), except that dorsal preparations were extended anteriorly and laterally to include the parotoid glands. Details of the sex, size and maturity of frogs used are given in Table 6.1.

Fresh flat mounts

Skin from the three regions was briefly examined and photographed in intact, double-pithed frogs using stereo microscopy, and then removed and mounted on microscope slides in amphibian Ringer. Fresh mounts were viewed from both inner and outer surfaces with a Polyvar research microscope (Reichert-Jung, Austria), using bright field and differential interference contrast viewing modes.

TABLE 6.1 Animals used in histological studies (SVLs in parentheses).

Species	Stereo microscopy and fresh flat mounts	Paraffin-embedded for light microscopy	Scanning electron microscopy
<i>Leiopelma archeyi</i>	Mature male (28.6mm)	Mature female (34.9mm) Mature male (26.0mm)	Mature female (34.9mm) Mature female (33.2mm)
<i>Leiopelma hochstetteri</i>	Immature female (32.7mm)	Mature female (41.2mm)	Mature female (41.2mm)
<i>Litoria aurea</i>	Unsexed juvenile (30.8mm)	Unsexed juvenile (33.4mm) Unsexed juvenile (32.9mm)	Unsexed juvenile (32.9mm)

Paraffin sections

Removed skin was rinsed in amphibian Ringer, and fixed in Zenker's fixative (10 h) or aqueous Bouin's fixative (24 h). Excess fixative was removed by rinsing in tap water (Zenker's-fixed material) or in 50% isopropyl alcohol (Bouin's-fixed material); the tissue was then dehydrated in an ascending alcohol series (70%, 95% and absolute isopropyl alcohol, absolute ethanol). Dehydrated tissue was cleared in cedarwood oil and embedded in paraffin wax. Transverse sections were cut at 10 μm using a rotary microtome, and stained with Ehrlich's haematoxylin and eosin or Masson's trichrome stain.

Mounted sections were viewed with the Polyvar microscope using bright field and differential interference contrast viewing modes. Total skin thickness (epidermis plus dermis) and epidermal thickness were measured in both the thickest (glandular) and the thinnest (non-glandular) regions of nine or more sections of each skin type, using a micrometer eyepiece.

Scanning electron microscopy (SEM)

Skin from pelvic, pectoral and dorsal regions was removed, rinsed in amphibian Ringer, and fixed in 4% glutaraldehyde in 0.025 mol l⁻¹ phosphate buffer. The phosphate buffer was prepared by mixing 11 ml of KH₂PO₄ (0.1 mol l⁻¹) and 39 ml of Na₂HPO₄ (0.1 mol l⁻¹) (pH of mixed solution 7.2), with 100 ml of deionised water. After fixation for 1-5 d at 4°C, pieces of tissue were rinsed in phosphate buffer and dehydrated in an ascending ethanol series (25%, 50%, 75%, 95% and absolute). They were then critical-point dried, sputter-coated with gold and viewed with a JEOL JSM-35 scanning microscope.

RESULTS

Leiopelma archeyi

Pelvic skin

Intact pelvic skin of *L. archeyi* is thin, smooth and semi-transparent. Pigmentation is provided by patches of melanophores and silvery, light-reflecting iridophores (Plate 6.1A). When the skin is removed the inner surface shows little evidence of vascularisation, either to the naked eye or at magnifications of up to 500 \times . Individual melanophores, iridophores and glands are evident in fresh flat mounts

Key to Plates 6.1 - 6.10

M	= melanophore(s)
I	= iridophore(s)
G	= unidentified gland
BV	= blood vessel
C	= capillary
E	= epidermis
D	= dermis
SCo	= stratum corneum
SS	= stratum spongiosum
SC	= stratum compactum
TS	= tela subcutanea
MG	= mucous gland
GG	= granular gland
Mo	= mound
H + E	= haematoxylin and eosin stain
MTC	= Masson's trichrome stain.

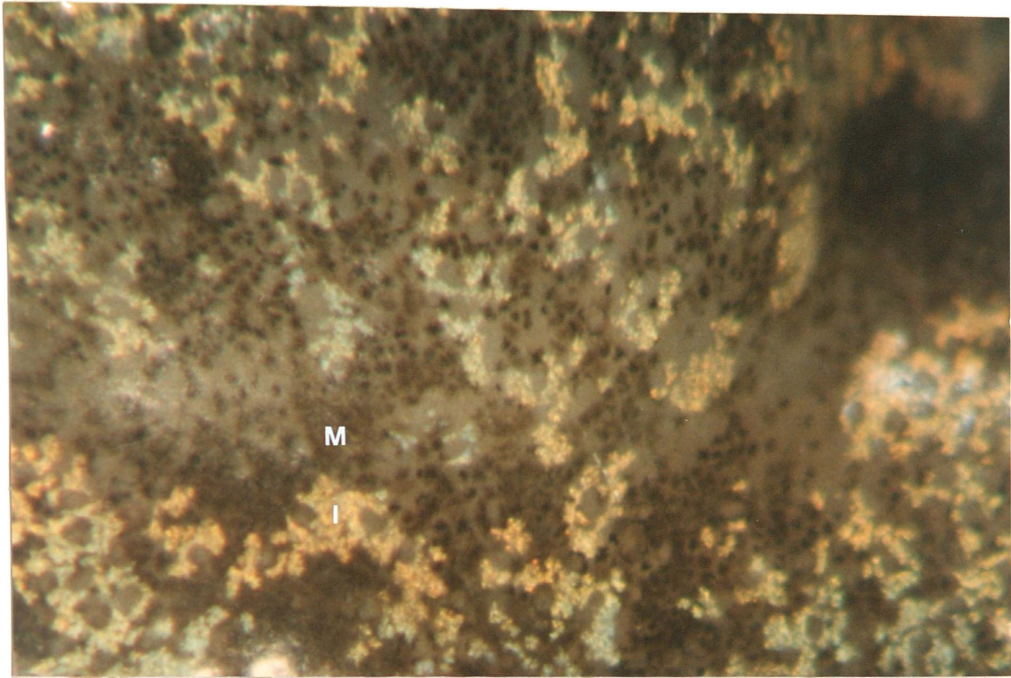
PLATE 6.1 Pelvic skin of *L. archeyi* (fresh).

A = stereo micrograph of intact skin.

B = light micrograph of fresh flat mount in a region where blood vessels were seen, viewed from inner surface (bright field, red filter).

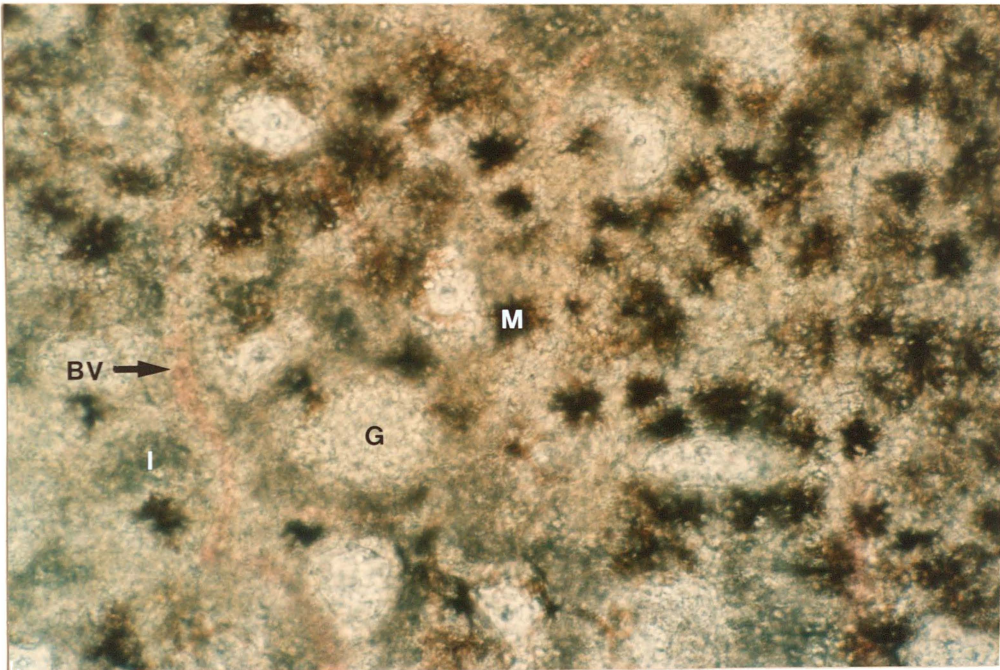
C = enlargement of B (bright field, neutral density filter).

Anuran iridophores appear silvery-gold in reflected light (A), but blue-green in transmitted light (B and C) (Bagnara 1976).



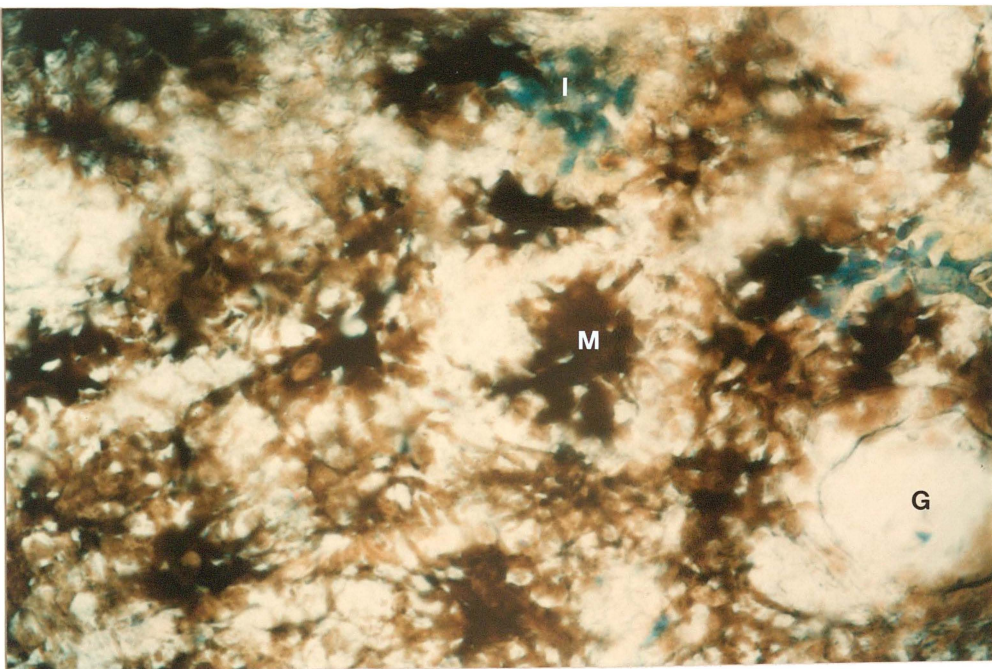
A

0.5 mm



B

100 μm



C

50 μm

viewed from the inner or outer surfaces, but the only blood vessels present are fine and sparsely distributed (Plate 6.1B and C).

The thin, sheet-like and poorly vascularised nature of pelvic skin of *L. archeyi* is confirmed in paraffin-embedded sections (Plate 6.2A and B). The epidermis is thin (2-3 cell layers deep), consistently measuring about 14 μm in thickness in both glandular and non-glandular areas of the specimen examined (Table 6.2). Immediately below the epidermis lies a discontinuous layer of melanophores; apart from this, the dermis consists almost entirely of the collagen fibres of the stratum compactum. Scattered mucous glands are present, but granular glands are infrequent. Very fine blood vessels are occasionally seen in the tela subcutanea, but in most sections capillaries penetrating the dermis could not be identified with certainty, even at magnifications of up to 1000 \times . Mean skin thickness (epidermis plus dermis) varied from 92 μm (in non-glandular areas) to 185 μm (in glandular areas) (Table 6.2); since most areas lack glands, the former value is more typical of pelvic skin in general.

Apart from some wrinkling of the epidermis due to dehydration, the structure of pelvic skin viewed by scanning electron microscopy appears similar to that seen in paraffin-embedded sections (Plate 6.2C). The epidermis is consistently thin, and the dermis is composed largely of stratum compactum with occasional glands. No blood vessels on the inner surface of the skin were seen.

Pectoral skin

Pectoral skin of *L. archeyi* is very similar in structure to pelvic skin. Fresh flat mounts are slightly less transparent than those of pelvic skin due to a greater density of melanophores and iridophores, but the same lack of prominent vascularisation is evident (Plate 6.3A, cf. Plate 6.1B and C). In paraffin-embedded sections the epidermis is 3-4 cell layers deep (Plate 6.3B) with a mean thickness of 19-28 μm (Table 6.2). The total thickness (epidermis plus dermis) of non-glandular areas of pectoral skin averaged 59 μm in the sections examined; this is considerably less than the value of 92 μm recorded for pelvic skin from a smaller animal (Table 6.2). In an SEM micrograph of pectoral skin a single blood vessel is present on the inner (dermal) surface (Plate 6.3C); however, in other respects the structure of this region is very similar to that in an SEM preparation of pelvic skin from the same animal (Plate 6.2C).

PLATE 6.2 Pelvic skin of *L. archeyi* (fixed).

A = light micrograph of paraffin-embedded transverse section (H + E, differential interference contrast, blue filter).

B = enlargement of same section as A, showing mucous gland and peeling stratum corneum (differential interference contrast, blue filter).

C = scanning electron micrograph of cut edge. This preparation shows features similar to that in B, but comes from a different animal.

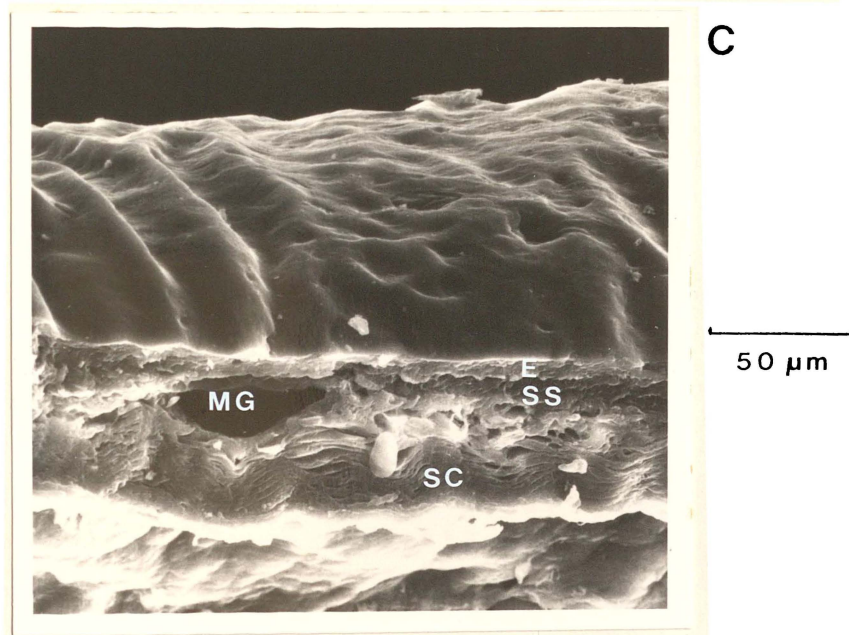
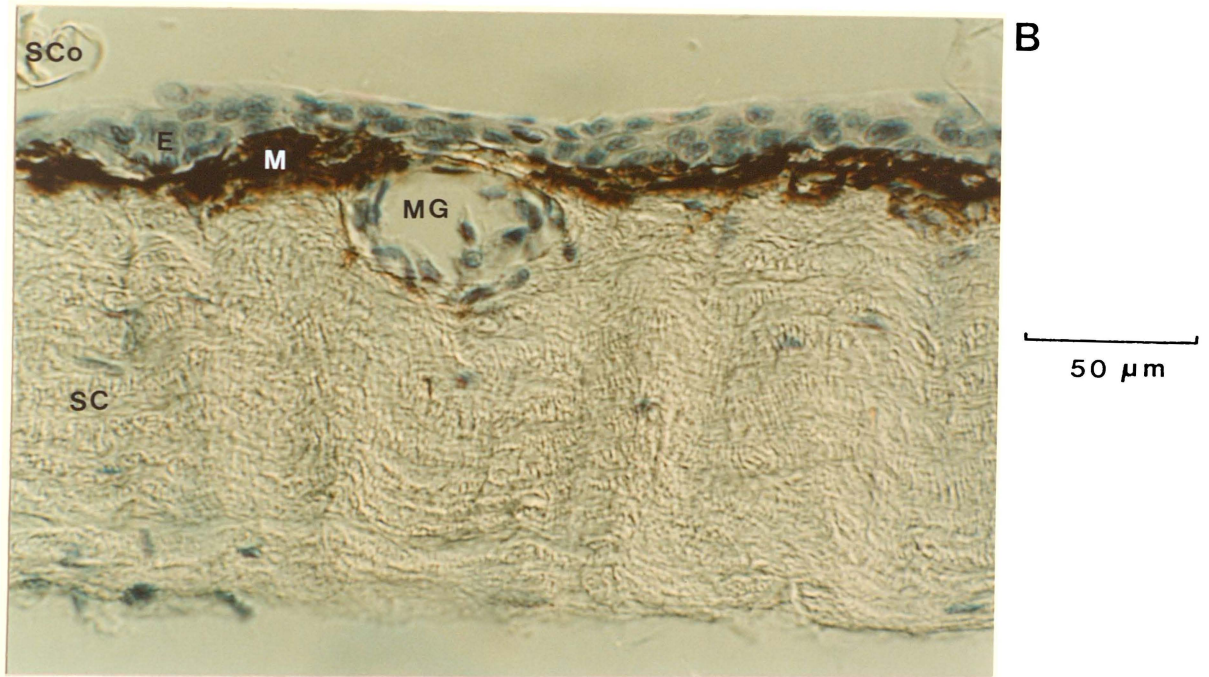
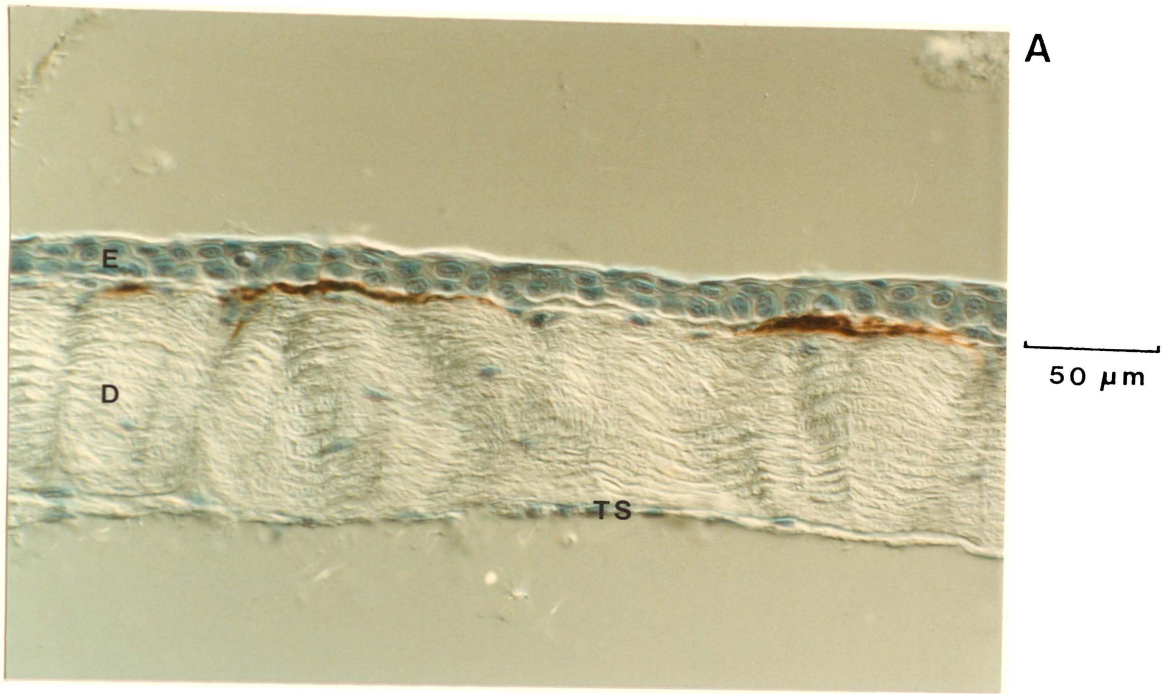


TABLE 6.2 Thickness of paraffin-embedded transverse sections of skin from *Leiopelma archeyi*, *L. hochstetteri* and *Litoria aurea* ($\bar{x} \pm SE$). Thickness was measured at two places on each of 9-10 sections: at the point of maximum skin thickness (in a glandular region) and at the point of minimum skin thickness (in a non-glandular region). Dorsal sections were from the upper dorsal region. Source codes: M = mature male, F = mature female, J = unsexed juvenile.

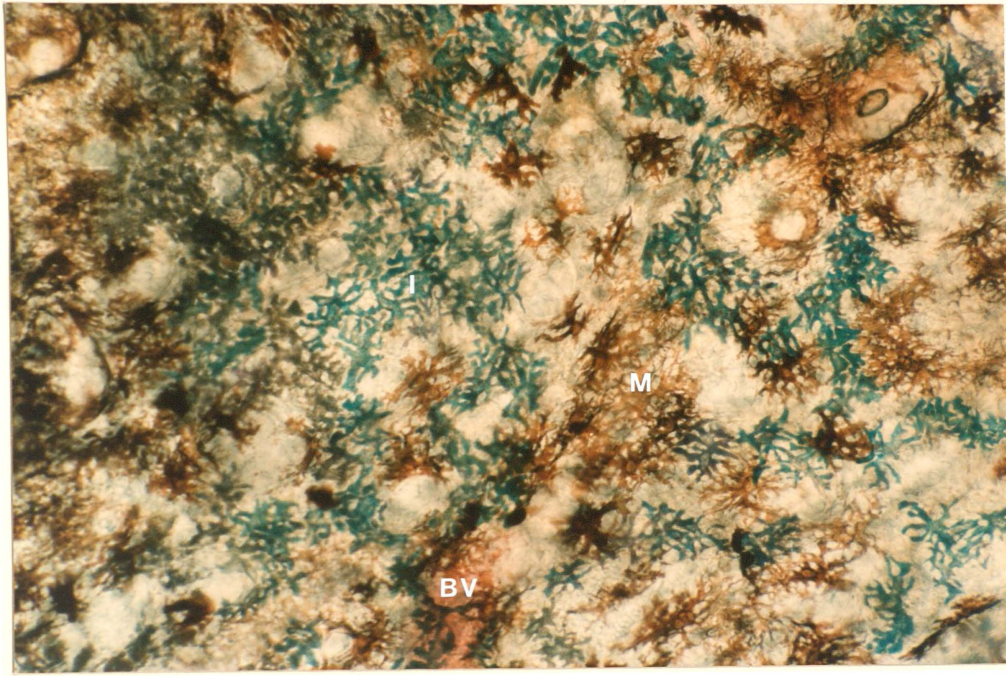
Area of skin	Source (SVL in mm)	Point of maximum thickness (glandular)		Point of minimum thickness (non-glandular)	
		Epidermis plus dermis (μm)	Epidermis (μm)	Epidermis plus dermis (μm)	Epidermis (μm)
<i>Leiopelma archeyi</i> :					
Pelvic	M(26.0)	185 \pm 18	14 \pm 1	92 \pm 3	14 \pm 1
Pectoral	F(34.9)	134 \pm 5	28 \pm 3	59 \pm 3	19 \pm 1
Dorsal	F(34.9)	640 \pm 29	41 \pm 2	96 \pm 4	16 \pm 1
<i>Leiopelma hochstetteri</i> :					
Pelvic	F(41.2)	210 \pm 13	61 \pm 6	115 \pm 3	47 \pm 2
Pectoral	F(41.2)	192 \pm 9	57 \pm 4	70 \pm 4	25 \pm 4
Dorsal	F(41.2)	297 \pm 3	30 \pm 2	96 \pm 2	24 \pm 1
<i>Litoria aurea</i> :					
Pelvic	J(32.9)	151 \pm 3	56 \pm 3	37 \pm 1	20 \pm 4
Pectoral	J(33.4)	183 \pm 12	66 \pm 1	87 \pm 4	31 \pm 2
Dorsal	J(33.4)	147 \pm 1	25 \pm 1	74 \pm 2	13 \pm 6

PLATE 6.3 Pectoral skin of *L. archeyi*.

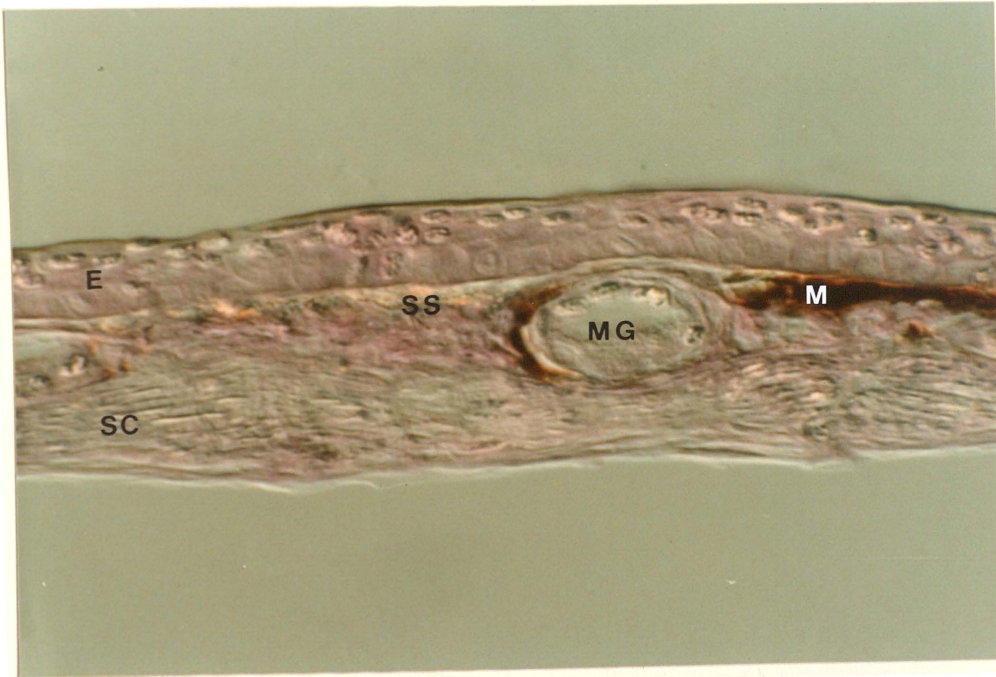
A = light micrograph of fresh flat mount in region of blood vessel.
viewed from outer surface (bright field, red filter).

B = light micrograph of paraffin-embedded transverse section (H + E,
differential interference contrast, red filter).

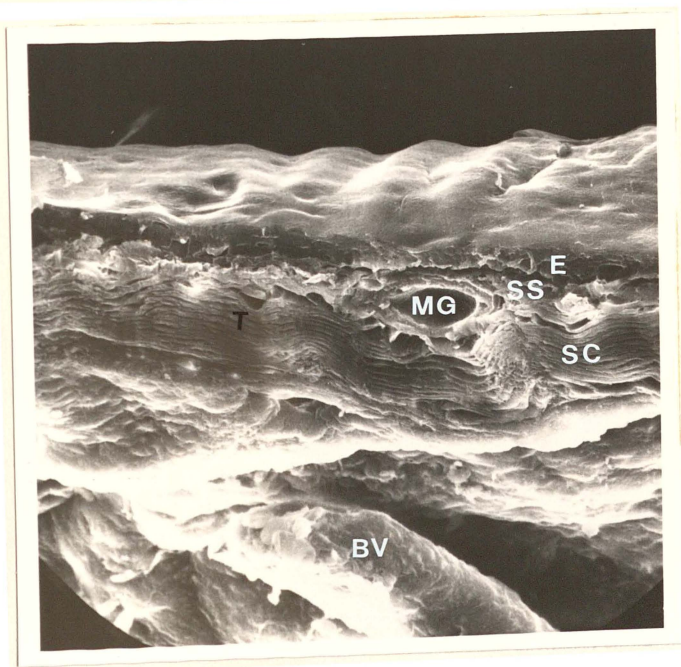
C = scanning electron micrograph of cut edge, in a region with a blood
vessel on the inner surface.



A



B



C

Dorsal skin

Intact dorsal skin of *L. archeyi* differs from pectoral and pelvic skin in its more intense and often green colouration (and thus reduced transparency), and in having raised glandular ridges (see Plate i.1B of General Introduction). Chromatophores (melanophores, iridophores, xanthophores and erythrophores) are densely packed in fresh flat mounts, but blood vessels are rarely seen. Cross-sections of paraffin-embedded or SEM preparations reveal that the glandular ridges contain one or more large granular glands (Plate 6.4A and C). The granular glands are flanked on each side by smaller mucous glands, and large glands of an unidentified type are also present (Plate 6.4A and B). The latter glands are at best only weakly eosinophilic, and have a characteristic pitted or raised appearance when viewed with differential interference contrast illumination (Plate 6.4B). Whether they represent pre-secretory granular glands or a third distinct type of gland is unknown.

The total thickness (epidermis plus dermis) of the glandular ridges of the upper dorsum averages 640 μm (Table 6.2), and the epidermis is 2-3 cell layers deep. In non-glandular areas, dorsal skin has a denser chromatophore layer but is otherwise similar in structure and thickness to pelvic skin (see left-hand edge of Plate 6.4A; cf. Plate 6.2A). The mean total thickness of epidermis plus dermis in non-glandular areas is 96 μm (Table 6.2).

*Leiopelma hochstetteri**Pelvic skin*

Pelvic skin of intact *L. hochstetteri* is in most parts smooth, although occasional small raised white spots are evident (Plate 6.5A; see also Plate i.3C of General Introduction). Pelvic skin of this species is less transparent than that of *L. archeyi*, due to a greater density of melanophores and iridophores.

Freshly removed pelvic skin of *L. hochstetteri* does not appear markedly vascularised to the naked eye. However, when viewed from the inner surface at low magnification (90 \times), blood vessels in the tela subcutanea and an extensive and regular network of capillaries are evident (Plate 6.5B). Virtually every gland is individually surrounded by capillaries arranged in a polygonal manner (Plate 6.5B).

PLATE 6.4 Dorsal skin of *L. archeyi* (fixed).

A = light micrograph of paraffin-embedded transverse section of lower dorsum, showing accumulation of granular glands, mucous glands and unidentified glands in the lateral glandular ridge (H + E, differential interference contrast, red filter).

B = enlargement of glandular ridge from a section similar to that shown in A (differential interference contrast, red filter).

C = scanning electron micrograph of cut edge of upper dorsum. Two large granular glands are evident in the lateral glandular ridge.



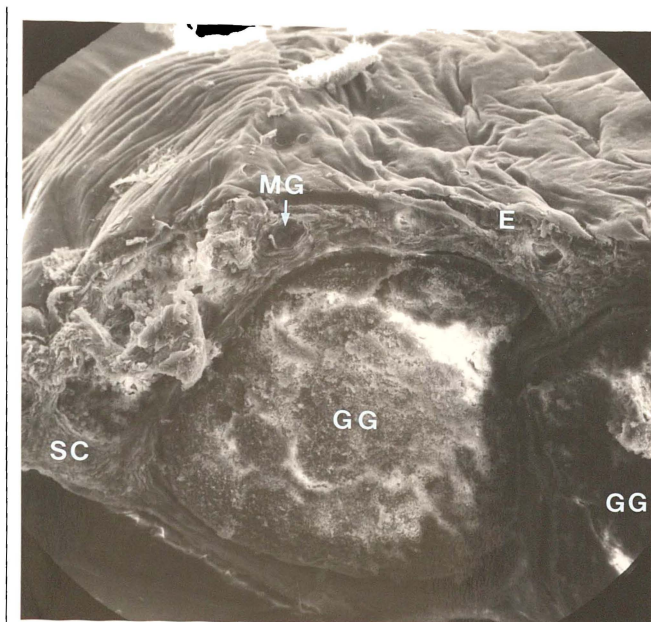
A

100 μm



B

50 μm



C

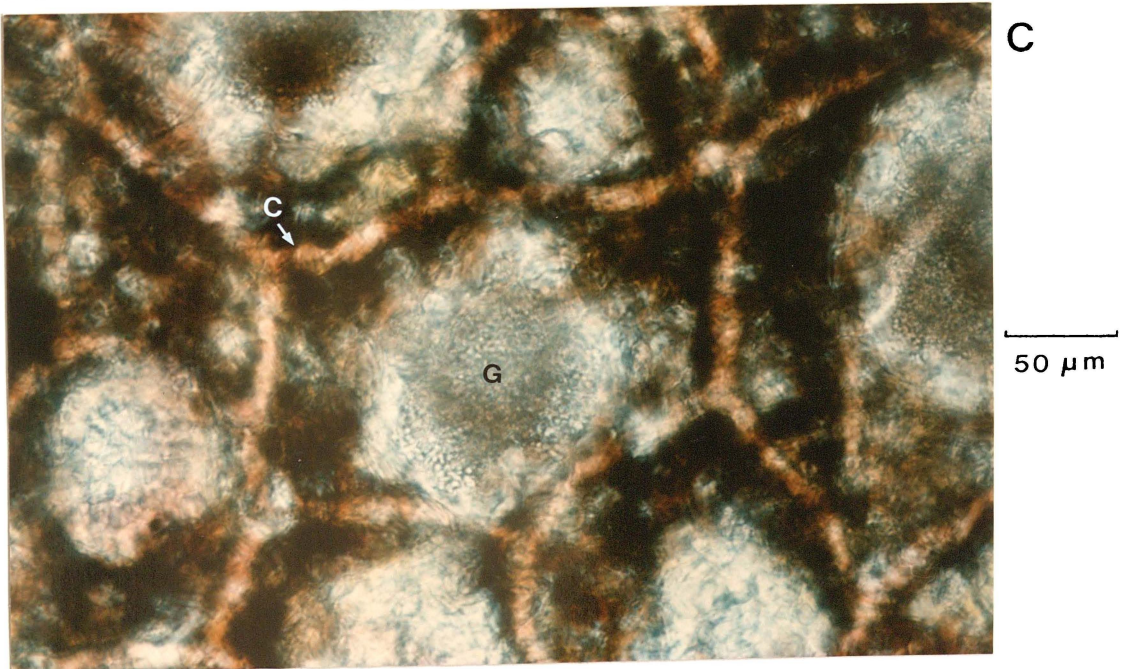
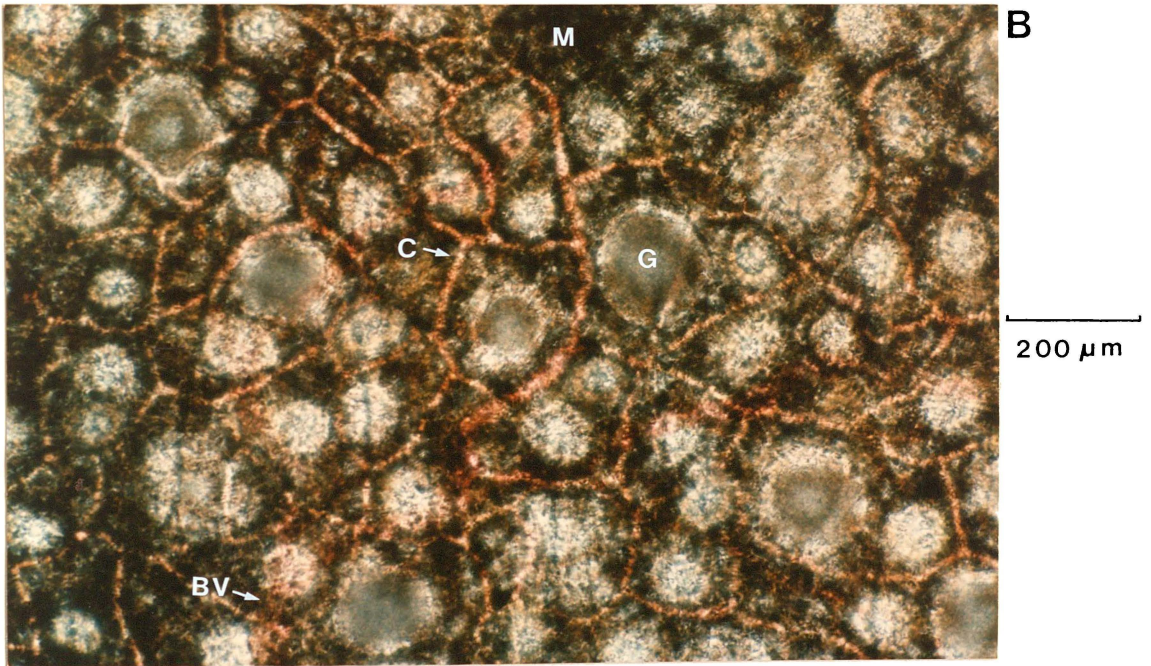
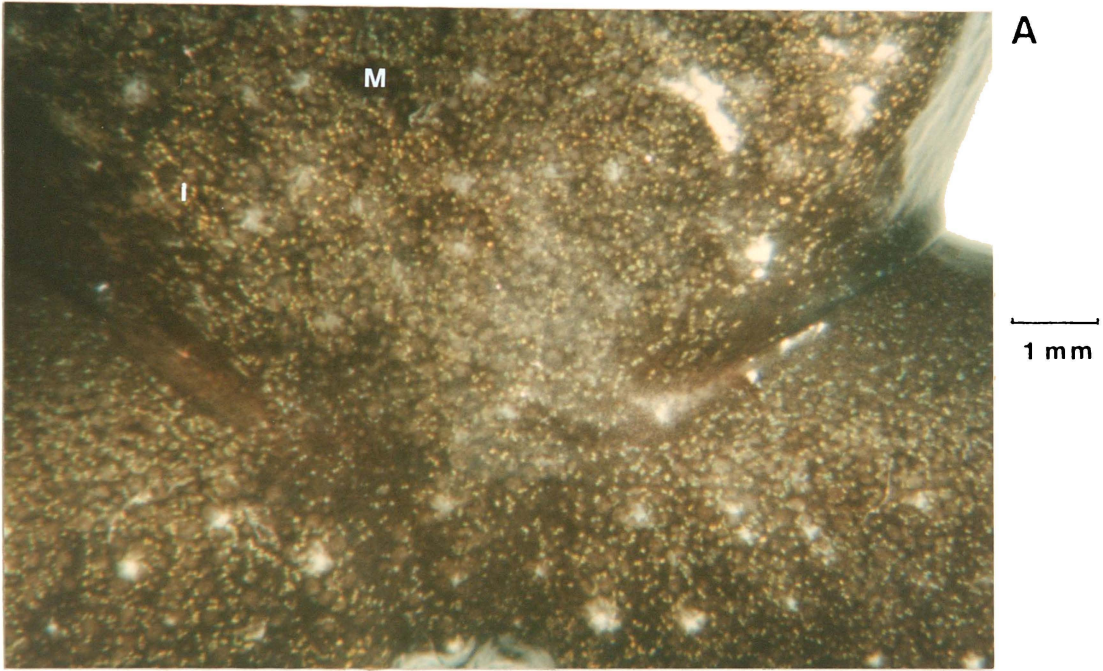
200 μm

PLATE 6.5 Pelvic skin of *L. hochstetteri* (fresh).

A = stereo micrograph of intact skin.

B = light micrograph of fresh flat mount, viewed from inner surface
(bright field, red filter). Note large blood vessel, and
regular network of capillaries around glands.

C = enlargement of B (bright field, blue filter).



and C).

Cross-sections of paraffin-embedded pelvic skin reveal a sheet-like epidermis about 5 cell layers deep (average thickness 47-61 μm ; Table 6.2), below which lies a discontinuous layer of chromatophores (Plate 6.6A and B). Many mucous glands and granular glands are present in the stratum spongiosum; these are also evident in the cut edges of skin viewed using scanning electron microscopy (Plate 6.6C). The average thickness of epidermis plus dermis in non-glandular areas of paraffin-embedded sections is 115 μm , increasing to 210 μm in glandular areas (Table 6.2). Individual red blood cells are obvious in blood vessels in the tela subcutanea, and also in capillaries which lie in the upper dermis just above the chromatophore layer (Plate 6.6B). These capillaries correspond with the capillary network seen in fresh flat mounts (Plate 6.5B and C).

Pectoral skin

Flat mounts, paraffin-embedded sections and scanning electron micrographs show that pectoral skin of *L. hochstetteri* is somewhat thinner but otherwise very similar in structure to pelvic skin (e.g., Plate 6.7A, cf. Plate 6.6C; Table 6.2). A capillary network is present in flat mounts, and in paraffin-embedded sections the epidermis is about 4-6 cell layers deep.

Dorsal skin

The dorsum of intact *L. hochstetteri* is characterised by the presence of small scattered warts, and lateral glandular ridges which extend posteriorly from behind each eye (see Plate i.3B of General Introduction). These glandular ridges are less pronounced than those of *L. archeyi*. Flat mounts viewed from the inner surface show that dorsal skin of *L. hochstetteri* is densely packed with chromatophores (melanophores, iridophores, xanthophores and occasional erythrophores). In areas where the chromatophore layer is thinner, a capillary network similar to that of pelvic and pectoral skin is visible.

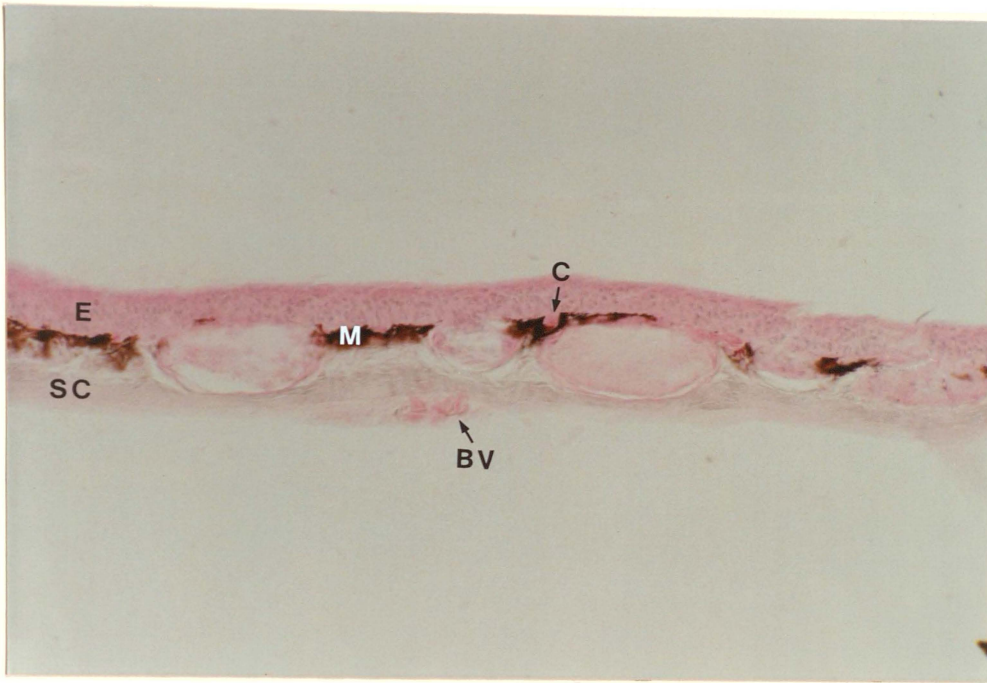
In cross-section, the dominant feature of dorsal skin is the presence of large granular glands in the lateral glandular ridges (Plate 6.7B). These glandular ridges are less than half as thick as those of *L. archeyi* (Table 6.2). The granular glands increase in size toward the centre of each lateral ridge, and ducts from the largest glands

PLATE 6.6 Pelvic skin of *L. hochstetteri* (fixed).

A = light micrograph of paraffin-embedded transverse section, showing
blood vessel in tela subcutanea, and subepidermal capillaries
(H + E, bright field, blue filter).

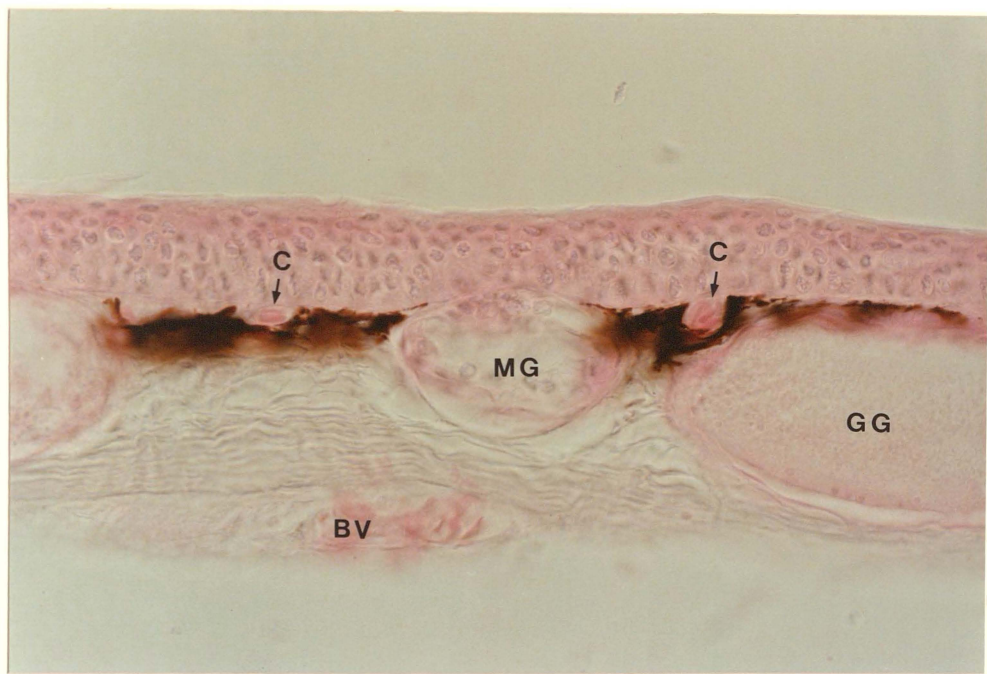
B = enlargement of A (bright field, neutral density filter).

C = scanning electron micrograph of cut edge.



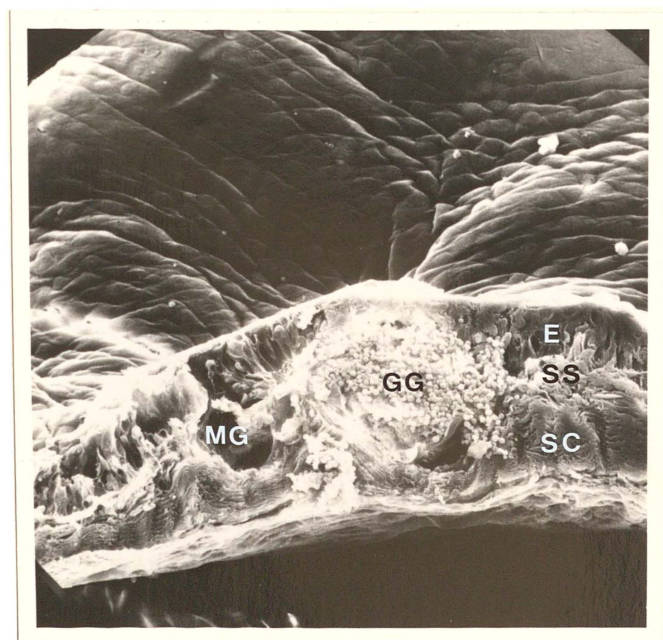
A

100 μ m



B

50 μ m



C

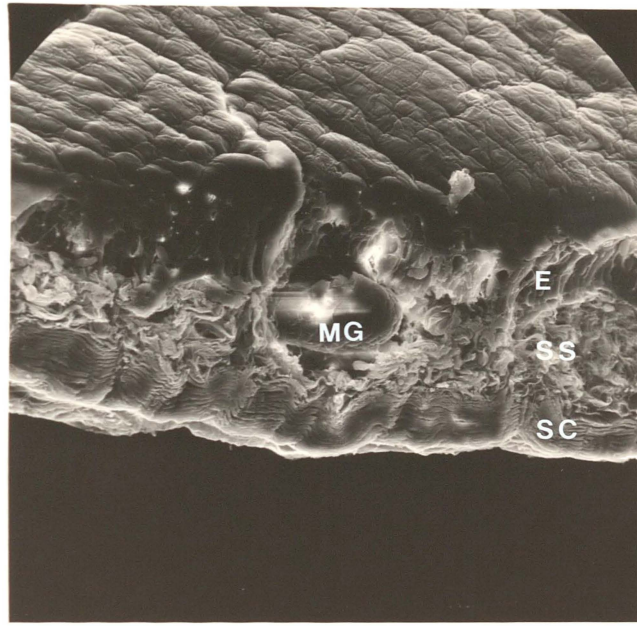
100 μ m

PLATE 6.7 Pectoral and dorsal skin of *L. hochstetteri* (fixed).

A = scanning electron micrograph of cut edge of pectoral skin.

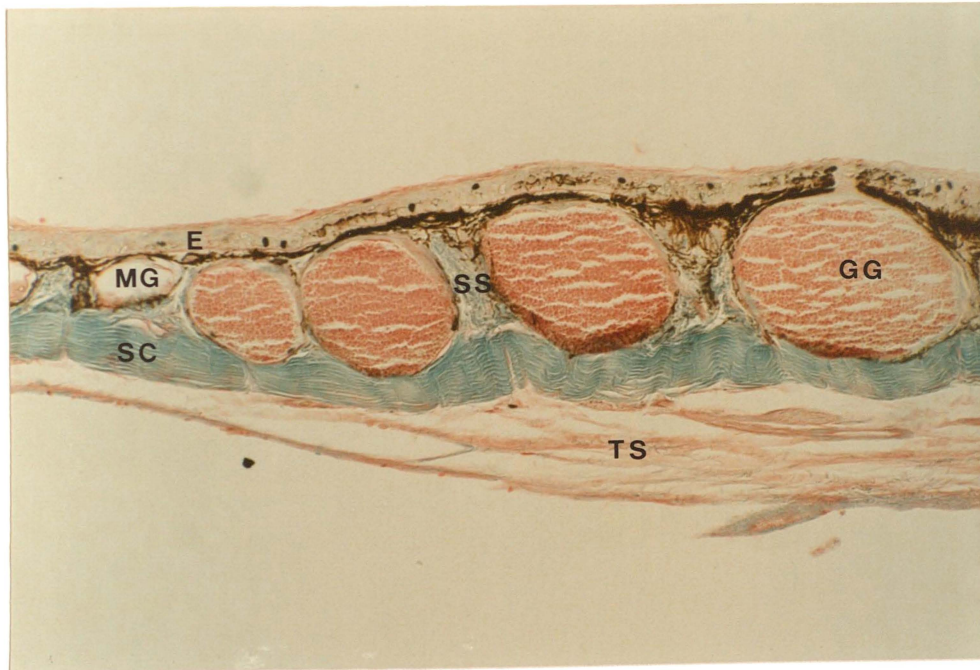
B = light micrograph of paraffin-embedded transverse section of upper
dorsum, showing aggregation of granular glands in the lateral
glandular ridge (MTC, bright field, blue filter).

C = enlargement of B, showing granular gland with duct to surface
(differential interference contrast, red filter).



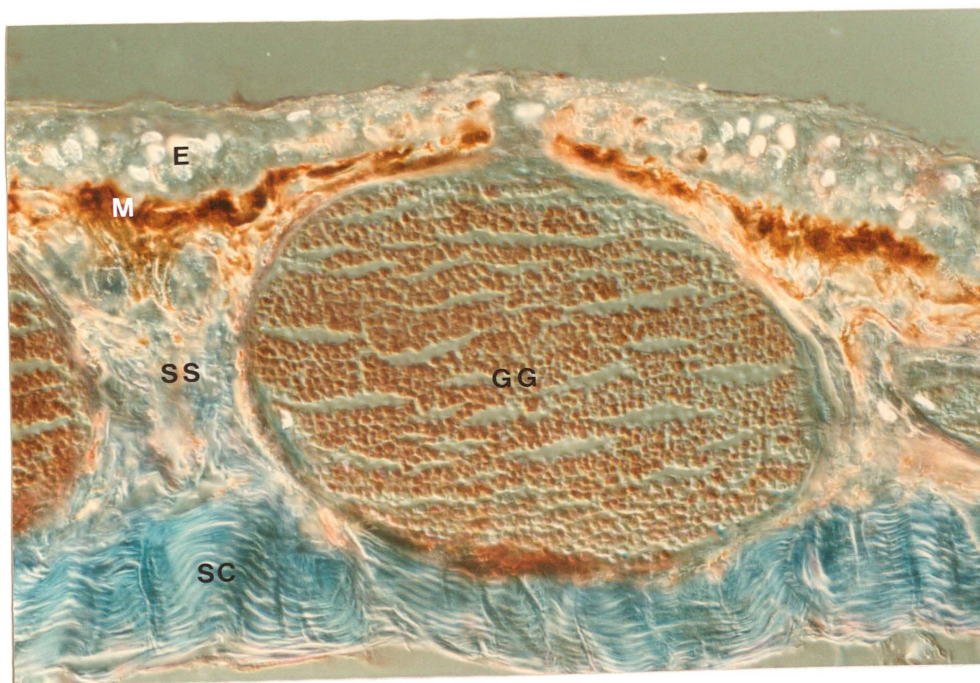
A

50 μm



B

100 μm



C

50 μm

penetrate the epidermis to the exterior (Plate 6.7B and C). Mucous glands are also present, but no glands comparable to the unidentified glands of *L. archeyi* were seen. In non-glandular areas, the total thickness (epidermis plus dermis) of dorsal skin averages 96 μm , a value intermediate between that for pectoral and pelvic skin (Table 6.2).

Litoria aurea

Pelvic skin

Skin covering the pelvic region and the ventral surface of the upper hind legs of *Litoria aurea* is characterised by the presence of closely packed warts or mounds, which produce a pebbled or granular appearance (Plate 6.8A). The inner surface of pelvic skin is extensively vascularised (Plate 6.8B), and blood vessels in the tela subcutanea are obvious to the naked eye. From the outer surface of fresh flat mounts, each mound is seen to be encircled by a blood vessel (Plate 6.8C).

Paraffin-embedded sections and scanning electron micrographs show that the mounds are produced by a thickening of the epidermis and the stratum spongiosum of the dermis (Plate 6.9A, B and C). In paraffin-embedded sections the mounds reach a maximum thickness (epidermis plus dermis) averaging 151 μm (Table 6.2), with each mound usually containing several mucous glands and one or more granular glands. Inter-mound areas have a mean thickness of only 37 μm and lack glands. The epidermis increases to a depth of about 7 cell layers (56 μm) over mounds, but is only about 3 cell layers deep (20 μm) between mounds (Table 6.2). The mounds have a basal diameter of $655 \pm 16 \mu\text{m}$ ($\bar{x} \pm \text{SE}$ for $n = 9$).

Paraffin-embedded sections confirm that the mounds are well vascularised. A blood vessel is present in the tela subcutanea beneath many mounds (Plate 6.9A), and smaller vessels branch from this to penetrate the dermis. Capillaries containing red blood cells are present in the stratum spongiosum close to the epidermis, both in mounds and in intermound areas. In mounds, cross-sections of capillaries are present in fingers of dermis which extend up between downward projections of the epidermis (Plate 6.9B).

PLATE 6.8 Pelvic skin of *Litoria aurea* (fresh).

A = stereo micrograph of intact skin.

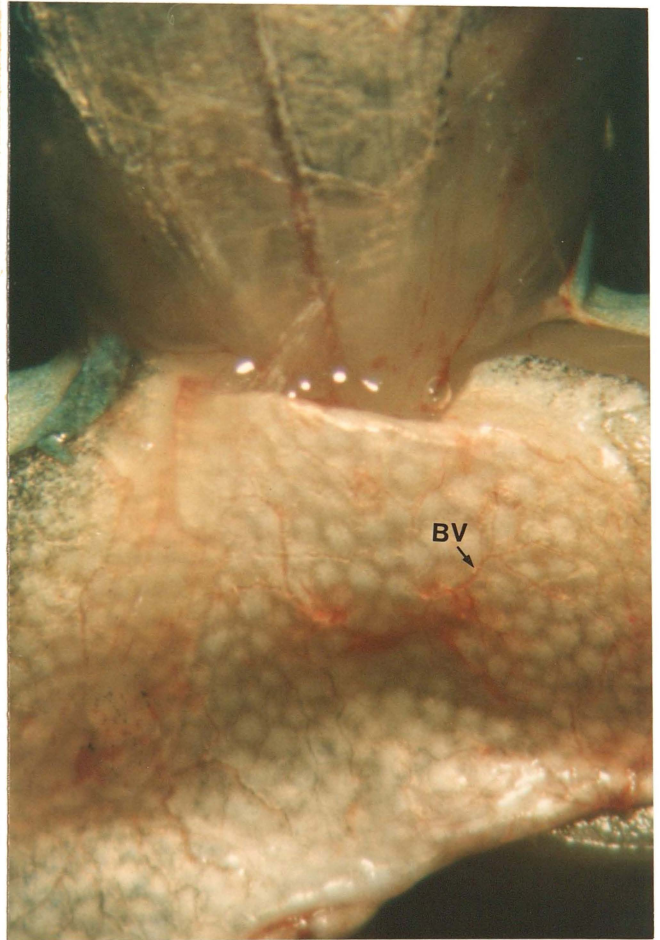
B = stereo micrograph of inner surface of pelvic skin, showing prominent blood vessels.

C = light micrograph of fresh flat mount viewed from outer surface. Each mound is surrounded by a blood vessel (bright field, neutral density filter).

A



B



C

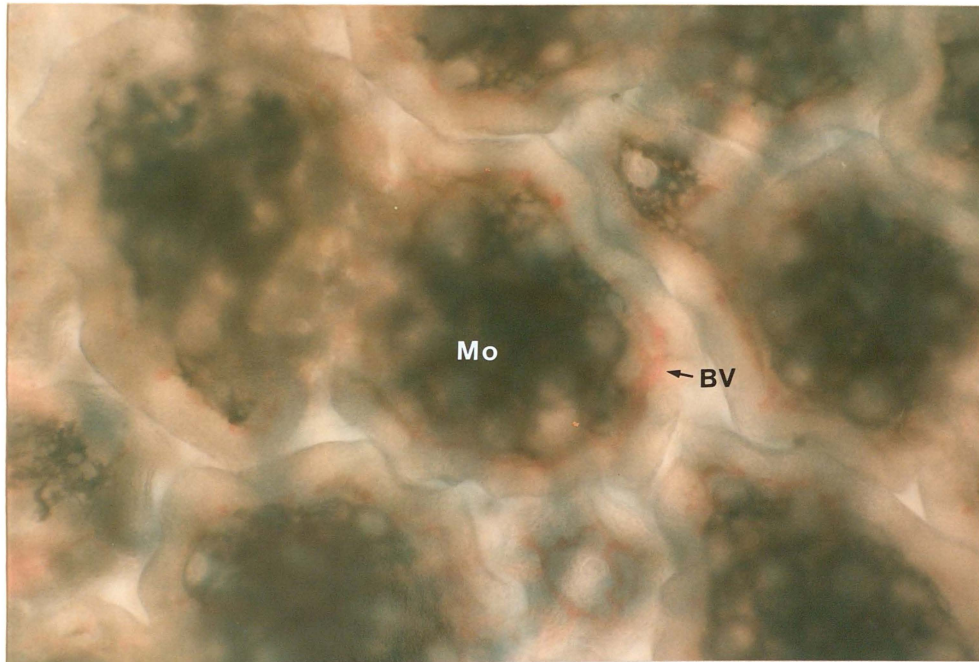
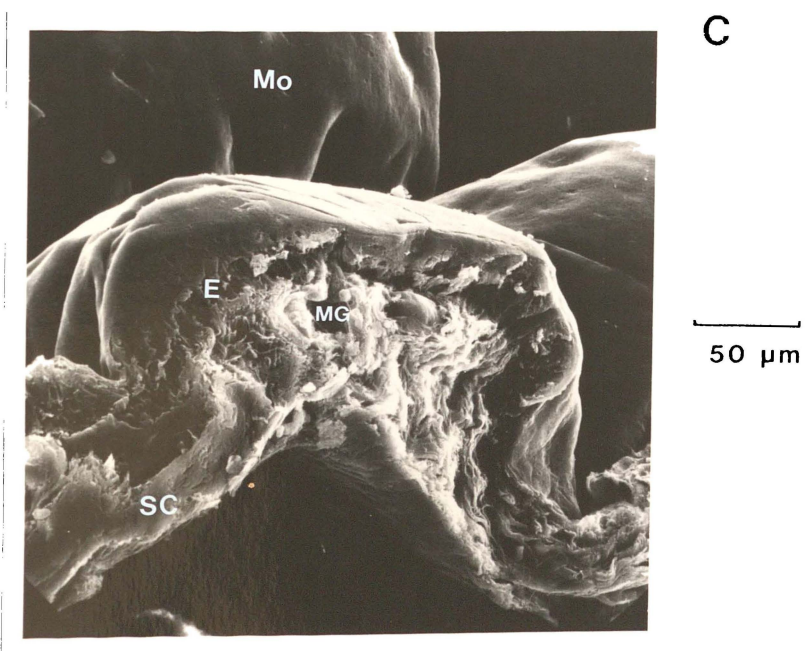
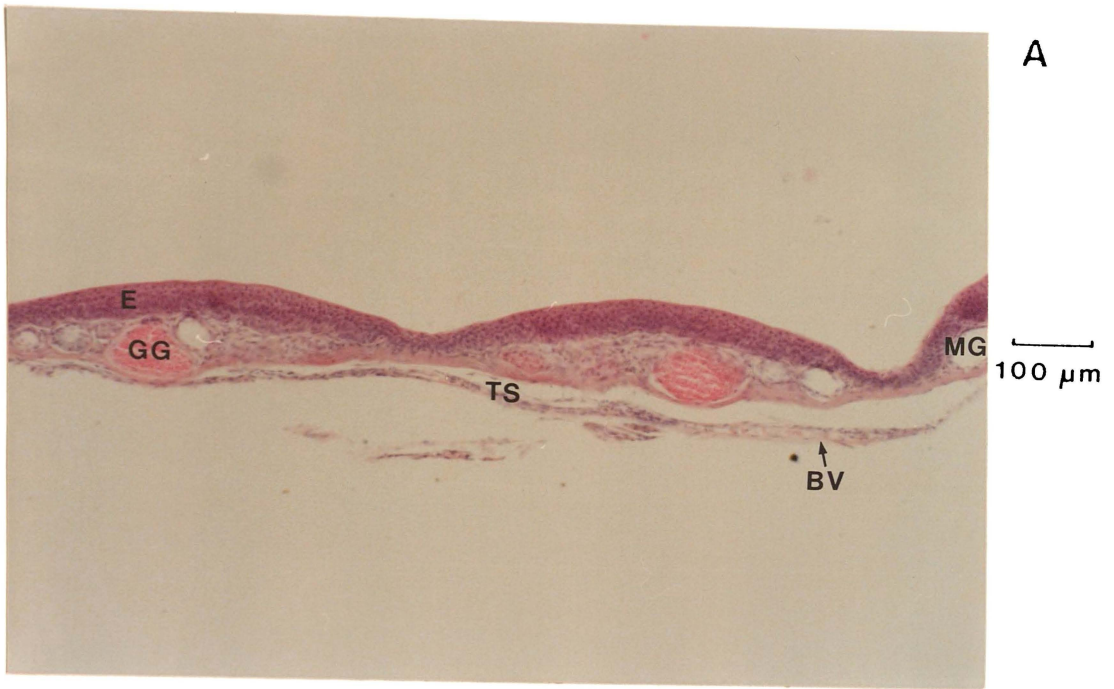


PLATE 6.9 Pelvic skin of *Litoria aurea* (fixed).

A = light micrograph of paraffin-embedded transverse section, showing mounds, thinner intermound areas, and blood vessel in tela subcutanea (H + E, bright field, blue filter).

B = enlargement of a similar section to A, showing cross-sections of capillaries between downward projections of epidermis (H + E, bright field, blue filter).

C = scanning electron micrograph of cut edge.



Pectoral skin

Pectoral skin of *Litoria aurea* is thicker than pelvic skin (Table 6.2) and has less pronounced mounds. Over the pectoral girdle of intact frogs, the mounds virtually disappear (Plate 6.10A). As in pelvic skin, the epidermis is thicker over mounds (up to 12 cell layers deep), but only about 5 cell layers deep between mounds. Pectoral skin is less densely vascularised than pelvic skin, although blood vessels in the tela subcutanea and capillaries in the dermis are still often seen. However, no finger-like dermal extensions carrying capillaries between downward projections of the epidermis were observed.

Dorsal skin

The mounds typical of pelvic and, to a lesser extent, pectoral skin of *Litoria aurea* are not present in dorsal skin (Plate 6.10B). In paraffin-embedded cross-sections dorsal skin appears relatively smooth, gradually increasing to an average thickness of 147 μm at the lateral ridges (Table 6.2), where granular glands are aggregated (Plate 6.10B). The epidermis is about 3 cell layers deep, and is thinner than the epidermis of pelvic or pectoral skin (Table 6.2). Dorsal skin is also less vascularised than pelvic or pectoral skin: blood vessels in the tela subcutanea are narrower and less often seen, and capillaries in the dermis are infrequent.

DISCUSSION

Hypervascularity of pelvic skin of anurans has been noted on many occasions. Noble (1925) reported finding superficial epidermal capillaries in mounds in the skin from the abdomen and undersurface of the thighs of arboreal hylids and rhacophorids, and suggested that these aided cutaneous respiration. However, Czopek (1965) considered that as these areas are normally pressed against inanimate surfaces, a role in water uptake seemed more likely. Roth (1973) reported a relationship between pelvic vascularity and water uptake in nine species of anurans. In the more terrestrial species (*Bufo* spp.), pelvic skin was hypervascular in comparison with other skin regions. However, *Xenopus laevis*, which shows no cutaneous hydroosmotic response, had the least extensive development of pelvic blood vessels among the species examined. Similar results were reported by Christensen (1974a), who compared vascularisation of pelvic skin from *X. laevis* with that from *Bufo bufo* and several ranids. A

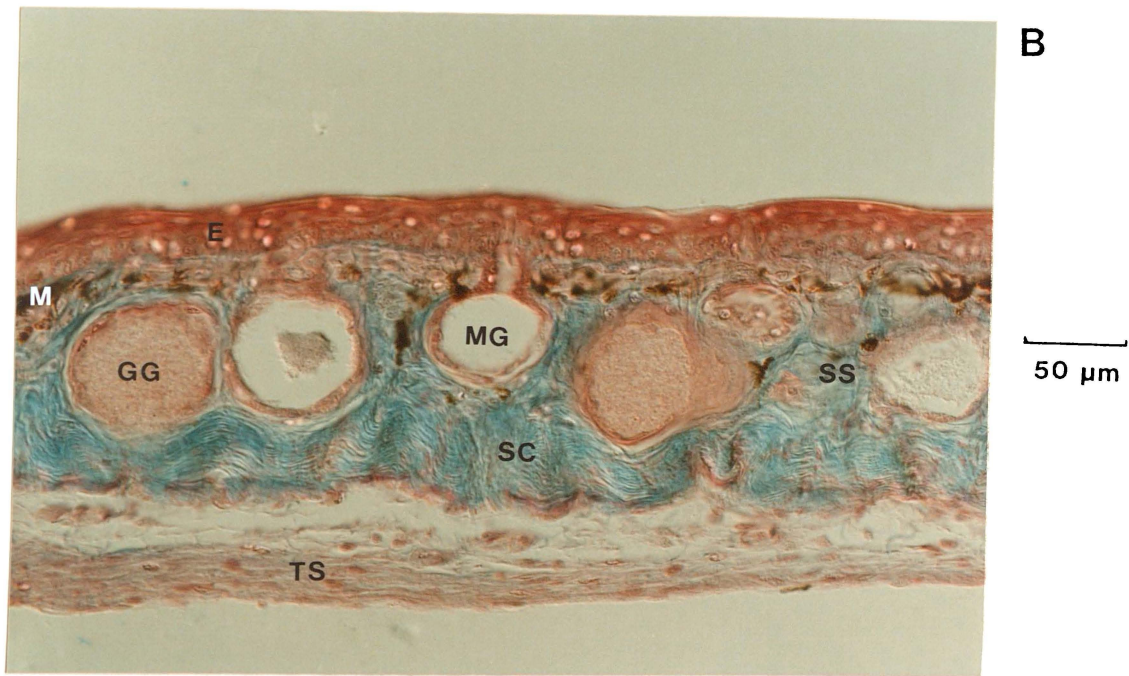
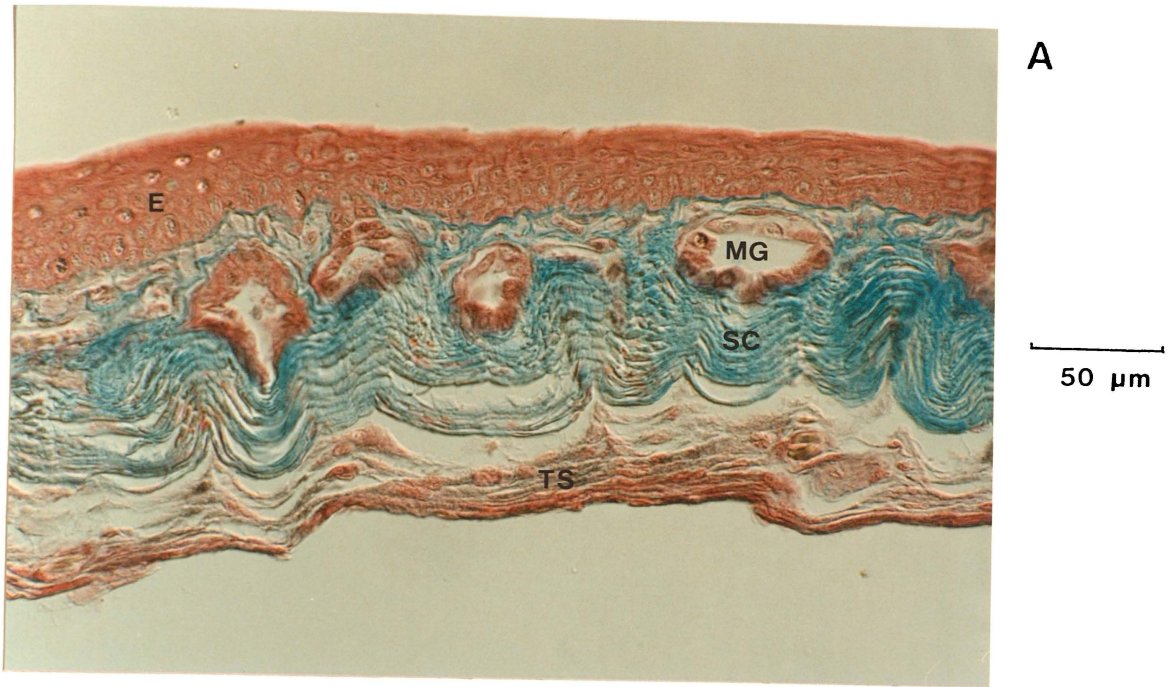


PLATE 6.10 Pectoral and dorsal skin of *Litoria aurea* (fixed).

A = light micrograph of paraffin-embedded transverse section of pectoral skin (MTC, differential interference contrast, red filter).

B = light micrograph of paraffin-embedded transverse section of dorsal skin in region of granular glands (MTC, differential interference contrast, red filter).

well-developed blood supply to the pelvic skin has also been reported for *Bufo punctatus* (McClanahan and Baldwin 1969), *Chiromantis petersi* (Drewes *et al.* 1977), *Bufo marinus* (Cameron 1980) and *Hyperolius viridiflavus* (Kobelt and Linsenmair 1986).

What is the significance of hypervascularisation for rapid water uptake? Christensen (1974b, 1975) clearly felt that a well-developed pelvic circulation was necessary for the removal of water absorbed during rapid rehydration, and experimental evidence suggests that the rate of osmotic water flow (OWF) through frog (*Rana pipiens*) skin is indeed related to the rate of skin circulation (Mahany and Parsons 1978). Christensen considered that water absorbed during rehydration passes directly into skin capillaries, preventing a decrease in osmotic concentration of fluid in the interstitial spaces of the skin, which would lead to a decline in water uptake. However, the very high rates of water uptake measured during rehydration or in response to AVT make it doubtful that the venous circulatory system can immediately accommodate such large volumes of water (Roth 1973). Instead, much of the absorbed water probably initially enters the lymph spaces (Carter 1979), either passing through the skin by diffusion or in lymph capillaries (Cameron 1980). Dehydrated toads (*B. marinus*) show elevated heart rates which may reflect increased peripheral circulation (Sherman 1980), and in several species of *Rana* and *Bufo*, dehydration causes the pelvic blood vessels to become engorged with blood (Christensen 1974a; Yokota and Hillman 1984). During rehydration, circulation of blood in the pelvic skin is very rapid (Overton 1904), and pulsations of the lymph hearts are observed (Stille 1958).

In addition to assisting in the rapid removal of absorbed water, capillaries which travel close to the surface of pelvic skin may aid in transporting endogenous AVT to epidermal cellular receptors. In isolated unperfused pelvic skin preparations, the presence of the dermis delays diffusion of hormone to the epidermis, thus delaying the hormone-induced increase in OWF (Rajerison *et al.* 1972).

In view of the extensive evidence supporting a relationship between blood supply and water uptake for pelvic skin, the poorly vascularised nature of pelvic skin of *L. archeyi* is surprising. Pelvic skin of this species shows a pronounced hydroosmotic response, whereas pectoral and dorsal skin do not (Chapter 5). However, the blood

supply to all three regions is poorly developed: vessels in the tela subcutanea are small and sparsely distributed, and capillaries in the dermis are virtually undetectable. In contrast, pelvic skin of *L. hochstetteri*, which shows no hydroosmotic response, is extensively vascularised.

Pelvic skin of *L. archeyi* is, however, characterised by a very thin epidermis when compared with that of other anurans. The number of cell layers (2-3) is at the lower extreme of the range of 2-10 cell layers reported for anurans in general by Elkan (1976). Czopek (1965) surveyed epidermal thicknesses in a variety of amphibians, and reported values for 20 anuran species ranging from 12.7 μm (for a 0.6 g *Rana pipiens sphenoccephala*) to 65.2 μm (for a 6.8 g *Bombina variegata*). In 20 urodele species, the ranges lay between 14.8 μm (for a 0.5 g lungless salamander, *Batrachoseps attenuatus*) and 110.5 μm (for a 245.0 g *Siren intermedia*). The value for the 2.1 g *L. archeyi* examined here (14 μm) is comparable to the lowest of these.

In addition to its very thin epidermis and lack of "sculpturing", pelvic skin of *L. archeyi* has a very simple dermal structure. Mucous and granular glands are infrequent, and in non-glandular areas the dermis largely comprises statum compactum. The average thickness (epidermis plus dermis) of the thinnest non-glandular regions was 92 μm . This value is comparable to or greater than that for pectoral or dorsal skin, and so cannot be regarded as evidence of regional specialisation (thinness) for rapid water uptake. Furthermore, the value is not unusual when compared with those for several other anurans. Elias and Shapiro (1957) reported skin thicknesses in five species of anurans ranging from 60 μm in *Hyla gratiosa* and *H. squirrella*, to 170 μm in *Microhyla carolinensis*. The minimum thickness of *L. archeyi* pelvic skin was also not greatly less than that seen here for *L. hochstetteri* (115 μm), and was considerably greater than that in the intermound areas of *Litoria aurea* pelvic skin (37 μm).

The well-developed subepidermal capillary network in pelvic, pectoral and dorsal skin of *L. hochstetteri* has been noted in a previous study (Czopek 1965), although apparently not by Cameron (1974). Its presence is presumably related to cutaneous respiration, since the lungs of this species are small and poorly vascularised (Czopek 1955) and the skin shows no hydroosmotic response to AVT. Similar capillary

networks are present in the skin of many urodeles and anurans (Czopek 1965). some of which also lack a cutaneous hydroosmotic response.

The lateral ridges on the dorsum of *L. archeyi*, and to a lesser extent *L. hochstetteri*, contain aggregations of large granular glands. Amphibian granular glands often contain irritants or toxins (Lindemann and Voûte 1976); whether this is also the case for leiopelmatid frogs is unknown. Further work is also required to establish the contents and function of the unidentified glands present in dorsal skin of *L. archeyi*. Amphibian skin typically contains only two types of alveolar glands (mucous glands and granular glands), although specialised types such as lipid glands (Blaylock *et al.* 1976) and hedonic glands (Truffelli 1954) have been reported for particular taxonomic groups.

Pelvic skin of the hylid *Litoria aurea* differs from that of *Leiopelma archeyi* and *L. hochstetteri* in containing prominent and well-vascularised mounds. These mounds become smaller and less vascularised towards the pectoral region and are absent from dorsal skin. Drewes *et al.* (1977) described similar structures in pelvic skin from *Chiromantis petersi* and proposed for them the term "verrucae hydrophilica", clearly implying a function in rapid water uptake. Well-vascularised pelvic mounds have also been reported for other hylids and rhacophorids, as well as leptodactylids, ranids and hyperoliids (Noble 1925; Cameron 1974; Blaylock *et al.* 1976; Kobelt and Linsenmair 1986). In *Hyperolius viridiflavus*, a small (0.3-2.5 g) hyperoliid from the dry African savannah, the capillary-to-surface distance at the base of the mounds is as little as 8-10 μm (Kobelt and Linsenmair 1986), less than seen here for *Litoria aurea* (20 μm). Pelvic skin of bufonid toads also has surface irregularities (Lillywhite and Licht 1974), although these may be evident as flattened areas separated by wrinkles or channels, rather than as raised mounds (Cameron 1980). In several anurans, capillaries in the mounds or non-channelled areas are said to penetrate into the epidermis (Noble 1925; Drewes *et al.* 1977; Cameron 1980). In *Litoria aurea*, the superficial capillaries are carried in finger-like, outward projections of dermis; these are surrounded almost, but not entirely, by downward thickenings of epidermis.

Although the mounds and intermound areas of pelvic skin of *Litoria aurea* are well supplied with superficial capillaries and with blood

vessels in the tela subcutanea, a functional circulatory system is clearly not essential for rapid water uptake *in vitro*. Isolated unperfused preparations of pelvic skin showed a high mean resting rate of OWF ($96 \text{ mg cm}^{-2} \text{ h}^{-1}$), which increased to $332 \text{ mg cm}^{-2} \text{ h}^{-1}$ when the skin was exposed to AVT ($3 \times 10^{-8} \text{ mol l}^{-1}$; Chapter 5). These values are higher than for pelvic skin of *L. archeyi* (resting $41 \text{ mg cm}^{-2} \text{ h}^{-1}$; stimulated $97 \text{ mg cm}^{-2} \text{ h}^{-1}$). Intact *Litoria aurea* also rehydrate more rapidly from shallow water than do *L. archeyi*: over the first 15 min of rehydration *Litoria aurea* absorbed water at a rate of $292 \text{ mg cm}^{-2} \text{ h}^{-1}$ (Appendix II) whereas *L. archeyi* gained $123 \text{ mg cm}^{-2} \text{ h}^{-1}$ (Chapter 3).

One factor which may contribute to more rapid water uptake through pelvic skin of *Litoria aurea* than that of *L. archeyi* may be the increased SA resulting from the presence of mounds in the former species. However, estimates of the magnitude of this increase suggest that it is relatively small and therefore unlikely to be the sole explanation. In paraffin-embedded sections, the mounds have a mean basal diameter of $655 \mu\text{m}$, and a mean height of $114 \mu\text{m}$ above the non-glandular intermound areas ($151 \mu\text{m}$ minus $37 \mu\text{m}$, Table 6.2). Using these data, and assuming that the mounds approximate in shape a portion of a sphere, the increase in SA resulting from the presence of mounds is calculated to be about 8%. In some bufonids, sculpturing of ventral skin further increases the area of skin participating in water absorption in intact animals, since water is channelled by capillary action to regions of skin not in immediate contact with surface moisture (Lillywhite and Licht 1974). Overton (1904) and Cameron (1974) have suggested that capillary transport of water may also occur between the mounds of hylids.

In conclusion, pelvic skin of *Litoria aurea* shows two structural features which may contribute to rapid water uptake from localised sources. These features (hypervascularisation and the presence of mounds) are not evident in pelvic skin of *L. archeyi*, which nevertheless shows a hydroosmotic response. Pelvic skin of *L. hochstetteri* is more densely vascularised than that of *L. archeyi*, but shows no cutaneous hydroosmotic response. Further differences between pelvic skin of these three species may well be present at the ultrastructural level.

CHAPTER 7

NITROGEN EXCRETION

INTRODUCTION

The metabolism of proteins in the diet of animals leads to formation of ammonia. Ammonia is a toxic substance, interfering with acid/base balance, oxidative metabolism and membrane transport (Campbell 1973), and is removed from the body in a form which is closely related to the availability of water in the environment (Schmidt-Nielsen 1983). Many aquatic animals excrete ammonia in highly dilute form as the end-product of protein metabolism. However, where water turnover is more restricted, ammonia is converted to less soluble and less toxic substances such as urea or uric acid. Such conversions require energy and the synthesis of appropriate enzymes (Balinsky 1970).

The major nitrogenous wastes of most anurans are ammonia and urea (Balinsky 1970). Aquatic larvae or tadpoles are often predominantly ammonotelic (Munro 1939, 1953; Cree 1985a), but during metamorphic climax (the period when the forelimbs emerge and the tail is reabsorbed) ammonia excretion decreases and the ability of the liver to synthesise urea is rapidly increased (Wixom *et al.* 1972). Most frogs and toads which spend at least part of their adult life out of water are ureotelic (Balinsky 1970), as are some larvae which develop in conditions of low or intermittent water supply (Shoemaker and McClanahan 1973; Jones 1980).

Some aquatic anurans remain ammonotelic as adults. These include *Xenopus laevis*, which is ammonotelic in water (Munro 1953) but ureotelic when water turnover is reduced (Balinsky *et al.* 1961). Other fully aquatic frogs may be obligatorily ammonotelic and suffer high mortality during water shortages (Shoemaker and McClanahan 1980).

A few anurans from xeric habitats are uricotelic as adults. These include *Chiromantis xerampelina* (Loveridge 1970) and *Phyllomedusa sauvagei* (Shoemaker and McClanahan 1975, 1982) which share the additional reptilian characteristic of low cutaneous evaporative water loss (Chapter 1). Uricotelism plays an important role in water conservation in these frogs, since uric acid is relatively insoluble

and can be excreted with loss of very little liquid urine (Shoemaker and McClanahan 1975).

The aim of the following experiments was to determine whether the proportions of ammonia, urea and uric acid excreted by adults of the three species of *Leiopelma* vary in accordance with habitat water availability. Other nitrogenous wastes such as amino acids, creatine and creatinine account for only a minor proportion of excreted nitrogen in anurans (Cragg *et al.* 1961) and were not examined here. In addition, a preliminary study of ammonia and urea excretion during development of the terrestrial eggs and larvae of *L. archeyi* was made, in conjunction with the water balance studies described in Chapter 8.

MATERIALS AND METHODS

1. Adults

Nitrogen excretion of adults was examined using two methods: collection of 24 h urine samples in bathing water (Shoemaker and McClanahan 1980) and direct collection of bladder urine (Espina *et al.* 1980). For collection of 24 h samples, animals with bladder urine removed were weighed and placed in glass jars containing 3.0 or 4.0 ml of tap water, sufficient to cover each frog's ventral surface to a depth of about 2 mm. Tap water contained no detectable urea or ammonia, and was acidified to pH 6.9 with a few drops of hydrochloric acid to prevent possible loss of excreted ammonia to the atmosphere. The jars containing the frogs were covered with cheesecloth, shielded with a sheet of black plastic from direct overhead lighting, and left for about 24 h at $16 \pm 1^\circ\text{C}$. The frogs' bladder contents were added to the bathing water at the end of this time. A urea standard which was left on a separate occasion in the same room showed no change in concentration over 24 h.

The above experiments were carried out using stock *L. archeyi* (2.3-4.9 g) and *L. hochstetteri* (3.5-9.1 g) in November 1984. Feeding was discontinued 1 wk prior to the experiment to help standardise the nutritional status of the animals. *L. hamiltoni* (4.4-8.2 g) were collected from Maud Island in May 1985 and held in Wellington for 1 wk at 15°C with access to woodlice (Dr B.D.Bell, pers. comm.). Upon arrival in Hamilton, they were held for only 24 h without food before

use in nitrogen excretion experiments, since some were already in lean condition.

Bladder urine samples were collected in polythene cannulae at the beginning of these experiments and at similar times from stock animals. Proportions of urea and ammonia nitrogen in these samples were compared with those in 24 h bathing water samples, since Espina *et al.* (1980) have suggested that the former method may yield erroneous results. Large bladder urine samples were also used for measurements of osmotic concentration (Appendix III).

2. Nitrogen excretion during development of *L. archeyi*

Patterns of ammonia and urea excretion during development of *L. archeyi* at 15-20°C were examined in conjunction with egg and larval water balance studies (Chapter 8). Developing eggs and larvae were staged according to the 20-point staging scheme given in Appendix IV, and ecological aspects of development in natural nests are described in Chapter 8. Nitrogen excretion studies focussed on three broad developmental stages (eggs, newly hatched larvae and larvae near the end of metamorphosis) and used material from four sources. Group 1 consisted of eggs kept separately in water during development. Eggs in Groups 2 and 3 were kept in dry, covered, plastic Petri dishes. Strips of moist paper towels were coiled around the inner edge of each dish to keep the humidity high, but the eggs did not contact these and were thus prevented from absorbing water. Group 4 consisted of larvae collected near the end of development and kept on moist paper towels. Groups 1, 2 and 4 were collected from the Tapu study area during the 1984 breeding season (October 1984-February 1985) and Group 3 in December 1985. Because *L. archeyi* egg clusters contain few eggs (1-13; Bell 1985b) and their collection is restricted by permit, sample sizes were necessarily small for all groups.

Proportions of urea and ammonia nitrogen in samples of water bathing three eggs kept about one-third immersed (Group 1) were examined. Each egg was kept from stage 3 of development in ~1.8 ml sterile deionised water changed every second day. Bathing water from an "early" stage of intracapsular development (4-6 d after collection, embryos at stages 4-5) and a "late" stage (2-6 d prior to hatching, larvae at stages 7-11) was analysed. Following the late stage collection period, a sample of intracapsular fluid (Martin and Cooper

1972; also known as perivitelline fluid - Salthe 1965) was obtained from the largest egg by piercing the capsule with a drawn-out capillary tube. After hatching, the three larvae were transferred to Petri dishes containing ~0.7 ml sterile deionised water (water depth 2-3 mm). Samples of bathing water from the first two 2 d periods after hatching were analysed.

Group 2 eggs were collected at stage 8. Nitrogen excretion of larvae hatching from these eggs was analysed in the same way as for larvae from Group 1, except that the volume of bathing water was reduced in some cases from 0.7 to 0.4 ml to increase the concentration analysed. A sample of intracapsular fluid released at hatching from the first egg to hatch was also analysed. The remaining two eggs released little or no separate fluid at hatching but a few drops adhered to or remained inside the ruptured capsules. Capsules were therefore stirred vigorously in 0.3 ml deionised water and samples of the water were analysed.

Group 3 eggs were used in an attempt to determine whether larvae store urea within their bodies. In addition, some samples of intracapsular fluid and of bathing water from the first 2 d period after hatching were analysed. The cluster contained four fertile eggs at stage 8 when collected. Larvae from two eggs were immediately removed from their capsules, intracapsular fluid was collected, and a preliminary trial was carried out to determine the urea content of the larvae. This indicated that urea was present in the supernatant from samples homogenised in water, but because of protein interference in the analysis, the absolute level could not be determined. The remaining two larvae were kept until hatching, and then their urea and ammonia nitrogen excretion over 2 d in 0.4 ml deionised water was measured. A sample of intracapsular fluid was also obtained from one ruptured egg at hatching. One larva died soon after, and the urea and ammonia nitrogen content of the remaining larva were analysed at stage 14. Tissue and yolk from the decapitated larva were separated and coarsely homogenised in 0.2 ml 10% trichloroacetic acid (TCA) to cause deproteinisation. Homogenisation was performed using a Kontes Micro-ultrasonic cell disrupter (Electro-mech Instrument Co., Perkasie, USA) and samples were held in icewater to prevent hydrolysis of urea. After centrifugation, the supernatant was treated with a quantity of sodium hydroxide calculated to restore pH to neutral, and 80 μ l samples were analysed for urea and ammonia nitrogen. Standards

and blanks were prepared with TCA and NaOH in the same manner.

Nitrogen excretion from six stage 17 larvae (Group 4) was examined about 2-3 wk before the completion of metamorphosis. These "late stage larvae" had been collected 6 d previously and used without apparent harm in dehydration/rehydration experiments 3 d previously (Chapter 8). Each larva was placed in 1.0 ml deionised water for 24 h, and the bathing water was later analysed.

3. Analysis

Ammonia and urea nitrogen concentrations in the bathing water of eggs, larvae and adults, intracapsular fluid of eggs, homogenates of larval tissue and bladder urine of adults were determined using a colorimetric urease-Berthelot reaction (Sigma Diagnostic Kit No. 640). Most samples were frozen at -18°C before analysis but some were analysed immediately. Some modifications to the procedure (Sigma Technical Bulletin No. 640) were made, depending on the concentrations present in each sample and the volume available for analysis. Sample volumes were between 10 and 100 μl and the volume of water added to the reaction mixture at Step 5 was reduced from 5.0 ml to 1.0-3.0 ml to produce stronger colour development. Standards and blanks were prepared accordingly. Bladder urine and intracapsular fluid samples were diluted by one-fifth or one-tenth with deionised water prior to analysis. Absorbance was read at 560 nm on a Spectronic 20 spectrophotometer (Bausch and Lomb, USA).

Uric acid was measured in 24 h bathing water collections from adult frogs. Samples of 0.2 ml were analysed using Sigma Diagnostic Kit No. 292-UV following the supplied procedure, and absorbance was read at 292 nm on a UV-120-02 spectrophotometer (Shimadzu, Japan).

RESULTS

1. Adults

Analysis of 24 h bathing water samples showed that adults of the three *Leiopelma* species excrete relatively large amounts of urea nitrogen, small amounts of ammonia nitrogen, and no uric acid above the minimum detection level of 1 mg 100 ml^{-1} (Table 7.1). Three samples of

TABLE 7.1 Nitrogen excretion of adult *Leiopelma* species ($\bar{x} \pm SE$). Nitrogen (N) excreted is the sum of urea N plus ammonia N (no uric acid was detected in 24 h bathing water samples).

Species	24 h bathing water		Bladder urine	
	N excreted ($\mu\text{g g}^{-1} \text{d}^{-1}$)	Percentage as ammonia N (%)	N excreted ($\text{mg } 100\text{ml}^{-1}$)	Percentage as ammonia N (%)
<i>L. archeyi</i>	49 ± 10 n = 7	5 ± 2	99 ± 23 n = 7	3 ± 1
<i>L. hamiltoni</i>	118 ± 15 n = 8	7 ± 2	232 ± 27 n = 8	5 ± 1
<i>L. hochstetteri</i>	44 ± 10 n = 8	7 ± 3	30 ± 10 n = 5	4 ± 2

L. archeyi bladder urine analysed for uric acid also gave negative results. Since no uric acid was detected for any species, the amount of nitrogen excreted is considered to be the sum of ammonia nitrogen plus urea nitrogen. The percentage of nitrogen excreted as ammonia in 24 h samples varied from $5 \pm 2\%$ ($\bar{x} + SE$) in *L. archeyi* to $7 \pm 3\%$ for *L. hochstetteri*. Proportions in urine collected directly from the bladder were similar. Values for the three species were compared by one-way analysis of variance and showed no significant differences, whether comparisons were for 24 h bathing water samples ($F = 0.25, P > 0.05$) or for bladder urine ($F = 0.65, P > 0.05$).

The concentration of excreted nitrogen in 24 h samples was very similar for stock *L. archeyi* and *L. hochstetteri* from which food had been withheld for 1 wk (Table 7.1). However, the concentration in bladder urine of *L. hochstetteri* was low compared with that of *L. archeyi*. Concentrations of waste nitrogen for *L. hamiltoni* were more than twice those for the other two species, both in 24 h samples and in bladder urine (Table 7.1).

2. Nitrogen excretion during development of *L. archeyi*

Excretion from eggs and newly hatched larvae

As no uric acid was detected in urine from adult *L. archeyi*, samples obtained from eggs and larvae were analysed for ammonia and urea nitrogen only. Changes in the proportion excreted as ammonia from Group 1 eggs kept in water and the larvae which hatched from these eggs are shown in Table 7.2. No urea or ammonia was detected in bathing water of early stage eggs, and only low concentrations (equivalent to mean values of $\sim 33 \mu\text{g}$ nitrogen (N) g^{-1} larval weight d^{-1} or less) were present in water bathing late stage eggs, hatched eggs and larvae which had hatched up to 4 d previously. The percentage of nitrogen excreted as ammonia declined from a mean value of 89% in water bathing late stage eggs to 19% for larvae which had hatched 3-4 d previously; however, because the concentrations were so low these percentages are only approximate.

Nitrogen excretion from larvae hatching from eggs kept in dry Petri dishes at high humidity (Groups 2 and 3) is shown in Table 7.3. The water in which ruptured capsules were washed contained $64 \pm 10 \mu\text{g}$ N g^{-1} larval weight, of which 89% was ammonia. Hatched larvae excreted similar amounts of nitrogen in each of the first two 2 d periods after

TABLE 7.2 Percentage of nitrogen excreted as ammonia N by eggs and newly hatched larvae of *L. archeyi* kept in water ($\bar{x} \pm SE$). Values for hatched larvae are for the first two 2 d periods after hatching (n = 3; larval weight = 63 ± 7 mg).

Late stage eggs Stages 7-11	Hatching water Stages 8-12	Hatched larvae	
		1st 2 d Stages 9-12	2nd 2 d Stages 9-12
89 ± 9	80 ± 2	49 ± 5	19 ± 3

TABLE 7.3 Nitrogen excretion of *L. archeyi* larvae hatching from eggs kept on dry plastic (Groups 2 and 3; see text) ($\bar{x} \pm SE$). Capsule wash = water in which ruptured capsules were washed. Larval weight is given in parentheses.

Parameter	Capsule wash Stage 15 (75 ± 1 mg) n = 2	Hatched larvae	
		1st 2 d Stages 10-15 (79 ± 5 mg) n = 5	2nd 2 d Stages 11-16 (74 ± 1 mg) n = 3
Nitrogen excreted	64 ± 10 $\mu\text{g g}^{-1}$	33 ± 8 $\mu\text{g g}^{-1} \text{d}^{-1}$	41 ± 17 $\mu\text{g g}^{-1} \text{d}^{-1}$
Percentage as ammonia N	89 ± 4	54 ± 11	44 ± 16

hatching (mean values 33 and 41 $\mu\text{g N g}^{-1} \text{d}^{-1}$), but the proportion excreted as ammonia declined from 54 to 44% over the same interval.

Nitrogen content of intracapsular fluid

Intracapsular fluid was collected at hatching (either spontaneous or deliberate) from four eggs kept on dry plastic, and by piercing the capsule of one egg kept in water (Table 7.4). In all cases, ammonia was the predominant form of nitrogen present and the percentage was lowest for the two least-developed eggs (stage 8). Three more developed eggs (stages 10-13) had higher percentages (84-90%). Concentrations of urea (as opposed to urea nitrogen) were fairly similar for the four eggs on dry plastic, showing a tendency to increase slightly as development progressed. Concentrations of ammonia showed more dramatic changes. In one egg which hatched at stage 13, the concentration of ammonia (4.93 mmol l^{-1}) was 8.6x the mean value for two eggs from the same cluster at stage 8. An even higher concentration of ammonia (6.04 mmol l^{-1}) was present in intracapsular fluid from a stage 10 egg, which, judging by its final weight, had absorbed the least amount of water during development of all eggs in Table 7.4. Intracapsular fluid from a single egg at stage 11 kept in water contained concentrations of urea and ammonia an order of magnitude lower than eggs of stages 10-13 kept on dry plastic.

Nitrogen content of larvae

The urea and ammonia nitrogen content of one hatched larva at stage 14 was measured. Urea was detected at a concentration of 36 $\mu\text{g N g}^{-1}$ larval weight, of which 29% was in larval tissue and the remainder in yolk. Ammonia was present at a concentration of 26 $\mu\text{g N g}^{-1}$ and was equally distributed between yolk and larval tissue.

Excretion from larvae near the end of metamorphosis

Larvae of *L. archeyi* at stage 17 (mean weight 104 ± 2 mg) excreted a relatively large amount of nitrogen in 24 h bathing water samples (162 ± 20 $\mu\text{g N g}^{-1} \text{d}^{-1}$). Ammonia accounted for less than one-quarter of this ($23 \pm 3\%$).

*Summary of changes in proportion of nitrogen excreted as ammonia during development of *L. archeyi**

Changes in the percentage of nitrogen excreted as ammonia during development of *L. archeyi* are summarised in Fig. 7.1. Apart from a relatively low proportion of ammonia in intracapsular fluid of eggs at

TABLE 7.4 Percentage of ammonia N and concentrations of ammonia and urea in intracapsular fluid of *L. archeyi* eggs ($\bar{x} \pm \text{SE}$). Intracapsular fluid was collected following spontaneous or deliberate hatching of eggs kept on dry plastic, or by piercing the capsule of an egg kept in water. Egg weight prior to hatching is given in parentheses. [Weights for eggs in columns 1 and 3 give mean egg weight for live eggs in this cluster at collection. When kept in a dry Petri dish at high humidity, little or no further weight change occurs (see FIG. 8.3, Chapter 8).]

Nitrogenous product	Eggs kept on dry plastic			Egg kept in water
	Stage 8 (0.43 g) n = 2	Stage 10 (0.26 g) n = 1	Stage 13 (0.43 g) n = 1	Stage 11 (0.99 g) n = 1
Percentage of nitrogen excreted as ammonia N	53 \pm 2	90	84	89
Urea (mmol l ⁻¹) ¹	0.31 \pm 0.03	0.33	0.48	0.03
Ammonia (mmol l ⁻¹) ¹	0.57 \pm 0.10	6.04	4.93	0.45

Note:

1. Concentrations of urea and ammonia (not urea N and ammonia N).

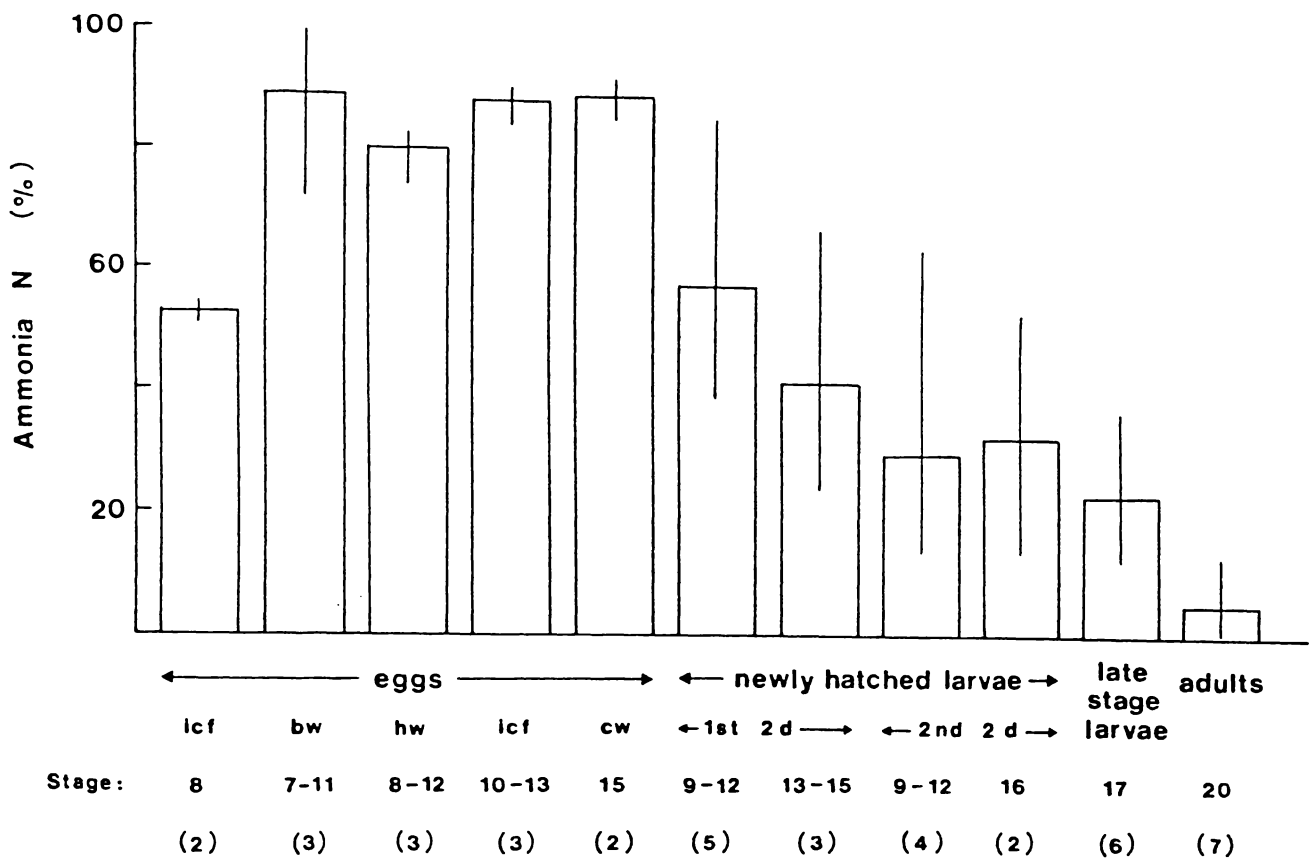


FIG. 7.1 Summary of changes in the proportion of nitrogen excreted as ammonia N during development of *L. archeyi* (\bar{x} and range). Data for newly hatched larvae are for the first and second 2 d periods after hatching, and have been grouped from data in TABLES 7.2 and 7.3 according to the stage of development (icf = intracapsular fluid, bw = bathing water, hw = hatching water, cw = water in which ruptured capsules were washed; sample sizes given in parentheses).

stage 8, a general decline during larval development is apparent. Bathing water from late stage eggs, intracapsular fluid of late stage eggs, and water in which eggs hatched or in which ruptured capsules were washed all contained similar proportions of ammonia nitrogen (mean values 80-89%). Newly hatched larvae of stages 9-12 excreted 58% of waste nitrogen as ammonia during the first 2 d after hatching, and the proportion was lower (42%) for more developed larvae (stages 13-15). The proportion declined for both groups of larvae during the second 2 d period after hatching. The proportion was further reduced for larvae near the end of metamorphosis (23%), and was lowest in fully metamorphosed adults (5%).

DISCUSSION

1. Adults

Adult *L. archeyi* and *L. hamiltoni* are strongly ureotelic, excreting little ammonia and no uric acid. Proportions of nitrogen excreted as ammonia in 24 h bathing water samples are 7% or less, values which are similar to those for other terrestrial or arboreal anurans but much lower than for highly aquatic, ammonotelic species (Table 7.5).

In common with many other semiaquatic species *L. hochstetteri* is also strongly ureotelic, excreting 7% of waste nitrogen as ammonia when in water. This proportion is similar to that for *L. archeyi* and *L. hamiltoni*, despite the fact that *L. hochstetteri* is more aquatic. Ureotelism is probably an important factor in allowing *L. hochstetteri* to move away from streams when conditions are wet (Chapter 2), since anurans which are obligatorily ammonotelic suffer high mortality when water turnover is reduced (Shoemaker and McClanahan 1980).

In all three *Leiopelma* species, urine collected directly from the bladder contained similar percentages of ammonia nitrogen to those present in 24 h bathing water samples. This was also the case in anurans examined by Shoemaker and McClanahan (1980, 1982) and indicates that analysis of directly collected bladder urine is a valid method for determining partition of nitrogenous excretory products. This conclusion contrasts with that of Espina *et al.* (1980), who reported that urine of the aquatic frog *Caudiverbera caudiverbera* contained 14% ammonia nitrogen when collected from the bladder by

TABLE 7.5 Nitrogen excretion of non-uricotelic adult anurans. Nitrogen excreted is the sum of ammonia N plus urea N (values for *Leiopelma* species are from 24 h bathing water samples; - = no data; habitat codes as in TABLE 4.6).

Species	Habitat	T (°C)	Period of prior fasting (d)	Nitrogen excreted ($\mu\text{g g}^{-1} \text{d}^{-1}$)	Percentage as ammonia (%)	Reference
Ammonotelic species (in water):						
<i>Pipa pipa</i>	A	-	-	109	93	Cragg <i>et al.</i> (1961)
<i>Hymenochirus sp.</i>	A	-	-	125	78	Cragg <i>et al.</i> (1961)
<i>Xenopus laevis</i>	A	-	-	273	62	Cragg <i>et al.</i> (1961)
<i>Rana kuhli</i>	A	25	~1-3	172	65	Shoemaker and McClanahan (1980)
<i>R. signata</i>	A/SA	25	~1-3	438	96	Shoemaker and McClanahan (1980)
Ureotelic species:						
<i>R. esculenta</i>	SA	-	-	53	9	Cragg <i>et al.</i> (1961)
<i>R. clamitans</i>	SA	-	-	-	8	Schmid (1968)
<i>R. pipiens</i>	SA	-	-	-	9	Schmid (1968)
<i>R. septentrionalis</i>	SA	-	-	-	16	Schmid (1968)
<i>Litoria raniformis</i>	SA	23	7-8	156	3	Cree (1985a)
<i>R. sylvatica</i>	T	-	-	-	7	Schmid (1968)
<i>R. temporaria</i>	T	-	-	102	8	Cragg <i>et al.</i> (1961)
<i>Bufo americanus</i>	T	-	-	-	6	Schmid (1968)
<i>B. boreas</i>	T	-	30	600	5	Shoemaker and Bickler (1979)
<i>B. bufo</i>	T	-	-	113	5	Cragg <i>et al.</i> (1961)
<i>B. calamita</i>	T	-	-	92	6	Cragg <i>et al.</i> (1961)
<i>B. hemiophrys</i>	T	-	-	-	18	Schmid (1968)
<i>B. woodhousei</i>	T	22	14	195	23	Jones (1982)
<i>Litoria ewingi</i>	T/ARB	23	7-8	176	3	Cree (1985a)
<i>Hyla arborea</i>	ARB	-	-	157	5	Cragg <i>et al.</i> (1961)
<i>H. versicolor</i>	ARB	-	-	-	4	Schmid (1968)
<i>Leiopelma archeyi</i>	T	16	7	49	5	Present study
<i>L. hamiltoni</i>	T	16	1	118	7	Present study
<i>L. hochstetteri</i>	SA	16	7	44	7	Present study

abdominal pressing. but 55% when collected over 24 h in a cloacal cannula. These authors interpreted the latter value as the "correct" one.

The amount of nitrogen excreted over 24 h by *L. archeyi* ($49 \mu\text{g g}^{-1} \text{d}^{-1}$) and *L. hochstetteri* ($44 \mu\text{g g}^{-1} \text{d}^{-1}$) was low compared with values for other anurans (Table 7.5). This suggests that these animals, which were kept in moderately cool conditions and had not been fed for 7-8 d, were metabolising little protein. Freshly collected *L. hamiltoni* which had been without food for only one day excreted a larger amount ($118 \mu\text{g g}^{-1} \text{d}^{-1}$). Balinsky and Baldwin (1961) have reported that nitrogen excretion from *X. laevis* is initially high for the first few days after a large meal but declines to reasonably constant levels after about 8 d without food, and the results obtained here are consistent with this observation.

The relationship between mode of nitrogen excretion and presence or absence of a cutaneous hydroosmotic response in anurans has been discussed by Shoemaker and McClanahan (1980). Of three species in their study which lacked a cutaneous response to dehydration, one (*Rana kuhli*) was obligatorily ammonotelic, one (*R. glandulosa*) was ammonotelic in water and ureotelic following a water shortage, whilst the third (*R. blythi*) was consistently ureotelic. *X. laevis*, which also lacks a cutaneous response, is facultatively ammonotelic (Balinsky *et al.* 1961) and the present study shows that *L. hochstetteri* is ureotelic. Thus, there is no single relationship between lack of a cutaneous hydroosmotic response and mode of nitrogen excretion: species lacking the first attribute may be obligatorily ammonotelic, facultatively ammonotelic or obligatorily ureotelic. This reflects the different selection pressures favouring the two characteristics: ureotelism is advantageous where water turnover is low, whereas the ability to rehydrate rapidly is advantageous only if dehydration actually occurs (Shoemaker and McClanahan 1980).

2. Nitrogen excretion during development of *L. archeyi*

Experiments examining changes in the proportions of urea and ammonia nitrogen excreted during larval development of *L. archeyi* produced initially surprising results. Bathing water of late stage eggs, intracapsular fluid, water into which larvae hatched and water in which ruptured capsules were washed all contained predominantly

ammonia nitrogen. Newly hatched larvae excreted very low levels of nitrogen [about one-fourth to one-thirtieth those reported for other feeding or non-feeding anuran larvae; e.g., Munro (1953); Shoemaker and McClanahan (1973, 1982); Jones (1980); Cree (1985a)], and this was approximately evenly distributed between ammonia and urea for the first 2 d after hatching.

Larvae inside the terrestrial eggs of *L. archeyi* were expected to be ureotelic for two reasons: conversion to urea would avoid high levels of toxic ammonia (Shoemaker and McClanahan 1973) and urea could also act as an osmotically active substance, enhancing water flow into the egg from surrounding soil or from the body fluids of a brooding parent (Taigen *et al.* 1984). One possibility which could explain the low levels of nitrogen excreted by newly hatched larvae and the low proportion of urea in intracapsular fluid is that urea or some other form of waste nitrogen is produced and stored within the body. Urea storage has been reported for larvae inside the terrestrial eggs of *Crinia georgiana* (Martin and Cooper 1972) and in terrestrial larvae of *Leptodactylus albilabrus* (Candelas and Gomez 1963). Larval *Leptodactylus bufonius* also probably store urea, since large amounts (more than $2000 \mu\text{g N g}^{-1} \text{d}^{-1}$) are excreted during the first day in water following removal from underground foam nests (Shoemaker and McClanahan 1973).

Compared with larvae of *Leptodactylus bufonius*, newly hatched *L. archeyi* larvae excreted very little urea (maximum mean value $23 \mu\text{g N g}^{-1} \text{d}^{-1}$). This suggests that if urea is stored within *L. archeyi* larvae it is not excreted immediately upon hatching. In one attempt to measure urea content, urea was detected in a stage 14 larva at a concentration of $36 \mu\text{g N g}^{-1}$. This is lower than expected as evidence of urea storage, since it represents only 51% of the amount excreted by the same larva in the 2 d period immediately after hatching. Possibly the fact that ultrasonic cell disruption failed to completely homogenise the sample caused an underestimate of true urea content. Further work is therefore needed to confirm whether significant urea storage occurs.

Regardless of whether or not larval *L. archeyi* store urea, ammonia is produced and may be present in the intracapsular fluid of the egg at surprisingly high concentrations. At least two factors probably interact to determine the concentration of nitrogenous wastes in

intracapsular fluid: the stage of larval development and the amount of water absorbed by the egg. Eggs containing well-developed larvae would be expected to have relatively high concentrations because of their greater total protein turnover, and eggs which had absorbed large amounts of water during development would have relatively low concentrations because of the diluting effect of absorbed water. Although the preliminary nature of this study and the small number of eggs available meant that only a few analyses of intracapsular fluid were made, the results obtained are consistent with the above predictions (Table 7.4). Concentrations of urea and ammonia were an order of magnitude lower in the single egg kept in water than in similarly developed eggs kept on dry plastic. Of those kept on dry plastic, concentrations were lowest in the least developed eggs.

The concentrations of ammonia in intracapsular fluid of the two most developed eggs kept on dry plastic ($4.9-6.0 \text{ mmol l}^{-1}$) are surprisingly high, being close to the lethal limit for two amphibian larvae developing in conditions where water is in short supply. Larvae removed from the "uterus" of the viviparous urodele *Salamandra salamandra* tolerate 5 mmol l^{-1} ammonia (as NH_4Cl) but die when placed in 10 mmol l^{-1} ammonia (Schindelmeiser and Greven 1981). Larval *Leptodactylus bufonius* cannot survive 5 mmol l^{-1} ammonia (as NH_4Cl), and since higher concentrations are sometimes present in the foam of natural nests it is possible that accumulation of ammonia limits larval survival (Shoemaker and McClanahan 1973).

If the tolerance of *L. archeyi* larvae to ammonia is similar to that of larval *S. salamandra* and *Leptodactylus bufonius*, then it is conceivable that the length of life within the egg capsule could be limited by rising ammonia concentrations. These concentrations would in turn be dependent on the availability of water for absorption during development. Eggs which absorbed large amounts of water would be able to dilute ammonia to a greater extent than eggs in dry conditions. Larvae inside eggs where little water uptake occurred might therefore die or be forced to hatch at an earlier stage of development and/or a smaller size to avoid lethal concentrations of ammonia.

To summarise, these observations on nitrogen excretion during development of *L. archeyi* show some general trends and suggest areas which need further examination. Larvae within egg capsules apparently

excrete more ammonia than urea. Hatched larvae rapidly become ureotelic, and the degree of ureotelism increases as development progresses. Fully metamorphosed adults are the most ureotelic of all stages examined. However, the effects of development and water absorption on ammonia and urea concentrations in intracapsular fluid need to be independently examined, and the possibility that nitrogenous wastes are stored within the body of the larva as urea or some other substance needs confirmation. In particular, the tolerance of larvae to ammonia and the hypothesis that rising concentrations of ammonia in intracapsular fluid of eggs unable to absorb water could affect survival or force hatching would make most interesting topics for future investigation.

CHAPTER 8

WATER BALANCE OF EGGS AND LARVAE OF *L. ARCHEYI*

INTRODUCTION

In comparison with the abundance of studies on water balance of adult anurans, relatively little attention has been given to the problems of water regulation facing anuran eggs and larvae which develop on land. Terrestrial anuran eggs lack an impermeable shell, and thus, like most adult anurans in dry surroundings, face the problem of preventing rapid desiccation. Many instances of parental care have evolved amongst anurans having non-aquatic oviposition, including egg attendance, egg and/or larval transport on or within a parent's body, and viviparity (see Wells 1981 for review). Prevention of egg desiccation has often been considered an important function of such activities (Salthe and Mecham 1974), but in most cases experimental evidence is scant (Wells 1981).

L. archeyi lays clusters of relatively few (1-13) large yolky eggs on land (Bell 1982a, 1982b, 1985b). These are deposited in secluded, moist terrestrial nest sites and are brooded by the male frog for several weeks until hatching. Once hatched, the larvae then continue development on the back of the male in the nest site. Development in the rarer *L. hamiltoni* is similar, but has only been observed in captive situations. Bell (1982a, 1985b) suggested that the primary function of parental care in *L. archeyi* and *L. hamiltoni* was to ensure adequate humidity or water supplies for the eggs and hatched larvae.

This chapter examines aspects of water balance in the terrestrial eggs and larvae of *L. archeyi*. Field and laboratory studies were undertaken to answer the following questions:

- (i) do eggs lose water by evaporation at the high rates typical of other anuran eggs?
- (ii) are eggs able to absorb water from moist or wet surfaces in the laboratory?
- (iii) do eggs in natural nest sites also absorb water during development, and if so, what are the likely sources of this?
- (iv) what effect does the amount of water absorbed by eggs during development have on hatching success?

(v) do hatched larvae share the same major features of water balance (lack of resistance to EWL, ability to rehydrate rapidly) as adult *L. archeyi*?

(vi) what effect does parental care (egg brooding and larval transport) have on water balance of eggs and larvae?

Since the extent of parental care in *L. archeyi* has only recently been discovered (Bell 1985b) and details for field populations remain incomplete, observations on egg brooding, larval development and larval transport are also included.

MATERIALS AND METHODS

1. Field studies

All observations and collections of eggs and larvae were made on the ridgetops of the Tapu study area (Chapter 2). Initial observations made during the spring-summer of 1983/84 (referred to as the 1983 season) showed that eggs and larvae were present between October and February in the same habitat as non-brooding adult *L. archeyi*, and that if their cover was carefully replaced, re-examination of brooding frogs and their clutch of eggs or larvae was possible. In this context, "clutch" refers to a discrete group of eggs or larvae present at a single nest site; each clutch is accompanied by a single adult frog. "Cluster" refers specifically to a clutch of eggs.

A more detailed study was accordingly carried out in the 1984 season. The ridgetops were searched for nest sites on six occasions between October 1984 and February 1985, and nest sites previously found in the same season were re-examined. Three of the eight egg clusters found (Nos 1/84, 2/84 and 8/84) and one clutch of larvae (9/84) were collected for laboratory studies of water balance (present chapter) and nitrogen excretion (Chapter 7). One cluster found in the 1985 season (1/85) was also collected.

In the 1984 and 1985 seasons, the number of eggs, stage of development, and presence of non-developing eggs were recorded for all clusters examined. Each cluster was weighed to the nearest 0.05 g with a spring balance (Pesola, Switzerland) and the maximum capsule diameter of eggs within it was measured with vernier calipers to the

nearest 0.1 mm. Capsule diameters were reproducible to within 0.3 mm (maximum difference between any two of ten consecutive measurements on each of two eggs). The type of substrate beneath the cluster, and the position, colour pattern and SVL of the brooding frog were also noted. Several clusters were found developing on soil, and in some cases a soil sample was returned to the laboratory in a sealed container for measurement of moisture content. Soil water potential was measured *in situ* with a "Quick Draw" soil moisture probe (Soilmoisture Equipment Corp., California, USA) in two instances. In clutches containing hatched larvae, the stage of development, body length (from tip of snout to posterior base of hind limbs) and combined weight of all larvae in the clutch were recorded.

2. Laboratory studies on water balance of eggs

Materials

Permits were obtained for the collection of five clusters over two seasons; these clusters were also used in nitrogen excretion studies (Chapter 7). As mentioned in that chapter, constraints on sample sizes and the number of experiments which could be carried out were inevitable. Attempts were made to supplement the collected material by inducing breeding in captive animals. Mature females and presumed males were treated with various doses of human chorionic gonadotrophin (HCG) or luteinising hormone-releasing hormone (des-Gly¹⁰, [D-Ala⁶]-LH-RH ethylamide) (both from Sigma Chemical Co.). Although these substances have been successful in inducing reproductive behaviour and/or oviposition in some anurans (Russell 1955; Wagner 1981), treatment of *L. archeyi* produced at best only infertile eggs and these were not used in experiments.

Water uptake and evaporative water loss

The ability of eggs to absorb water and to resist evaporative water loss (EWL) was examined using two fertile egg clusters (1/84 and 2/84). These were collected on 22/10/84 and kept on moist moss during transport to the laboratory; the frogs brooding these clusters were also collected.

For water uptake experiments, each cluster was half-immersed in sterile deionised water for one hour. Changes in cluster weight (blotted wet weight) and in egg capsule and yolk diameters were recorded. Weight was measured to the nearest 0.1 mg, and capsule and

yolk diameters to the nearest 0.1 mm. Yolk diameter was estimated by placing the calipers against the outside of the transparent egg capsule, and was reproducible to within 0.3 mm (maximum difference between any two of ten consecutive measurements on each of two eggs).

Each blotted cluster was then transferred to a dry glass Petri dish, and placed in a desiccator over partly dry silica gel for measurement of EWL. Relative humidity and temperature inside the desiccator were recorded using a quick-response hygrometer connected to a CR-21 micrologger (Campbell Scientific Inc., USA). Values recorded at the start and end of each experimental period were averaged.

EWL was measured as the rate of weight loss under the following conditions:

- (i) first 15 min: each cluster was exposed to the air inside the desiccator;
- (ii) second 15 min: three fertile eggs were separated from clutch 2/84 with their capsules intact, and were then placed in separate dishes in the desiccator for 2 h 15 min. Weight loss over the first 15 min was measured to compare with that obtained in (i) above for intact clusters. Changes in weight, capsule diameter and yolk diameter over the entire 2 h 15 min period were also recorded.

To determine whether anuran eggs have any resistance to EWL, rates of EWL from real eggs can be compared with those from replicas of the same size and shape made from 2% agar, which have no resistance to EWL (Spotila and Berman 1976; Taigen *et al.* 1984). In experiments with *L. archeyi* eggs and egg clusters, rates of EWL from model agar eggs or egg clusters were therefore measured simultaneously. Each real egg was matched to an agar egg of similar shape and diameter, which had been fashioned using a plasticene mould and then soaked overnight in deionised water to ensure saturation (Taigen *et al.* 1984). Agar clusters contained the same number of eggs arranged in the same manner as in real clusters.

The effect of brooding by male *L. archeyi* on EWL from eggs was examined using anaesthetised frogs and agar clusters. Real eggs were not used because of the possibility that residues of anaesthetic on the frogs might affect subsequent egg development. Frogs were anaesthetised in 0.03 or 0.06% ethyl m-aminobenzoate (MS-222, Sandoz Ltd. Basle, Switzerland) for 7-15 min until their limbs became

flaccid. Bladder urine was then removed and each frog was placed in the brooding position over a cluster of six agar eggs. Weight loss from the clusters placed in the desiccator for 20 min periods with and without brooding frogs was then recorded.

Further development of eggs in water and on soil

Following EWL experiments, the three eggs separated from clutch 2/84 were each one-third immersed in ~1.8 ml sterile deionised water in a covered glass Petri dish and allowed to develop further at $18 \pm 3^\circ\text{C}$. The intact cluster 1/84 was also kept in shallow water, but the eggs in this cluster failed to develop further for unknown reasons. Each dish was wrapped in aluminium foil and kept in the dark, except when examined. About every second day the water was changed, the eggs were blotted dry and weighed, capsule diameters were measured and the development of embryos was examined to assist in producing a staging scheme. Once hatched, each larva was transferred to ~0.7 ml sterile deionised water changed every second day (water depth 2-3 mm). Samples of bathing water from eggs and hatched larvae were frozen for urea and ammonia analysis (Chapter 7).

The remaining five eggs from clutch 2/84 were placed on moist soil in an unsuccessful attempt to determine whether eggs could absorb soil moisture. Unfortunately, when the cluster was removed for weighing 2 d later, the base of the cluster adhered tightly to the soil and then ruptured, causing the yolks to fall out of their capsules.

Osmotic concentration of intracapsular fluid

One sample of intracapsular fluid was obtained from an egg developing in the laboratory in shallow water, using a drawn-out capillary tube to pierce the gelatinous capsule. Additional samples were taken from three eggs in cluster 1/85, which was collected on 15/12/85 and transported to the laboratory in a dry Petri dish. Intracapsular fluid was obtained from two of these eggs by piercing their capsules immediately upon arrival at the laboratory. The remaining egg was kept in a dry covered Petri dish. Coiled strips of moistened paper towel were placed around the inner edge of the Petri dish to keep the humidity high, but the egg did not contact these and was thus prevented from absorbing water. Intracapsular fluid was collected from the egg when it hatched spontaneously. Osmotic concentrations of intracapsular fluid samples were determined using a vapour pressure osmometer (Wescor Model 5100-C, Utah, USA). Urea and ammonia

concentrations were also determined for samples of intracapsular fluid (Chapter 7).

Effect of moisture on egg development

The effect of water absorption on development of the terrestrial eggs of *Eleutherodactylus coqui* was recently examined by Taigen *et al.* (1984). These workers found that coquí eggs which were prevented from absorbing water produced significantly smaller hatchlings with a lower body water content than those hatching from eggs kept on moist substrates.

An experiment aimed at examining the effect of moisture on development of *L. archeyi* eggs was therefore carried out. Cluster 8/84, which contained seven developing eggs, was collected on 29/11/84 and transported to the laboratory on moist moss. The following day, the eggs were separated with their capsules intact. Three eggs were placed an equal distance apart in a dry plastic Petri dish. Strips of moistened paper towel around the inner edge of the Petri dish kept the humidity high, but the eggs did not contact these. The remaining four eggs were placed an equal distance apart on filter paper saturated with water in a Petri dish. The dishes were covered with lids, wrapped in aluminium foil and kept in the dark at $18 \pm 1^\circ\text{C}$. The eggs were checked daily for hatching, and every 2 d their weights and capsule diameters were measured before the eggs were transferred to a fresh dish. The following details were recorded for larvae upon hatching: blotted weight, stage of development, and body length. Samples of intracapsular fluid released at hatching from the three eggs kept in the dry dish, and of bathing water from the same larvae for the first two 2 d periods after hatching, were kept for nitrogen excretion studies (Chapter 7).

3. Laboratory studies on water balance of hatched larvae

Dehydration and rehydration rates of hatched larvae were examined using larvae which had hatched from eggs kept in the laboratory, and larvae collected from the field (clutch 9/84, collected on 15/1/85). The larvae were dehydrated using a similar procedure to that described for adult frogs in Chapter 3. Each larva was blotted dry, weighed and placed in a dry Petri dish covered with a lid of plastic mesh. Dehydration was carried out inside an open black plastic bag at room conditions (still air, $72 \pm 3\%$ RH, $20 \pm 1^\circ\text{C}$) to about 10% weight loss.

Dehydrated larvae were then half-immersed in deionised water and weighed at intervals during rehydration.

In a second experiment, the effect of transport on an adult frog's back on larval EWL was examined. A preliminary trial was made using three larvae dehydrated for 30 min to 6% weight loss and then placed on the backs of unanaesthetised adult frogs. Although EWL from the larvae fell by 80% over the next 30 min (from 123 ± 10 to 24 ± 5 $\text{mg g}^{-1} \text{h}^{-1}$; $\bar{x} \pm \text{SE}$), movements of the adult frogs caused the larvae to slip from their original position to other parts of the adult, thus possibly influencing larval water exchange.

The final experiment was performed using anaesthetised frogs. This allowed EWL from larvae on the adults' backs to be measured over a longer period without the larvae slipping. Adults were first anaesthetised in MS-222 and then placed on filter paper moistened with additional MS-222 whilst the larvae were on their backs. Each larva was weighed every 30 min, and checked to ensure that it stayed on the adult's back without coming into contact with the moist filter paper. After 2 h on the adults, larvae were half-immersed in deionised water to rehydrate.

4. Developmental stages

Eggs and larvae were staged according to the 20-point staging scheme given in Appendix IV.

RESULTS

1. Field studies

Observations

Field observations were made on three clutches in the 1983 season (each examined twice during development), nine in the 1984 season (examined 1-5 times) and two in the 1985 season (examined once or twice). Eggs were seen between late October and mid-December and hatched larvae between early and late January. By early February, most nest sites were empty. Details of observations for individual clutches in the 1984 and 1985 seasons are summarised in Appendix V.

Nest sites

All clutches of eggs or larvae were found under the cover of single rocks. Apart from the presence of a shallow depression or hollow in which the egg cluster lay, nest sites did not differ obviously from the retreat sites occupied by nearby non-brooding frogs (Chapter 2). The two closest nest sites were about 2 m apart, and no non-brooding frogs were found under the same rocks as clutches.

Most egg clusters lay in shallow depressions (Plate 8.1) although several were in distinct hollows about 20-30 mm deep (Plate 8.2). Substrates beneath egg clusters included soil, twigs, leaf litter, fine tree roots and rock, but when brooding frogs were removed, clusters typically contacted the substrate only lightly (Plate 8.3). Two newly-laid clusters were partially off the ground (loosely adhering to twigs or tree roots) when first seen, but on subsequent observations were on the ground (clutches 3/84 and 4/84, Appendix V). Soil moisture content measured for four clusters developing on soil ranged from 91-283%, and in two instances where water potential was measured, the substrate was essentially saturated (-2 to -16 kPa).

None of the nest sites occupied by clutches 1/84 - 8/84 was occupied by frogs or eggs in the 1985 season (the site of clutch 9/84 was not checked).

Brooding of eggs

On all but one occasion that egg clusters were examined, an adult frog was brooding the cluster. In the exceptional case, a frog was present about 50 mm away; possibly it had been disturbed when the covering rock was lifted. The frog was returned to the egg cluster, and when viewed the following day and on subsequent occasions it was brooding its eggs or carrying hatched larvae in the normal manner (clutch 5/84, Appendix V). SVLs for brooding frogs examined in the 1984 season were 26.0-31.7 mm, values which are comparable with the known size range of preserved adult males (25-31 mm; Bell 1978).

In the brooding position the frog crouches over the egg cluster with his ventral surface tightly apposed to the uppermost surface of the eggs (Plates 8.4 and 8.5). Forelimbs and hindlimbs are held out to the sides of the cluster, and all four feet usually contact the ground. Clutch 7/84 contained seven very large (swollen) eggs when examined on 16/12/84, and the frog brooding this cluster was barely



PLATE 8.1 *L. archeyi* egg cluster 1/85 at stage 8 in the field. Note wet rock surface and smaller, non-developing egg (arrowed).



PLATE 8.2 *L. archeyi* egg cluster 6/84 at stage 6 in the field. Note distinct nest hollow.



PLATE 8.3 *L. archeyi* egg cluster 3/84 at stage 8 in the field. The eggs have separated into two strings; these may represent eggs from separate ovaries (Bell 1982a).



PLATE 8.4 Adult male *L. archeyi* brooding egg cluster 3/84. This photograph was taken just prior to PLATE 8.3.



PLATE 8.5 Adult male *L. archeyi* brooding egg cluster 7/84. These seven stage 9 eggs attained the largest mean weight and capsule diameter of any eggs seen in the field (see FIG. 8.1)



PLATE 8.6 Two hatched early-stage 16 larvae on the back of an adult *L. archeyi*. This clutch was from the 1983 season. Note moist back of adult. Ring (11.5 mm id) is included for scale.

able to cover it (Plate 8.5), but in other cases brooding males largely obscured their clusters from view when the covering rock was lifted (Plate 8.4). Brooding frogs were rarely disturbed when exposed, sometimes remaining motionless for up to 10 min whilst photographs were taken. Most apparently resumed brooding when returned to their clusters after examination, but desertion may have occurred in the cases of clutches 4/84 and 7/84 since their nest sites became empty before development was expected to be complete (Appendix V).

Clutches 3/4, 5/84, 6/84 and 7/84 were examined at night on 15/12/84 (2130 h NZ Standard Time) to see whether the brooding frogs had moved off their egg clusters. Daytime rain had saturated the vegetation, and in the nearby R3 site many emerged frogs were seen (Chapter 9). However, all four brooding frogs remained on their clusters.

Development and water absorption in field egg clusters

Changes in egg weight, capsule diameter and stage of development of clusters examined in the 1984 and 1985 seasons, including those collected or apparently deserted during development, are shown in Fig. 8.1. Four clusters examined on 22/10/84 were all at stage 1 and had presumably been laid within the previous few days. Five weeks later, six clusters examined were at stages 6-8, and nearly three weeks after this four clusters seen were at stages 9-11.

Previous workers have noted that *L. archeyi* eggs kept on moist surfaces in the laboratory increase in diameter during development, implying that water absorption occurs (Archey 1922; N.G. Stephenson 1951a; Bell 1985b). The results of this study show that fertile eggs in natural nests also swell during development (Fig. 8.1A and B). Mean egg weights of clusters examined in the 1984 season were 0.11-0.18 g for stage 1 eggs, 0.13-0.31 g for eggs at stages 6-8, and 0.19-0.47 g for eggs at stages 9-11. Mean capsule diameters of fertile eggs showed parallel increases to those recorded for weight: diameters were 5.6-6.8 mm for stage 1 eggs, 6.0-8.9 mm for eggs at stages 6-8 and 7.1-10.6 mm for eggs at stages 9-11 in the 1984 season (Fig. 8.1B).

Where particular clusters were examined more than once, weight and capsule diameter consistently increased as development progressed. For instance, clutch 3/84 increased in weight by 1.05 g (94%) over 57

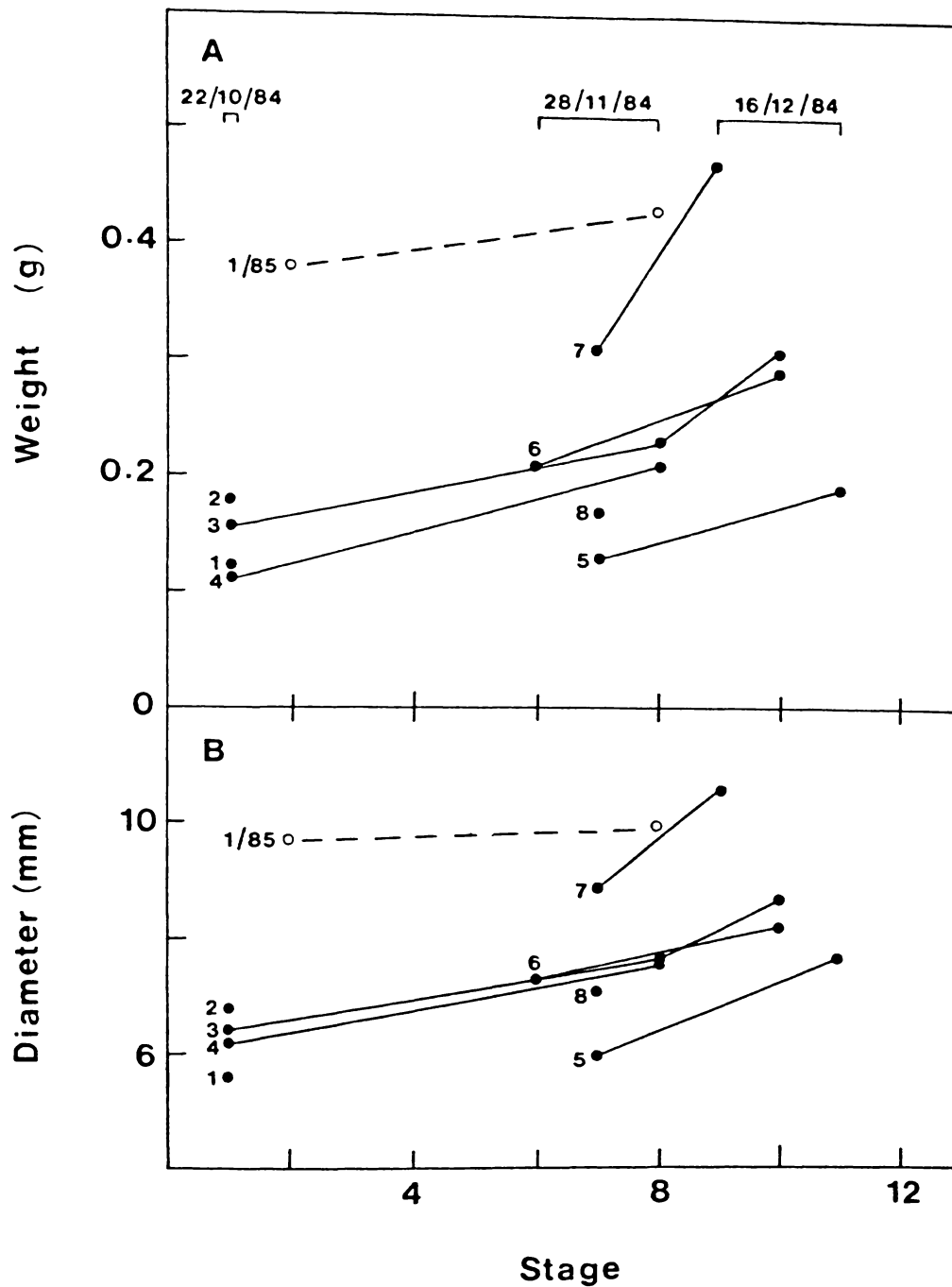


FIG. 8.1 Increase in egg weight (A) and capsule diameter (B) during development of *L. archeyi* eggs in natural nest sites. Each point represents the mean value for one cluster (capsule diameters are means for developing eggs only). Dashed or solid lines connect consecutive observations of the same cluster. Clutch numbers 1-8 (●) indicate clusters examined in the 1984 season; dates examined are indicated at the top of A. One cluster from the 1985 season (○, clutch 1/85) was examined on 3/11/85 and 15/12/85.

d between stage 1 and stage 10. Clutch 7/84 showed the most rapid weight gain (52% over 18 d. between stages 7 and 9) and eggs within it attained the greatest weight and diameter of those seen for any field cluster (Plate 8.5). Total weight increases from the time of laying may have been greater than those recorded. If one assumes a "presumed egg weight at laying" equal to the lowest mean egg weight recorded for a stage 1 cluster (0.11 g), eggs at stages 9-11 had absorbed sufficient water to increase their mass 1.7-4.3 \times during development.

Non-developing and presumably infertile eggs did not swell to the same extent as fertile eggs. For example, clutch 3/84 contained one non-developing egg, which had a capsule diameter of 6.6 mm at the same time as developing eggs in the same cluster had diameters of 8.3-9.1 mm. Similarly, a non-developing egg in clutch 1/85 had a capsule diameter of only 8.1 mm, at the same time as developing eggs in the same cluster had diameters of 9.3-10.2 mm (Plate 8.1).

The single egg cluster observed in 1985 (clutch 1/85) contained stage 2 eggs which were much heavier and larger than stage 1 eggs in the previous season (Fig. 8.1). These eggs had increased only slightly in weight and capsule diameter when re-examined at stage 8. The high initial weight of eggs in this cluster correlates with the very wet substrate beneath the cluster on both occasions it was examined (Plate 8.1). Rainfall was much higher at the NZ Meteorological Service recording station at Waiau (Fig. 2.1A, Chapter 2) in the early stages of the 1985 season than in the 1984 season, and visually this appeared to be the case in the Tapu study area also. At Waiau, rainfall over the 2 wk period prior to which eggs were first seen and weighed was 28.2 mm for the 1984 season and 96.7 mm for the 1985 season.

Larval development

Following hatching, brooding frogs remained at their nest sites and carried their hatched larvae on their backs (Plates 8.6, 8.7, 8.8). Adults usually remained inactive when the covering rock was lifted, but larvae often wriggled on the moist surface of the adults' backs. Clutches of up to eight larvae were seen, and in these cases the larvae were piled in a wet wriggling mass (Plate 8.7). Mean weights of larvae in clutches examined in the 1984 and 1985 seasons ranged from 0.11-0.15 g, and larvae within one clutch (6/84) had the same mean weight (0.13 g) when weighed 13 d apart at stages 17 and 18 (Appendix V).



PLATE 8.7 Adult male *L. archeyi* carrying eight stage 17 larvae (clutch 6/84) (cf. PLATE 8.2).



PLATE 8.8 Adult male *L. archeyi* carrying two stage 18 larvae (clutch 3/84, cf. PLATES 8.3 and 8.4). Apart from a tail stump, the larvae appear fully metamorphosed from above.

Larvae remain on or close to the adult until the end of metamorphosis (Plate 8.8). Stage 17-18 larvae are quite mobile and active, and often jump off the adult to escape under nearby root or rock crevices when disturbed. Soon after the anticipated end of metamorphosis in the 1984 season (late January-early February 1985) two nest sites were empty, but a third (clutch 6/84) still contained the parent and four of the original eight larvae when examined on 28/1/84 (Appendix V). Eight days later, only two newly metamorphosed juvenile frogs remained at this nest site.

2. Laboratory studies on water balance of eggs

Water uptake

Egg clusters collected from the field at stage 1 increased in weight by 14-21% when kept on damp moss for 2 d. Further water uptake occurred when the two clusters were half-immersed in water for 1 h (Table 8.1). Both clusters absorbed water at a similar rate (210-270 mg g⁻¹ h⁻¹, equivalent to weight increases of 21-27%). Mean capsule diameters also increased but by a more variable amount (11-29%). Mean yolk diameters did not change appreciably ($\leq \pm 4\%$).

Evaporative water loss

L. archeyi egg clusters lost water by evaporation at a rate similar to that of agar egg clusters of the same size, shape and egg number (Table 8.2). The same was true of individual eggs. The rate of weight-specific EWL from individual *L. archeyi* eggs at 45% RH and 15°C was 110 ± 4 mg g⁻¹ h⁻¹ ($\bar{x} \pm$ SE), 4.1x the rate measured for eggs in clusters of 7-8 (27 ± 6 mg g⁻¹ h⁻¹).

Measurements of EWL were also made for three non-developing and presumably infertile eggs of *L. hochstetteri* laid in captivity. At 50% RH and 18°C, individual *L. hochstetteri* eggs lost water at a rate of 39.2 ± 2.8 mg egg⁻¹ h⁻¹, essentially identical to the rate for matched agar eggs (39.2 ± 0.8 mg egg⁻¹ h⁻¹). To compare the rate of EWL from *L. hochstetteri* eggs with that from the smaller *L. archeyi* eggs, rates were calculated in terms of exposed surface area (SA). Eggs were assumed to be spherical with 90% of their SA exposed, and egg diameter was taken as the mean of maximum and minimum diameter. Rates of EWL calculated in this way were similar for the two species: 10.9 ± 1.4 mg cm⁻² h⁻¹ for *L. hochstetteri* eggs and 11.2 ± 0.4 mg cm⁻²

TABLE 8.1 Water uptake by *L. archeyi* egg clusters half-immersed in water for 1 h. Egg capsule and yolk diameters are $\bar{x} \pm \text{SE}$. Percentage changes are given in parentheses.

Cluster No.	Cluster weight (g)	Initially		After 1 h in water		
		Capsule diameter (mm)	Yolk diameter (mm)	Cluster weight (g)	Capsule diameter (mm)	Yolk diameter (mm)
1/84 (7 eggs)	0.973	6.4 \pm 0.2	4.4 \pm 0.2	1.177 (+ 21%)	7.1 \pm 0.4 (+ 11%)	4.3 \pm 0.2 (- 2%)
2/84 (8 eggs)	1.749	6.8 \pm 0.2	5.2 \pm 0.2	2.226 (+ 27%)	8.8 \pm 0.5 (+ 29%)	5.4 \pm 0.2 (+ 4%)

TABLE 8.2 Evaporative water loss (EWL) from egg clusters and individual eggs of *L. archeyi*, and from matched agar eggs and egg clusters of similar size and shape. EWL is given in $\text{mg egg}^{-1} \text{h}^{-1}$ ($\bar{x} \pm \text{SE}$) for eggs held at 45% RH and 15°C for 15 min.

Egg clusters		Individual eggs	
Real n = 2	Agar n = 2	Real n = 3	Agar n = 3
5.2 ± 0.0	4.8 ± 0.4	24.4 ± 0.8	23.2 ± 2.8

TABLE 8.3 EWL and changes in capsule and yolk diameter during dehydration of individual *L. archeyi* and *L. hochstetteri* eggs ($\bar{x} \pm \text{SE}$). *L. archeyi* eggs and their matched agar eggs were dehydrated at 45% RH and 15°C for 2.25 h. *L. hochstetteri* eggs and their matched agar eggs were dehydrated at 50% RH and 18°C for 5.25 h.

Species	EWL ($\text{mg egg}^{-1} \text{h}^{-1}$)		Change in diameter of real eggs	
	Real eggs n = 3	Agar eggs n = 3	Capsule (mm)	Yolk (mm)
<i>L. archeyi</i>	20.2 ± 1.0	18.5 ± 1.3	-1.0 ± 0.2	+0.1 ± 0.1
<i>L. hochstetteri</i>	26.1 ± 2.1	24.6 ± 1.2	-1.7 ± 0.4	0.0 ± 0.1

h^{-1} for *L. archeyi* eggs.

Changes in capsule and yolk diameter during dehydration over several hours were recorded for individual eggs of both species (Table 8.3). Eggs showed a reduction in mean capsule diameter of 1.0 mm or greater, but little or no change in yolk diameter. EWL remained similar for real eggs and matched agar eggs throughout this longer period of dehydration.

The effect of brooding by male *L. archeyi* on EWL from brooded eggs was examined using four agar egg clusters, each containing six eggs. Anaesthetised frogs remained over their clusters in the brooding position throughout the experiment. At 45% RH and 19°C, mean cluster EWL was $3.9 \pm 0.3 \text{ mg egg}^{-1} \text{ h}^{-1}$ when clusters were brooded, and $9.0 \pm 1.1 \text{ mg egg}^{-1} \text{ h}^{-1}$ when they were not. Thus, brooding reduced EWL from clusters by $5.1 \text{ mg egg}^{-1} \text{ h}^{-1}$ (57%).

Development of eggs in shallow water

Changes in weight, capsule diameter and embryo or larval length of three *L. archeyi* eggs kept in shallow water until hatching are shown in Fig. 8.2. Hatching was recorded between Days 26-39 when larvae were at stages 8-12. Embryo or larval length is only approximate, since the larvae rotate as they grow and their tails curl inside the capsule, making precise measurements impossible.

Large increases in weight occurred during development (Fig. 8.2B). Eggs weighed $260 \pm 23 \text{ mg}$ ($\bar{x} \pm \text{SE}$) when placed in water at the end of EWL experiments on Day 0, and their weight increased nearly three-fold to $755 \pm 29 \text{ mg}$ by Day 22, shortly before the first egg hatched. The enclosed larvae rotated vigorously in their capsules, which became large and fluid-filled during development (Fig. 8.2C, see also Plate IV.1B of Appendix IV). Following Day 22, the remaining two eggs continued to increase in weight and by Day 34 weighed $970 \pm 13 \text{ mg}$ (equivalent to 5.4 \times the mean weight of eggs in this cluster when first collected). The capsule of one egg was pierced on Day 34 with the tip of a drawn-out capillary tube to obtain a sample of fluid for analysis of osmotic concentration, and of urea and ammonia content (Chapter 7). As the tip was withdrawn, fluid continued to drain out rapidly and over about 3 min the weight of the egg declined by 56%, to 549 mg. This loss of fluid did not adversely affect the larva and when weighed 3 d later, the egg had nearly returned to its former weight and

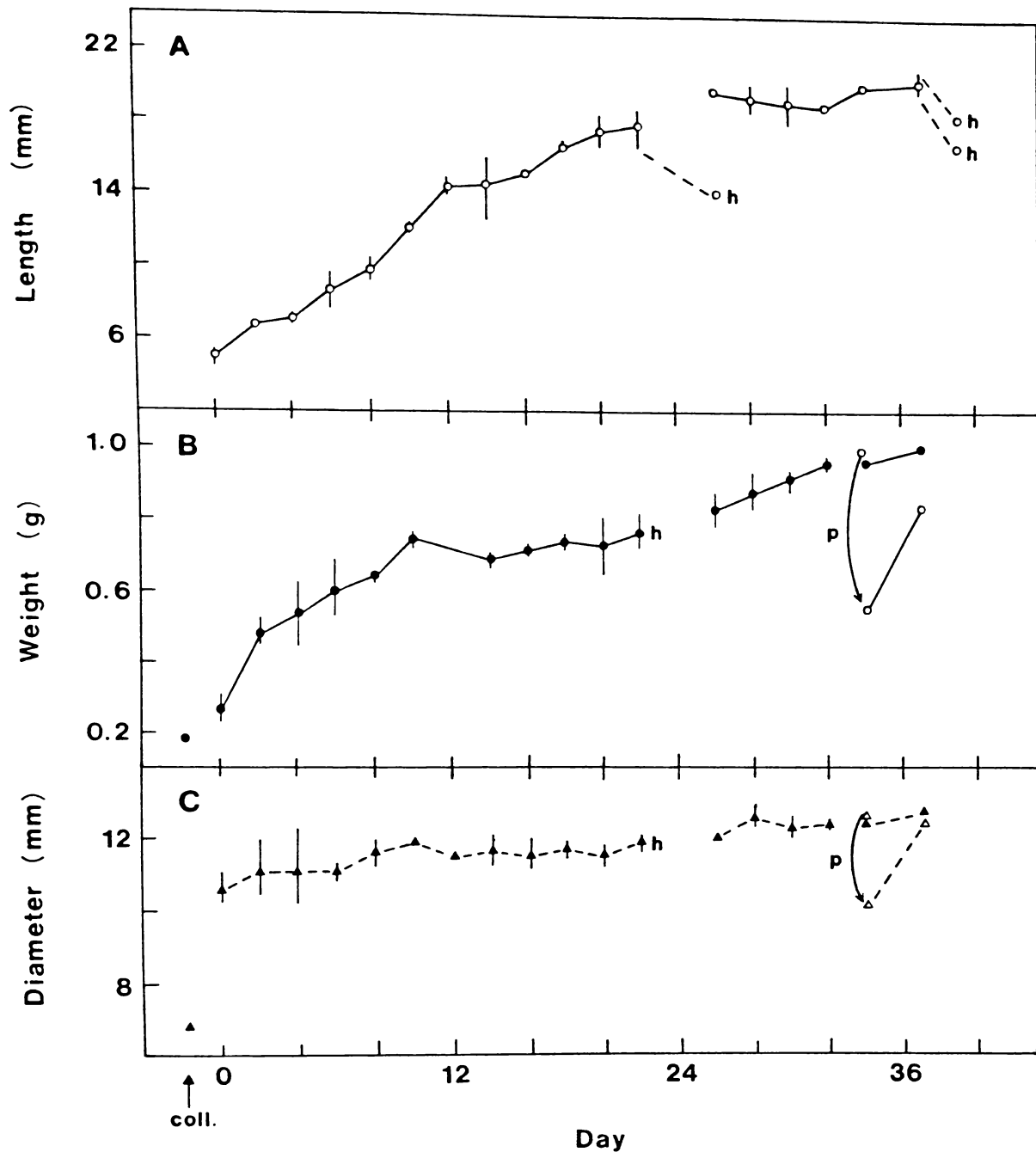


FIG. 8.2 Changes in total embryo or larval length (A), weight (B) and capsule diameter (C) of three *L. archeyi* eggs kept in shallow water from stage 3 (Day 0). Figure shows mean and range for $n = 3$ until Day 22, following which the first egg hatched (h). Subsequent values are for $n = 2$ until Day 34. In A, larval lengths measured after hatching are connected by dashed lines to less precise estimates made before hatching. In B and C, mean egg weight and capsule diameter at the time of collection are also shown for the cluster from which these eggs were taken. On Day 34, the capsule of one egg (open symbols) was pierced (p) to obtain a sample of intracapsular fluid, causing a reduction in weight and capsule diameter over about the next 3 min (indicated by arrow).

diameter. Both this and the remaining egg hatched within the next 2 d. Mean weight of the three hatched larvae was 63 ± 7 mg, equivalent to 7.4% of mean egg weight immediately prior to hatching.

Osmotic concentration of intracapsular fluid

Samples of intracapsular fluid were obtained from two stage 8 eggs (mean weight 0.43 g), one stage 11 egg (0.99 g), and one stage 13 egg (0.43 g). Despite differences in degree of development and the amount of water previously absorbed, intracapsular fluid from all four eggs was similar in osmotic concentration ($53\text{--}59$ mOsm kg^{-1}). The mean value was 56 ± 1 mOsm kg^{-1} .

Effect of moisture on egg development

By late November when clutch 8/84 was collected for this experiment, development had already proceeded to stage 7 (Fig. 8.1). Some water absorption had also occurred, probably both prior to collection and also during transport to the laboratory on moist moss. Subsequently, three of these eggs which were kept on the base of a dry Petri dish at high humidity (Plate 8.9) declined slowly but steadily in weight (Fig. 8.3A). Capsule diameter fluctuated more widely but tended to decline slightly also (Fig. 8.3B). Hatching was recorded between Day 6 and Day 20.

The remaining four eggs from the same cluster were kept on wet filter paper. For the first 4 d all increased in weight and capsule diameter, but on the sixth day the smallest egg declined in weight and diameter to less than at the start of the experiment, and to less than recorded for eggs on dry plastic (Fig 8.3). This egg increased only slightly in weight and diameter over the next 4 d and hatched on Day 12. The remaining three eggs on wet filter paper generally continued to increase slightly in weight and diameter until 2-4 d before hatching, which occurred 14-24 d after the start of the experiment. The declines in weight and diameter which occurred 2-6 d prior to hatching of eggs on wet filter paper resulted from loss of intracapsular fluid. Intracapsular fluid loss was not detected in eggs kept on dry plastic, and the reason for it is unknown.

Despite wide variations in time till hatching within each group, eggs on wet filter paper reached a greater maximum capsule diameter and showed significant increases in weight compared with those on dry plastic (Table 8.4). Stage at hatching varied considerably within

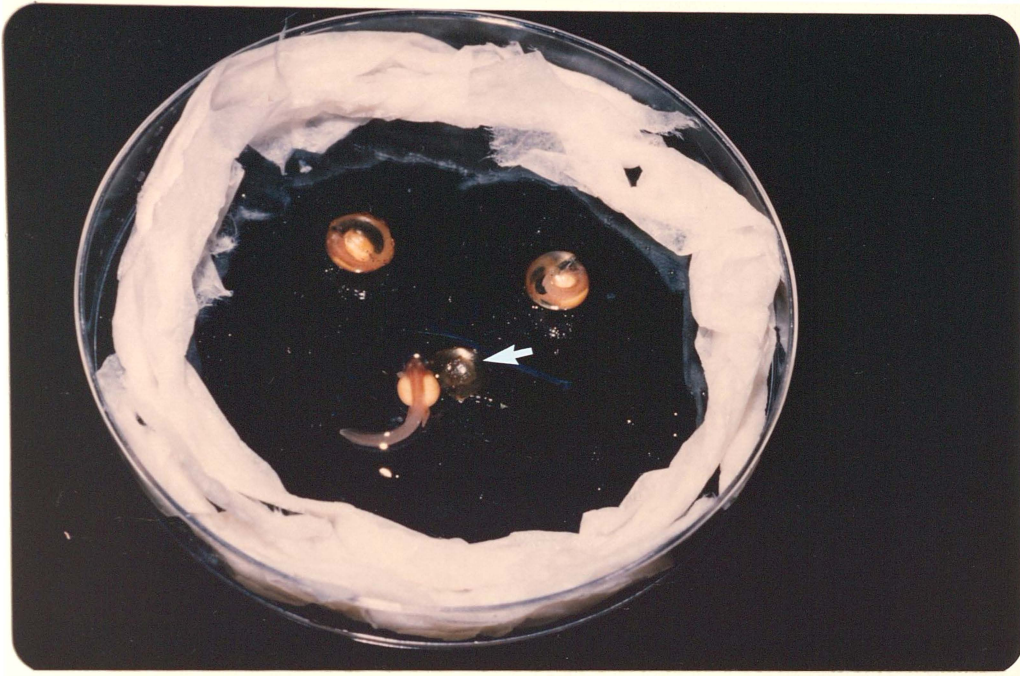


PLATE 8.9 *L. archeyi* eggs kept without access to water in the laboratory. The eggs in the dry Petri dish are surrounded by strips of moist paper towel to maintain a high humidity. Hatched larva is at stage 10; note ruptured capsule (arrowed).



PLATE 8.10 *L. archeyi* larva placed on the back of an adult frog for measurement of larval EWL (larva is at stage 17, but final experiment was carried out with stage 16 larvae).

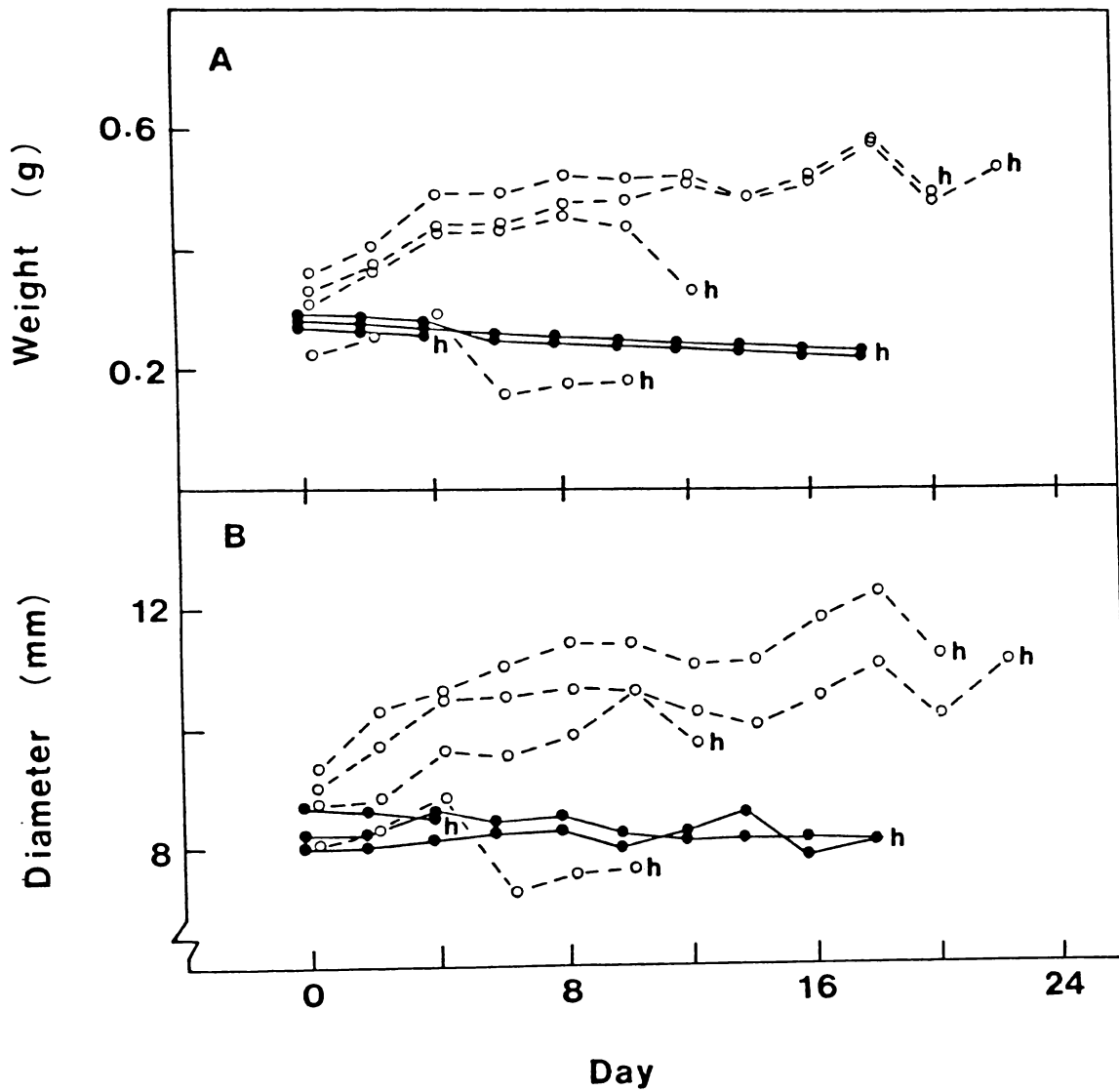


FIG. 8.3 Changes in weight (A) and capsule diameter (B) of three *L. archeyi* eggs kept on dry plastic (●) and four eggs kept on wet filter paper (○). Eggs were at stage 8 on Day 0. An "h" indicates that hatching occurred during the following 2 d period.

TABLE 8.4 Effect of substrate moisture on development of *L. archeyi* eggs. Three eggs from a clutch of seven eggs at stage 8 were kept on dry plastic and the remaining four were kept on wet filter paper ($\bar{x} \pm SE$; * = $P < 0.05$, ** = $P < 0.01$).

Variable	Dry n = 3	Wet n = 4	Significance
Maximum increase or decrease in egg weight prior to hatching (mg g ⁻¹ initial weight)	-139 ± 60	+551 ± 105	t = 5.697 **
Maximum capsule diameter attained (mm)	8.7 ± 0.1	10.7 ± 0.7	t = 2.670 *
Time till hatching (d)	14.7 ± 4.4	18.0 ± 2.6	t = 0.654 P > 0.5
Stage of larva at hatching	13.3 ± 1.7	13.8 ± 1.3	t = 0.196 P > 0.5
Weight of larva at hatching (mg)	74.2 ± 0.7	82.8 ± 4.0	t = 2.108 P > 0.05
Body length of larva at hatching (mm)	7.6 ± 0.3	7.9 ± 0.6	t = 0.464 P > 0.5

each group and mean values for the two groups were similar. Although larvae hatching from eggs kept on dry plastic were slightly smaller in mean body length and weight than those hatching from eggs kept on wet filter paper, the differences are not statistically significant.

3. Laboratory studies on water balance of hatched larvae

Larvae at stages 15-18 dehydrated at rapid and constant rates (Fig. 8.4 A-C). Rates of EWL showed a consistent relationship with body size, being lowest ($68 \pm 2 \text{ mg g}^{-1} \text{ h}^{-1}$) for the heaviest larvae at stage 16 ($W_0 = 107 \pm 3 \text{ mg}$), intermediate ($83 \pm 1 \text{ mg g}^{-1} \text{ h}^{-1}$) for larvae at stage 18 ($W_0 = 75 \pm 6 \text{ mg}$) and highest ($111 \text{ mg g}^{-1} \text{ h}^{-1}$) for the lightest larva at stage 15 ($W_0 = 68 \text{ mg}$) (Fig. 8.4 B, C and A). The stage 15 larva also had a relatively longer tail and less compact body shape than those at stages 16 or 18. This gave it a very high SA:volume ratio, which undoubtedly contributed to the high rate of weight-specific EWL measured.

When dehydrated for 1 h, the tip of the tail and the digits on all four limbs of the stage 15 larva became stuck to the dish, and despite struggling the larva was unable to free them. When half-immersed in water for rehydration, the larva's tail and digits came free but remained pink and distorted in shape. The larger and more mobile stage 16-18 larvae did not adhere tightly to the substrate during dehydration.

Rehydration occurred at initially rapid rates for all larvae. Rates of water uptake over the first 30 min were $76 \text{ mg g}^{-1} \text{ h}^{-1}$ for the stage 15 larva, $49 \pm 6 \text{ mg g}^{-1} \text{ h}^{-1}$ for stage 16 larvae and $130 \pm 24 \text{ mg g}^{-1} \text{ h}^{-1}$ for stage 18 larvae (Fig. 8.4 A, B and C). The stage 15 larva regained its original weight within 1.75 h but continued to absorb water, reaching 109% of its original weight 4 h after beginning rehydration. By this time the groin of the motionless larva was engorged with blood, and the larva died soon after. Water uptake of stage 16 larvae declined as rehydration progressed. After 2 h they had reached $95 \pm 0\%$ of their original weight, and since they were voluntarily climbing out of the water and up the container walls the experiment was ended at this point. Stage 18 larvae regained their original weight in 1.5 h. Unlike the stage 15 larva, stage 16 and 18 larvae showed no adverse reactions to the experiment and were still alive several days later.

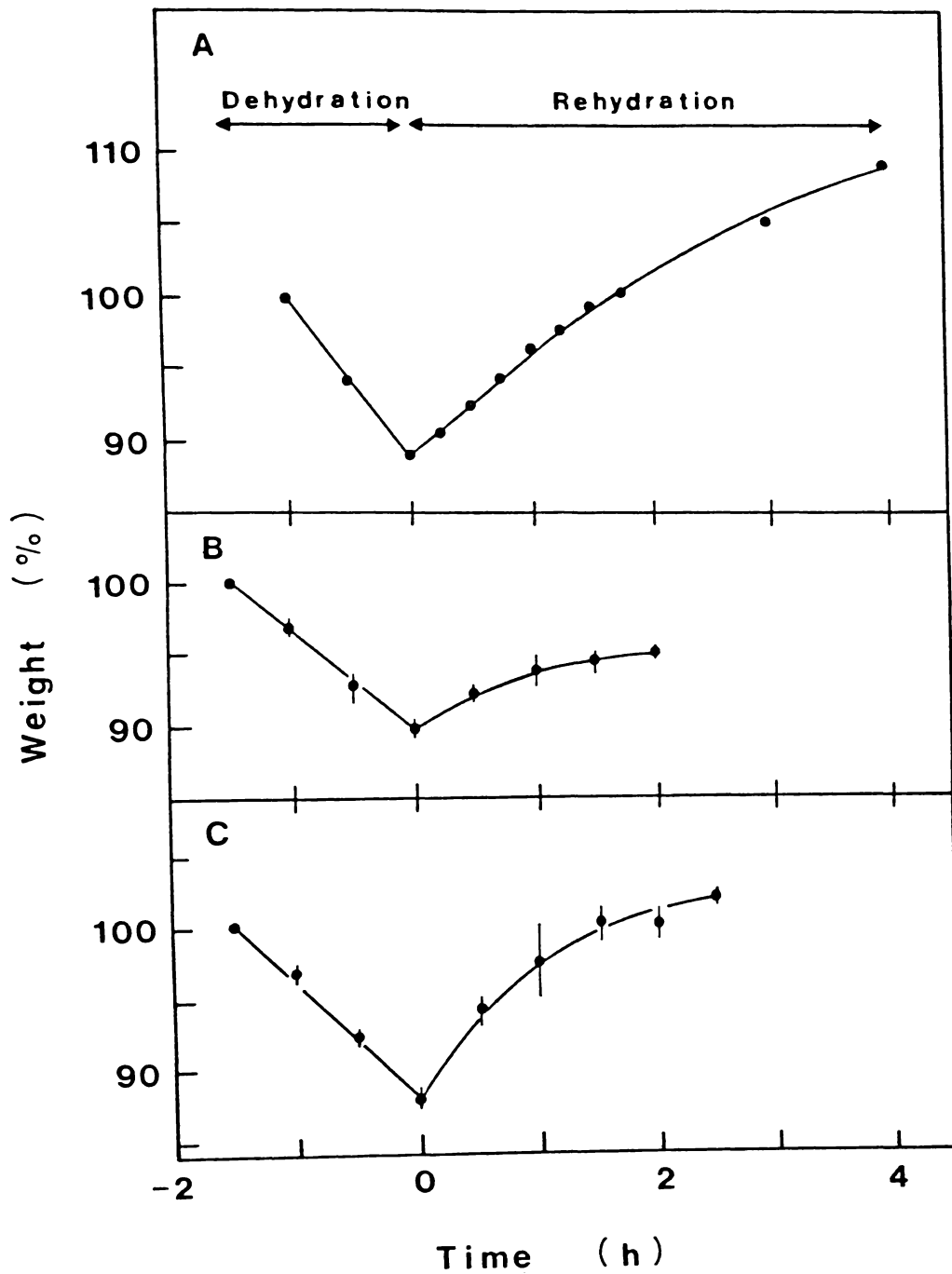


FIG. 8.4 Dehydration and rehydration of *L. archeyi* larvae. A = one stage 15 larva. B = four stage 16 larvae (\bar{x} and range). C = two stage 18 larvae (\bar{x} and range). Animals were dehydrated in a dry dish until 0 h, when they were half-immersed in water. Initial weights were: 68 mg (A); 107 ± 3 mg ($\bar{x} \pm SE$) (B); 75 ± 6 mg ($\bar{x} \pm SE$) (C).

In the remaining experiment, four stage 16 larvae (initial weight 105 ± 1 mg) were dehydrated for 1.75 h to a weight loss of $12 \pm 0\%$, and then each was placed on the back of an anaesthetised frog for 2 h (Plate 8.10, Fig. 8.5). EWL from the larvae during the first 1.75 h averaged 71 ± 3 mg g⁻¹ h⁻¹, but fell to a negligible amount (2 ± 2 mg g⁻¹ h⁻¹) when the larvae were on the adult's backs. The relatively large standard error indicates that some larvae lost no weight when on the adults. When transferred to shallow water, the larvae rehydrated in the same manner as other stage 16 larvae (Fig. 8.5, cf. Fig. 8.4B). Water uptake during the first 30 min in water averaged 47 ± 11 mg g⁻¹ h⁻¹. After 2 h in water both groups of stage 16 larvae were transferred to moist paper towels. When reweighed 3 d later, their mean weight was 99% of their initial weight before dehydration, indicating that virtually complete rehydration did eventually occur.

DISCUSSION

Here I return to the questions raised in the Introduction to this chapter.

(i) *Do eggs of L. archeyi lose water by evaporation at the high rates typical of other anuran eggs?*

Like those of other anurans, the terrestrial eggs of *L. archeyi* lack an impermeable membrane and lose water freely by evaporation. Rates of EWL are as high as those of matched agar eggs or clusters of the same size and shape, as previously reported for eggs of *Eleutherodactylus coqui* (Taigen *et al.* 1984). Rates of EWL measured for *L. archeyi* eggs cannot be directly compared with those for other species examined in the literature because of differences in temperature, humidity, egg size and degree of clustering. However, rapid EWL has previously been reported for terrestrial eggs of the anurans *E. portoricensis* (Heatwole *et al.* 1969) and *Crinia victoriana* (Martin and Cooper 1972), and the urodeles *Plethodon cinereus* (Heatwole 1961) and *Ambystoma opacum* (Marangio and Anderson 1977).

Individual eggs of *L. archeyi* lose water by evaporation at more than 4x the weight-specific rate measured for eggs in intact clusters under the same conditions. This shows the value of clustering in reducing exposed SA, and hence weight-specific EWL. Despite being laid in a terrestrial nest site, individual *L. archeyi* eggs have no greater

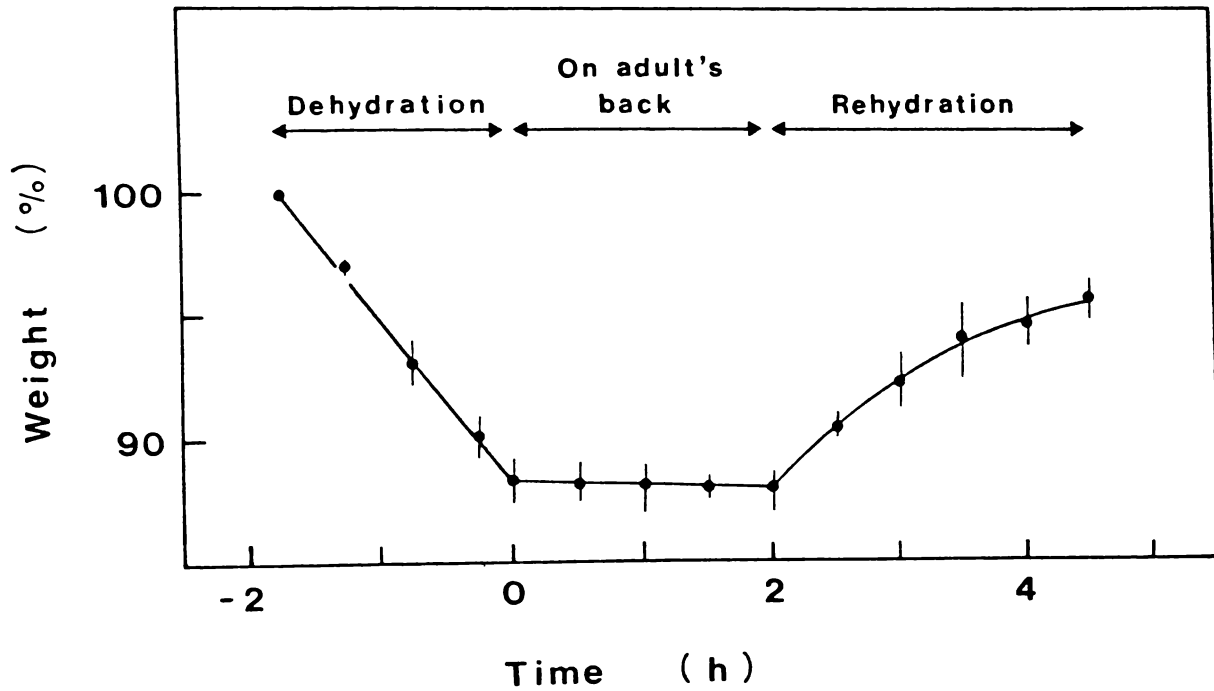


FIG. 8.5 Effect of transport on the backs of adult frogs on EWL from four *L. archeyi* larvae (stage 16, \bar{x} and range). Each larva was dehydrated for 1.75 h in a dry dish, then placed on an adult's back for 2 h, then half-immersed in water for 2.5 h. Initial weight of larvae was 105 ± 1 mg ($\bar{x} \pm$ SE).

resistance to evaporation than the semiaquatic (infertile) eggs of *L. hochstetteri*. However, *L. archeyi* eggs are normally packed in tighter clusters than those of *L. hochstetteri* (Bell 1985b), and adult *L. archeyi* brood their egg clusters whilst *L. hochstetteri* do not. Brooding by male *L. archeyi* reduces mean weight-specific EWL from agar egg clusters by 57%.

(ii) Are eggs able to absorb water from moist or wet surfaces in the laboratory?

Despite being vulnerable to potentially high rates of EWL, eggs of *L. archeyi* are able to absorb water rapidly from moist or wet surfaces. In short-term laboratory experiments (2.25 h or less), water uptake and EWL were accompanied by increases and decreases respectively in capsule diameter. Since yolk diameters did not change appreciably, water loss and uptake initially occur from or into the gelatinous capsule. The capsule thus acts as a buffer between the developing embryo or larva and the surrounding air, protecting the larva from unfavourable water exchanges. Martin and Cooper (1972) discussed the value of the egg capsule to the terrestrial eggs of *Crinia victoriana* and concluded that it has both structural and physiological advantages: the flaccid outer jelly layer and adhesive capsule surface allow eggs to be tightly packed together into a mass with relatively small SA (this is also the case for *L. archeyi* egg clusters), and the capsule provides the larva with a copious reservoir of water, most of which can be lost without affecting viability. The value of the capsule to *C. victoriana* was demonstrated by comparing the dehydration tolerance of intact eggs, with the tolerance of eggs from which the outer of the two capsule layers had been removed. Dehydration rates were similar over 7 h at 65% RH for both groups, but all intact eggs survived whereas all treated eggs died.

In long-term experiments over many days, *L. archeyi* eggs kept in contact with water continued to swell during development. As the larvae develop, absorbed water accumulates not only in the capsule, but also in intracapsular fluid. This continued water uptake presumably occurs in response to the osmotic concentration gradient between intracapsular fluid and the water surrounding the egg. Intracapsular fluid of *L. archeyi* eggs contains nitrogenous wastes (urea and ammonia, Chapter 7), which would contribute at least partially to the osmotic concentration measured. Impermeant proteins and polysaccharides may also be secreted into intracapsular fluid by

developing anuran larvae (Salthe 1965; Pyburn 1980).

(iii) *Do eggs in natural nest sites also absorb water during development?*

Like those incubated in the laboratory on wet surfaces, eggs brooded by adult *L. archeyi* in natural nests absorb water as they develop. In the 1984 season, clusters containing eggs at stages 9-11 had mean egg weights of 0.19-0.47 g, 1.7-4.3x the "presumed egg weight at laying" (0.11 g). Non-developing eggs do not swell to the same extent as fertile eggs, and this is consistent with the observation that larvae accumulate osmotically active substances in intracapsular fluid as they develop.

The source of water absorbed during development of *L. archeyi* eggs in natural nests was not examined experimentally. However, studies on terrestrial eggs of other amphibian species have inferred or demonstrated water uptake from at least four sources. (1) Absorption of water released from the bladder of one of the parents over the egg cluster. This occurs during oviposition in several species of leaf-breeding *Agalychnis* (Pyburn 1970). (2) Osmotic water uptake from the brooding frog. This is presumed to occur during development of *E. coqui* eggs, which are laid on dry surfaces isolated from rainwater, yet increase 3-4x in weight during development. Since coquí eggs have a lower (more negative) water potential than the osmotic potential of the blood of brooding adults, an osmotic gradient theoretically favouring water flow from adult to eggs exists (Taigen *et al.* 1984). (3) Absorption of water from moist substrates at the nest site - believed, although not proven, to occur for eggs of the salamander *Plethodon cinereus* (Heatwole 1961). (4) Absorption of water from fluid-filled, yolkless egg capsules - demonstrated for eggmasses of *Phyllomedusa hypochondrialis* (Pyburn 1980).

Of these four possibilities, circumstantial evidence suggests that substrate moisture is an important source of water for *L. archeyi* eggs developing in natural nests. Laboratory experiments show that eggs in contact with moist or wet surfaces are able to absorb water rapidly, and since substrates beneath egg clusters in natural nest sites were always moist or wet (saturated soil, wet litter or rock), water uptake from these is likely. Rainfall during October-December when eggs are present can be extremely high (Fig. 2.7, Chapter 2), and the greater availability of substrate moisture in the 1985 season explains why

cluster 1/85 had heavier, more swollen eggs at stage 2 than those seen at a similar stage of development in the 1984 season. If this hypothesis about the importance of substrate moisture is correct, then brooding male *L. archeyi* may make an important contribution to the water balance of their eggs by bringing and keeping the clusters in close contact with the substrate. Selection of a suitably moist oviposition site is also probably an important factor to water balance of the egg cluster, but whether this is carried out by the male or the female parent is unknown (Bell 1985b).

In contrast, there is no evidence that adult urine, adult blood, or non-developing capsules contribute water to developing eggs of *L. archeyi*. Egg clusters do not contain fluid-filled eggless capsules, and although transfer of water from capsules of non-developing eggs to developing eggs has not been ruled out, this cannot account for water uptake in clusters containing only fertile eggs. Adult urine or body fluids remain possible sources of moisture, but seem unlikely to be important for several reasons. First, the osmotic gradient between intracapsular fluid and the urine or plasma of adult frogs is not in a direction to favour water movement into the intracapsular fluid. Where measured, intracapsular fluid had osmotic concentrations of 53-59 mOsm kg⁻¹, whereas urine of captive adult *L. archeyi* had a mean osmotic concentration of 110 mOsm kg⁻¹ (range 69-189, n = 7) and plasma 215 mOsm kg⁻¹ (range 190-240, n = 2) (Appendix III). Second, although urinary secretions might still contribute to the initial swelling of the gelatinous capsule, brooding frogs were never seen urinating over their eggs, and when in the brooding position the vent is directed away from the egg cluster. Third, the large amounts of water gained by some egg clusters (up to 2.52 g above the "presumed weight at laying") are equivalent to as much as 73% of a brooding frog's bladder-empty weight, and water losses of this magnitude could not be survived unless the brooding frog left the cluster to rehydrate. Although data are few, four clusters examined on one wet night, when males could be expected to leave their eggs to replenish body water reserves, were all covered by brooding frogs.

(iv) *What effect does the amount of water absorbed by eggs during development have on hatching success?*

Laboratory experiments demonstrate that stage 8 eggs weighing 0.26-0.29 g do not need to absorb additional water to hatch

successfully. Eggs within this weight range and subsequently kept on dry plastic at high humidity lost water slowly by evaporation for up to 20 d before hatching. Eggs from the same cluster kept on wet filter paper increased in weight until 2-6 d before hatching. The date and stage at which hatching occurred in both groups was unexpectedly variable, and showed no relationship to treatment. The amount of water absorbed beyond stage 8 also had no significant effect on body length or weight of larvae at hatching.

These results do not mean that the amount of water absorbed by the eggs since oviposition has no effect on larval development. In retrospect, it is clear from the results of the field study that the eggs collected for this experiment had already absorbed sufficient water before the experiment began to more than double their weight above the "presumed egg weight at laying". To conclusively test the hypothesis that water uptake by eggs affects larval development will require that eggs are collected immediately after laying, before significant water uptake has occurred. This would mean collecting clusters with mean egg weights of less than about 0.11 g (or capsule diameters of less than about 5.6 mm, although this is less reliable than weight as an indication of water absorption).

(v) *Do hatched larvae share the same major features of water balance (lack of resistance to EWL, ability to rehydrate rapidly) as adult L. archeyi?*

Because of their small size and high SA:volume ratio, hatched larvae are susceptible to even higher weight-specific rates of EWL than adult *L. archeyi*. Under the relatively mild conditions used in this study, larvae at stages 15-18 lost 10% of their initial weight by dehydration in only 1-1.5 h, with the weight-specific rate of EWL being highest ($111 \text{ mg g}^{-1} \text{ h}^{-1}$) for the smallest and least-developed larva (68 mg). At the same humidity and a temperature 6°C cooler, the predicted rate for adult *L. archeyi* is $4 \text{ mg g}^{-1} \text{ h}^{-1}$ (Fig. 3.2, Chapter 3). Because of their elongate shape and therefore very high SA:volume ratio, newly hatched larvae are likely to be vulnerable to higher weight-specific rates of EWL than late stage larvae. Newly-hatched larvae are also less mobile and therefore less able to escape dehydrating conditions, since body extremities rapidly become stuck to dry surfaces.

The water balance response seen in rehydrating adult *L. archeyi* (Chapter 3) is present to some degree in hatched larvae from at least

stage 15 onwards (Figs 8.4 and 8.5). Dehydrated larvae at or beyond this stage of development rehydrate at initially rapid but declining rates when half-immersed in water, suggesting that, as in adults, water uptake through the ventral skin increases as a consequence of dehydration. The water balance response may, however, be imperfectly regulated in the earlier of these larval stages, since the single stage 15 larva examined continued to absorb water beyond its original weight. Additional study is needed to confirm this trend.

Development of a cutaneous hydroosmotic response allowing rapid rehydration before the end of metamorphosis is conceivably advantageous to *L. archeyi* larvae in their terrestrial environment. To my knowledge, rates of dehydration and rehydration have not previously been reported for anuran larvae, but studies examining the effects of neurohypophysial peptides (NHPs) on larval water uptake suggest that more conventional aquatic anuran larvae (tadpoles) do not acquire a pronounced cutaneous hydroosmotic response until the final stage of metamorphosis. For instance, tadpoles of *Bufo bufo* (Howes 1940), *Rana catesbeiana* (Alvarado and Johnson 1966; Bentley and Greenwald 1970) and *R. ridibunda* (Goldenberg and Warburg 1977) do not show large amounts of water retention in response to injected NHPs until metamorphic climax (when the forelimbs emerge and tail resorption occurs), although very small responses in these and *Heleioporus eyrei* tadpoles (Bentley 1959) may be seen at earlier stages. It is possible that developing *L. archeyi* acquire a cutaneous response to AVT at a relatively early stage of larval development compared with more aquatic tadpoles, and *in vivo* injection experiments could be carried out to test this hypothesis. It would also be extremely interesting to compare the rehydration ability of the semiaquatic larvae of *L. hochstetteri* with the results obtained here for larval *L. archeyi*.

(vi) *What effect does parental care have on water balance of eggs and larvae?*

As discussed in preceding sections, brooding of eggs by male *L. archeyi* reduces EWL from egg clusters by more than half. Brooding may also increase contact between the cluster and moist substrates, enhancing water uptake into the eggs. The extreme sensitivity of larvae to dehydration when on a dry surface suggests that transport by the adult of larvae on his back makes an important contribution to larval water balance also. In the experiment shown in Fig. 8.5, EWL

from stage 16 larvae fell to 3% of its former value when the larvae were placed on the backs of adult frogs. Had the larvae not been transferred onto the adults, EWL would probably have continued at a rate similar to that seen during the initial period of dehydration, since in adult anurans EWL generally shows only a slight and gradual reduction in rate with progressive dehydration towards the lethal end-point, when variation in activity is taken into account (Heatwole *et al.* 1969). Although EWL was expected to fall when larvae were transferred to the high humidity boundary layer surrounding the adult, the fact that it declined to a negligible amount ($2 \text{ mg g}^{-1} \text{ h}^{-1}$) raises the possibility that larvae are able to absorb water osmotically from the body fluids or secreted mucus of the adult frog beneath them. This possibility appears even more likely when field data are considered. Transported larvae would be subject to continuous EWL from their dorsal surfaces even in the high humidity of the nest site, yet after being transported for several weeks show no evidence of dehydration.

Such a contribution to larval water balance is a novel indication of the value of parental care in an anuran. Indeed, it is possible that the presence of male *L. archeyi* at the nest site is more critical to the water balance of hatched larvae than to the water balance of eggs. Because of the presence of a gelatinous capsule and intracapsular fluid, eggs are almost certainly tolerant of much greater dehydrational water losses than are hatched larvae. Furthermore, mean monthly rainfall at Waiiau is heavier during October-December than in January (Fig. 2.7, Chapter 2), which suggests that nest site substrates are more likely to become dry when larvae have hatched than when they are still encapsulated. Because of their low mobility, newly-hatched larvae abandoned on a dry substrate would be unable to move to reach moister surfaces. Perhaps the phenomena of egg brooding and larval transport have evolved for largely independent reasons: egg brooding may, for instance, have relatively greater significance for antipathogenic and antipredatory reasons (Salthe and Mecham 1974; Bell 1985b), whereas transport of larvae is probably crucial to maintain larval water balance.

CHAPTER 9

EFFECT OF ENVIRONMENTAL CONDITIONS ON EMERGENCE OF *L. ARCHEYI*,
AND ABSORPTION OF SOIL MOISTURE BY *L. ARCHEYI* AND *L. HOCHSTETTERI*.

INTRODUCTION

Availability of moisture is widely assumed to limit the temporal and spatial activity of permeable-skinned amphibians in natural habitats, and thus their ability to carry out such important ecological activities as feeding and reproduction (Keen 1984). When confronted with a water shortage, many amphibians remain in or rapidly seek moist microhabitats (e.g., Heatwole 1962; Jaeger 1971; Degani and Warburg 1978), but the South American frog *Eleutherodactylus coqui* is considered an exception (Pough *et al.* 1983). *E. coqui* inhabits subtropical rainforests and emerges by night to engage in foraging, territorial defence and reproductive activity. Despite losing water by evaporation, coquís continue to emerge on dry nights for at least the first 7 d without rain, occupying the same arboreal perches as they do on wet nights. Behavioural adjustments are made on dry nights to minimise EWL and bladder water is reabsorbed, thus keeping plasma osmotic pressure and tissue water content stable. By day, coquís return to their retreat sites and make up for water lost overnight by absorbing moisture from their retreat site substrates (van Berkum *et al.* 1982; Pough *et al.* 1983).

Like *E. coqui*, *L. archeyi* is a terrestrial frog which inhabits high rainfall forest and usually remains in cool, humid retreat sites by day (Chapter 2). However, apart from reports that *L. archeyi* (Turbott 1942) or leiopelmatid frogs in general (Bell *et al.* 1985) climb trees and vegetation on wet nights, little is known about factors affecting emergence. This chapter examines the relationship between environmental conditions and nocturnal activity of *L. archeyi* in a field population. Is emergence of this species restricted to times when EWL would be negligible and/or surface water widely available for absorption, or does it continue during periods of dry weather, as reported for *E. coqui*?

In addition, the ability of *L. archeyi* to absorb water from two natural moisture sources which frogs encounter either when emerged

(wet vegetation) or in retreat sites (moist soil) was examined. *L. hochstetteri* was included in laboratory soil water uptake experiments to see if this species differs from *L. archeyi* in its ability to absorb water from soil.

MATERIALS AND METHODS

1. Effect of environmental factors on nocturnal emergence of *L. archeyi*

The number of frogs emerged in a 10 × 8 m² area within the R3 site of the Tapu study area (Chapter 2) was examined on 21 nights between 2/6/84 and 2/2/86. On each night, a specific path through the search area was followed, and the ground and vegetation up to 3 m high were searched for frogs using a hand-held white light torch. Searches were conducted during the first hour of darkness in the forest and took 25-40 min.

The following environmental conditions were noted at the end of each search: relative humidity (RH), temperature, windspeed, wetness of vegetation, heaviness of rainfall during the previous daylight hours, and occurrence of rain during the search. Relative humidity was measured ~0.5 m above ground level with a Sundo hair hygrometer (Stacker and Olms, Hamburg, West Germany) and temperature at ground level with a spirit thermometer, in a defined spot at the end of each search. Vapour pressure deficits were calculated from RH and temperature data, using the saturated vapour pressure tables of Campbell (1977). The speed of windgusts was estimated using a five-point qualitative scale, ranging from 0 (no wind) to 4 [equivalent to gusts of 16 km h⁻¹ in the forest, measured with a "Sims" Model K/KG anemometer (R.A.Simerl Instrument Div., Maryland, USA)]. Rainfall during the previous daylight hours was recorded as none, light or heavy, and wetness of vegetation during the search as dry, moist or wet. "Moist" nights are defined as those on which the vegetation was only slightly wet, or wet in some patches of the search area and dry in others; on "wet" nights the vegetation was saturated throughout the search area. For each frog seen, height above ground was noted as zero (on the ground), above ground up to or including 1 m, or higher than 1 m. Apart from three counts in June 1984 (mid winter), all counts were made between spring and late summer

(October-March). Counts were not made on nights following daytime searches of the same area (Chapter 2).

To determine that frogs do emerge during the first hour of darkness if conditions are suitable, and that this is therefore an appropriate time to compare the number of frogs emerged on different nights, repeated counts were made during three nights following daytime rain. On each of these nights 6-9 counts were made at intervals 30 min - 5 h apart.

2. Rehydration of *L. archeyi* on wet vegetation

The ability of *L. archeyi* to absorb moisture from wet kiekie fronds (*Freycinetia baueriana banksii*, a plant favoured by frogs emerged during night searches) was examined in the field on a moist spring night. Six *L. archeyi* (0.7-3.0 g) were removed from their retreat sites in the early afternoon, dehydrated over silica gel to about 10% weight loss, and then placed on kiekie fronds at night to rehydrate. Rain falling in the afternoon had initially wet the vegetation, but subsequent evening winds had nearly dried it. About 200 ml of additional water was therefore poured over the plant and allowed to drain through. The frogs were then placed on the wet fronds. To minimise the frogs' chances of escape, the crown of the kiekie was enclosed within a stretchable plastic mesh tube fashioned from a commercial garlic bag. The top and bottom of the tube were tied, and the kiekie fronds were pulled through slits cut in the mesh sides. Every 2 h, frogs were removed from the mesh enclosure and weighed to the nearest mg, using an electronic balance connected via a 12 V DC/240 V AC generator to a 12 V car battery. Temperature and RH measured about 2 m away from the rehydrating frogs were 15°C and 96% respectively.

3. Absorption of soil moisture by *L. archeyi* and *L. hochstetteri*

Animals and procedure

The ability of *L. archeyi* (1.4-5.1 g) and *L. hochstetteri* (1.6-8.1 g) to absorb water from soil was examined in the laboratory at $17 \pm 1^\circ\text{C}$ using stock animals. The first series of experiments examined the ability of hydrated frogs to maintain weight on laboratory soil of either 20%, 30% or 40% moisture content (MC). Weighed frogs with bladders emptied were placed on a tray of prepared soil and weighed at

intervals of 1-7 h. Between weighings, the trays were kept in an open black polythene bag at humidities of 63-80% RH (soil at 20% MC), 70-80% RH (30% MC) or 75-95% RH (40% MC). Before weighing, each frog was momentarily dipped in water to remove adhering soil and then blotted dry (simply wiping the frogs with dry or damp tissue was insufficient to remove adhering soil). Bladder urine was removed after each weighing to minimise fluctuations in weight due to spontaneous urination onto the soil. Each frog on the tray of soil was enclosed within a perspex ring (67 mm id) covered by a plastic mesh lid. Thus, frogs were not buried in the soil, did not contact each other and were simultaneously losing water by evaporation from their dorsal surface.

In a second experiment, frogs were dehydrated to about 10% weight loss and then placed on soil of 40% MC. They were weighed at hourly intervals to determine their ability to rehydrate from this soil. Bladder urine was not removed during rehydration.

Preparation of laboratory soil

Soil was collected from the Tapu ridgetop and dried in the laboratory at ~105°C to constant weight. Large lumps were removed by sieving the soil through a 2.36 mm mesh. Distilled water equivalent to 20%, 30% or 40% of the dry soil weight was added, and the soil was left in sealed containers for several hours to equilibrate.

Soil samples ranging in MC from 10-70% were used to determine the soil moisture tension curve for laboratory soil. Water potential of the samples was measured using a SC-10A thermocouple psychrometer (Decagon Devices Inc., Washington, USA).

RESULTS

1. Field emergence of *L. archeyi*

Temporal variation in emergence

The number of emerged *L. archeyi* was counted at intervals during three nights following days when rain had fallen (Fig. 9.1 A-C). On each occasion, frogs had emerged by dusk, and remained present in moderate, fairly constant numbers (13-25) throughout the night. Most disappeared at or soon after dawn. Frogs were not disturbed by the

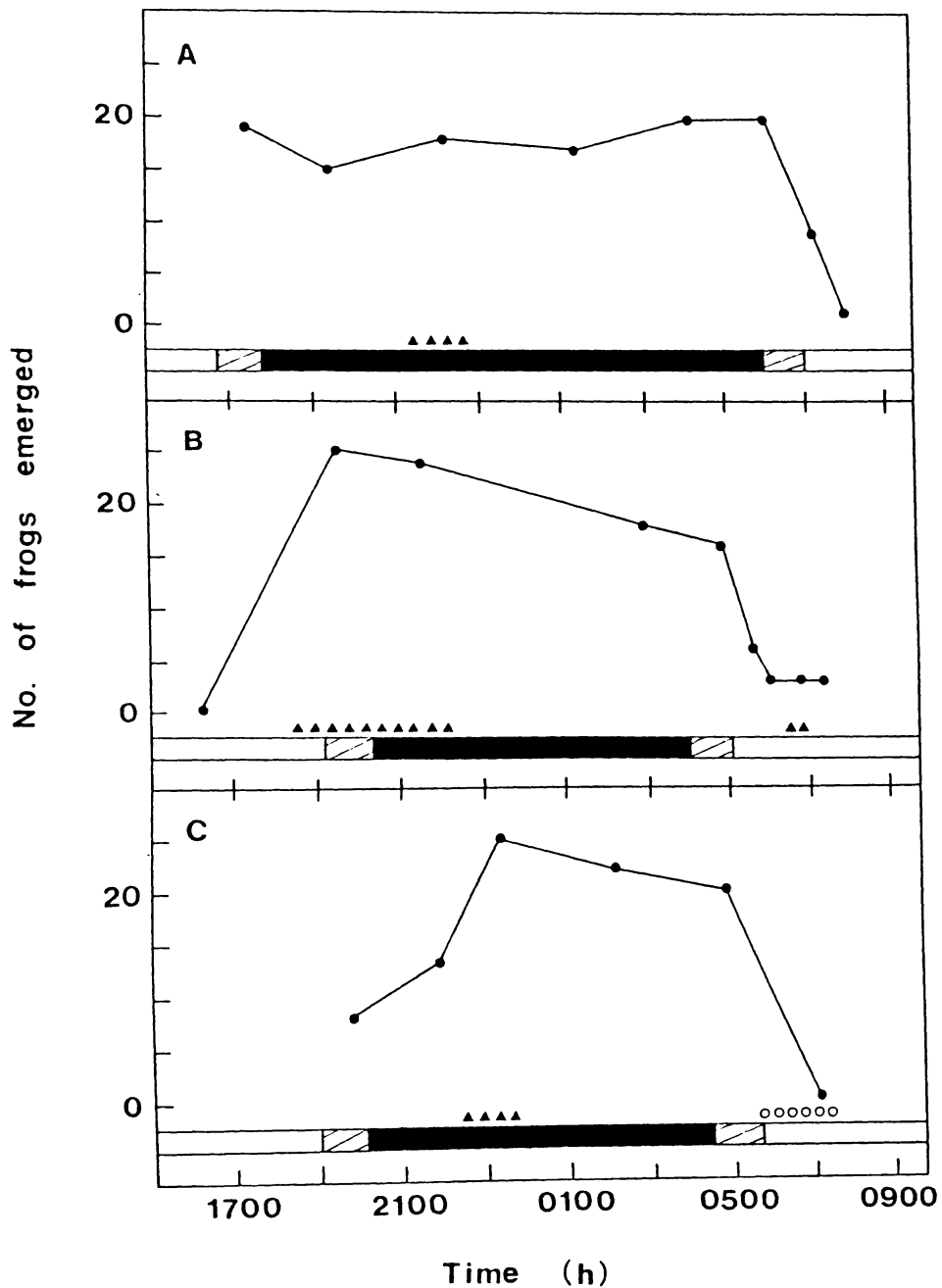


FIG. 9.1 Number of *L. archeyi* emerged in the search area during three nights following daytime rain. A = night beginning 4/6/84. B = night beginning 16/12/84. C = night beginning 8/2/85. Times are in NZ Standard Time. Horizontal bar indicates daylight (no shading), dusk or dawn (hatched) and night (solid). Rainfall is indicated by triangles, and open circles indicate a fine, sunny morning.

torch beam passing quickly over them, and the number counted during each search did not appear to be affected by earlier searches.

On a wet winter night (4/6/84), frogs had already emerged by dusk when the first count was made, but by full daylight at 0800 h only one frog remained emerged (Fig. 9.1A). On a wet summer night (16/12/84) no emerged frogs were seen during late afternoon, but at dusk, during torrential rain and a heavy electrical storm, 25 were counted (Fig. 9.1B). Three were still emerged well into daylight the following morning, which was overcast with intermittent drizzle. Relatively few frogs (13 or less) were seen during the first 2 h of darkness on a summer night when the vegetation was initially only moist (8/2/85; Fig. 9.1C). However, light rain between 2215 and 0000 h appeared to stimulate more frogs to emerge.

These observations confirm that if conditions are suitable, frogs will already be emerged within the first hour of darkness; this is therefore an appropriate time to compare numbers emerged on different nights.

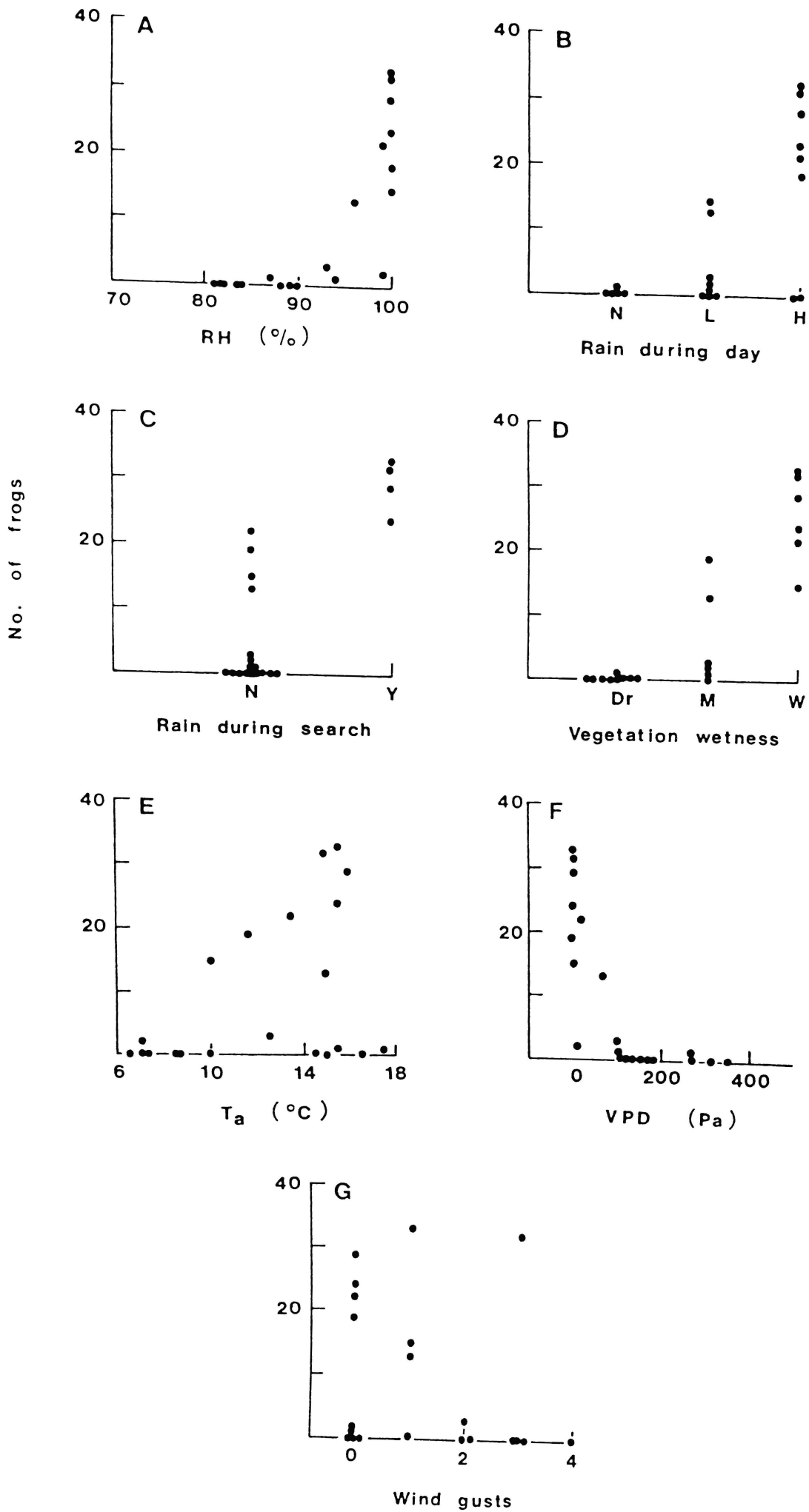
Effect of environmental factors on emergence

The number of frogs emerged during the first hour of darkness on 21 nights was examined in relation to RH, vegetation wetness, rainfall during the previous day, rainfall during the search, temperature, vapour pressure deficit and strength of windgusts (Fig. 9.2).

Relative humidity. Even on warm dry summer nights, no humidity less than 81% RH was recorded at night in the forest (Fig. 9.2A). At humidities between 81 and 90% RH, no frogs emerged except on one night (at 87% RH) when one was seen. Between 93 and 100% RH the number of frogs emerged showed a positive relationship with RH, the largest numbers (15-33) being consistently seen on nights when the air was essentially saturated (99-100% RH).

Rainfall during the previous day. On nights following days when no rain had fallen, one or no frogs were seen (Fig. 9.2B). Following light daytime rain up to 15 frogs were emerged at night, and following heavy daytime rain up to 33. However, large numbers of frogs were not always emerged following heavy daytime rainfall; on two occasions when heavy rain fell during the previous morning but afternoon winds dried the vegetation before nightfall, no frogs were seen.

FIG. 9.2 Relationship between environmental conditions and the number of *L. archeyi* emerged during the first hour of darkness in the search area (data for 21 nights). A = relative humidity; B = heaviness of rain falling during the previous daylight hours (N = none, L = light, H = heavy); C = occurrence of rain during the search (N = no, Y = yes); D = wetness of vegetation (Dr = dry, M = moist, W = wet); E = ambient temperature; F = vapour pressure deficit (VPD); G = strength of wind gusts. In each graph, the vertical axis indicates the number of frogs emerged. Values for Spearman rank correlation coefficients are given in the text.



Rain during the search. The largest numbers of frogs were counted on nights when rain was falling (Fig. 9.2C). However, moderate numbers (up to 22) were sometimes seen even if rain was not falling during the search.

Vegetation wetness. When the vegetation was consistently dry throughout the search area, one or no frogs emerged (Fig. 9.2D). On moist nights, up to 19 frogs were counted. The largest numbers (15-33) were consistently seen on nights when all the vegetation in the search area was thoroughly wet.

Temperature. Emerged frogs were seen at temperatures between 7.0 and 17.5°C (essentially the entire temperature range over which searches were made) (Fig. 9.2E). The largest numbers were seen at temperatures between 10.0 and 16.0°C but there was no clear relationship with temperature.

Vapour pressure deficit. Highest numbers of emerged frogs were seen on nights of little or no vapour pressure deficit (Fig. 9.2F). On 10 nights vapour pressure deficits ≥ 110 Pa were recorded, but only one frog was seen emerged in these conditions.

Wind. The largest numbers of frogs were usually seen on nights of little or no wind (wind gusts 0-1) (Fig. 9.2G). None was seen on nights with strong dry westerly winds (wind gusts 3-4), but on one night when wet easterly wind (wind gust 3) blew rain onto the study area, a large number of frogs (32) was counted.

Analysis. The significance of the relationship between each environmental factor and the number of emerged frogs was determined by calculating the non-parametric Spearman rank correlation coefficient (r_s). All moisture-related factors showed a strong correlation with the number of emerged frogs. The relationship was a positive one for RH ($r_s = 0.907$, $P < 0.001$), vegetation wetness ($r_s = 0.858$, $P < 0.001$), rainfall during the search ($r_s = 0.768$, $P < 0.001$), and rain during the previous day ($r_s = 0.653$, $P < 0.005$), and a negative one for vapour pressure deficit ($r_s = -0.786$, $P < 0.001$). Since all these factors are obviously at least partly correlated with each other, a key factor affecting emergence cannot be distinguished. Temperature showed a poor and barely significant correlation with emergence ($r_s = 0.453$, $P < 0.05$), and strength of wind gusts no significant

correlation ($r_s = -0.187, P > 0.1$).

Number of previous searches. Nearly half (48%) of all counts were made on nights when no count had been made the previous night, but on occasions searches were made up to four nights in a row. To ensure that emergence of frogs was not affected by searches made on previous nights, the number emerged was tested for correlation with the number of previous consecutive nightly counts; however, no significant correlation was found ($r_s = 0.120, P > 0.1$).

Activity of emerged L. archeyi

Within the first hour of darkness, 96% of all frogs seen on wet nights were above ground level (Table 9.1), climbing or sitting on the stems of shrubs, on tree trunks or on foliage. Frogs were seen up to 2 m above ground level. Some frogs which were readily identifiable by their colour pattern and size remained in virtually the same spots in the vegetation throughout the night. For instance, of nine recognisable frogs seen on at least five consecutive occasions spanning 10 h or more on the night of 4/6/84, six remained in the same place with only slight changes in position, and the remaining three moved less than 40 cm. Other frogs disappeared between successive observations, new ones appearing in different spots to keep the total number counted reasonably stable. Sites favoured by adults included the fronds or frond bases of kiekie (Plate 9.1) and nikau palms (*Rhopalostylis sapida*), silver fern trunks (ponga, *Alsophila tricolor*) and the bare trunks or branch forks of trees. Juveniles usually remained closer to the ground in clumps of bush rice grass (*Microlaena avenacea*) or hook sedge (*Uncinia uncinata*), or on the fronds of various ferns (see Frontispiece).

Despite lacking toe pads these frogs are excellent climbers, using their long slender digits and particularly the terminal phalanges to grasp vegetation. The typical movement pattern of a frog on the forest floor was to climb or walk for a few paces or make a single jump, then to remain motionless for several minutes. Once in the vegetation, the most conspicuous feature of emerged frogs was their lack of movement. Individuals were never seen feeding, despite the presence of small insects in the vegetation or on the ground, or being fed upon. The only interaction seen between individual frogs occurred when one climbed over the top of another, which produced essentially no reaction from either. At dawn, several frogs were seen returning

TABLE 9.1 Frequency distribution for height above ground of emerged *L. archeyi*. Values are given as a percentage of the total number of frogs seen.

Conditions	No. of nights	Total no. of frogs	Height		
			on ground (%)	>0 - 1 m (%)	>1 m (%)
Wet nights	6	155	4	79	17
Moist or dry nights	15	39	13	79	8



PLATE 9.1 Emerged adult *L. archeyi* on a kiekie frond at night.

to retreat sites, about 1 m away from the base of the vegetation they had climbed or jumped down from.

On dry or moist nights fewer frogs emerged, and these tended to remain on or closer to the ground than on wet nights (Table 9.1). Frogs which were seen on dry nights (either in or out of the search area) emerged at or soon after dusk, but most disappeared within 1 h. Frogs which were in the vegetation on dry nights were not more closely concealed than on wet nights.

2. Rehydration of *L. archeyi* on wet vegetation

Dehydrated *L. archeyi* were able to rehydrate from wet vegetation in the field. When placed on wet kiekie fronds for 4 h, five frogs increased in weight from $92 \pm 1\%$ to $99 \pm 2\%$ of W_0 ($\bar{x} \pm SE$) (Fig. 9.3). The remaining frog showed the highest rate of water uptake of all six frogs during the first 2 h period ($61 \text{ mg g}^{-1} \text{ h}^{-1}$) but could not be found at the fourth hour and had probably escaped. Experimentally dehydrated frogs were less settled than other frogs spontaneously emerged on the same night and did not continuously sit flat on the plant fronds; faster rates of rehydration could therefore be expected for undisturbed, naturally rehydrating frogs.

3. Absorption of soil moisture by *L. archeyi* and *L. hochstetteri*

Soil moisture tension curve

The soil moisture tension curve for laboratory soil is given in Fig. 9.4. Soil of 20% MC, which appeared dry, had a water potential of -850 kPa. Soils with moisture contents of 30% and 40% were closer to saturation (-360 and -240 kPa respectively).

Hydrated frogs

Hydrated *L. archeyi* and *L. hochstetteri* were unable to maintain weight on soil with a water potential of -850 kPa (20% MC, Fig. 9.5A). Both species dehydrated at nearly constant rates from 4 h until the end of the experiment at 28 h, and although the rate of mean weight loss over this interval was lower for *L. archeyi* ($1.9 \text{ mg g}^{-1} \text{ h}^{-1}$) than for *L. hochstetteri* ($2.9 \text{ mg g}^{-1} \text{ h}^{-1}$) the total weight loss at 28 h did not differ significantly between the two species (*L. archeyi* = $79 \pm 10 \text{ mg g}^{-1}$, *L. hochstetteri* = $90 \pm 14 \text{ mg g}^{-1}$; $t = 0.644$, $P > 0.5$).

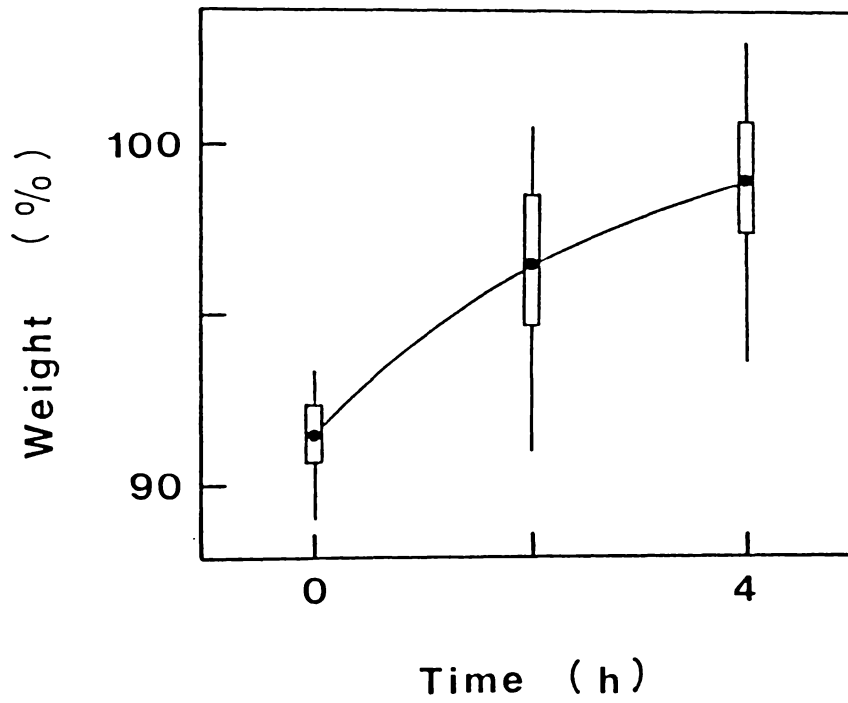


FIG. 9.3 Rehydration of experimentally dehydrated *L. archeyi* from wet kiekie fronds in the field at 15°C. Weight is given as a percentage of W_0 ($\bar{x} \pm SE$ and range for $n = 5$).

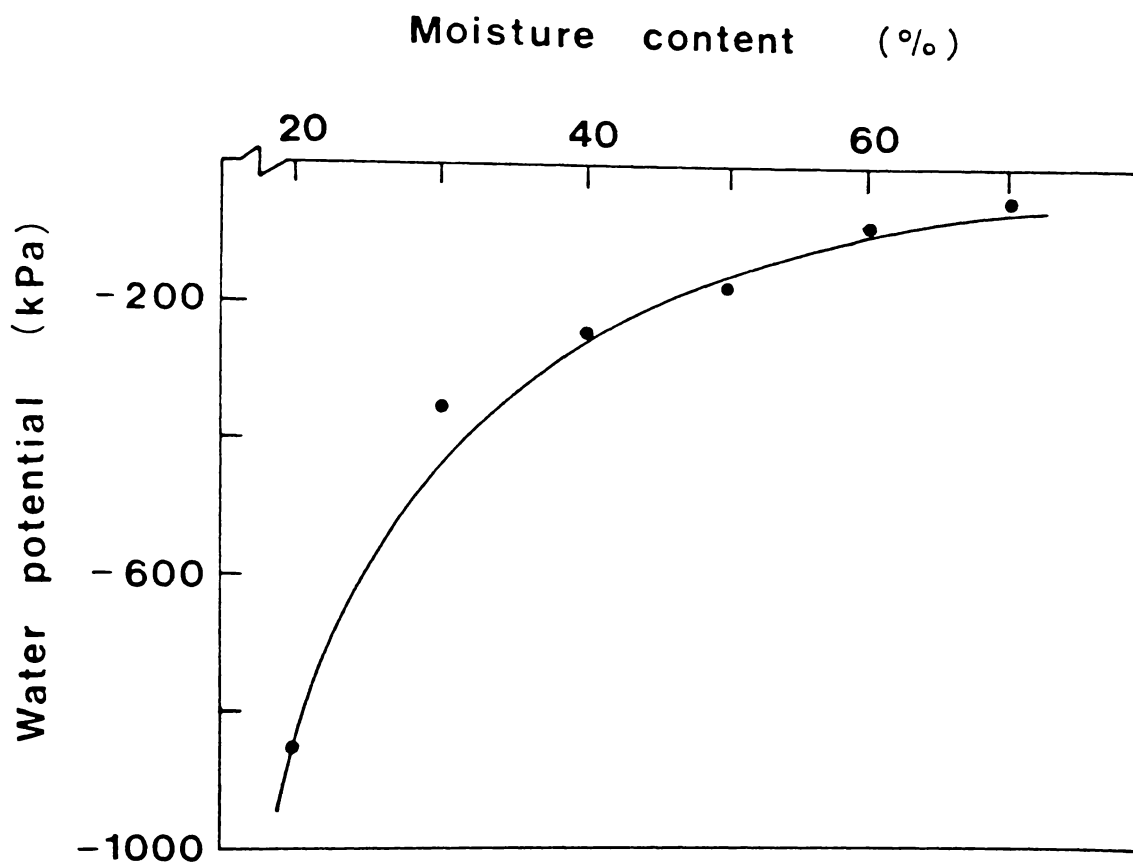


FIG. 9.4 Soil moisture tension curve for laboratory soil. Moisture content (MC) is given as a percentage of dry soil weight. The water potential for soil of 10% MC was off-scale (-17 958 kPa).

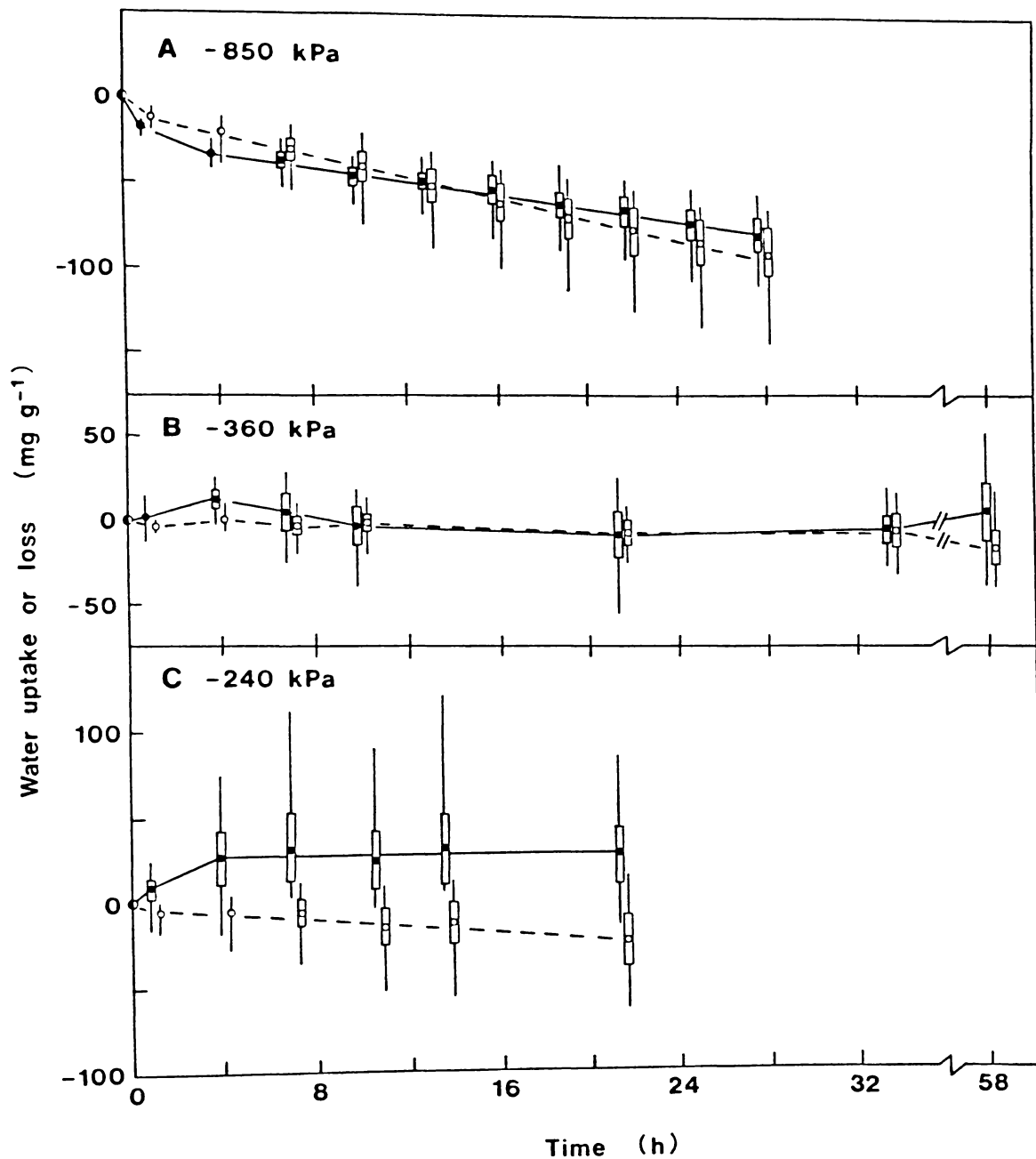


FIG. 9.5 Weight changes due to water uptake or loss for *L. archeyi* (●) and *L. hochstetteri* (○) on laboratory soil. Soil moisture contents were 20% (A), 30% (B) and 40% (C) (water potentials indicated on figure). Water uptake is given in $\text{mg g}^{-1} W_0$ ($\bar{x} \pm \text{SE}$ and range for $n = 5$ in each experiment; SE deleted where necessary for clarity). Weight changes due to urine removal following each weighing are not included.

On moister soils (-360 and -240 kPa, or 30% and 40% MC respectively), at least some individuals of both species were able to maintain essentially constant weight (Fig. 9.5B and C). Individual *L. archeyi* on soil with a water potential of -360 kPa remained within $\pm 55 \text{ mg g}^{-1}$ of W_0 over 58 h. On wetter soil (-240 kPa) most *L. archeyi* absorbed water from the soil during the first 4 h and then maintained a nearly constant weight, although there was considerable variation in the amount of moisture absorbed between individual frogs. *L. hochstetteri* was also able to maintain weight on soil with a water potential of -360 kPa. On wetter soil (-240 kPa), a slight decline in weight averaging $1 \text{ mg g}^{-1} \text{ h}^{-1}$ was apparent, but given this species' ability to maintain weight on soil at -360 kPa, this trend is probably fortuitous. Final values for water uptake or loss do not differ significantly between the two species on soil of either -360 kPa [*L. archeyi* = $+7 \pm 19 \text{ mg g}^{-1}$ (a weight gain), *L. hochstetteri* = $-16 \pm 10 \text{ mg g}^{-1}$ (a weight loss); $t = 1.052$, $P > 0.1$] or -240 kPa (*L. archeyi* = $+27 \pm 18 \text{ mg g}^{-1}$, *L. hochstetteri* = $-24 \pm 15 \text{ mg g}^{-1}$; $t = 2.175$, $P > 0.05$).

The lowest level of soil moisture at which an amphibian can absorb water from soil is defined as the absorption threshold (Heatwole and Lim 1961). These experiments indicate that the absorption threshold for hydrated *L. archeyi* and *L. hochstetteri* lies between -360 and -850 kPa, in the sense that frogs on soil with a water potential of -850 kPa are unable to absorb sufficient moisture to counteract simultaneous EWL.

Dehydrated frogs

Dehydrated *L. archeyi* and *L. hochstetteri* were able to absorb moisture from soil with a water potential of -240 kPa (Fig. 9.6). *L. archeyi* gained weight at a rate of $18 \pm 2 \text{ mg g}^{-1} \text{ h}^{-1}$ over the first hour, and this rate declined steadily as rehydration progressed. *L. hochstetteri* absorbed $6 \pm 2 \text{ mg g}^{-1} \text{ h}^{-1}$ over the first hour and the rate remained nearly constant for the next 5 h. Rates of water uptake over the first hour differ significantly between the two species ($t = 3.580$, $P < 0.01$).

DISCUSSION

In the Tapu search area, *L. archeyi* emerged in greatest numbers on wet

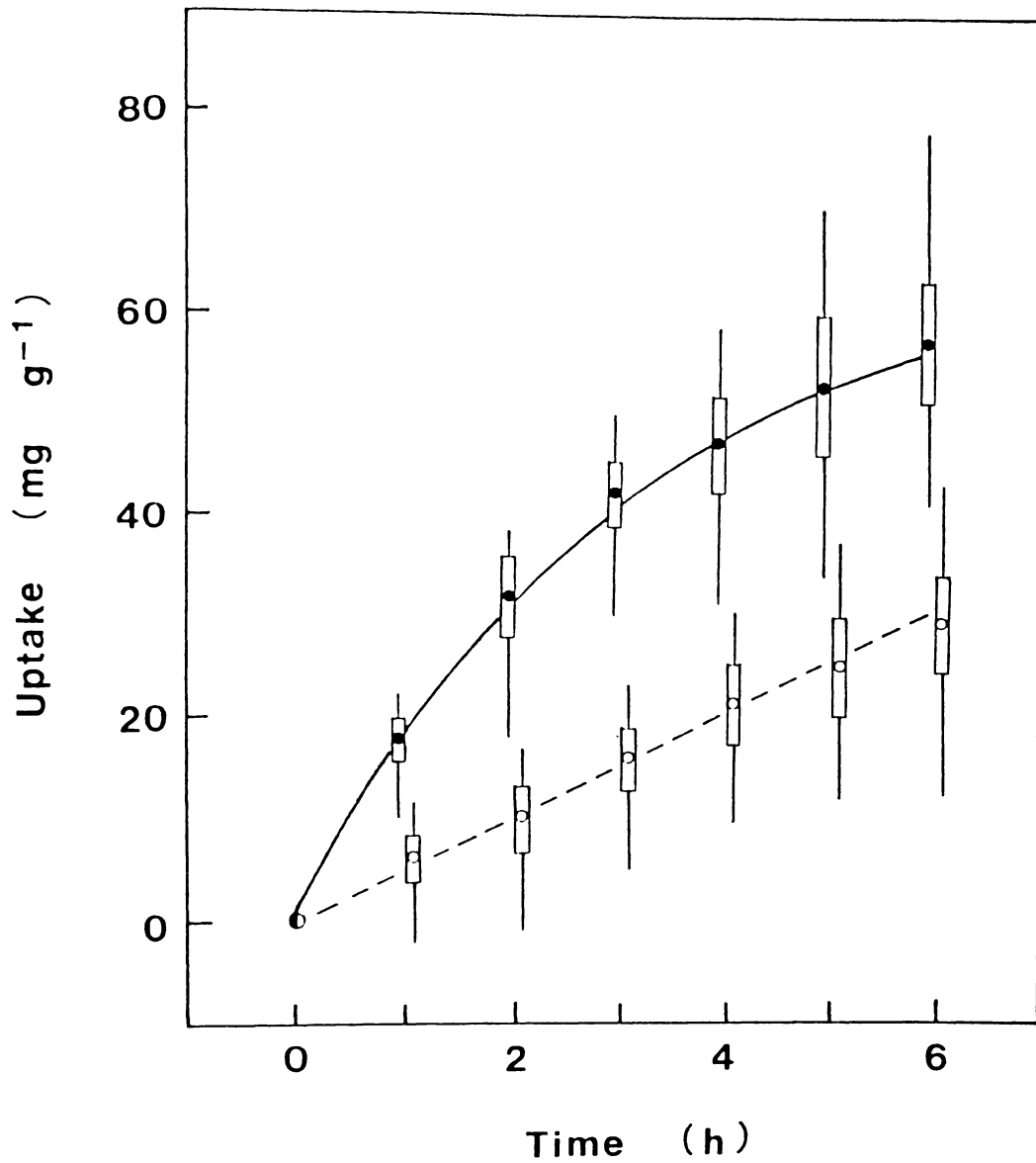


FIG. 9.6 Water uptake by dehydrated *L. archeyi* (●) and *L. hochstetteri* (○) from laboratory soil with a water potential of -240 kPa. Water uptake is given in mg g⁻¹ W₀ ($\bar{x} \pm SE$ and range for n = 5).

nights during or following heavy rain, when the vegetation was soaking and the air was saturated with water vapour (99-100% RH; vapour pressure deficit 0-10 Pa). Under these conditions, EWL would be negligible and water is widely available for absorption. Fewer frogs emerged on nights of lower humidity (87-99% RH) and higher vapour pressure deficit (70-110 Pa), when the vegetation was only moist. These conditions occurred when heavy daytime rain was followed by strong dry westerly winds, or when light daytime rain was followed by light or no wind. Virtually no frogs emerged at night following rainless days (RH \leq 87%, vapour pressure deficit \geq 110 Pa), when all vegetation in the search area was dry. In this respect *L. archeyi* differs from *Eleutherodactylus coqui*, which continues to emerge on dry nights for periods up to 7 d without rain (Pough *et al.* 1983).

All moisture-related factors considered here (RH, daytime rainfall, rain during search, vegetation wetness, vapour pressure deficit) showed a strong correlation with the number of emerged frogs. Temperature over the range searches were made (6.5-17.5°C) and strength of wind gusts had relatively less effect on emergence. Newman (1977) reported similar results for *L. hamiltoni* on Stephens' Island: numbers emerged were significantly and positively correlated with relative humidity and rain, but not with temperature or wind. A close relationship between relative humidity and nocturnal activity has also been reported for terrestrial phase *Salamandra salamandra* (Degani and Warburg 1978). However, this urodele continues to show some activity at humidities as low as 60% RH, whereas *L. archeyi* was not found emerged at humidities below 87% RH.

Frogs which emerge and climb when the air is not saturated and the foliage is dry undoubtedly suffer some water loss by evaporation. However, the high humidity and cool temperatures recorded inside the Tapu forest would minimise this. For instance, the greatest vapour pressure deficit at which emerged *L. archeyi* were seen (259 Pa) occurred on a still night at 87% RH and 17.5°C. At the same humidity and a slightly cooler temperature (14°C), predicted EWL is 1.8 mg g⁻¹ h⁻¹ (Fig. 3.2, Chapter 3). A frog which emerged for an entire night (10 h) under these conditions would therefore lose only 1.8% of W_0 by dehydration, a relatively small amount. Potential EWL from frogs which emerge on dry nights is minimised by the fact that frogs remain closer to the ground where air flow is probably less and humidity higher. Bell (1978) reported a greater difference between height

above ground on wet and dry nights for *L. hamiltoni* on Maud Island than observed here for *L. archeyi*: the mean height above ground of *L. hamiltoni* on dry nights was 0.5 m, and on wet nights in heavy rain 3.3 m.

On wet nights, *L. archeyi* emerge at or about dusk and climb. Individual frogs may remain all night in the same spot in the vegetation, only returning to their retreat sites at dawn. If the following day is also wet, some may stay above ground until well into daylight, confirming earlier reports of daytime activity (present study, Chapter 2; Bell 1978). Thus, although traditionally regarded as terrestrial and nocturnal (E.M. Stephenson 1961; Bell 1982a, 1985a), on the basis of the activity observed in the present study these frogs can be considered at least partly arboreal and diurnal.

Except when seen climbing, emerged *L. archeyi* were almost always motionless, regardless of weather conditions. Possible activities engaged in by frogs above ground include foraging and/or predator avoidance, although nothing is known of the preferred prey, or predators (if any) of *L. archeyi* in wild populations. Frogs which climb also come into contact with wet vegetation from which water can be absorbed, and the rehydration experiment carried out shows that experimentally dehydrated *L. archeyi* are able to rehydrate from wet kiekie fronds. To my knowledge, this represents the first time that rapid rehydration has been demonstrated for an anuran in a field situation, using a naturally available moisture source. Although the fastest rate of water uptake recorded on kiekie ($61 \text{ mg g}^{-1} \text{ h}^{-1}$ over 2 h) was slightly less than the mean rate recorded for frogs rehydrating in shallow water in the laboratory ($73 \text{ mg g}^{-1} \text{ h}^{-1}$ over 2 h, calculated from data in Fig. 3.3), the rate observed is nevertheless sufficiently high that dehydrated frogs would need to sit on wet vegetation for only a portion of a night to regain a dehydration deficit of 10%.

Dehydrated *L. archeyi* are also able to absorb moisture from soil. Laboratory experiments indicate that the absorption threshold of *L. archeyi* lies between -240 and -850 kPa, and since moisture-retaining substrates (soil, humus) on which this species was found in the field were almost always wetter than this (Chapter 2), absorption of water from these substrates is possible at most, if not all, times of the year. The lowest soil water potential on which a

frog was found was < -100 kPa, but wetter substrates were available nearby. Whether or not water potential ever falls below the absorption threshold for *L. archeyi* remains unknown.

Several authors have suggested that the relative ability of various species of amphibians to absorb moisture from soil may be a factor accounting, at least in part, for differences in geographical distribution (Dole 1967; Claussen 1969; Walker and Whitford 1970). Differences in absorption threshold amongst six species of anurans examined by Walker and Whitford (1970) support this suggestion. Thresholds were closest to saturation (between -81 and -122 kPa) for the most aquatic species (*Rana pipiens*), intermediate for two species from mesic environments (*Hyla cinerea*: between -122 and -152 kPa; *Bufo americanus*: between -152 and -253 kPa), and lowest (between -253 and -274 kPa) for three fossorial toads from xeric habitats (*Scaphiopus couchi*, *S. hammondi* and *Bufo cognatus*). After several months buried in soil, *S. couchi* and *S. hammondi* may reduce their absorption thresholds to values lower than -1013 kPa as a consequence of urea storage (Ruibal *et al.* 1969). However, Spight (1967b) found no evidence of differences in absorption threshold between six species of salamanders ranging in habitat from fully aquatic to fully terrestrial: in each case the threshold lay between -203 and -405 kPa.

The results of the present study show no evidence for a difference in absorption threshold between *L. archeyi* and *L. hochstetteri*, at least in short-term experiments, despite the fact that *L. archeyi* is usually found on less saturated substrates (Chapter 2). Thresholds for both species lie between -360 and -850 kPa, values which are further from saturation than those reported by Walker and Whitford (1970) but consistent with the values reported for salamanders by Spight (1967b) and for *Eleutherodactylus coqui* (-620 to -650 kPa) (van Berkum *et al.* 1982). These results suggest that *L. hochstetteri* is capable of absorbing moisture from the soil found in *L. archeyi* retreat sites on the Tapu ridgetop, at least at most times of the year. The only difference between the two species shown by these experiments is in the rate at which they are able to absorb soil moisture: *L. archeyi* rehydrated from soil at -240 kPa at an initial rate three times that measured for *L. hochstetteri*. This difference is attributable to the fact that *L. archeyi* possesses a cutaneous hydroosmotic response whilst *L. hochstetteri* does not (Chapter 3). One possible consequence

of this is that on soil just slightly wetter than the absorption threshold, *L. archeyi* may be able to absorb moisture sufficiently rapidly from the soil to compensate for simultaneous EWL through the dorsal skin, whilst *L. hochstetteri* might not and would thus slowly dehydrate. If this is true, such a difference could influence the ability of *L. hochstetteri* to remain on the ridgetops during periods of low rainfall.

CHAPTER 10

GENERAL DISCUSSION

The theme throughout this study has been to describe physiological and ecological aspects of the water relations of New Zealand's endemic frogs. While most emphasis has been placed on *Leiopelma archeyi* and *L. hochstetteri*, valuable comparisons with the rarer *L. hamiltoni* have also been made. Here, the results from field and laboratory studies are reviewed, and their implications for theories about the evolution of the cutaneous hydroosmotic response amongst anuran amphibians are also discussed.

Habits and habitats of *L. archeyi* and *L. hochstetteri*

The study began with a survey of the habitats of *L. archeyi* and *L. hochstetteri* in an area of native forest near Tapu on the Coromandel Peninsula (Chapter 2). These two species have long been known to live in cool, humid and usually forested habitats; however, until the present study little quantitative information on microclimatic conditions in these habitats or on the extent of overlap in the distributions of the two species was available.

The results from 13 monthly searches showed that *L. archeyi* and *L. hochstetteri* have nearly disjunct distributions in the Tapu study area. *L. archeyi* is a completely terrestrial frog which remains on the ridgetops year-round, occupying cool, humid retreat sites under rocks and logs on the forest floor by day. Although permanent free-standing water is absent from this habitat, conditions are often moist. Annual rainfall during the study was high (2729 mm), ambient air temperatures were cool ($< 23^{\circ}\text{C}$), ambient relative humidity (RH) was often high ($> 80\%$), and soil or humus substrates within retreat sites usually had a high moisture content ($> 100\%$ of dry soil weight) and water potential close to saturation (> -36 kPa). In these conditions frogs probably suffer little or no water shortage, since in similar situations in the laboratory moisture is absorbed sufficiently rapidly from soil to counteract evaporative water loss (Chapter 9).

Periods of up to several weeks without rain occasionally occurred on

the ridgetops. During dry weather RH fell to values as low as 56%, and substrate moisture content and water potential also declined (to 32% and < -100 kPa respectively). Some *L. archeyi* were then found on substrates which appeared dry, and from which their ability to absorb water is uncertain. Whether or not substrate water potential ever declines to values lower than the soil moisture absorption threshold of *L. archeyi* (which laboratory experiments indicate lies between -360 and -850 kPa; Chapter 9) is unknown.

The presence of suitable retreat sites is almost certainly crucial to the survival of *L. archeyi* on the ridgetops during dry periods. Retreat sites are cooler and more humid than ambient air, often contain substrates of saturated soil, and are protected from the dehydrating effects of wind. The difference in RH alone is of considerable survival value. Based on rates of evaporative water loss (EWL) measured in the laboratory (Chapter 3), a frog exposed to still air at 56% RH and 14°C , without access to substrate moisture, would reach a potentially lethal water loss in less than 3 d. At 99% RH (the estimated maximum retreat site humidity; Chapter 2), the same water loss would require 37 d to reach.

L. hochstetteri showed only a small amount of overlap in distribution with *L. archeyi* at the Tapu study area. Most *L. hochstetteri* were found at lower altitudes along the edges of streams, either on waterlogged substrates or partly immersed in stream water. Streamside habitats always had high relative humidities ($> 80\%$), and during dry summer weather, evaporation rates from wet surfaces at streamside sites were lower than at ridgetop sites. *L. hochstetteri* were only found on wet or moist substrates (water potential > -6 kPa) from which water uptake is always possible (Chapter 9), and laboratory experiments confirmed that this species has a greater preference for water than does *L. archeyi* (Appendix I). A small percentage (4%) of *L. hochstetteri* found in the Tapu study area occurred on the ridgetops in the same habitats as *L. archeyi*. This possibly indicates that some *L. hochstetteri* move away from streams and onto the ridgetops during wet weather (which sometimes leads to stream flooding). However, mark-recapture studies are required to confirm this hypothesis.

Further studies on *L. archeyi* showed that this species is behaviourally adapted to living in an environment with only intermittent above-ground surface moisture. Emergence in

the field at night was strongly correlated with indices of moisture (RH, rainfall, wetness of vegetation, vapour pressure deficit; Chapter 9), and emerged frogs were occasionally also seen during wet daytime weather (Chapter 2). Frogs which emerge during or following rain are able to make up any water deficit previously incurred, by rehydrating from wet vegetation. The habitats and emergence behaviour of *L. archeyi* appear similar to those previously described for the allopatric *L. hamiltoni* (Newman 1977; Newman *et al.* 1978), although no information on substrate moisture conditions is available for the latter species. In both species, the consequences of sporadic emergence on ecological activities such as foraging, mate selection and other social interactions are unknown.

Laboratory studies on water balance

The field studies described above confirmed that *L. archeyi* is, like *L. hamiltoni*, a completely terrestrial frog, whilst *L. hochstetteri* is semiaquatic. Laboratory studies examined aspects of water balance in the three species, to determine whether *L. archeyi* and *L. hamiltoni* have adaptations for reducing water loss or enhancing water uptake which are not present in *L. hochstetteri*. Rates of dehydration and rehydration, responses to the neurohypophysial hormone arginine vasotocin (AVT), and patterns of nitrogen excretion were compared. Skin structure in *L. archeyi* and *L. hochstetteri* was also examined.

Dehydration and rehydration

The three species showed no appreciable differences in surface area (SA)-specific rates of evaporative water loss (EWL) (Chapter 3), despite their differences in habitat. Calculated mean values for resistance to EWL in still air were between 5 and 10 s cm⁻¹ for all species, similar to the value of 12 s cm⁻¹ recorded for a dish of water. These results are consistent with those from many previous studies of EWL in anurans, which show that terrestrial species from mesic environments usually have no greater resistance to water loss than do semiaquatic or aquatic species.

The three species do, however, fall into two distinct groups with respect to their ability to absorb water rapidly after dehydration. Dehydration of *L. archeyi* and *L. hamiltoni* caused the rate of water uptake through ventral skin to increase markedly, so that when

dehydrated frogs were placed in shallow water their original body weight was rapidly restored (in 1-2 h, for individuals dehydrated by 9-15% of W_0). This increase in cutaneous water uptake operated in conjunction with a temporary reduction or cessation of urine release. Similar cutaneous and antidiuretic water balance responses have been reported for many other terrestrial, arboreal or semiaquatic anurans. However, a cutaneous response was not seen in *L. hochstetteri*: rehydration occurred slowly (at a rate of about 1% of W_0 per hour), and was apparently due solely to a reduction in the rate of urine output.

Responses to AVT

The ability of many anurans to rehydrate rapidly has been attributed, at least in part, to release of the neurohypophysial hormone AVT during dehydration (Chapter 1). Circulating AVT is known to reduce water loss from dehydrating anurans and increase water uptake, by increasing the osmotic permeability of the ventral skin, reducing the rate of urine production and increasing the rate of bladder water reabsorption. This evidence suggested that the differing rehydration rates of the two groups of *Leiopelma* might be caused by differing responses to circulating AVT.

Results from experiments in which hydrated frogs were treated with exogenous AVT were consistent with this hypothesis (Chapter 4). *L. archeyi* and *L. hamiltoni* showed rapid gains in weight (about 30% in 4-5 h) following treatment with 7×10^{-11} mol g^{-1} AVT; this water retention was due largely to an increase in the rate of water uptake through the ventral skin (cutaneous hydroosmotic response). In contrast, *L. hochstetteri* absorbed water at a slow and linear rate, gaining only 9% in weight over 9 h. No increase in the rate of cutaneous water uptake occurred, and the rate of weight gain observed was consistent with a solely antidiuretic hormonal effect (at the kidneys and/or bladder).

Further experiments were carried out to examine the effects of AVT on the skin, kidneys and bladder independently (Chapter 5). Isolated preparations of skin from ventral pelvic, ventral pectoral and dorsal regions were exposed to an osmotic gradient (inner surface hyperosmotic). AVT (3×10^{-8} mol l^{-1}) at the inner surface caused a marked increase (2.4-7.8x) in osmotic water flow (OWF) through pelvic skin of *L. archeyi* and *L. hamiltoni*, but not through pectoral or

dorsal skin. Some preparations of pectoral skin from *L. hamiltoni* (but not *L. archeyi*) showed moderate responses after long periods at increased concentrations, and the difference between the two species may have reflected the more recently caught condition of *L. hamiltoni*. However, skin from all three regions of *L. hochstetteri* showed no increase in OWF, even when exposed to pharmacological doses of AVT (up to 3×10^{-6} mol l⁻¹). This confirmed the absence of a cutaneous hydroosmotic response in this species.

The effect of AVT on glomerular filtration rate (GFR) at the kidneys was examined by measuring creatinine clearance (C_{CR}) before and after treatment with AVT. C_{CR} in *L. archeyi* and *L. hamiltoni* fell from 24 and 21 $\mu\text{l g}^{-1} \text{h}^{-1}$ to 0 and 1 $\mu\text{l g}^{-1} \text{h}^{-1}$ respectively following AVT treatment, implying that filtration at the kidneys essentially ceased. Mean C_{CR} also fell in six AVT-treated *L. hochstetteri* (from 22 to 10 $\mu\text{l g}^{-1} \text{h}^{-1}$); this reduction was not statistically significant, but was sufficiently large to account for the rate of weight gain observed in AVT-treated animals. Four *L. hochstetteri* did show a marked reduction in C_{CR} (from 30 to 5 $\mu\text{l g}^{-1} \text{h}^{-1}$), but the remaining two animals showed an increase in clearance and this may have reflected a technical failure to completely remove bladder urine at the end of the prior control period. This would lead to an underestimate of control C_{CR} and an overestimate of C_{CR} following AVT treatment. Further studies are therefore required to confirm the effect of AVT on GFR in *L. hochstetteri*.

Bladder water reabsorption does not seem to be an important method of water conservation in any of the *Leiopelma* species. Maximum voluntary bladder urine volumes in captive animals were small compared with those of many other anurans, values ranging from an equivalent of 4% of W_0 for *L. hochstetteri*, to 12% for *L. archeyi*. AVT (3×10^{-8} mol l⁻¹) also had a relatively small effect on OWF through isolated bladder lobes. After 50 min exposure, the stimulated rate of OWF (in mg h^{-1}) was 1.6 \times the resting value for *L. archeyi*, 1.5 \times for *L. hamiltoni* and 2.1 \times for *L. hochstetteri*. In comparison, the large toad *Bufo marinus* has a bladder holding fluid equivalent to at least 25% of W_0 (Shoemaker 1964) and stimulated rates of OWF through isolated bladder lobes are up to 100 \times resting values (Eggens 1972). It seems that in small terrestrial frogs such as *L. archeyi* and *L. hamiltoni*, which have a large SA:volume ratio, the bladder plays a relatively small role compared with the skin in the overall water

balance response.

The effects of AVT on the skin, kidneys and bladder of the three species have been summarised in models (Fig. 10.1). These models combine results from Chapters 3, 4 and 5 to illustrate the water movements occurring in hydrated frogs before and after treatment with AVT; they are based on similar models developed by Brown and Brown (1977) for the urodele *Notophthalmus viridescens*. All numerical values have been converted to $\mu\text{l g}^{-1} \text{h}^{-1}$ (assuming that 1 mg of fluid has a volume of 1 μl). Although the numerical values do not balance exactly, the models nevertheless help to illustrate the difference between the water balance response of *L. hochstetteri* and the responses of *L. archeyi* and *L. hamiltoni*.

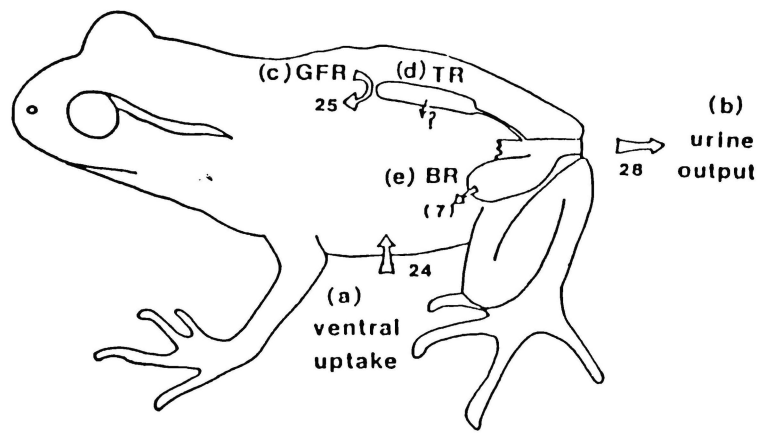
In the models of "control" animals, water uptake through ventral skin is relatively slow and essentially balanced or exceeded by urine output (differences of 4-8 $\mu\text{l g}^{-1} \text{h}^{-1}$ can be accounted for by the minimum reproducibility of weighing). Thus, body weight stays reasonably constant or even declines slightly. However, following treatment with AVT, *L. archeyi* and *L. hamiltoni* show a four-fold increase in the rate of water uptake through ventral skin and a simultaneous drop in GFR and urine output. The net effect is a fluid gain of 90 $\mu\text{l g}^{-1} \text{h}^{-1}$ and 47 $\mu\text{l g}^{-1} \text{h}^{-1}$ respectively (equivalent to weight gains of 9.0% and 4.7% of W_0 per hour respectively). Although the rate of fluid retention was lower for *L. hamiltoni* than for *L. archeyi* in the experiment from which these values are taken, this was not the case in all experiments and is not evidence of an interspecific difference. The models suggest that in both species, bladder reabsorption contributes little, if at all, to the water balance response, particularly since GFR simultaneously declines. Even if both bladder lobes were full at the time of AVT treatment, their small volume and poor hydroosmotic response *in vitro* suggests that they would reabsorb water equivalent to a total of only 11 $\mu\text{l g}^{-1} \text{h}^{-1}$ in *L. archeyi* (9 $\mu\text{l g}^{-1} \text{h}^{-1}$ for *L. hamiltoni*). This is much lower than the rate of fluid retention contributed by increased water uptake through the skin, or to a lesser extent by reduced GFR. In AVT-treated *L. archeyi*, the increase in cutaneous water uptake is equivalent to 69 $\mu\text{l g}^{-1} \text{h}^{-1}$ (39 for *L. hamiltoni*), and the reduction in GFR 25 $\mu\text{l g}^{-1} \text{h}^{-1}$ (20 for *L. hamiltoni*).

Unlike those of *L. archeyi* and *L. hamiltoni*, models of AVT-treated

FIG. 10.1 Models of water movements in hydrated *Leiopelma* species sitting in shallow water before and for first hour after AVT treatment. Water movements are given in $\mu\text{l g}^{-1} \text{h}^{-1}$ and the sizes of arrows indicate the relative rates.

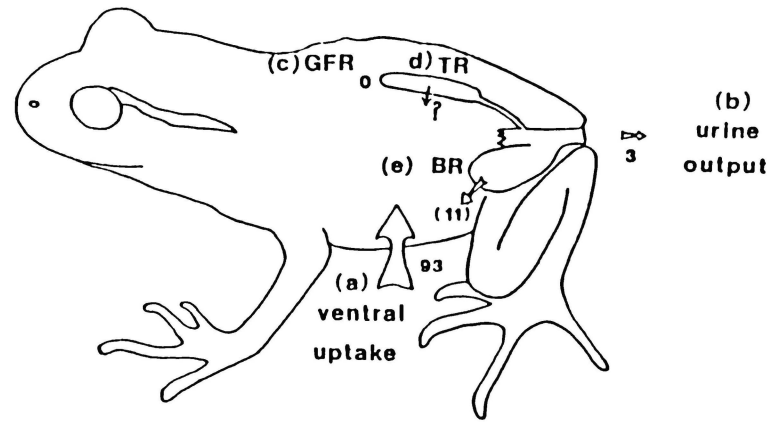
Rates of (a) water uptake through ventral skin and (b) urine output for control animals are from hydrated water uptake experiments of Chapter 3 (FIGS 3.3, 3.4 and 3.5). Rates of (a) and (b) for AVT-treated animals come from experiment in which AVT ($7 \times 10^{-11} \text{ mol g}^{-1}$) was injected *in vivo* (Chapter 4, FIGS 4.1, 4.2 and 4.3). Glomerular filtration rates or GFR (c) are from data in TABLE 5.2. Tubular reabsorption or TR (d) was not measured. Values for bladder reabsorption or BR (e) are given in parentheses and are calculated from data in TABLE 5.4, assuming that the rate of reabsorption through an intact bi-lobed bladder is equal to twice the rate of OWF through a single isolated bladder lobe. These values for BR estimate the amount of reabsorption possible from full bladders. In reality, bladders were emptied at the start of all *in vivo* experiments so that actual reabsorption is probably less than indicated.

[Models based on an idea from Brown and Brown (1977).]



Control

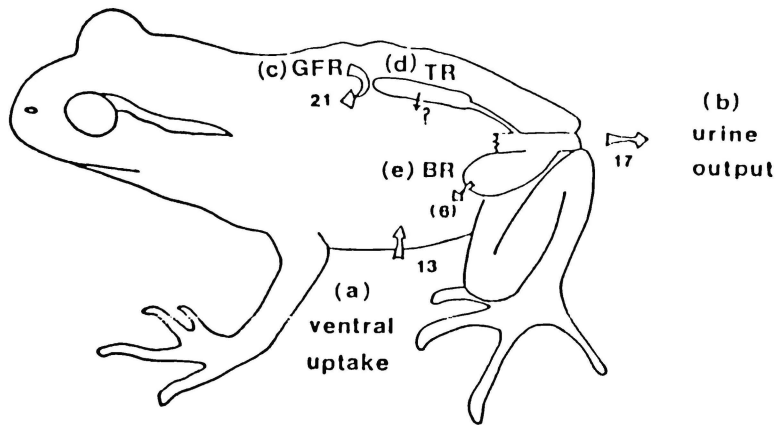
No weight gain
uptake \approx loss



AVT-treated

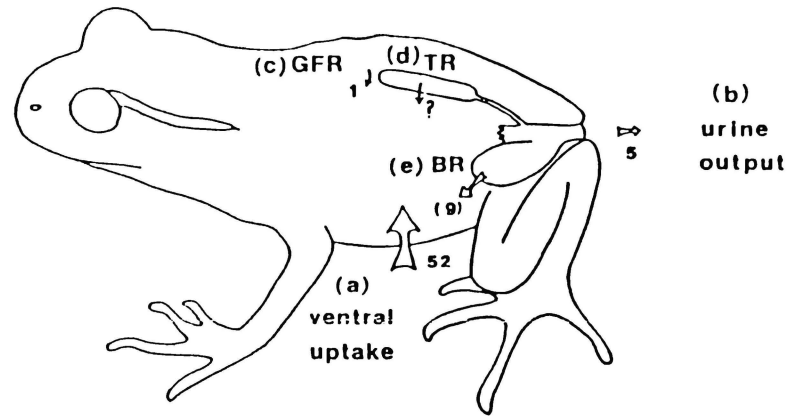
Weight gain $\approx 90 \mu\text{l g}^{-1} \text{h}^{-1}$

L. archeyi



Control

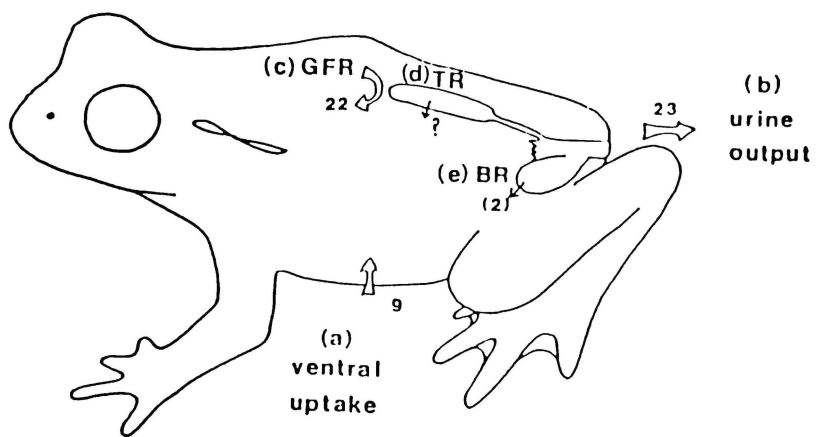
No weight gain
uptake \approx loss



AVT-treated

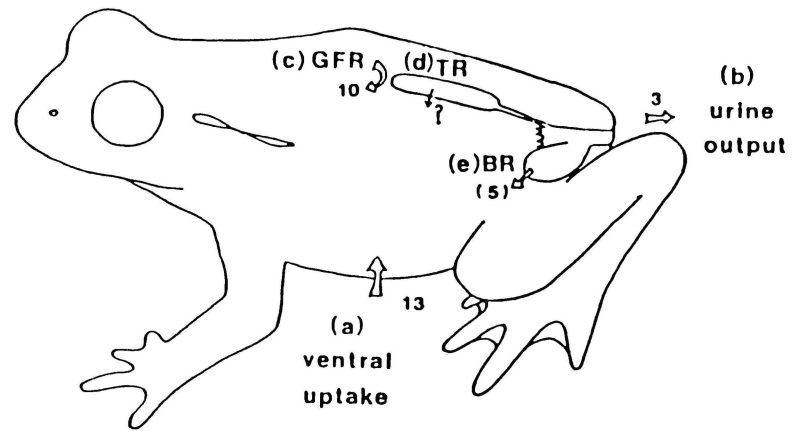
Weight gain $\approx 47 \mu\text{l g}^{-1} \text{h}^{-1}$

L. hamiltoni



Control

No weight gain
uptake \leq loss



AVT-treated

Weight gain $\approx 10 \mu\text{l g}^{-1} \text{h}^{-1}$

L. hochstetteri

L. hochstetteri show no significant increase in cutaneous water uptake. However, GFR is reduced by $12 \mu\text{l g}^{-1} \text{h}^{-1}$, which is sufficient to account for the observed net weight gain of 1.0% of W_0 per hour. If both bladder lobes were full, reabsorption of bladder water could account for an increase of $5 \mu\text{l g}^{-1} \text{h}^{-1}$ (i.e., about half the rate of water retention observed).

No attempt was made to measure the effects of AVT on tubular reabsorption in this study, and this is one aspect which could be addressed in future studies of water balance in these species. However, the models suggest that tubular reabsorption may contribute little to the water balance response. In control animals, urine output is within $4 \mu\text{l g}^{-1} \text{h}^{-1}$ of the amount filtered by the kidneys. In AVT-treated *L. archeyi* and *L. hamiltoni*, the effect on GFR is so potent that there is little or no primary urine from which water could be reabsorbed. Filtration apparently continues in AVT-treated *L. hochstetteri*, but the amount of urine excreted plus that potentially reabsorbed by the bladder accounts for within $2 \mu\text{l g}^{-1} \text{h}^{-1}$ of the amount filtered.

Skin structure

The relationship between the structure of the skin and its function in rapid water uptake was investigated for *L. archeyi* and *L. hochstetteri*, and comparisons were made with the hylid *Litoria aurea* (Chapter 6). Fresh flat mounts, paraffin-embedded transverse sections and scanning electron microscope preparations of pelvic, pectoral and dorsal skin were examined. Pelvic skin of *L. archeyi* had a smooth and thin epidermis ($14 \mu\text{m}$, or 2-3 cell layers deep in the specimen examined) and lacked the well-vascularised mounds characteristic of pelvic skin of *Litoria aurea* and many other anurans, which have been implicated in rapid water uptake. Pectoral skin of *L. archeyi* was thinner and less transparent, but not otherwise morphologically distinct from pelvic skin. Dorsal skin had a greater density of chromatophores and contained aggregations of large granular glands, but in non-glandular areas was similar in structure and thickness to pelvic skin. Skin from all three regions of *L. archeyi* was poorly vascularised.

Pelvic skin of *L. hochstetteri* was also smooth. Although the epidermis from the specimen examined was more than three times as thick as that from *L. archeyi*, the total skin thickness (epidermis

plus dermis) in non-glandular regions did not differ greatly between the two species (92 μm for *L. archeyi*, 115 μm for *L. hochstetteri*). Unexpectedly, pelvic skin from *L. hochstetteri* was much more densely vascularised than that of *L. archeyi*. The extensive network of subepidermal capillaries seen in pelvic skin of *L. hochstetteri* was also present in pectoral and dorsal skin. These results contrast with those of previous studies suggesting that pelvic skin of anurans with a cutaneous hydroosmotic response is often "sculptured", and hypervascularised in comparison to other skin regions or in comparison to pelvic skin from species without such a response (e.g., Roth 1973; Christensen 1974a).

Nitrogen excretion

No differences were observed between adults of the three *Leiopelma* species in terms of predominant mode of nitrogen excretion (Chapter 7). All are ureotelic, as are many other terrestrial or semiaquatic anurans. The mean proportion of urea plus ammonia nitrogen excreted as ammonia nitrogen in bladder urine or in 24 h bathing water samples was between 3 and 7% for all species, and no uric acid was detected in bathing water samples.

Reproductive adaptations of *L. archeyi* to terrestriality

Aspects of nitrogen excretion and water balance during embryonic and larval development were further examined in *L. archeyi*, which lays large, yolky, terrestrial eggs (Chapters 7 and 8). The developing embryo is surrounded by a gelatinous egg capsule, which protects the embryo from unfavourable rates of water exchange. The capsule has no resistance to EWL, but absorbs water rapidly when the egg comes into contact with moist surfaces. Experiments with agar egg models showed that the brooding posture of male *L. archeyi* reduced weight-specific EWL from egg clusters by more than half, and brooding may also enhance water uptake into real eggs by bringing the eggs into close contact with moist substrates. Eggs in natural nests absorbed water during development, but it is uncertain whether this is necessary for successful hatching to occur. Substrate moisture is probably an important source of water for eggs in natural nests, but further experiments are necessary to quantify the role of other potential sources, including excreted urine or water transferred osmotically through the skin of the brooding frog.

Intracapsular fluid collected from eggs close to or at hatching contained more ammonia nitrogen than urea nitrogen, suggesting that larvae are ammonotelic whilst inside the egg. However, the possibility that intracapsular larvae store urea within their body tissues has not been eliminated. Newly-hatched larvae are or rapidly become ureotelic, and complete the final weeks of development on the back of the male frog. The larvae are tiny (≤ 110 mg), tailed and elongate in shape, with consequently a very high SA:volume ratio. Although they dehydrated rapidly when placed on dry surfaces, those on the backs of adult frogs did not lose weight. This suggests that larvae absorb sufficient water osmotically from the male frog beneath them to replace that simultaneously lost by evaporation. The presence of a continuously moist substrate such as an adult's back is probably crucial to the survival of the poorly mobile newly-hatched larvae in natural nests. Hatched larvae rehydrated rapidly from shallow water, implying that a cutaneous hydroosmotic response to secreted AVT is at least partly developed before the end of metamorphosis.

Evolution of the cutaneous hydroosmotic response amongst anuran amphibians

A significant finding of this thesis is the presence of a cutaneous hydroosmotic response in adult *L. archeyi* and *L. hamiltoni*, but not in *L. hochstetteri*. In additional studies the response was also found in the hylid *Litoria aurea* (Chapter 5 and Appendix II), but not in *Xenopus laevis* or a single *Ascaphus truei* (Appendix II). Several previous reviews have reported the response to be absent in only two adult anurans, *X. laevis* and *Rana cancrivora* (Bentley 1971a, 1974, 1982b).

Two explanations have been proposed to account for the presence of a cutaneous hydroosmotic response in some anurans but not in others (Chapter 1). According to the first (the "ecological" hypothesis), cutaneous responses are best developed in species which live in habitats of reduced water availability where dehydration may occur (Ewer 1952; Shoemaker and McClanahan 1980). Rapid rehydration is advantageous in such habitats, since it enables animals to make use of transient water sources (Bentley 1971a; Alvarado 1979), quickly regaining lost water prior to any subsequent dehydration. This hypothesis implies that a cutaneous response has been lost in

X. laevis (Sawyer *et al.* 1978), which lives in an aquatic environment where dehydration is unlikely to occur. An alternative explanation is that development of the cutaneous hydroosmotic response varies according to the phylogenetic position of the species concerned (Heller and Bentley 1965; Cameron 1974). According to this "phylogenetic" hypothesis, the response is best developed in more advanced anurans but may be absent or poorly developed in archaic forms.

The results obtained for the *Leiopelma* species support the ecological hypothesis but not the phylogenetic hypothesis. Clearly, implications that cutaneous responses might be poorly developed or absent in archaic frogs in general (Heller and Bentley 1965) and absent in leiopelmatid frogs in particular (Cameron 1974) are incorrect, since a well-developed response is found in *L. archeyi* and *L. hamiltoni*. Both are terrestrial species. However, *L. hochstetteri* lacks the response, and this result is consistent with its more aquatic habitat.

These results, plus the large number of publications on water balance of anurans in recent years, suggest that a wider review of the evidence for the two hypotheses amongst anurans in general is timely. A survey of the distribution of the cutaneous hydroosmotic response amongst anuran amphibians has therefore been carried out, and the results related to both habitat and phylogenetic position of the species concerned (Appendix VI). The analysis shows that of 82 species for which data were available, a cutaneous hydroosmotic response is apparently absent in nine: *Leiopelma hochstetteri* (present study; Cameron 1974), *Ascaphus truei* (present study), *Xenopus laevis* (present study; Ewer 1952; Heller and Bentley 1965; Bentley 1969b), *Ceratophrys ornata* from South Chaco (Canziani and Cannata 1980), *Calyptocephalella gayi* (Garcia *et al.* 1970), *Rana cancrivora* (Dicker and Elliott 1970), and *R. blythi*, *R. glandulosa* and *R. kuhli* (Shoemaker and McClanahan 1980). These include representatives of both the advanced frogs (suborder Neobatrachia: *Ceratophrys*, *Calyptocephalella* and *Rana*) as well as archaic frogs (suborder Archaeobatrachia: *Ascaphus*, *Xenopus* and *L. hochstetteri*).

In contrast, there is good evidence for a cutaneous response in over 60 anuran species, representing both the Archaeobatrachia (Leiopelmatidae) and Neobatrachia (Myobatrachidae, Leptodactylidae, Bufonidae, Hylidae, Ranidae, Hyperoliidae and Rhacophoridae). A

weaker response may also be present in members of the Pelobatidae and Discoglossidae (Archaeobatrachia).

When these results are considered in relation to the presumed phylogenetic relationships of present-day anuran families (Duellman 1975), it is clear that many of the species which lack a cutaneous response are not closely related. Assuming a monophyletic origin for the anurans, a parsimonious explanation of this distribution pattern is that the cutaneous response was present in the ancestral anuran lineage and has been lost independently on at least five occasions (the number of families containing species lacking the response). An alternative explanation is that the response was not present in the original ancestral anuran lineage and has evolved independently on as many as 10 occasions. This seems a less attractive hypothesis, both in terms of the number of times an evolutionary change would need to occur, and because there are probably many more genetic mutations which could lead to a loss of response (e.g., a change in the protein structure of a membrane receptor) than there are mutations which could produce the acquisition of a response (which would probably require relatively specific genetic changes).

The data summarised in Appendix VI thus provide little support for the phylogenetic hypothesis. However, there is considerable evidence to favour the ecological hypothesis. Of the nine species lacking the response, five (*L. hochstetteri*, *A. truei*, *X. laevis*, *C. gayi* and *R. kuhli*) are partially or completely restricted to freshwater, two (*R. blythi* and *R. glandulosa*) are large and live in Bornean rainforest of high humidity where dehydration is unlikely to occur (Shoemaker and McClanahan 1980), and one (*R. cancrivora*) inhabits brackish water, avoiding "dehydration" by elevating its plasma urea concentration to keep body fluids hyperosmotic to the surrounding water (Gordon *et al.* 1961).

The ninth species, the South American burrowing frog *Ceratophrys ornata*, forms an apparent exception to the ecological hypothesis. Rehydration rates of two populations of this frog were examined by Canziani and Cannata (1980). Members of the South Chaco population rehydrated at a slow and constant rate, which suggests that the response is absent, whereas frogs from the Tigre population rehydrated at an initially rapid rate, suggesting that a response is present. However, Tigre is the more humid region (Canziani and Cannata 1980).

This apparent anomaly may be a reflection of a trend towards only weak development of the cutaneous response amongst burrowing anurans in general, as judged by their relatively slow rehydration rates (Claussen 1969; Loveridge and Withers 1981) and poor responses to neurohypophysial peptides (Warburg 1971a; Hillyard 1975). Several burrowing frogs, including *C. ornata* (McClanahan *et al.* 1976), are able to resist dehydration by cocoon formation, whilst others do so by raising the urea content (and thus osmotic concentration) of their body fluids (Ruibal and Hillman 1981). Thus, the selective advantage of rapid rehydration in a fossorial habitat may be low. Furthermore, in burrowed, non-cocooned anurans a large cutaneous response would clearly be inappropriate, since it could potentially lead to an increased rate of water loss from a burrowed toad to surrounding dry soil.

Amongst species possessing a definite cutaneous response, the response is most pronounced in members of the families Rhacophoridae, Hyperoliidae and Hylidae. This is evident from their rapid rehydration rates (Appendix II; Shoemaker and McClanahan 1975, 1980; Drewes *et al.* 1977; Degani and Warburg 1984; Geise and Linsenmair 1986), and in the case of hylids, rapid and large responses to neurohypophysial peptides *in vivo* (Appendix II; Warburg 1971a) and *in vitro* (Chapter 5; Yorio and Bentley 1977). The presence of large cutaneous responses in these families is consistent with the ecological hypothesis, since many of the species examined are small and arboreal, occupying habitats which are conceivably amongst the most desiccating of those of any anurans.

In summary, this review suggests that a cutaneous hydroosmotic response may have been present in the ancestral anuran lineage and then lost independently on several occasions. Most species lacking the response live in habitats where dehydration is unlikely to be rapid. The response is relatively weak in burrowing frogs where it could potentially increase the rate of water loss to dry soils, and is most pronounced in arboreal frogs of the families Hylidae, Hyperoliidae and Rhacophoridae. According to this analysis, the absence of the response in *L. hochstetteri* cannot be considered a primitive characteristic. Finally, one can note that its presence in both *L. archeyi* and *L. hamiltoni* is consistent with previous studies indicating closer morphological, ecological and genetic similarities between these two species, than between either and *L. hochstetteri*.

(Bell 1978, 1982a, 1985a; Daugherty *et al.* 1981, 1982).

Relationship between the cutaneous hydroosmotic response, resistance to EWL and mode of nitrogen excretion

When the three aspects of water balance examined in this thesis (presence of a cutaneous hydroosmotic response, resistance of the skin to EWL, and predominant mode of nitrogen excretion) are considered together, it is apparent that none of the *Leiopelma* species shows unique adaptations when compared with other anurans. Each has a combination of characteristics in common with at least one other unrelated species from a similar environment. *L. hochstetteri* has little or no resistance to EWL, no cutaneous hydroosmotic response and is ureotelic; the same features are evident in *Rana blythi* (Shoemaker and McClanahan 1980) and *Ascaphus truei* (Appendix II and additional unpubl. obs.), both of which remain close to water. This combination is indicative of lesser adaptation to a fully aquatic environment than seen in species such as *X. laevis* or *R. kuhli*, which also have little or no resistance to EWL and no cutaneous hydroosmotic response but are facultatively or obligatorily ammonotelic (Balinsky *et al.* 1961; Shoemaker and McClanahan 1980). *L. archeyi* and *L. hamiltoni* are also ureotelic and have little or no resistance to EWL, but do have a cutaneous hydroosmotic response. Such a combination is typical of many semiaquatic, terrestrial or arboreal species from mesic environments, including *Rana pipiens* (Cragg *et al.* 1961; Tracy 1976), *Rhacophorus pardalis* (Shoemaker and McClanahan 1980), *Bufo marinus* (Cragg *et al.* 1961; Bentley and Ferguson 1967), and *Litoria ewingi* and *Litoria raniformis* (Cree 1985a).

Final conclusion

This study has demonstrated a difference in water balance between the three species of leiopelmatid frogs which relates to their differences in habitat: *L. hochstetteri* has no cutaneous hydroosmotic response and is the most aquatic, *L. archeyi* and *L. hamiltoni* have a clear cutaneous hydroosmotic response and are the most terrestrial. The presence or absence of the response is likely to be a factor involved in determining the distributions of the three species. In terms of other aspects of water balance (resistance to EWL, predominant mode of nitrogen excretion), no differences were observed. Although leiopelmatid frogs are "primitive" or "archaic" in the sense of being

anatomically similar to Jurassic fossils, they cannot be considered maladapted to the environments in which they presently live. Gans (1983) came to a similar conclusion when examining aspects of the environmental physiology of another archaic element of the New Zealand herpetofauna, the tuatara *Sphenodon punctatus*.

SUMMARY

This study compared aspects of the habitats and water balance physiology of members of New Zealand's archaic and endemic frog genus *Leiopelma*. The study aimed to determine whether features of water balance varied in an adaptive manner between the three species, *L. archeyi* (terrestrial), *L. hamiltoni* (terrestrial) and *L. hochstetteri* (semiaquatic). In addition, reproductive and behavioural adaptations of *L. archeyi* to a fully terrestrial life were examined.

Habitats of *L. archeyi* and *L. hochstetteri*

The habitats of the partially sympatric species *L. archeyi* and *L. hochstetteri* were compared, because of uncertainty about the overlap in distribution of these species and the limited climatic information available for their habitats. The Tapu study area in the Coromandel Range ranged in altitude from ~150-500 m asl and was covered in warm-temperate mixed podocarp-broadleaf forest.

During 13 monthly searches, individual *L. archeyi* were observed on 215 occasions and *L. hochstetteri* on 204. The two species had nearly disjunct distributions: 99% of *L. archeyi* were found in terrestrial habitats on the forested ridgetops, whereas 94% of *L. hochstetteri* were found at lower altitudes, in semiaquatic habitats along the edges of streams. *L. archeyi* were found further from water and on qualitatively and quantitatively drier retreat site substrates than *L. hochstetteri*. Where substrate water potential could be determined, *L. hochstetteri* substrates were always waterlogged (water potential > -6 kPa). *L. archeyi* substrates were often close to saturation (annual mean -17 kPa), but during dry weather, water potential varied to < -100 kPa. Whether substrate water potential on the ridgetops ever declines to below the soil moisture absorption threshold of either species (which laboratory experiments indicate lie between -360 and -850 kPa) is unknown. Annual rainfall in the study site was high (2729 mm over one year), but periods of up to 16 d without rain were recorded. Ambient relative humidity (RH) was often high for both species (annual mean ≥ 90%), but varied to lower levels for *L. archeyi* (56%) than for *L. hochstetteri* (82%). During dry summer weather, evaporation rates from Piché evaporimeters were higher in *L. archeyi*

habitats (up to 19 mm d^{-1}) than in *L. hochstetteri* habitats (up to 4 mm d^{-1}). Ambient temperatures were similar and cool in the habitats of both species ($< 23^\circ\text{C}$).

A small proportion (4%) of *L. hochstetteri* occurred on the ridgetops in the same habitat as *L. archeyi*. It is suggested that such individuals move onto the ridgetops during wet weather, and back to streams as conditions dry out. *L. hochstetteri* streams are prone to flooding, but frogs can recolonise previously flood-damaged streams.

The presence of retreat sites is almost certainly crucial to the survival of *L. archeyi* on the ridgetops during dry summer weather. Retreat sites were cooler and more humid than ambient air by day, often contained substrates with a high moisture content and water potential, and protected frogs from the desiccating effects of wind. Climatic conditions in the habitat of *L. archeyi* resembled those previously described for the allopatric species *L. hamiltoni* (Newman 1977; Newman *et al.* 1978).

Laboratory studies of water balance

Laboratory studies examined rates of dehydration and rehydration, substrate moisture preference, absorption of soil moisture, responses to the neurohypophysial peptide arginine vasotocin (AVT), skin structure and nitrogen excretion in some or all of the three species.

Dehydration

All three species dehydrated rapidly in dry surroundings. Weight loss during dehydration was due to constant rates of evaporative water loss (EWL) and declining rates of urinary water loss (UWL). Resistance to EWL was low in all species ($5\text{-}10 \text{ s cm}^{-1}$ in still air) and similar to that for a free water surface (12 s cm^{-1}). Rate of EWL varied inversely and linearly with RH in *L. archeyi* and *L. hochstetteri*.

Rehydration

The three species fell into two groups in terms of rehydration rate. When dehydrated by 9-15% of initial weight (W_0) and then placed in shallow water, *L. archeyi* and *L. hamiltoni* rehydrated fully in 1-2 h. In contrast, *L. hochstetteri* rehydrated very slowly and failed to regain original weight within 16 h. Rehydrating frogs of all species

had initially reduced rates of bladder accumulation in comparison with control (hydrated) animals. However, only *L. archeyi* and *L. hamiltoni* showed increased rates of cutaneous water uptake. Mean rates of water uptake through the ventral skin during the first 15 min of rehydration in shallow water (with control rates in parentheses) were 123 (24), 109 (13) and 14 (9) $\text{mg g}^{-1} \text{h}^{-1}$ for *L. archeyi*, *L. hamiltoni* and *L. hochstetteri* respectively.

L. archeyi also rehydrated rapidly when immersed. However, the surface area (SA)-specific rate of water uptake ($27 \text{ mg cm}^{-2} \text{ h}^{-1}$) was much lower than that for frogs in shallow water ($69 \text{ mg cm}^{-2} \text{ h}^{-1}$), indicating that ventral skin is specialised for rapid water uptake. *L. hamiltoni* had a similarly rapid rate of water uptake through ventral skin ($78 \text{ mg cm}^{-2} \text{ h}^{-1}$). In contrast, the rate for immersed *L. hochstetteri* ($7 \text{ mg cm}^{-2} \text{ h}^{-1}$) was similar to that for frogs in shallow water ($10 \text{ mg cm}^{-2} \text{ h}^{-1}$), suggesting uniformly low permeability of all skin regions.

The results indicate that rapid rehydration of *L. archeyi* and *L. hamiltoni* is due to both cutaneous and antidiuretic water balance responses, whereas the slow rehydration of *L. hochstetteri* is caused solely by antidiuresis.

Substrate moisture preference and absorption of water from soil

L. hochstetteri showed a greater preference for water to wet, moist or dry soils than did *L. archeyi*. Both species were able to maintain weight on soil with water potentials of -240 and -360 kPa, but dehydrated at similar rates when on drier soil (-850 kPa). Following dehydration to about 10% weight loss, *L. archeyi* absorbed water from moist soil (-240 kPa) 3x as rapidly as *L. hochstetteri*. This difference is attributable to the presence of a cutaneous water balance response in *L. archeyi* but not in *L. hochstetteri*.

Responses to AVT in vivo

The water balance responses produced by injection of AVT were similar to those seen during rehydration in shallow water. The highest dose ($7 \times 10^{-11} \text{ mol g}^{-1}$) caused significant water retention in all three species. In *L. archeyi* and *L. hamiltoni*, mean weights of 31% and 29% above W_0 respectively were reached 4-5 h after treatment, and water retention was attributed to both cutaneous and antidiuretic responses. *L. hochstetteri* showed a much slower and constant rate of weight

increase, reaching 9% above W_0 9 h after injection. No significant increase in cutaneous water uptake occurred in *L. hochstetteri*, indicating that water retention was due solely to antidiuresis.

A lower and possibly physiological dose of AVT (7×10^{-13} mol g^{-1}) also produced significant water retention in *L. archeyi* (24%) and *L. hamiltoni* (26%), but not in *L. hochstetteri* (2%). A dose of 7×10^{-15} mol g^{-1} led to minor water retention (6%) in *L. archeyi*, but the effect on *L. hamiltoni* (5%) was not statistically significant.

Effects of AVT on skin, kidneys and bladder

Results from experiments examining the independent effects of AVT on the skin, kidneys and bladder confirmed that increased osmotic water flow (OWF) through ventral pelvic skin is the major cause of water retention in AVT-treated *L. archeyi* and *L. hamiltoni*. *In vitro*, pelvic skin showed AVT-stimulated rates of osmotic water flow (OWF) which were $2.4\times$ (*L. archeyi*) and $7.8\times$ (*L. hamiltoni*) those of control rates (AVT concentration 3×10^{-8} mol l^{-1}). However, pectoral and dorsal skin showed no response. Glomerular filtration rate (GFR, assessed by creatinine clearance) fell following AVT treatment *in vivo* in both species (from 25 to 0 $\mu l g^{-1} h^{-1}$ in *L. archeyi*, and from 21 to 1 $\mu l g^{-1} h^{-1}$ in *L. hamiltoni*). The bladders of both species were small (voluntary bladder urine volumes were equivalent to $\leq 12\%$ of W_0 in *L. archeyi* and $\leq 8\%$ of W_0 in *L. hamiltoni*) and showed only small hydroosmotic responses to AVT *in vitro*.

Water retention in AVT-treated *L. hochstetteri* probably results from both reduced urine production and increased bladder water reabsorption. No hydroosmotic response to AVT was seen in isolated pelvic, pectoral or dorsal skin (maximum AVT concentration 3×10^{-6} mol l^{-1}). GFR fell following AVT treatment *in vivo* (from 22 to 10 $\mu l g^{-1} h^{-1}$), and although the effect was not statistically significant, it was sufficiently large to account for the rate of water retention observed. The bladder of *L. hochstetteri* was very small (voluntary bladder urine volumes $\leq 4\%$ of W_0) and showed significant but small increases in OWF in response to AVT *in vitro*.

Skin structure

Pelvic, pectoral and dorsal skin from *L. archeyi* and *L. hochstetteri* was compared using scanning electron and light microscopy. No evidence of regional specialisation in structure for rapid water

uptake was seen in pelvic skin from *L. archeyi*. Pelvic skin of this species was smooth, and of a similar simple structure to pectoral skin, or to dorsal skin away from glandular ridges. The epidermis of pelvic skin was thinner in *L. archeyi* (14 μm) than in *L. hochstetteri* (47 μm), but total skin thickness in non-glandular areas did not vary greatly between the two species (92 μm cf. 115 μm respectively). Pelvic skin of *L. archeyi* was markedly less vascularised than that of *L. hochstetteri*, in contrast to previous studies suggesting a relationship between hypervascularisation and presence of a cutaneous hydroosmotic response in other anurans.

Comparisons with the hylid frog *Litoria aurea* showed that pelvic skin from this species differed morphologically from that of *L. archeyi*; it also demonstrated a more pronounced hydroosmotic response. Pelvic skin of *Litoria aurea* was well vascularised and had surface irregularities (mounds) which increased the external SA. These mounds (151 μm thick) were separated by very thin (37 μm), non-glandular channels. The minimum capillary-to-surface distance was 20 μm . *In vitro*, the AVT-stimulated rate of OWF through pelvic skin of *Litoria aurea* exposed to $3 \times 10^{-8} \text{ mol l}^{-1}$ AVT was $332 \text{ mg cm}^{-2} \text{ h}^{-1}$ (cf. $97 \text{ mg cm}^{-2} \text{ h}^{-1}$ in *L. archeyi*), and during rehydration, the rate of water uptake through ventral skin of *Litoria aurea* was $292 \text{ mg cm}^{-2} \text{ h}^{-1}$ (cf. $123 \text{ mg cm}^{-2} \text{ h}^{-1}$ in *L. archeyi*).

Nitrogen excretion

The predominant nitrogenous waste excreted by *L. archeyi*, *L. hamiltoni* and *L. hochstetteri* was urea. Urine collected directly from the bladder and in 24 h bathing water samples contained between 3 and 7% of waste nitrogen (urea plus ammonia nitrogen) as ammonia. No uric acid was detected.

Reproductive and behavioural adaptations of *L. archeyi* to a terrestrial habitat

Nitrogen excretion, water balance and ecological aspects of development in the terrestrial eggs and larvae of *L. archeyi* were examined. Clutches of newly-laid eggs were present in nest sites by late October and were brooded by male frogs for several weeks until hatching. Hatched larvae were carried on the backs of adult frogs in the nest site. By early February, metamorphosis was complete and most

nest sites were empty. A 20-point scheme for staging *L. archeyi* eggs and larvae is presented.

Water bathing well-developed eggs in the laboratory, water into which larvae hatched, water in which ruptured capsules were washed and intracapsular fluid from eggs close to hatching all contained predominantly ammonia nitrogen (80-89%), indicating that unhatched larvae are ammonotelic. Following hatching, the proportion of waste nitrogen excreted as ammonia rapidly declined, and by 3-4 d after hatching most larvae were ureotelic.

Eggs in dry conditions lost water by evaporation at the same rate as agar egg models, indicating that the gelatinous capsule surrounding real eggs has no resistance to EWL. Clustering of eggs reduces exposed SA, and hence weight-specific EWL. Eggs placed in shallow water absorbed water rapidly, increasing their weight up to 5x before hatching. Eggs in natural nests also increased in weight during development, and substrate moisture is probably an important source of water. Hatched larvae are tiny (≤ 110 mg) and tailed, with a very unfavourable SA:volume ratio and consequently very high rates of weight-specific EWL in dry conditions. Newly-hatched larvae were relatively immobile and unable to escape dehydrating conditions. When placed in shallow water, dehydrated larvae absorbed water rapidly, suggesting that a cutaneous hydroosmotic response may be at least partly developed before the end of metamorphosis.

Male parental care contributes to water balance of *L. archeyi* eggs and larvae in several ways. Brooding reduced the weight-specific EWL from egg clusters by more than half, as a consequence of reducing the exposed SA. Brooding may also help bring clusters into close contact with moist substrates from which water can be absorbed. Hatched larvae carried on the backs of adults had negligible rates of EWL ($2 \text{ mg g}^{-1} \text{ h}^{-1}$) compared with those on dry surfaces ($71 \text{ mg g}^{-1} \text{ h}^{-1}$).

The nocturnal activity of adult *L. archeyi* in the field was investigated, in relation to environmental factors potentially affecting water balance. The number of frogs emerged in a region of the Tapu study area during the first hour of darkness was strongly dependent on moist conditions. Emergence was positively correlated with RH, rainfall during the previous day, rainfall during the search, and wetness of vegetation, and negatively with vapour pressure

deficit. Emergence showed a relatively weak correlation with temperature, and none with windspeed or the number of previous searches.

On wet nights, frogs climbed up to 2 m high in the vegetation. Experimentally dehydrated *L. archeyi* were able to rehydrate from this natural moisture source, increasing in weight from 92% to 99% of W_0 over 4 h on wet kiekie fronds.

Distribution of the cutaneous hydroosmotic response among *Leiopelma* species

The presence of a cutaneous hydroosmotic response in *L. archeyi* and *L. hamiltoni* but not in *L. hochstetteri* is consistent with the more terrestrial habitats of the two former species. These results support an ecological hypothesis for the distribution of this response among anuran amphibians, and the absence of the response in *L. hochstetteri* is not considered a primitive characteristic. The results are also consistent with previous reports indicating a closer ecological similarity and evolutionary relationship between *L. archeyi* and *L. hamiltoni*, than between either and *L. hochstetteri*.

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APPENDIX I

SUBSTRATE MOISTURE PREFERENCES OF *L. ARCHEYI* AND *L. HOCHSTETTERI*Introduction

The field studies described in Chapter 2 show that *L. archeyi* and *L. hochstetteri* occupy nearly disjunct habitats: *L. archeyi* is found on the forest floor on ridgetops above stream headwaters, whilst *L. hochstetteri* is usually seen on waterlogged substrates within 1 m of streams. This difference in distribution suggests that the two species differ in substrate moisture preference. The following experiment was designed to test this hypothesis.

Materials and Methods

Preference chambers were constructed from white plastic trays (360 × 280 × 65 mm deep), which were bisected twice with perspex partitions to form four water-tight compartments (180 × 140 × 40 mm deep). In each chamber, one compartment ("water over pebbles") was nearly filled with black aquarium pebbles, which were then covered by shallow stream water. The depth of water over the pebbles was about 2 mm at the edges of the compartment, increasing to 5 mm at the centre. The remaining three compartments were filled in a clockwise fashion with soils of the following moisture contents: 20% ("dry" soil), 40% ("moist" soil) and 60% ("wet" soil). Soil was collected from the Tapu study area, dried at about 105°C to constant weight, and sieved through a 2.36 mm mesh to remove large lumps. Stream water equivalent to 20%, 40% or 60% of the dry soil weight was added, and the soils were left for about 5 h to equilibrate before being placed in the compartments. Water potential of the prepared soils was measured with a "Quick Draw" tensiometer (Soilmoisture Equipment Corp., California, USA): soils with 40% and 60% moisture contents were essentially saturated (-7 and -4 kPa respectively), but soil with a 20% moisture content had a water potential which was lower than the range of the instrument used (-100 kPa). The soil moisture tension curve for this experiment is similar to that for soil used in soil moisture uptake experiments (Chapter 9), but not identical because of differences in soil particle size distribution.

Glass lids covered by a sheet of black polythene were held about 2 mm above the trays by matchsticks; this arrangement provided adequate ventilation whilst still maintaining high humidities [> 80% RH, measured with a quick-response humidity sensor (Vaisala, Finland)] inside the chambers. The low lid and dark conditions inside the chamber simulated conditions inside a natural retreat site, but still allowed ample clearance for a frog to move freely between compartments.

Experimental trials were conducted at $18 \pm 2^\circ\text{C}$ on a 12 h L: 12 h D photoperiod. The frogs (eight *L. archeyi* and seven *L. hochstetteri*) were used individually 13-19 d after collection. Bladder urine was removed, and then each animal was placed in the centre of a chamber on the intersection between the four compartments. The frogs were left overnight to explore their surroundings. Their positions within the chambers were then recorded at 4 h intervals for the following 36 h, by lifting the polythene sheet. Observations at night were made with the aid of a red light. Successive observations 10-15 min apart showed that frogs were not disturbed by viewing.

Results

Nine position records were obtained for each frog. These provided sufficient observations for each species to analyse statistically [$n = 70$ for *L. archeyi* (two instances where frogs were observed on the walls of the chamber were excluded); $n = 63$ for *L. hochstetteri*].

L. archeyi spent most time (67% of observations) on the soil compartments, with 23% of observations being for frogs on the driest soil (Fig. I.1). The observed distribution differs only slightly from a random expected distribution of 25% on each of the four compartments, when tested for goodness-of-fit by chi-square analysis ($\chi^2 = 10.5$, $0.05 > P > 0.01$), indicating that there was no strong preference for any particular compartment. In contrast, *L. hochstetteri* showed a marked preference for the "water over pebbles" compartment (84% of observations), and a total avoidance of "dry" soil. Furthermore, whereas *L. archeyi* in the "water over pebbles" compartment were only seen at the edges where the water was shallowest, *L. hochstetteri* stayed in the centre or moved the pebbles aside to create a deeper pool in which they half-immersed themselves,

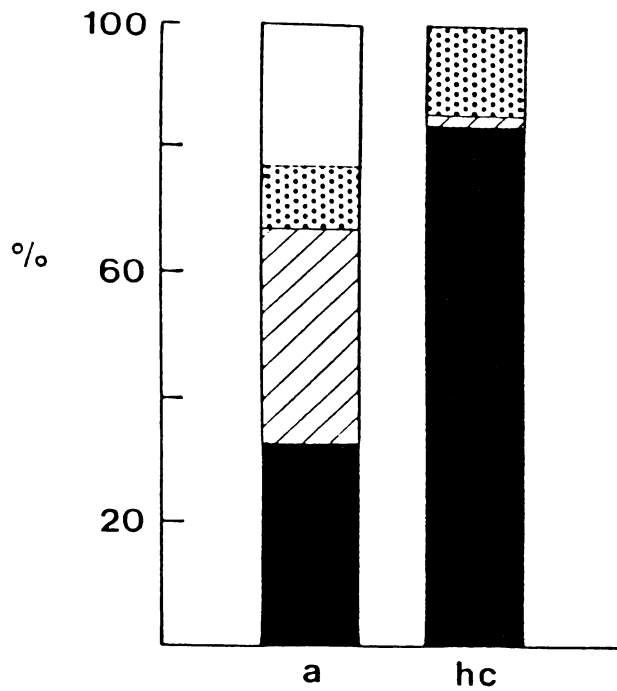


FIG. I.1 Substrate moisture preferences of *L. archeyi* (a) and *L. hochstetteri* (hc). The figure shows the frequency with which frogs were observed on "water over pebbles" (solid), "wet" soil (60% MC, hatched), "moist" soil (40% MC, stippled) and "dry" soil (20% MC, open) (n = 70 observations for *L. archeyi*, n = 63 for *L. hochstetteri*).

a behaviour not observed for *L. archeyi*. The frequency distribution for *L. hochstetteri* (Fig. I.1) differs significantly from a random distribution ($\chi^2 = 120.6$, $P < 0.001$) and also differs significantly from that observed for *L. archeyi* ($\chi^2 = 84.3$, $P < 0.001$).

Discussion

This experiment confirms that *L. hochstetteri* has a greater preference for water than *L. archeyi*. This is consistent with its streamside habitat and saturated retreat site substrates (Chapter 2) and its lack of a cutaneous hydroosmotic response (Chapters 3, 4 and 5). *L. hochstetteri* is unable to rehydrate rapidly, and presumably therefore tends to remain on wet substrates where it will not become dehydrated.

Similar differences in substrate moisture preference have previously been demonstrated for anurans from different habitats. Claussen (1973) compared preferences of *Ascaphus truei* with those of *Hyla regilla*, an arboreal frog which can be found hundreds of metres from the nearest standing water. *A. truei* had a greater preference for water than did *H. regilla*, and was also unable to absorb moisture from a wet surface in a dehydrating environment (moving air) as rapidly as *H. regilla*. Like *L. hochstetteri*, *A. truei* lacks a cutaneous hydroosmotic response (Appendix II). Carlisky *et al.* (1970) demonstrated an inverse correlation between preference for water and dryness of habitat for three anurans, *Calyptocephalella gayi* (aquatic), *Leptodactylus ocellatus* (semiaquatic) and *Bufo arenarum* (terrestrial).

However, Jaeger (1971) was unable to demonstrate differences in substrate moisture preference between two urodeles from habitats of differing aridity. *Plethodon cinereus cinereus* and *P. richmondi shenandoah* have contiguous but largely non-overlapping distributions: *P. r. shenandoah* lives in talus (rock) slopes, whilst *P. c. cinereus* occurs in areas of soil surrounding the talus, a generally moister environment. Both species show no preference between soil and rock when the substrates are moist, but prefer soil to rock as the substrates dry out. Jaeger concluded that differences in substrate moisture preference could not explain the observed distributions, and suggested that *P. r. shenandoah* was competitively excluded from areas of soil by *P. c. cinereus*.

APPENDIX II

WATER BALANCE RESPONSES OF *ASCAPHUS TRUEI*, *XENOPUS LAEVIS*
AND *LITORIA AUREA*Introduction

This appendix describes the water balance responses of *Ascaphus truei*, *Xenopus laevis* and *Litoria aurea* (Plate II.1) to dehydration and/or exogenous AVT. The responses of these locally available species were examined for comparison with those of the *Leiopelma* species, and the results have also been used in Appendix VI to help distinguish the features of the water balance response in species with and without the cutaneous component of the response.

X. laevis is well known to lack a cutaneous hydroosmotic response; it therefore provides an ideal species for comparison with *L. hochstetteri*. Skin of *X. laevis* shows no response to NHPs either *in vivo* (Ewer 1952; Heller and Bentley 1965) or *in vitro* (Bentley 1969b), and also shows no increase in water uptake following dehydration (Ewer 1952). However, it is not clear whether *X. laevis* possesses an antidiuretic response to dehydration, since Ewer (1952) did not indicate whether dehydrated animals returned to their original weight when placed in water. I re-examined the water balance response of *X. laevis* to dehydration, to compare the results with those obtained for *L. hochstetteri* in Chapter 3, and to determine whether rehydrating animals return to their original weight.

A. truei is found in or near cold, swift mountain streams of the northwestern United States (Metter 1964). On the basis of habitat one would expect *A. truei* to have features of water balance similar to those of *X. laevis* and *L. hochstetteri*, and the few previous studies made on *A. truei* are consistent with this suggestion. Mullen and Alvarado (1976) found that cutaneous water uptake of hydrated *A. truei* was low compared with that of more terrestrial anurans, and attributed this to low serum osmolarity and low skin permeability. Claussen (1973) observed that *A. truei* placed on a wet surface and simultaneously exposed to a stream of dry air slowly dehydrated, whilst the tree frog *Hyla regilla* increased in weight. These comparisons suggest that the cutaneous component of the water balance

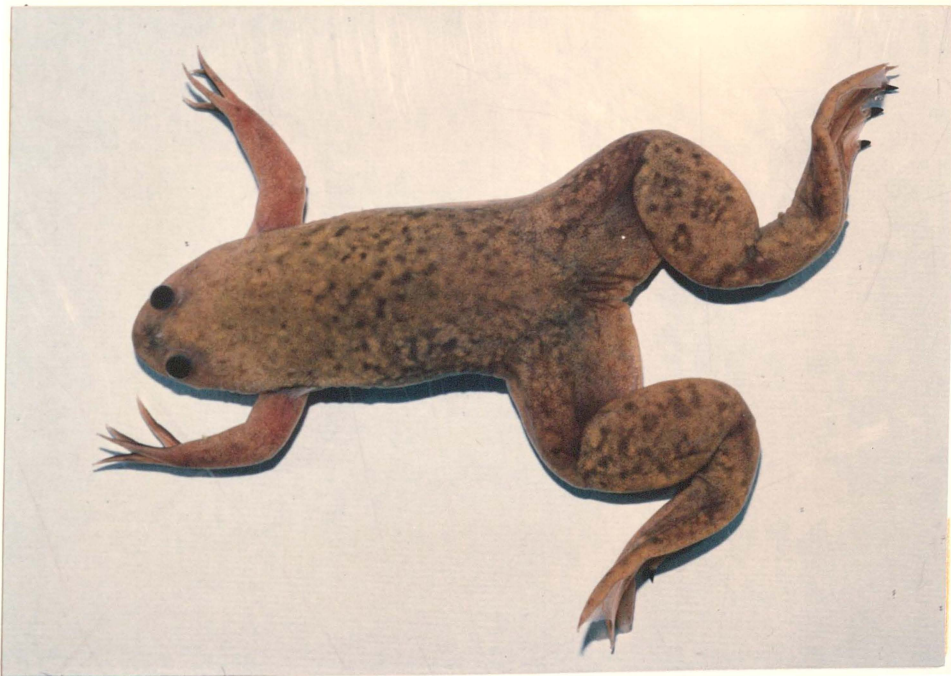
PLATE II.1 *Ascaphus truei* (A), *Litoria aurea* (B), and *Xenopus laevis* (C).



A



B



C

response might be absent or poorly developed in *A. truei* also, and this prediction was tested by examining the rate of rehydration and the extent of water retention occurring following AVT treatment *in vivo*.

Pelvic skin of *Litoria aurea* showed a very large hydroosmotic response to AVT *in vitro* in Chapter 5, suggesting that the water balance response of this species might be more pronounced than that of either *Leiopelma archeyi* or *L. hamiltoni*. This was examined by determining rates of rehydration and responses to AVT *in vivo*.

Materials and Methods

Animals

A. truei. The single female (11.6 g) used in this study was lent by Dr B.D.Bell, Victoria University of Wellington. It had been collected from Oregon, USA and for several years had been kept outdoors in Wellington in a vivarium containing soil, leaf litter and water seepages. Upon arrival in Hamilton it was kept overnight at 15°C in an aquarium containing pebbles and a pool of stream water. The frog was not fed during the week that experiments were conducted, and was returned to its aquarium for 2-3 nights between each experiment.

X. laevis. Three unsexed *X. laevis* (22.2-32.3 g) previously kept in tap water were lent by Dr V.B.Meyer-Rochow, University of Waikato. These were kept in stream water at 15°C for 5 d before use and were not fed.

Litoria aurea. Newly metamorphosed, unsexed *Litoria aurea* (1.5-3.1 g) were collected from pond banks in Rotorua and near Raglan, Waikato in February 1985. They were housed at 17°C in a terrarium containing sand, logs and a dish of water, and were fed houseflies and mealworms.

Photoperiod in the laboratory was 12 h L: 12 h D for all species.

Experiments

Experiments were carried out in May and June 1985 at 15 ± 1°C. Rehydration experiments were performed in a similar manner to those described for the *Leiopelma* species in Chapter 3. All species were first dehydrated in still air to 8-15% weight loss. Rates of water

uptake and urine accumulation for *Litoria aurea* in shallow water were measured for rehydrating frogs, and later for hydrated frogs. Rehydration rates were also determined for immersed *Litoria aurea*. *A. truei* and *X. laevis* were kept in shallow water for the first 4 h of rehydration and then were immersed. Urine was not removed from the bladders of rehydrating *A. truei* and *X. laevis*, and hydrated water uptake was not examined. The area of ventral skin in water during rehydration of *Litoria aurea* and *A. truei* in shallow water was measured.

The effect of exogenous AVT (7×10^{-11} mol g⁻¹) on water retention in *A. truei* and *Litoria aurea* was determined as previously described for *Leiopelma* species (Experiment 1 of Chapter 4). Separate controls were used for *Litoria aurea*, but the single *A. truei* served as its own control.

Results

Rehydration

A. truei and *X. laevis*. These two species rehydrated at slow and constant rates (Fig. II.1). *A. truei* took 16 h to replace a dehydration deficit of 13% and water uptake during rehydration averaged 8 mg g⁻¹ h⁻¹ (equivalent to 11 mg cm⁻² h⁻¹ when in shallow water). *X. laevis* rehydrated at a mean rate of 5 mg g⁻¹ h⁻¹ to reach 96% of W_0 after 12 h in water, but subsequent defaecation obscured any further water uptake. Assuming that defaecation had not occurred, the extrapolated time required for *X. laevis* to complete rehydration is 20 h (for a deficit of 9%). In neither species did the rate of rehydration change appreciably when frogs were immersed at 4 h.

Litoria aurea. Water uptake and urine accumulation of hydrated frogs in shallow water averaged 42 ± 5 mg g⁻¹ h⁻¹ and 46 ± 8 mg g⁻¹ h⁻¹ respectively ($\bar{x} \pm SE$); these do not differ significantly ($t = 0.367$, $P > 0.4$). Following dehydration, frogs in shallow water rehydrated rapidly and replaced a mean dehydration deficit of 13% within 45 min (Fig. II.2A). Water uptake during the first 15 min of rehydration occurred at a rate of 292 ± 63 mg g⁻¹ h⁻¹ ($\bar{x} \pm SE$) which differs significantly from the rate seen in hydrated frogs ($t = 3.982$, $P < 0.01$). Frogs which had rehydrated for 15 min had increased in weight to $94 \pm 2\%$ of W_0 and had already accumulated urine in their bladders.

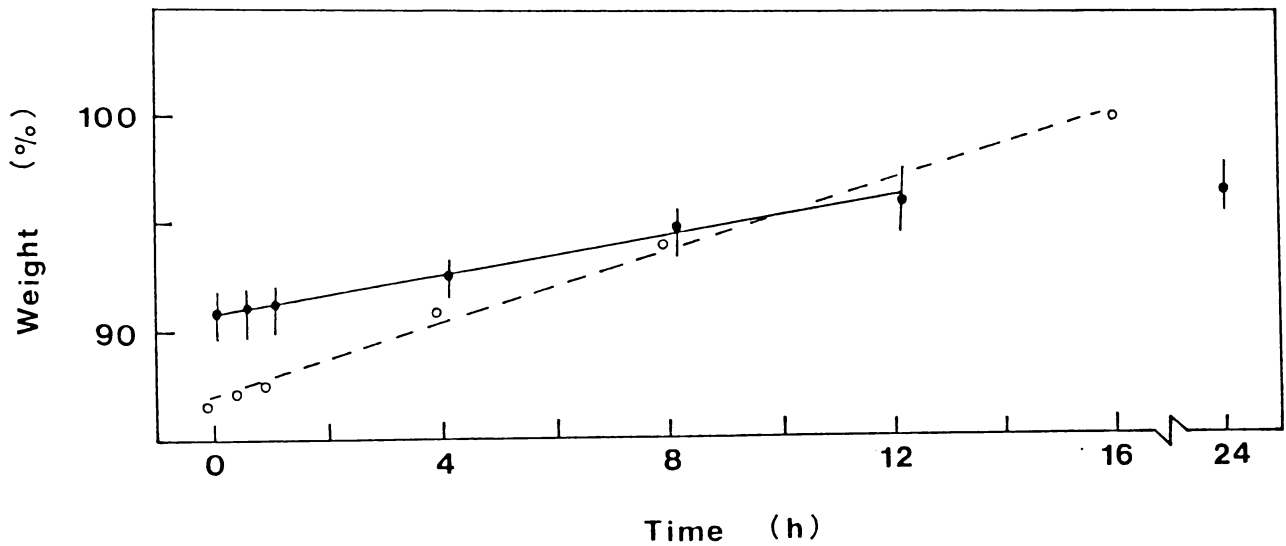


FIG. II.1 Rehydration of *Ascaphus truei* (o, n = 1) and *Xenopus laevis* (●, n = 3). Weight is expressed as a percentage of W_0 . For the first 4 h of rehydration frogs were in shallow water; following this they were immersed. Values for *X. laevis* show mean (●) and range (bars). This species defaecated between 12 and 24 h, producing a lower than expected weight gain during this period.

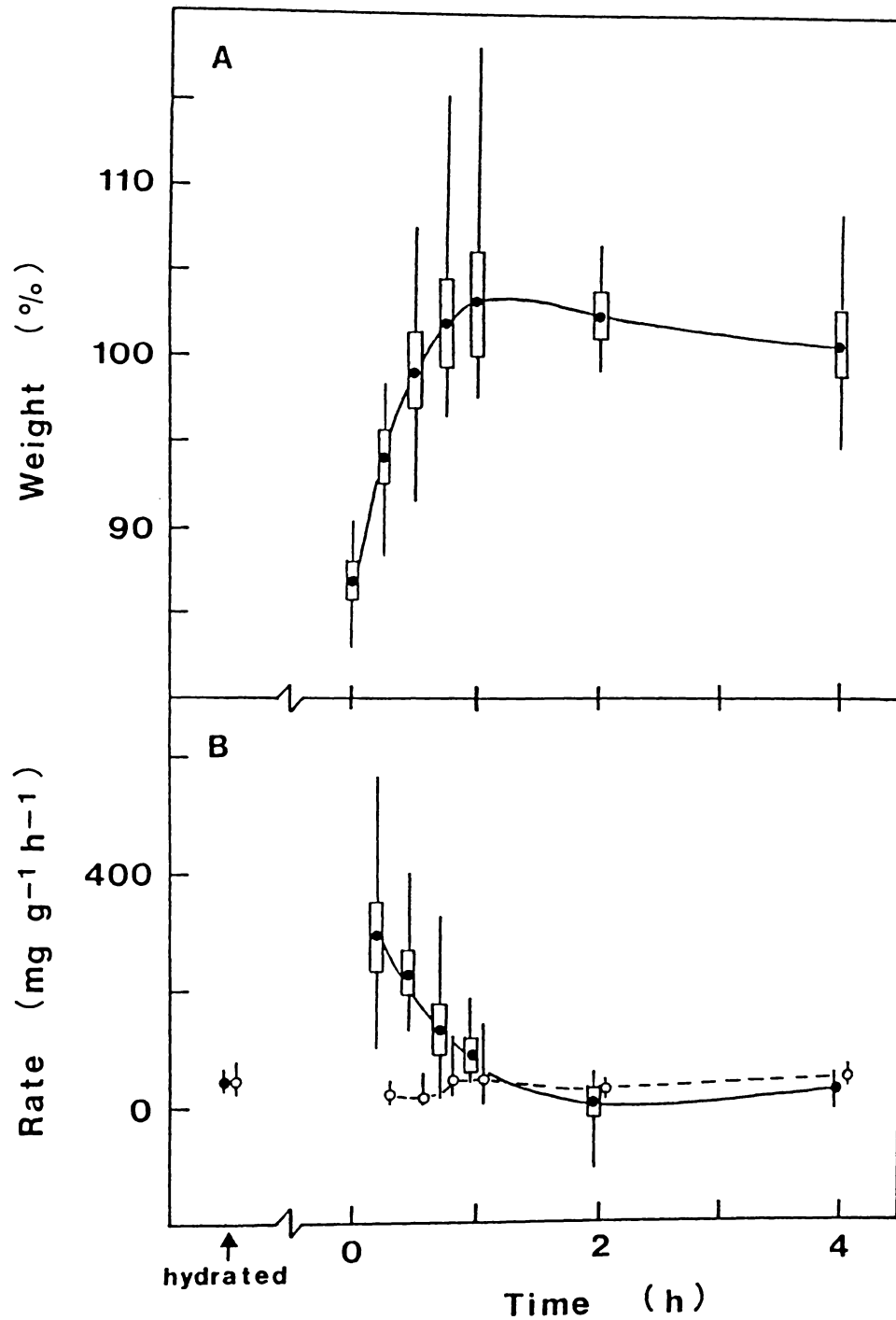


FIG. II.2 Rehydration of *Litoria aurea* in shallow water. A = weight prior to urine removal as a percentage of W_0 . B = rate of water uptake (\bullet) and urine accumulation (\circ). Values for hydrated frogs are included for comparison in B ($\bar{x} \pm SE$ and range for $n = 6$; SE deleted where necessary for clarity).

On a surface area (SA) basis, the rate of water uptake during the first hour of rehydration in shallow water was $153 \pm 21 \text{ mg cm}^{-2} \text{ h}^{-1}$.

Immersed *Litoria aurea* also rehydrated rapidly and regained an 11% dehydration deficit within 30 min (Fig. II.3). Water uptake during the first 15 min of rehydration (not shown in Fig. II.3) was $265 \pm 44 \text{ mg g}^{-1} \text{ h}^{-1}$.

Effect of AVT on water retention

A. truei. No weight gain greater than 1% occurred during the 8 h following AVT treatment (Fig. II.4). After treatment with the control solution, weight declined by 3.5% over the same period. Rates of water uptake and urine accumulation measured hourly were $< 9 \text{ mg g}^{-1} \text{ h}^{-1}$ during both experiments.

Litoria aurea. AVT-treated *Litoria aurea* showed a large and rapid weight gain, with mean weight reaching a maximum of $27 \pm 3\%$ above W_0 4 h after injection (Fig. II.5A). Controls remained within -3 to $+9\%$ of W_0 . Water uptake of AVT-treated frogs increased to $125 \pm 13 \text{ mg g}^{-1} \text{ h}^{-1}$ (equivalent to $84 \pm 11 \text{ mg cm}^{-2} \text{ h}^{-1}$) 1 h after treatment (Fig. II.5B). Urine accumulation dropped following treatment with AVT, and remained low for the next 5 h when compared with controls (Fig. II.5C).

Discussion

A. truei and *X. laevis* rehydrate very slowly and show no evidence for increased cutaneous water uptake as a consequence of dehydration. Assuming that the area of skin in water for immersed *A. truei* is given by the formula $SA \text{ (cm}^2\text{)} = 6.48 \times W_0 \text{ (g)}^{0.66}$ (Mullen and Alvarado 1976), then the rate of water uptake during immersed rehydration is equivalent to $3 \text{ mg cm}^{-2} \text{ h}^{-1}$. This is very close to the value of $3.98 \text{ mg cm}^{-2} \text{ h}^{-1}$ measured for hydrated *A. truei* in comparable conditions (Mullen and Alvarado 1976). Dehydrated *X. laevis* absorbed water at a rate of $5 \text{ mg g}^{-1} \text{ h}^{-1}$ at 15°C , a lower rate than that reported for hydrated animals ($19 \text{ mg g}^{-1} \text{ h}^{-1}$) of a similar size range (26-50 g) at 26°C (Ewer 1952). Although both *A. truei* and *X. laevis* apparently lack a cutaneous response to dehydration, the fact that both species tend to return to their original weight when placed in water implies that an antidiuretic response occurs.

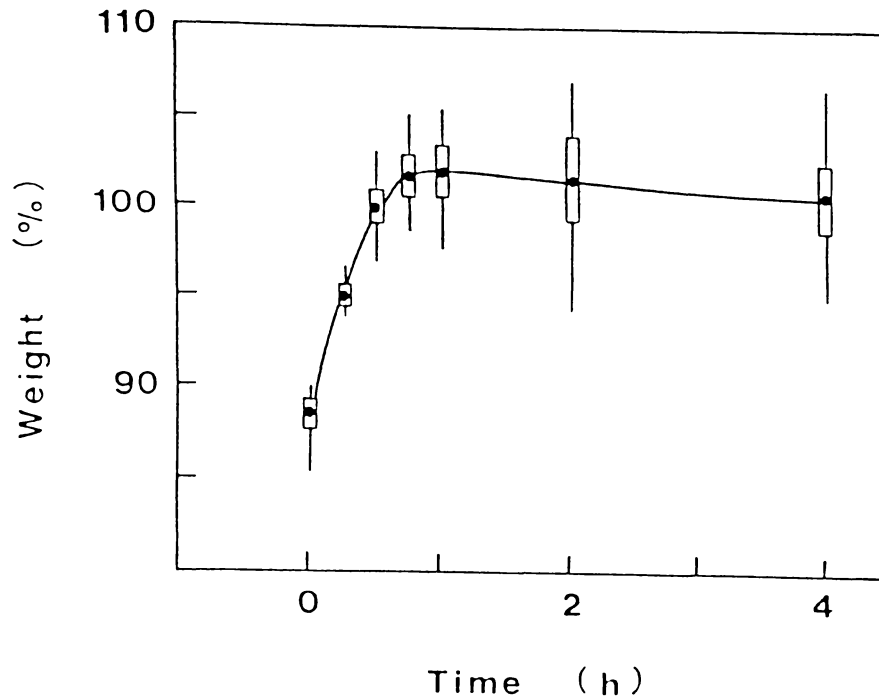


FIG. II.3 Rehydration of *Litoria aurea* when immersed. Weight prior to urine removal is given as a percentage of W_0 ($\bar{x} \pm SE$ and range for $n = 5$).

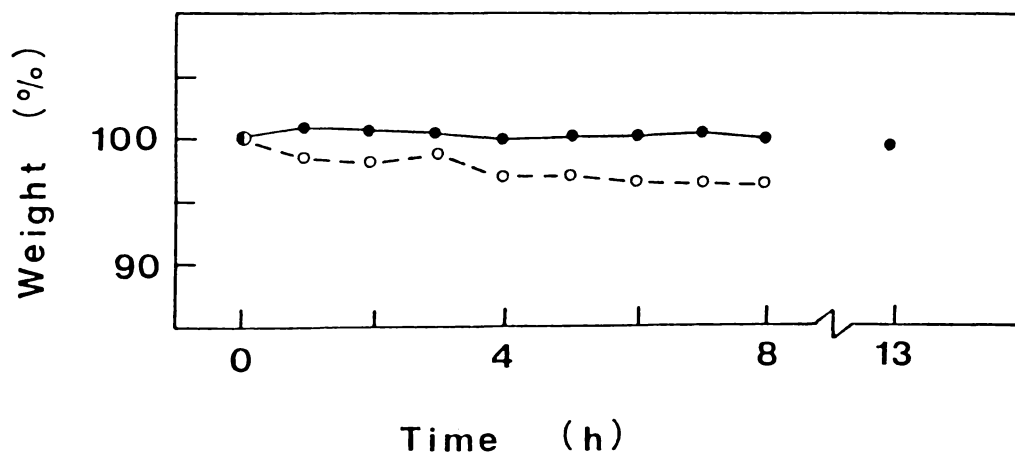


FIG. II.4 Effect of AVT ($7 \times 10^{-11} \text{ mol g}^{-1}$) on water balance of *A. truei*. Weight prior to urine removal is expressed as a percentage of W_0 . AVT was injected into the frog at 0 h after the frog had spent the previous hour in shallow water (● = AVT-treated, $n = 1$; ○ = control, $n = 1$).

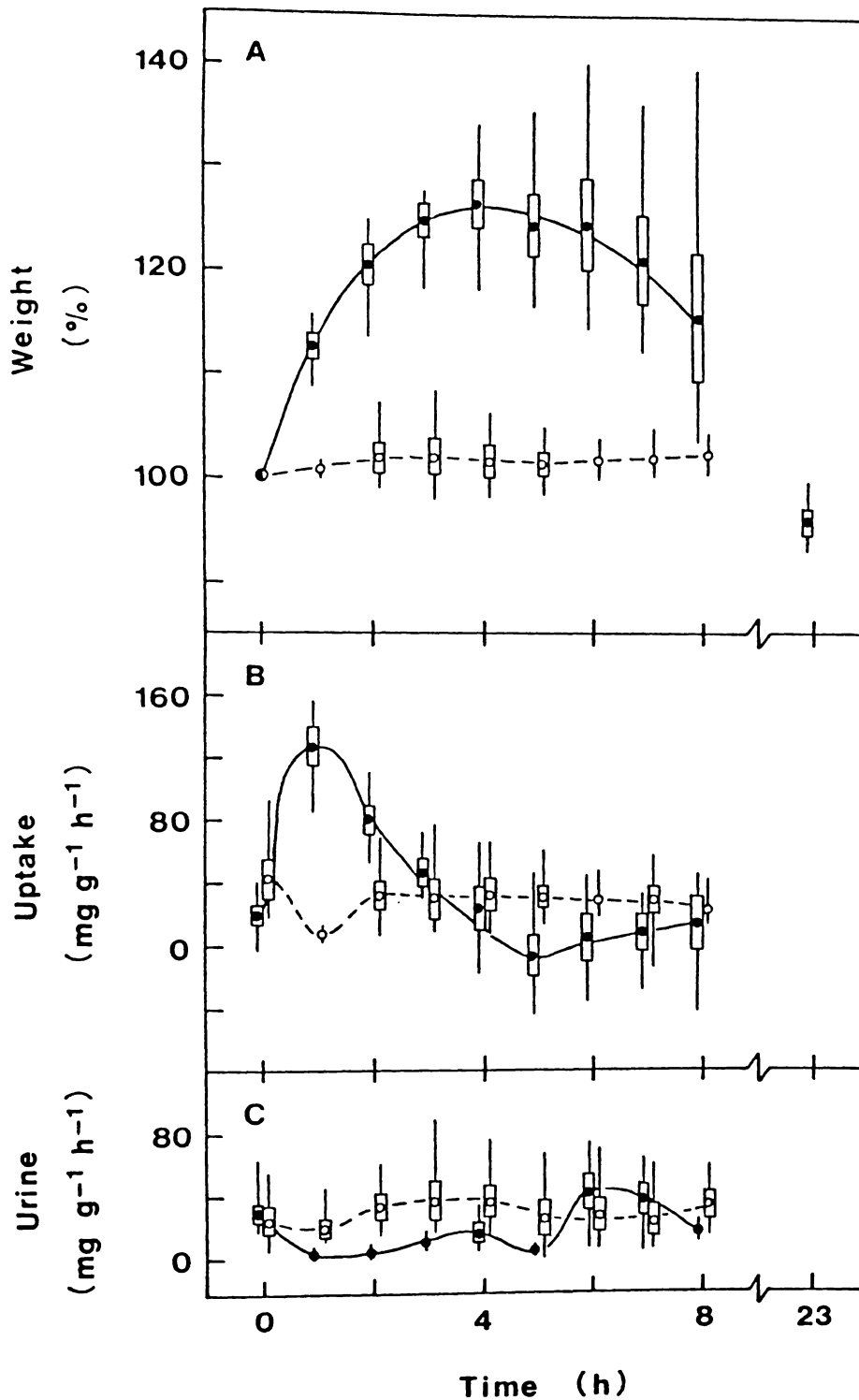


FIG. II.5 Effect of AVT (7×10^{-11} mol g⁻¹) on water balance of *Litoria aurea*. A = weight prior to urine removal as a percentage of W_0 , except at 0 and 23 h where weights are after urine removal. B = rate of water uptake. C = rate of urine accumulation. AVT was injected into frogs at 0 h after they had spent the previous hour in shallow water; animals were returned to their holding cages at 8 h ($\bar{x} \pm$ SE and range, SE deleted where necessary for clarity; ● = AVT-treated, n = 5; ○ = controls, n = 5).

Treatment with AVT produced no weight gain in *A. truei*, confirming that the peptide had no effect on cutaneous water uptake in the individual examined. Whether the small loss of weight of the control (3.5% of W_0 over 8 h) indicates that the hormone did have some water-retaining (antidiuretic) effect is unknown and requires further study.

The water balance responses of rehydrating *A. truei* and *X. laevis* are very similar to that described in Chapter 3 for *L. hochstetteri*. All rehydrate at slow and constant rates, and although water uptake in shallow water is slightly faster on a weight basis for *L. hochstetteri* ($14 \text{ mg g}^{-1} \text{ h}^{-1}$) than for either *A. truei* ($8 \text{ mg g}^{-1} \text{ h}^{-1}$) or *X. laevis* ($5 \text{ mg g}^{-1} \text{ h}^{-1}$), this probably simply reflects differences in body size and hence relative SA. When calculated in terms of SA, the rates for *L. hochstetteri* and *A. truei* in shallow water are very similar (10 and $11 \text{ mg cm}^{-2} \text{ h}^{-1}$ respectively).

In contrast to *A. truei*, *X. laevis* and *L. hochstetteri*, *Litoria aurea* demonstrates a clear cutaneous response to dehydration and to exogenous AVT. Mean rates of water uptake during the first 15 min of rehydration were $292 \text{ mg g}^{-1} \text{ h}^{-1}$ in shallow water and $265 \text{ mg g}^{-1} \text{ h}^{-1}$ when immersed. Following AVT treatment, water uptake increased to $125 \text{ mg g}^{-1} \text{ h}^{-1}$. These rates are 3-7x greater than the hydrated rate ($42 \text{ mg g}^{-1} \text{ h}^{-1}$) and cannot be explained by antidiuresis alone, since hydrated animals accumulated urine at a rate of only $46 \text{ mg g}^{-1} \text{ h}^{-1}$. The similarity between weight-specific rates of water uptake during rehydration in shallow water and when immersed indicates that the increase in cutaneous water uptake is confined to the ventral skin. This is consistent with results obtained in Chapter 5, where AVT increased osmotic water flow through isolated pelvic skin but not through pectoral or dorsal skin.

The water balance responses of *Litoria aurea* thus resemble those of *Leiopelma archeyi* and *L. hamiltoni* (Chapters 3 and 4). All three species rehydrate rapidly and show large amounts of water retention in response to exogenous AVT. The maximum mean weight gains recorded following treatment with the same dose of AVT ($7 \times 10^{-11} \text{ mol g}^{-1}$) are similar (27, 31 and 29% above W_0 for *Litoria aurea*, *L. archeyi* and *L. hamiltoni* respectively), and maximum rates of water uptake in response to AVT are also comparable (125 , 127 and $99 \text{ mg g}^{-1} \text{ h}^{-1}$ or 85 , 86 and $68 \text{ mg cm}^{-2} \text{ h}^{-1}$ respectively). However, the maximum rate of

water uptake was reached more rapidly for *Litoria aurea* (1 h after AVT treatment) than for *L. archeyi* and *L. hamiltoni* (2 h after treatment). Dehydrated *Litoria aurea* also rehydrated twice as rapidly as either of the two *Leiopelma* species (see Discussion of Chapter 3 for comparisons of rates of water uptake during rehydration). These results are consistent with the greater hydroosmotic response of pelvic skin of *Litoria aurea in vitro* compared with that of either *L. archeyi* or *L. hamiltoni* (Chapter 5).

APPENDIX III

OSMOTIC CONCENTRATIONS OF URINE AND PLASMA

Materials and methods

Samples of plasma were obtained from hydrated animals used in Experiment 1 of Chapter 5, using the procedure outlined in Experiment 2 of the same chapter. Urine was obtained from the bladders of stock animals removed from their vivaria (Experiment 4, Chapter 5). Osmotic concentrations of the samples were determined using a vapour pressure osmometer (Wescor Model 5100C, Utah, USA). All urine samples were analysed in duplicate or triplicate and the mean taken for each animal, but because of lower sample volumes most plasma samples were analysed only as single replicates.

TABLE III.1 Osmotic concentrations of urine and plasma from *Leiopelma* species.

Species	Osmotic concentration (mOsm kg ⁻¹)	
	plasma	urine
<i>L. archeyi</i>	215 ± 25 n = 2 ¹	110 ± 15 n = 7
<i>L. hamiltoni</i>	211 ± 7 n = 3	127 ± 13 n = 8
<i>L. hochstetteri</i>	215 ± 0 n = 2	64 ± 4 n = 6 ¹

Note:

1. One sample was pooled from two animals.

APPENDIX IV

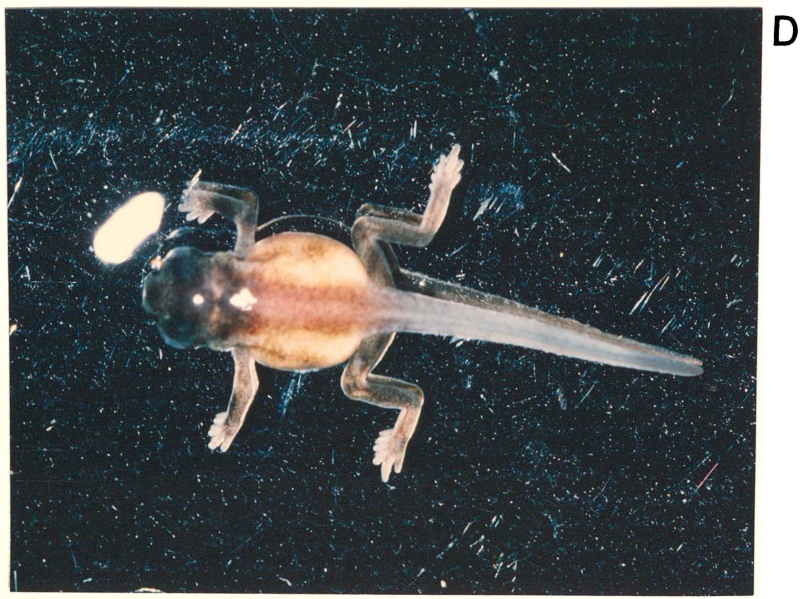
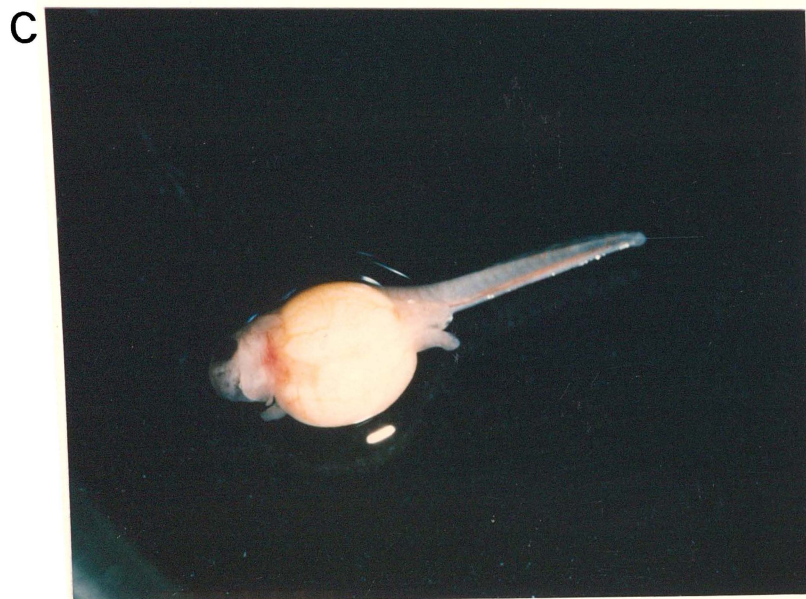
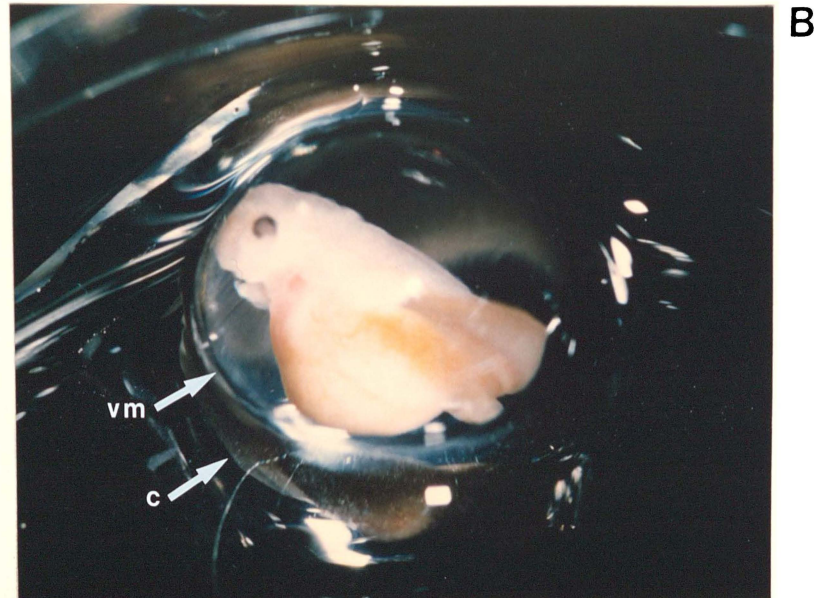
DEVELOPMENTAL STAGES OF *L. ARCHEYI*Introduction

No detailed and complete staging scheme has yet been published for *L. archeyi*, and because of the gradual and unusual manner in which development occurs, conventional anuran staging schemes (e.g., Gosner 1960) are inappropriate. The 20-point staging scheme developed for use in this study is summarised in Table IV.1, and general aspects of development are discussed briefly below. Developmental stages are recognised on the basis of my own field and laboratory observations of the morphology and behaviour of live material, supplemented by information from Archey (1922), N.G. Stephenson (1955) and Bell (1978 and pers. comm.).

Fertile and presumably newly-laid eggs of *L. archeyi* consist of a large, spherical creamy-pale yellow yolk about 4-5 mm in diameter, enclosed by a transparent gelatinous capsule (Plate IV.1A). The outer capsule surface is sticky and eggs adhere into a cluster. From about stage 6 the embryo makes occasional movements, and eye pigment appears at stage 7. Stages 8-15 are characterised mainly by further development of the limbs, including the appearance of limb digits, and the initial development of adult pigmentation patterns on the limbs (Plates IV.1B, C and D). Whilst in the egg the embryo is enclosed within a vitelline membrane lying inside the gelatinous capsule, although this transparent membrane may be difficult to see in intact eggs.

The stage at which hatching occurred in laboratory eggs was extremely variable, occurring between stages 8 and 16. The free-swimming, free-feeding stages of conventional anuran tadpoles are traditionally defined as larvae (e.g., Gosner 1960); neither of these features occurs during development of *L. archeyi* and the earliest stage at which hatching occurred (stage 8) is here considered to be the first larval stage. Hatching probably takes several hours, and newly hatched larvae remained close to their ruptured capsules and vitelline membranes (Plate 8.9; Chapter 8). Following transfer to dishes containing about 0.7 ml water (2-3 mm in depth), hatched larvae of

PLATE IV.1 Developing eggs and larvae of *L. archeyi* in the laboratory. These eggs and larvae were used in the water balance experiments described in Chapter 8. A = a cluster containing eight stage 3 eggs; neurulae are not visible in this photograph (cluster 1/84 prior to water uptake experiment of Chapter 8). B = stage 8 egg kept one-third immersed in water (egg from cluster 1/84 on Day 21 of FIG. 8.2). Note fluid-distended capsule (c) and vitelline membrane (vm). C = hatched larva at stage 9. Note prominent heart, open mouth and limb buds without obvious digits. D = hatched larva at stage 15. Note banding on limbs, black streaks on dorso-lateral surface of yolk between forelimbs and hindlimbs, and blunt snout.



stages 8-15 were usually immobile, lying (sometimes upside down) in the water with only occasional tail flicks or weak limb movements (Plate IV.1C).

Tail resorption began at about stage 16 in all but one laboratory-reared larva; the exception showed rapid tail resorption from stage 13. During stages 16-18, the yolk and tail gradually become resorbed, adult pigmentation develops on the body, and the mouth begins to move forward to the anterior ventral edge of the snout. In addition to these morphological changes, features of adult behaviour become evident: gular fluttering develops and the larva is quite mobile, walking, jumping and climbing readily. When the mouth reaches its final position and the yolk and tail are fully resorbed at stage 19, transformation into a fully metamorphosed juvenile frog is complete. Attainment of sexual maturity probably requires at least 3-4 y (Bell 1978).

TABLE IV.1 Developmental stages of *L. archeyi*. Descriptions refer to morphological and behavioural features observable with the naked eye. Times are approximate for eggs and larvae developing in the laboratory at 15-21°C (field observations suggest that those brooded by adults in natural nest sites develop at about the same rate). Plates refer to those in this appendix and Chapter 8.

Stage	Description	Day
1	Fertile, newly-laid egg with no signs of development, yolk pale yellow-cream and spherical, gelatinous capsule transparent and spherical [non-developing eggs (presumably infertile) have a more intense orange-yellow or yellow-brown yolk, which is often asymmetrical].	0
2	Yolk plug	1?
<u>Embryonic stages</u>		
3	Neural plate to neural tube: presumptive head and tail become distinguishable (PLATE IV.1A).	2
4	Early head development: cream-coloured embryo raised above yolk sac, head and tail ends free of yolk, tip of tail beginning to curl to one side, no tail fin.	4
5	Early forelimb and hindlimb development: forelimbs appear as white rounded buds at anterior margin of yolk below neck of embryo, initially directed away from sides of embryo at approximately right angles; hindlimb buds less obvious, arise at posterior margin of yolk beneath base of tail, initially directed posteriorly beneath tail. Tail fin evident, yolk beginning to flatten dorso-ventrally.	6

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TABLE IV.1 (cont.)

6	Gular fold (= branchial fold, branchial membrane, opercular fold) apparent. Forelimb buds about 2 mm long, embryo moves head and tail and rotates within capsule.	13
7	Pigmented eyes (large and black with white centre); elbow joint in forelimbs; incipient heart developing below anterior end of larva at intersection with yolk.	17
<u>Larval stages</u>		
8	Heart large, engorged with blood and beats regularly; blood vessels on yolk below tail and on sides of yolk. Mouth visible ventrally as an open crescent-shaped depression. Tail as long as or longer than body of embryo. Hatching may occur between now and stage 16 (PLATES IV.1B, 8.1, 8.3).	21
9	Faint nostrils appear as small dark spots anterior to the eyes. Body of embryo developing faint grey pigmentation (PLATE IV.1C, 8.5).	26
10	Limb digits appear as faint rounded buds on end of forelimbs and hindlimbs. Body of embryo grey; eyes fully dark (PLATE 8.9).	33
11	Knee and ankle joints appear on hind limbs; elbow joints on forelimbs are pronounced. Hind limbs about 5 mm long when nearly straight. Grey smudges on yolk posterior to forelimbs, and on ventral surface of yolk.	40
12	Eyes bulge prominently from sides of body when larva is viewed dorsally. Hatched larva can right itself, wriggles and flicks tail, raises and lowers head, moves forelimbs.	43

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TABLE IV.1 (cont.)

13	<p>Digits are well-defined and obvious to naked eye. Hindlimbs lie bent at groin (i.e., femurs directed at right angles to tail), blood vessels apparent at ankles, heart becoming obscured.</p>	47
14	<p>Very faint banding (alternating light and dark stripes) on forelimbs and hindlimbs; snout blunt (no longer rounded). Larva crawls using both sets of limbs, and can hold head raised.</p>	53
15	<p>Beginnings of adult pigmentation on body and stronger banding on limbs. A band of black pigmentation runs laterally from just behind eyes to just above forelimbs. Developing parotoid ridges slightly raised (PLATE IV.1D).</p>	57
16	<p>More pronounced adult pigmentation: darker, more obvious banding on limbs, black dorsal band between eyes, lateral band of black pigmentation extends from just behind eyes along upper surface of yolk to about halfway between forelimbs and hindlimbs. Digits more or less fully formed, tapered, fourth digit of hindlimb easily recognisable as the longest hindlimb digit. Tail shortening apparent. Walks using forelimbs and hindlimbs, holding head raised. Gular fluttering (PLATE 8.6).</p>	61
17	<p>Adult pigmentation nearly complete: body pigmentation lightening in places and green tinge develops on dorsum of those larvae which will appear green as metamorphosed frogs. Black markings on snout (from eyes to nostrils) obvious. Top edge of eye gold. Lateral band of black pigmentation extends from just behind eyes nearly to base of hindlimbs. Parotoid and other glandular ridges on dorsal surface of body are raised and wavy. Larva active, jumps, climbs up walls of container (PLATES 8.7, 8.10).</p>	65

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TABLE IV.1 (cont.)

18	Tail stump up to 4 mm long: apart from this, larva appears fully metamorphosed from above. Ventrally, a circle of yolk still evident in middle of abdomen, mouth not yet fully forward to ventral anterior edge of snout (PLATE 8.8).	74
<u>Adult Stages</u>		
19	Juvenile frog: metamorphosis complete.	90
20	Mature adult frog.	several years

APPENDIX V

DEVELOPMENT OF *L. ARCHEYI* EGGS AND LARVAE IN NATURAL NESTS

TABLE V.1 Development of *L. archeyi* eggs and larvae in natural nests in the 1984 and 1985 seasons (MC = moisture content; Ψ = substrate water potential; mean weight and mean body length of larvae in each clutch given in parentheses).

Clutch No.	Total no. of eggs	No. of fertile eggs	Substrate below egg cluster	Dates examined during egg development			Dates examined after hatching		
				22/10/84	28/11/84 or 29/11/84	16/12/84	15/1/85	28/1/85	6/2/85
1984 season:									
1/84	7	6	On soil MC = 283% Ψ = -2 kPa	Male brooding egg cluster (both collected) (Plate IV.1A)	Empty	-	-	-	-
2/84	8	7	In hollow, on scant layer of soil over rock. MC = 213% Ψ = -16 kPa	Male brooding egg cluster (both collected)	Empty	-	-	-	-
3/84	7	6	Initially half in air attached to twigs and tree roots, then on soil and litter	Male brooding egg cluster	Male brooding egg cluster (Plates 8.3, 8.4)	Male brooding egg cluster	Male carrying two stage 18 larvae (0.15 g, 9.2 mm) (Plate 8.8)	Empty (completed development?)	-
4/84	6	5	Initially half in air attached to tree roots, then on rock	Male brooding egg cluster	Male brooding egg cluster	Empty (deserted?)	-	-	-
5/84	6	6	On soil, in hollow	-	Male ~ 50 mm from egg cluster	Male brooding egg cluster	Male carrying five stage 17 larvae (0.14 g, 9.5 mm)	Empty (completed development?)	Empty
6/84	8	8	On soil, in hollow MC = 91%	-	Male brooding egg cluster (Plate 8.2)	Male brooding egg cluster	Male carrying eight stage 17 larvae (0.13 g, 9.0 mm) (Plate 8.7)	Male with four stage 18 larvae (two on back, two alongside) (0.13 g, 9.1 mm)	Two stage 19 juvenile frogs on ground
7/84	7	7	On wet leaf fragment in hollow	-	Male brooding egg cluster	Male brooding egg cluster (Plate 8.5)	Empty (deserted?)	-	-
8/84	7	7	On soil, in hollow MC = 231%	-	Male brooding egg cluster (cluster collected)	-	-	-	-
9/84	-	-	On soil, in hollow	-	-	-	Male carrying eight stage 16 larvae (larvae collected) (0.11 g, 8.3 mm)	-	-
1985 season:									
1/85	5	4	On wet rock and litter, in a slight wedge between two rocks	3/11/85 Male brooding egg cluster	-	15/12/85 Male brooding egg cluster (Plate 8.1) (cluster collected)	-	-	-
2/85	-	-	On soil, under overhang of adjacent rock	-	-	-	-	22/1/86 Male carrying six stage 16 larvae (0.12 g; length not measured)	-

APPENDIX VI

DISTRIBUTION OF THE CUTANEOUS HYDROSMOTIC RESPONSE AMONGST
ANURAN AMPHIBIANSIntroduction

The results presented in Chapters 3, 4 and 5 and Appendix II show that *Leiopelma archeyi*, *L. hamiltoni*, *L. hochstetteri*, *Ascaphus truei*, *Xenopus laevis* and *Litoria aurea* fall into two distinct groups in terms of water balance response. Those species with a cutaneous hydrosmotic response (*L. archeyi*, *L. hamiltoni* and *Litoria aurea*) have water balance responses to dehydration or treatment with exogenous AVT of the types shown in Fig VI.1. During rehydration, weight gain initially follows a parabolic curve, with weight overshooting W_0 and then dropping back to near W_0 as urine production increases (Fig. VI.1A). Following treatment with AVT a similar but temporary increase in weight is seen, the excess water eventually being excreted (Fig. VI.1B). Significant increases in osmotic water flow (OWF) also occur when isolated ventral pelvic skin is exposed to AVT.

In contrast, species which lack a cutaneous response (*L. hochstetteri*, *X. laevis* and *A. truei*) rehydrate at a slow, linear rate (Fig. VI.2A). The rate of water uptake is unchanged from that seen in hydrated animals, and weight gain is attributed to antidiuresis. When these species are treated with AVT a similar slow, constant weight increase attributed to antidiuresis may occur (*L. hochstetteri*), or there may be no weight gain at all [*A. truei* in the present study; *X. laevis* in Heller and Bentley (1965)] (Fig. VI.2B). Isolated skin from the two species examined [*L. hochstetteri* in Chapter 5 and Cameron (1974); *X. laevis* in Bentley (1969b)] also shows no increase in OWF when exposed to AVT or other neurohypophysial peptides (NHPs).

Two hypotheses have been proposed to account for the presence of a cutaneous hydrosmotic response in some anurans but not in others (Chapter 1). According to the "ecological" hypothesis, the presence of the response varies according to habitat and the response may be absent in species which are unlikely to become dehydrated. According to the "phylogenetic" hypothesis, the response is absent or poorly

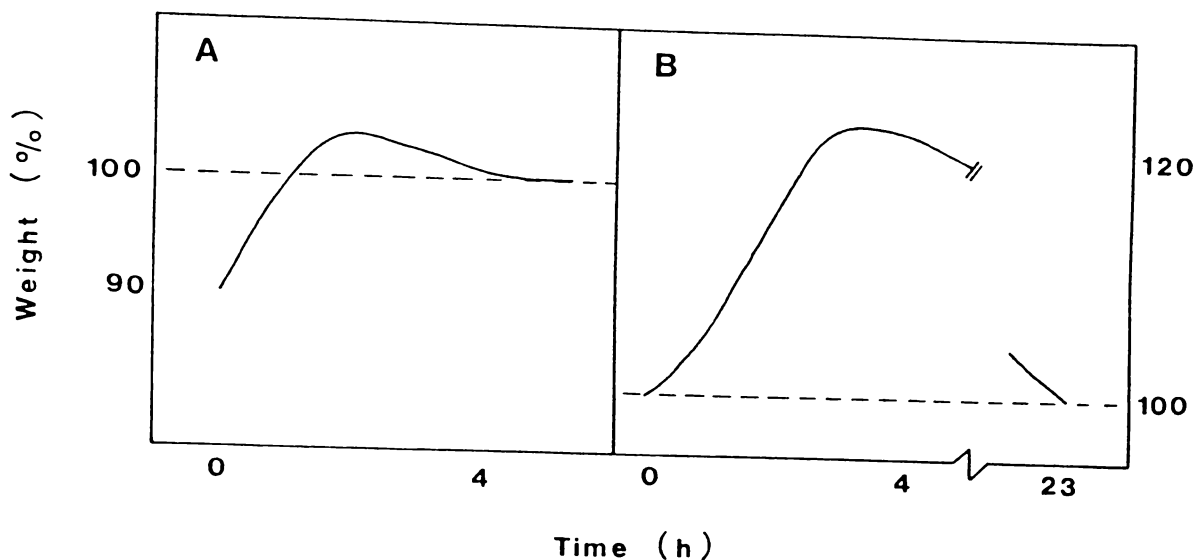


FIG. VI.1 Diagrammatic representation of the water balance responses to dehydration (A) and exogenous AVT (B) in anurans possessing a cutaneous hydroosmotic response. In each case the dashed line indicates the original weight (W_0). Weight and time scales are only approximate and are included to draw attention to the differences between these responses and those of FIG. VI.2.

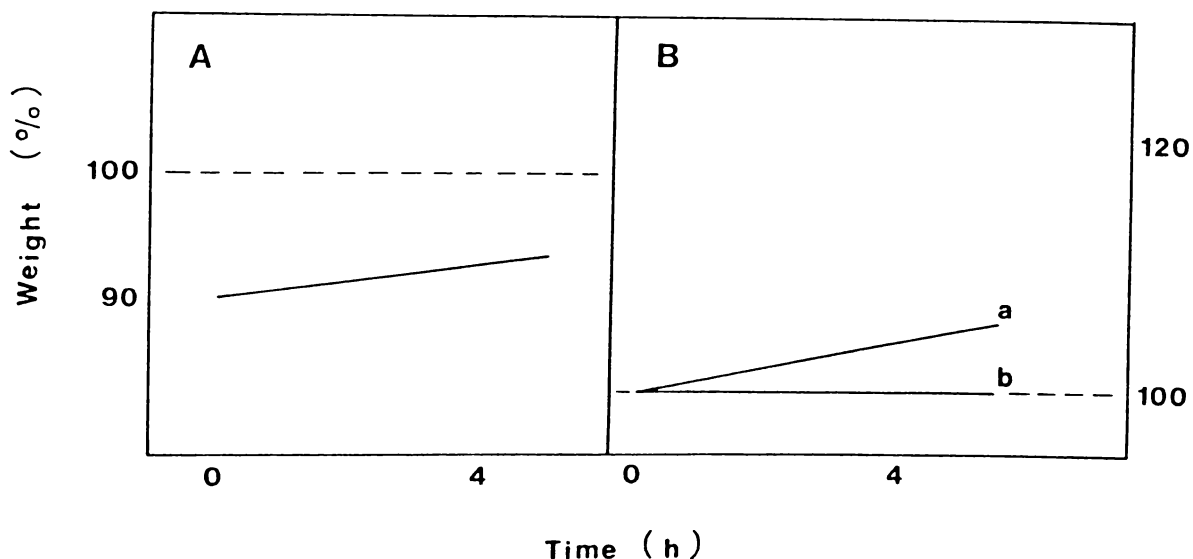


FIG. VI.2 Diagrammatic representation of the water balance responses to dehydration (A) and exogenous AVT (B) in anurans lacking a cutaneous hydroosmotic response. Response type a to exogenous AVT is demonstrated by *Leiopelma hochstetteri* and b by *Ascaphus truei* and *Xenopus laevis*. As in FIG. VI.1, weight and time scales are only approximate.

developed in archaic frogs but pronounced in advanced ones. A review of the evidence for these two hypotheses was made by examining the distribution of the response amongst anuran species, in relation to both habitat and phylogenetic position.

Criteria for recognising a cutaneous response in species examined in the literature were considered as any of the following:

(i) a rehydration pattern similar to that shown in Fig. VI.1A, where water uptake following dehydration occurs at a significantly faster rate than seen in hydrated animals. In studies where rates of water uptake in hydrated animals were not given, water uptake in excess of $69 \text{ mg cm}^{-2} \text{ h}^{-1}$ during rehydration in shallow water or $27 \text{ mg cm}^{-2} \text{ h}^{-1}$ when immersed was assumed to be evidence of a cutaneous response (these were the lowest of the mean values obtained for *L. archeyi*, *L. hamiltoni* or *Litoria aurea*. In view of the small dehydration deficit and cool temperatures used, these rates are likely to provide minimal estimates of rehydration rates for species having a cutaneous response);

(ii) a response to exogenous NHPs similar to that shown in Fig. VI.1B;

(iii) a significant increase in cutaneous OWF *in vitro* in response to NHPs.

Conversely, the absence of a cutaneous response was inferred from:

(i) a rehydration pattern similar to that shown in Fig. VI.2A;

(ii) a response to exogenous NHPs similar to that shown in Fig. VI.2B;

(iii) lack of increase in OWF through isolated skin preparations following exposure to NHPs.

The results of a literature survey carried out in this way are given in Table VI.1, and the interpretation of the results is discussed in Chapter 10 (General Discussion).

TABLE VI.1 Distribution of the cutaneous hydroosmotic response (CHR) amongst anurans. Families are listed according to the phylogenetic scheme of Duellman (1975), except that the additional family Ascaphidae is recognised (Green *et al.* 1980). Habitat codes are as in TABLE 4.6, with the addition of BW = brackish water. A cutaneous response is considered to be present (+) or absent (-) on the basis of evidence from rehydration rates (1), responses to NHPs *in vivo* (2), or responses to NHPs *in vitro* (3). A question mark indicates that the response is uncertain or weak.

Species	Habitat	Presence or absence of CHR	Evidence from	Reference
ARCHAEOBATRACHIA				
Leiopelmatidae:				
<i>Leiopelma archeyi</i>	T	+	1,2,3	Present study
<i>L. hamiltoni</i>	T	+	1,2,3	Present study
<i>L. hochstetteri</i>	SA	-	1,2,3	Present study; Cameron (1974)
Ascaphidae:				
<i>Ascaphus truei</i>	SA	-	1,2	Present study
Discoglossidae:				
<i>Discoglossus pictus</i>	A	+?	2	Heller and Bentley (1965)
Pipidae:				
<i>Xenopus laevis</i>	A	-	1,2,3	Ewer (1952); Maetz (1963); Heller and Bentley (1965); Bentley (1969b); present study
Pelobatidae:				
<i>Pelobates cultripes</i>	F	+?	2	Heller and Bentley (1965)
<i>P. syriacus</i>	F	+?	1,2	Warburg (1971a); Goldenberg and Warburg (1983)
<i>Scaphiopus couchi</i>	F	+?	1,2,3	Claussen (1969); Hillyard (1976); Jones (1978)
NEOBATRACHIA				
Myobatrachidae:				
<i>Crinia signifera</i>	SA/T	+	1	Warburg (1965)
<i>C. georgiana</i>	SA/T	+	2,3	Bentley and Main (1972b)
<i>Cyclorana platycephalus</i>	F	+	1	Main and Bentley (1964)
<i>Heleioporus albopunctatus</i>	F	+	1,2	Bentley <i>et al.</i> (1958)
<i>H. australiacus</i>	F	+	1,2	Bentley <i>et al.</i> (1958)
<i>H. eyrei</i>	F	+	1,2	Bentley <i>et al.</i> (1958)
<i>H. inornatus</i>	F	+	1,2	Bentley <i>et al.</i> (1958)
<i>H. psammophilus</i>	F	+	1,2	Bentley <i>et al.</i> (1958)
<i>Neobatrachus centralis</i>	F	+	1,2	Bentley <i>et al.</i> (1958)
<i>N. pelobatoides</i>	F	+	1,2	Bentley <i>et al.</i> (1958)
<i>N. pictus</i>	F	+	1	Warburg (1965)
<i>N. sutor</i>	F	+	1,2	Bentley <i>et al.</i> (1958)
<i>N. wilsmorei</i>	F	+	1,2	Bentley <i>et al.</i> (1958)
<i>Notaden nichollsi</i>	F	+	1	Main and Bentley (1964)
<i>Taudactylus diurnus</i>	SA	+	1	Johnson (1971)
Leptodactylidae:				
<i>Eleutherodactylus antillensis</i>	T/ARB	+	1	van Berkum <i>et al.</i> (1982)
<i>E. coqui</i>	T/ARB	+	1	van Berkum <i>et al.</i> (1982)
<i>Ceratophrys ornata</i> (Tigre)	F	+	2	Canziani and Cannata (1980)
<i>C. ornata</i> (South Chaco)	F	-	2	Canziani and Cannata (1980)
<i>Calyptocephalella gayi</i>	A	-	2	Garcia <i>et al.</i> (1970)

(cont. over page)

TABLE VI.1 (cont.)

Species	Habitat	Presence or absence of CHR	Evidence from	Reference
Bufonidae:				
<i>Bufo americanus</i>	T	+	2	Steggerda (1937)
<i>B. asper</i>	SA	+	1	Shoemaker and McClanahan (1980)
<i>B. boreas</i>	T	+	1,3	Claussen (1969); Baldwin (1974); Kent and McClanahan (1980)
<i>B. bufo</i>	T	+	1,2,3	Howes (1940); Jørgensen and Rosenkilde (1956); Morel <i>et al.</i> (1958); Christensen (1974a, 1974b)
<i>B. carens</i>	T	+	2	Ewer (1952)
<i>B. debilis</i>	T	+	1	Claussen (1969)
<i>B. marinus</i>	T	+	2,3	Bentley and Ferguson (1967); Bentley and Main (1972a); Cameron (1980); De Sousa and Grosso (1982)
<i>B. quadriporcatus</i>	SA	+	1	Shoemaker and McClanahan (1980)
<i>B. punctatus</i>	T	+	1,3	Claussen (1969); McClanahan and Baldwin (1969); Baldwin (1974)
<i>B. regularis</i>	T	+	1,2	Ewer (1951); Cloudsley-Thompson (1967)
<i>B. viridis</i>	T/BW	+	1,2	Warburg (1971a); Katz and Graham (1980); Goldenberg and Warburg (1983); Degani (1985)
<i>B. woodhousei</i>	T	+	3	Marrero and Hillyard (1985)
Hylidae:				
<i>Hyla arborea</i>	ARB	+	1,2	Overton (1904); Warburg (1971a) Degani and Warburg (1984)
<i>H. arenicolor</i>	ARB	+	3	Yorio and Bentley (1977)
<i>H. femoralis</i>	ARB	+	3	Yorio and Bentley (1977)
<i>H. septentrionalis</i>	ARB	+	1	Claussen (1969)
<i>H. squirrella</i>	ARB	+	3	Yorio and Bentley (1977)
<i>Phyllomedusa sauvagei</i>	ARB	+	1	Shoemaker and McClanahan (1975)
<i>P. pailona</i>	ARB	+	1	Shoemaker and McClanahan (1975)
<i>P. iherengi</i>	ARB	+	1	Shoemaker and McClanahan (1975)
<i>P. hypochondrialis</i>	ARB	+	1	Shoemaker and McClanahan (1975)
<i>Pachymedusa dacnicolor</i>	ARB	+	1	Shoemaker and McClanahan (1975)
<i>Agalychnis annae</i>	ARB	+	1	Shoemaker and McClanahan (1975)
<i>A. dacnicolor</i>	ARB	+	3	Yorio and Bentley (1977)
<i>Litoria aurea</i>	SA	+	1,2,3	Present study
<i>Litoria caerulea</i>	ARB	+	1	Main and Bentley (1964); Johnson (1971)
<i>Litoria ewingi</i>	T/ARB	+	1,3	Cameron (1974); Cree (1985a)
<i>Litoria latopalmata</i>	ARB	+	1	Main and Bentley (1964)
<i>Litoria moorei</i>	ARB	+	1,3	Main and Bentley (1964); Bentley and Main (1972a)
<i>Litoria raniformis</i>	SA	+	1,3	Cameron (1974); Cree (1985a)
<i>Litoria rubella</i>	ARB	+	1	Main and Bentley (1964)
Ranidae:				
<i>Pyxicephalus adspersus</i>	F	?	1	Loveridge and Withers (1981)
<i>Rana arvalis</i>	SA/T	+	1 <i>in vitro</i>	Christensen (1974a)
<i>R. blythi</i>	SA	-	1	Shoemaker and McClanahan (1980)
<i>R. cancrivora</i>	BW	-	2	Dicker and Elliott (1970)
<i>R. catesbeiana</i>	SA	+	1,2,3	Alvarado and Johnson (1966); Claussen (1969); Bentley (1969b, 1973)
<i>R. chalcinota</i>	SA/ARB	+	1	Shoemaker and McClanahan (1980)
<i>R. clamitans</i>	A/SA	+	2	Steggerda (1937)
<i>R. esculenta</i>	SA	+	2,3	Morel <i>et al.</i> (1958); Heller and Bentley (1965); Johnsen and Nielsen (1984)
<i>R. glandulosa</i>	T	-	1	Shoemaker and McClanahan (1980)
<i>R. kuhli</i>	A	-	1	Shoemaker and McClanahan (1980)
<i>R. limnocharis</i>	SA/T	+	1	Shoemaker and McClanahan (1980)
<i>R. nicobariensis</i>	ARB	+	1	Shoemaker and McClanahan (1980)
<i>R. pipiens</i>	SA	+	1,2,3	Steggerda (1937); Bentley and Main (1972a); Tracy (1976); Parsons <i>et al.</i> (1978)
<i>R. ridibunda</i>	SA	+	1,2	Warburg (1971a); Katz and Graham (1980); Goldenberg and Warburg (1983)
<i>R. septentrionalis</i>	SA	+	2	Boyd and Brown (1938)
<i>R. temporaria</i>	SA/T	+	1,2	Jørgensen and Rosenkilde (1956); Christensen (1974a)
Hyperoliidae:				
<i>Hyperolius viridiflavus</i>	ARB	+	1	Geise and Linsenmaier (1986)
Rhacophoridae:				
<i>Chiromantis petersi</i>	ARB	+	1	Drewes <i>et al.</i> (1977)
<i>C. xerampelina</i>	ARB	+	1	Loveridge (1970)
<i>Rhacophorus leucomystax</i>	ARB	+	1	Shoemaker and McClanahan (1980)
<i>R. otitophus</i>	ARB	+	1	Shoemaker and McClanahan (1980)
<i>R. pardalis</i>	ARB	+	1	Shoemaker and McClanahan (1980)