Volume 2, Number 1

January 1992 ISSN 0268-0130

THE HERPETOLOGICAL JOURNAL



Published by THE BRITISH HERPETOLOGICAL SOCIETY

Indexed in Current Contents *The Herpetological Journal* is published quarterly by the British Herpetological Society and is issued free to members. Applications to purchase copies and/or for details of membership should be made to the Hon. Secretary, British Herpetological Society, The Zoological Society of London, Regent's Park, London, NW1 4RY, UK.

Instructions to authors are printed inside the back cover. All contributions should be addressed to the Editor (address below).

Editor:

Richard A. Griffiths, The Durrell Institute of Conservation and Ecology, University of Kent, Canterbury, Kent, CT2 7NX, UK.

Assistant Editor:

Margaret M. Bray, Department of Biochemistry and Biological Sciences, Wye College (University of London), Nr. Ashford, Kent, TN25 5AH, UK.

Editorial Board:

Pim Arntzen (Leicester) Donald Broadley (Zimbabwe) John Davenport (Millport) Tim Halliday (Milton Keynes) Michael Klemens (New York) Colin McCarthy (London) Andrew Milner (London) Henk Strijbosch (Nijmegen) Richard Tinsley (London)



Copyright

It is a fundamental condition that submitted manuscripts have not been published and will not be simultaneously submitted or published eleswhere. By submitting a manuscript, the authors agree that the copyright for their article is transferred to the publisher if and when the article is accepted for publication. The copyright covers the exclusive rights to reproduce and distribute the article, including reprints and photographic reproductions. Permission for any such activities must be sought in advance from the Editor.

ADVERTISEMENTS

The Herpetological Journal accepts advertisements subject to approval of contents by the Editor, to whom enquiries should be addressed.

AQUATIC OXYGEN COMFORMITY IN THE MEXICAN AXOLOTL, AMBYSTOMA MEXICANUM

H.I. GRIFFITHS¹ AND D.H. THOMAS²

¹ Environment Lab., School of History and Archaeology, University of Wales College of Cardiff, P.O. Box 909, Cardiff, CF1 3XU, U.K.
² School of Pure and Applied Biology, University of Wales College of Cardiff, P.O. Box 915, Cardiff, CF1 3TL, U.K.

(Accepted 5.5.90.)

ABSTRACT

The Mexican axolotl, *Ambystoma mexicanum* is an aquatic-phase oxygen conformer with a close correlation between rates of aquatic oxygen consumption and aquatic oxygen tension. Rates of oxygen consumption at normoxic oxygen tensions were 18.2μ l O_{2.g}⁻¹h⁻¹ at 20°C and 13.8μ l O_{2.g}⁻¹h⁻¹ at 30°C. Air-breathing (i.e. rising to the water surface to gulp air) increases in hypoxia, associated with a decrease in gill ventilatory movements. Therefore, oxygen compensation during aquatic hypoxia appears to occur in the aerial phase, whilst aquatic gas-exchange surfaces show little ability to compensate for changes in ambient oxygen tension.

INTRODUCTION

Three major strategies for respiration have been indentified for primarily aquatic urodeles by Guimond and Hutchison (1973). These are the development of elaborate gills with poorly developed and vascularised lungs as in the Proteidae, air-breathing with elaborate lungs but with the gills vestigial or absent as in the Sirenidae and the Amphiumatidae, or a lack of either elaborate gills or lungs but with gas-exchange taking place primarily across a highly vascularised and expanded skin surface as in the Cryptobranchidae. The facultatively paedomorphic members of the Ambystomatidae would seem to represent a fourth option however, having primitive but functional lungs (Brunst, 1955) and well-developed external gills (McIndoe and Smith, 1984). Both aerial and aquatic respiration have been shown to contribute significantly to the animals needs (e.g. Whitford and Sherman, 1968; Lenfant, Johansen and Hanson, 1970; Heath, 1976).

The relative importance of the different gas-exchange sites (gills, lungs, skin) varies between amphibians. There is much evidence that cutaneous respiration can be the most significant aquatic gas-exchange surface in many adult urodeles; in A. mexicanum the skin of the back and flanks is thin and well-vascularised (Czopek, 1957), whilst the tail is expanded dorsally and ventrally into fins, these adaptations serving to reduce diffusive distances and to increase the surface area available for aquatic gas-exchange (see Feder and Burggren, 1985). Bucco-pharyngeal mucous membranes are not usually considered competent for gas-exchange in urodeles because of the poor development of the intraepithelial capillaries (Elkan, 1955, 1958). In larval and paedomorphic forms the gills appear to function primarily in acid-base regulation and in carbon dioxide exchange (Baldwin and Bently, 1982; Lewinson, Rosenburg, Goldenburg and Warburg, 1987; Boell, Greenfield and Hille, 1963).

The object of this study was to observe the effects of a variety of aquatic oxygen tensions on the rate of cutaneous uptake of oxygen by *A. mexicanum*, to determine the role of aquatic gas-exchange under different ecophysiological conditions.

MATERIALS AND METHODS

Ten paedomorphic albino A. mexicanum of similar age (weight range 85g-106g) were obtained from a local breeder. Animals were kept in two large aquaria and maintained at ambient temperature on a 12 hour light: 12 hour dark photoperiod. Animals were starved for three days prior to use and rested for several days between experimental runs. Identification of individual animals was facilitated by marking with a fish-tattoo panjet (F.H. Wright, Dental Manufacturing Co. Ltd., Dundee) loaded with alcian blue dye (Wisniewski, Paull, Merry and Slater, 1980). Animals were acclimated to experimental temperatures overnight before use. Stocks of hypoxic and hyperoxic maintenance media were made up from oxygen-saturated and oxygen-free stock prepared by bubbling gaseous oxygen or nitrogen through bulk water samples under liquid paraffin (B.D.H. Ltd.).

Respirometry was carried out in a closed system within a heated water bath at 20°C or 30°C with media (1 litre) of various initial oxygen tensions made by mixing hypoxic and hyperoxic stocks in the appropriate proportions. Animals were placed in experimental chambers and the air/water interface sealed with a thin layer of liquid paraffin so as to isolate the water phase from atmospheric oxygen without restricting the animals access to air.

After allowing animals to settle, water samples were taken at thirty minute intervals over a period of two hours. Three 20ml water samples were removed in 25ml disposable syringes adapted to allow the addition of titration reagents, whilst excluding atmospheric oxygen. Sample volumes were replaced with oxygen-purged water introduced through the paraffin layer. The oxygen content of the samples was measured by a Winkler titration method modified from that of Hoar and Hickman (1967). Aquatic oxygen concentrations were converted to $P_{O2(w)}$ using the α -values for freshwater from Randall (1970). The coefficient of variation of dissolved oxygen concentrations obtained by this method during control runs was 5.6%. Experimental runs in which animals showed clear escape reactions were excluded from data analysis. Virtually all respirometry was carried out between 09.00 and 13.00 hours to reduce diel effects in oxygen consumption,

previous observations having shown that *A. mexicanum* displays diel variation in ventilatory cycles (Griffiths and Thomas, unpublished data).

Oxygen consumption rates were calculated from slopes of oxygen concentration against time (determined by least squares regression) and expressed as μ l O₂(S.T.P.D.).gram wet body mass '.hour'.

The effect of aquatic oxygen concentration on the frequency of lung and gill ventilations was also examined by direct observation. Animals were held at 20° C in a constant temperature room and the total number of lung ventilations per hour recorded. Gill ventilatory movements were recorded as the mean number of gill beats per minute in each of four random one minute periods within each hour.

The relationships between aquatic oxygen tension $(P_{O2(w)})$ and the rate of oxygen consumption $(\dot{V}_{O2(w)})$, gill ventilation frequency (f_{gill}) and lung ventilation frequency (f_{ung}) were examined by linear least-squares regression analysis.

RESULTS

There was a strong positive correlation between the rate of oxygen consumption and the oxygen tension of the water at both experimental temperatures. The relationship between $\dot{V}_{O2(w)}$ and initial oxygen tension at 20°C and 30°C is shown in Fig. 1 and regressions at each temperature are shown below. The strongly positive slopes show that *A. mexicanum* is an oxygen conformer for its aquatic-phase respiration, at least in this temperature range:

At 20°C:

 $\dot{V}_{O2(w)} = (-2.29 \pm 1.99) + (0.13 \pm 0.03) \cdot P_{O2(w)(initial)}$

Units: μ l O₂.g^{-'}.h^{-'} & mmHg; r = 0.92, df = 14, P < 0.001.

At 30°C:

 $V_{O2(w)} = (-1.08 \pm 1.02) + (0.09 \pm 0.03).P_{O2(w)(initial)}.$

Units: μ l O2.g⁻¹.h⁻¹ & mmHg; r = 0.99, df = 8, P < 0.01.



 $f_{\text{lung}} = (6.12 + 2.43) + (0.03 + 0.02) \cdot \text{Poly(withinitial)}.$

Units: $f_{lung.hr'}$ & mmHg; r = 0.48. df = 13, P > 0.05.



Fig. 2 Frequency of gill ventilation $(f_{gull}.minute')$ and lung ventilation $(f_{lung}.hour')$ of axolotls under different aquatic oxygen tensions at 20°C. Regressions are given in the text.



Fig. 1 Rates of oxygen consumption of axolotls in water of differing initial aquatic oxygen tensions at 20° C (broken line and open circles) and 30° C (solid line and closed circles). Regressions are at 20° C and 30° C are given in the text.

Axolotls appear to compensate for aquatic hypoxia by increasing aerial respiration. Fig. 2 shows apparent trends for f_{tell} to decrease with decreasing aquatic oxygen tension and for f_{lung} to increase, although neither of these regressions is statistically significant:

DISCUSSION

The results presented here show that A. mexicanum is an aquatic-phase oxygen conformer: in other words, in water oxygen uptake is determined by external Po2(w), and the animal has little ability therefore to regulate its O₂ gains. Previous similar studies of paedomorphic aquatic salamanders are sparse, although Heath (1976) has reported aquatic-phase oxygen conformity in paedomorphic Ambystoma tigrinum. On the other hand, Branch and Taylor (1977) found A. maculatum to be oxygen conformers only before metamorphosis, whilst post-metamorphic animals were oxygen regulators (i.e. the animal itself can take measures to compensate for variable external Po2(w) by increasing the effectiveness of O2 uptake processes when Po2(w) is low). Ultsch (1973) reported Siren lacertina of a variety of size classes to be oxygen conformers at oxygen tensions below 80mmHg, above which they became oxygen regulators. From our data there is no evidence of aquatic oxygen regulation in A. mexicanum over a broad range of aquatic oxygen tensions, therefore any compensation for environmental hypoxia must occur in the aerial phase.

Rose, Armentrout and Roper (1971) have shown that paedomorphs of *A. tigrinum* are remarkably resistant to enforced aquatic anoxia and are apparently capable of anaerobiosis, as indicated by reduced cardiac glycogen stores and increased blood lactate and glucose levels. *A. mexicanum* may also be capable of withstanding environmental anoxia and studies of muscle histology that report poor capillarisation of the white muscle fibres lend support to this view (Totland, 1984). However, behavioural avoidance, if possible, appeared to over-ride physiological compensation; in severe hypoxia a number of our animals showed profound escape responses, to the extent of climbing out of the experimental chambers into the surrounding water-bath.

Our value for the rate of (normoxic) aquatic oxygen consumption at 20°C (18.2μ l O₂.g⁻¹.h⁻¹, Po₂(w) = 155mmHg) is consistent with values of 22µl O₂.g⁻¹.h⁻¹ for *A mexicanum* at 20°C reported by Gahlenbeck and Bartels (1970). The apparent interspecific difference in $\dot{V}_{O2}(w)$ is probably a simple effect of body mass: assuming a scaling exponent of -0.34 for mass-specific metabolism in aquatic urodeles (cf. Ultsch, 1974), $\dot{V}_{O2}(w)$ for animals of body mass 44g (Gahlenbeck and Bartels, 1970) and 100g (this study) would be expected in proportion of 24:18, which is close to the observed proportions in the two studies. More precise comparisons are not possible since Gahlenbeck and Bartels (1970) did not provide Po₂(w) values for which due allowance must be made (Fig. 1).

As increasing temperature leads to increased metabolic demand in poikilotherms, the value of 13.8μ l O_{2.g⁻¹}.h⁻¹ at 30°C (Po_{2(w)} = 152mmHg) for aquatic respiration is lower than might be expected, and doubtless reflects a switch to aerial respiration as we have shown at reducing Po_{2(w)} (Fig. 2), (in our system,

animals could and did push through the supernatant paraffin-layer to gulp air). At any given Po2(w) the availability of dissolved oxygen will be lower at 30°C than at 20°C; however, because of problems inherent to closed-system respirometry (Po2(w) decreasing and Pco2(w) increasing with time), it is impossible to state with certainty that any switch to air-breathing at this temperature is mediated by aquatic hypoxia. In some air-breathing fish, aquatic hypercarbia will also lead to a switch to lung-breathing, presumably by creating conditions that are more favourable to aerial CO2exchange (Hughes and Singh, 1971). However, during our study individual slopes for A. mexicanum showed no evidence of threshold effects, supporting the contention that if Pco2-mediated switching to lung-breathing does occur, the relevant response threshold was not attained during the experimental period. Although an aquatic temperature of 30°C is somewhat in excess of that recommended for captive maintenance of axolotls (British Herpetological Society Captive Breeding Committee, 1978), experimental animals showed no signs of distress or abnormality either during or after exposure to this temperature regime.

West and Burggren (1982) working on tadpoles of Rana catesbeiana, suggested that aquatic oxygen tension exerted an effect on gill and lung ventilation which interact so that a high lung ventilation frequency in hypoxic waters acts to supress gill ventilation cycles. This is consistent with our present observations on A. mexicanum, that seem to show that air-breathing increased and gill ventilation decreased under hypoxic conditions. At some as yet undefined threshold, severe environmental hypoxia will act to deplete systemic venous blood arriving at the gills. Furthermore, at low ambient oxygen tensions gill ventilation will become decreasingly efficient energetically, as the amount of work done increases in direct proportion to the decrease in the availability of dissolved oxygen. In paedomorphs of A. tigrinum, Malvin and Heisler (1988) have shown that an increase in the fraction of cardiac output flowing to the lungs and a decrease in the fraction to the gills accompanies aquatic hypoxia, the latter having a protective function. This is consistent with the view that in general the gills of perennibranchiate urodeles are of limited use as oxygen exchangers, though they may be important in CO2-exchange and acid-base regulation owing to the higher permeability of CO2.

It would appear from these studies that the axolotl is quite well adapted to ecologically realistic levels of hypoxia that might arise due to changes in temperature or biological oxygen demand in its normal habitat of a shallow, warm-belt lake at an altitude in excess of 2200m. Bimodal respiratory adaptations are characteristic of aquatic vertebrates inhabiting waters subject to periodic hypoxia and hypercarbia (Dehadrai and Tripathi. 1976: Ultsch, 1976). Unfortunately, few details of the natural history and habitat of A. mexicanum have been published (see Griffiths and Thomas. 1989). Even more unfortunate is the fact that the sole surviving wild population of the Mexican axolotl. in one lake near Mexico City, appears vulnerable so that it may soon become impossible to study the axolotl in its normal habitat (Griffiths and Thomas, 1989).

ACKNOWLEDGEMENTS

The authors would like to thank Doug Paul for advice on husbandry and Mark Simmonds (Queen Mary College, London) for marking the animals; also thanks are due to Dr. A. John Morgan (University College, Cardiff) for advice in other areas of the work. We would also like to thank Professor G. M. Hughes (University of Bristol) and two anonymous reviewers for their helpful comments.

REFERENCES

- Boell, E.J., Greenfield, P. and Hille, B. (1963). The respiratory function of the gills in the larvae of *Ambystoma punctatum*. *Developmental Biology* 7, 420-431.
- Branch, L.C. and Taylor, D.H. (1977). Physiological and behavioural responses of larval spotted salamanders (Ambystoma maculatum) to various concentrations of oxygen Comparative Biochemistry and Physiology 58A, 269-274.
- British Herpetological Society Captive Breeding Committee (1978). Notes on Axolotls. London: British Herpetological Society. (Mimeograph).
- Brunst, V.V. (1955). The Axolotl (Siredon mexicanum). II. Morphology and pathology. Laboratory Investigation 4, 429-449.
- Czopek, J. (1957). The vascularisation of respiratory surfaces in Ambystoma mexicanum (Cope) in ontogeny. Zoologica Poloniae 8, 131-149
- Dehadrai, P.V. and Tripathi, S.D. (1977). Environment and ecology of freshwater air-breathing teleosts. In: *Respiration* of amphibious vertebrates. 39-72. Hughes, G.M. (Ed). London: Academic Press.
- Elkan, E. (1955). The buccal and pharyngeal mucous membranes in urodeles. *Proceedings of the Zoological Society of London* 125, 685-710.
- Elkan, E. (1958). Further contributions on the buccal and pharyngeal mucous membranes in urodeles. *Proceedings of the Zoological Society of London* 131, 335-355.
- Feder, M.E. and Burggren, W.W. (1985). Cutaneous gas exchange in vertebrates; design, patterns, control and implications. *Biological Reviews* 60, 1-45.
- Gahlenbeck, H. and Bartels, H. (1970). Blood gas transport properties in gill and lung forms of the axolotl *Ambystoma mexicanum. Respiration Physiology* **9**, 175-182.
- Griffiths, H.I. and Thomas, D.H. (1989). What is the status of the Mexican Axolotl? British Herpetological Society Bulletin 26, 3-5.
- Guimond, R.W. and Hutchison, V.H. (1973). Aquatic respiration; an unusual strategy in the Hellbender Cryptobranchus alleganiensis alleganiensis (Daudin). Science (N.Y.) 182, 1263-1264.
- Heath, A.G. (1976). Respiratory responses to hypoxia by *Ambystoma tigrinum* larvae, paedomorphs and metamorphosed adults. *Comparative Biochemistry and Physiology* 55A, 45-49.

- Hoar, W.S. and Hickman Jnr., C.P. (1967). A laboratory companion for general and comparative physiology, 286-289. New Jersey: Prentice Hall Inc.
- Hughes, G.M. and Singh, B.N. (1971). Gas exchange with air and water in an air-breathing catfish. Saccobranchus (= Heteropneustes) fossilis. Journal of Experimental Biology 55, 667-682.
- Lenfant, C., Johansen, K. and Hanson, D. (1970). Bimodal gas exchange and ventilation-perfusion relationships in lower vertebrates. *Federation Proceedings* 29, 1124-1129.
- Lewinson, D., Rosenburg, M., Goldenburg, S. and Warburg, M.R. (1987). Carbonic anhydrase cytochemistry in mitochondria-rich cells of salamander gill epithelium as related to age and H⁺ and Na⁺ concentrations. *Journal of Cellular Physiology* 130, 125-132.
- McIndoe, R. and Smith, D.G. (1984). Functional morphology of gills in larval amphibians. *Perspectives in Vertebrate Science* 3, 55-69.
- Malvin, G.M. and Heisler, N. (1988). Blood perfusion patterns in the salamander *Ambystoma tigrinum*, before, during, and after metamorphosis. *Journal of Experimental Biology* 137, 53-74.
- Randall, D.J. (1970). Gas exchange in fish. In: Fish physiology Vol. IV, 253-292. Hoar, W.S. and Randall, D.J. (Eds.). London: Academic Press.
- Rose, F.L., Armentrout, D. and Roper, P. (1971). Physiological responses of paedogenic *Ambystoma tigrinum* to acute anoxia. *Herpetologica* 27, 101-107.
- Totland, G.K. (1984). Capillary distribution in the lateral muscle of Axolotl (*Ambystoma mexicanum* Shaw). Acta Zoologica (Stockholm) 65, 221-225.
- Ultsch, G.R. (1973). A theoretical experimental investigation of the relationships between metabolic rate, body-size and oxygen-exchange capacity. *Respiration Physiology* 18, 143-160.
- Ultsch, G.R. (1974). Gas exchange and metabolism in the Sirenidae (Amphibia: Caudata)-1. Oxygen consumption of submerged sirenids as a function of body size and respiratory surface area. *Comparative Biochemistry* and *Physiology* **47A**, 485-498.
- Ultsch, G.R. (1976). Ecophysiological studies of some metabolic and respiratory adaptations of sirenid salamanders. In: *Respiration of amphibious vertebrates*, 287-312. Hughes, G.M. (Ed). London: Academic Press.
- West, N.H. and Burggren, W.H. (1982). Gill and lung ventilatory responses to steady-state aquatic hypoxia and hyperoxia in the Bullfrog tadpole. *Respiration Physiology* 47, 165-176.
- Whitford, W.G. and Sherman, R.G. (1968). Aerial and aquatic respiration in axolotl and transformed *Ambystoma tigrinum*. *Herpetologica* 24, 233-237.
- Wisniewski, P.J., Paull, L.M., Merry, D.G. and Slater, F.M. (1980). Studies on the breeding migration and intermigratory movements of the Common Toad (Bufo hufo) using panjet dye-marking techniques. British Journal of Herpetology 6, 71-74.

INSEMINATION AND EGG LAYING DYNAMICS IN THE SMOOTH NEWT, TRITURUS VULGARIS, IN THE LABORATORY.

ANNA PECIO

Jagiellonian University, Department of Comparative Anatomy, Karasia 6, 30-060 Krakow, Poland,

(Accepted 17.5.90)

ABSTRACT

Female smooth newts begin egg deposition in spring after hibernation even if they have not been inseminated. All these eggs are unfertilized. This indicates that the sperm from the previous years are either not retained in the seminal receptacles or are incapable of fertilization. A large proportion of females do not lose receptivity after the first insemination and may collect several spermatophores during the egg-deposition period. There seems to be a positive correlation between the number of eggs deposited and the number of spermatophores transferred.

INTRODUCTION

In the genus Triturus, as in most other urodele amphibians, eggs are fertilized in the female's body the sperm being released from a specialized recess of her cloaca, the spermatheca. At the start of the breeding season a female newt is inseminated by a spermatophore, deposited by the male on the bottom of the water body, having been transferred to her cloaca. This happens during an elaborate and relatively long courtship. My own observations (Pecio, unpublished) indicate that the number of sperm in one spermatophore of the smooth newt, Triturus vulgaris, is so high that one insemination should provide the female with enough sperm to fertilize all the eggs laid in one season. One spermatophore on average contains several hundred thousand sperms, as I have found by counting the concentration of sperm isolated from single spermatophores.

One might expect then that a female should lose her receptivity after the first insemination in a season, since engaging in further courtship may be costly in terms of time and energy invested, and result in a higher risk of predation. Indeed, Verrell (1984) reported that *T. vulgaris* females lose receptivity after insemination.

The genetic analysis of the progeny collected from individual females of *T. alpestris* and *T. montandoni* has shown, however, that the progeny of a large proportion of females is sired by more than one male (Rafiński, 1981: and unpublished). This means that either a female is inseminated by sperm from several males during one breeding season or that sperm capable of fertilization survive in the female's spermatheca for more than one breeding season. Retention of sperm capable of fertilization for more than one breeding was described for *Salamandra salamandra* (Joly, 1966).

Here I consider two questions: (1) Does the female newt use sperm from the previous season for fertilization? (2) What is the influence of the number of inseminations on the receptivity of the female and the number of eggs she lays?

MATERIALS AND METHODS

On 5 April 1986, 37 female smooth newts were collected in the garden of the Cracow Institute of

Zoology on their way to the pond. Each female was placed in a 1.5 l glass bowl with 0.5 l water and fragments of aquatic plants (*Elodea sp., Fontinalis sp.*) for deposition of eggs. The animals were fed with tubifex and plankton three times a week.

Two or three days before each experiment, courting males were netted from the pond adjacent to the area where the females had been collected. The males were placed in an aquarium isolated from the females, with plants, and fed with tubifex.

Observations were carried out every 2-3 days between 08.00hr and 14.00hr. Each female was placed with a male in an aquarium ($80 \times 30 \times 15$ cm) with a sand substrate. If no sexual activity was noted after 15 minutes the observation was terminated. Negative or positive response of females to males and the number of spermatophores transferred were noted. After each encounter with a male the female was returned to her glass bowl.

Every second day all the eggs laid were collected, and the number of fertilized and unfertilized eggs from each female recorded using a microscope.

The experiment was finished on 3 June, when all the females had stopped laying eggs and the males no longer exhibited breeding activity. All females were anaesthetised with MS 222, fixed in formalin, for further analysis of the spermatozoa content of the seminal receptacle.

RESULTS

Of the 37 females used in the experiment, 12 produced eggs before they were inseminated. All eggs produced by these females were not fertilized. Only one noninseminated female had not been courted by a male. All other non-inseminated females exhibited a positive or negative response to a male, or both sexes did not show any reciprocal interest. The number of eggs laid by noninseminated females varied between 2 and 35 (Table 1). Four of those females picked up spermatophores later, at the end of the experiment.

No. of spermatophores	e	No. of ggs lai	f d	% of unfertilized No eggs fem		
	max.	min.	mean			
0	35	2	12.7	100	8	
1	180	14	53.2	31.8	8	
>1	216	47	136.7	11.9	6	

TABLE 1. Number of eggs laid in relation to the number of spermatophores transferred.

No.	No. of spermatophores/no. of males									
	1/1	2/1	3/1	>2/>2	Total					
No. of females inseminated	10	1	1	6	18					
% of females inseminated	55.5	5.5	5.5	33	100					
No. of sperm. picked up	10	2	3	17	32					

TABLE 2. Number of females inseminated with one or several spermatophores from one or more males.

During 144 (35 successful) encounters observed, 18 females (48.6%) became inseminated with at least one spermatophore (Table 2). During the first encounter 12 females transferred one spermatophore to their cloaca, one transferred two spermatophores, and another female three. Some inseminated females remained receptive during further exposure to courting males some time after the first insemination: four females transferred spermatophores from two different males (in 6, 14, 19 and 26 days after the initial insemination), and two females transferred spermatophores from three different males (2 + 11 and 17 + 8 days after initial)insemination, Table 2). One of the females inseminated three times exhibited a further positive response to male courtship within 16 days after the third insemination, which induced spermatophore deposition but not transfer to the cloaca.



Fig. 1. Relationship between spermatophore transfer (day 0) and the start of egg laying (days 3-14). Each square represents one female. Open squares are females which initially laid unfertilized eggs.

The first eggs (6, 18, 24 in a clutch) laid by three of the inseminated females were not fertilized. Without transfer of additional spermatophores those females later laid only fertilized eggs. The remaining females produced fertilized eggs three days after initial insemination at the earliest, most of them started egg deposition between days 4 and 6 (Fig. 1). Females inseminated with one spermatophore laid on average 52.2 eggs per season, the number of eggs ranging from 14 to 180. On average females inseminated more than once laid 136.7 eggs (Table 1).

There was no significant difference (Mann-Whitney U-test) between numbers of eggs laid by females which had collected one spermatophore and numbers of eggs laid by females which had collected several spermatophores. The data indicates, however, that there might be a causative relationship between the number of eggs deposited and the number of spermatophores collected by a female. Small sample size might be the cause of the non-significant result of the statistical test.

Five to thirty-nine days after insemination females started to produce non-fertilized eggs, the total number of eggs laid per female diminished during the course of the experiment, with an increase in the number of nonfertilized eggs. After subsequent insemination these females increased egg production: most of these eggs were fertilized.

DISCUSSION

All females that were not inseminated failed to produce fertilized eggs. This suggests that the spermatozoa are not stored from season to season in female *T. vulgaris. Salamandra salamandra* eggs can be fertilized by spermatozoa which have survived for two years in the spermatheca (Baylis, 1939; Joly, 1966). In *T. vulgaris* even if the spermatozoa survived in the spermatheca, they apparently are not capable of fertilization or they undergo resorption after the end of the breeding season.

My data indicate that the beginning of egg-laying in a season does not depend on insemination since both the inseminated and non-inseminated females laid eggs. The process may depend on external factors, e.g. temperature, and/or the physiological state of the female. Bell (1977) showed that egg deposition is determined by the length of time spent by the female in water: a female has to spend about 40 days in water before she begins laying eggs. My data indicates, however, that this is not true in the population I studied. In my experiment, 12 females began laying eggs 10 days after being placed in bowls of water; one female inseminated a day after being placed in water laid seven eggs three days after picking up the first spermatophore. Verrell and Halliday (1985) also found that females may oviposit about 10 days after entry into water. The discrepancy between Bell's (1977) and my data and observations by Verrell and Halliday (1985) might be explained by the local differences in trophic conditions or either environmental factors.

The beginning of egg deposition after initial insemination confirms earlier data of Verrell (1984), who observed egg deposition between two and six days after initial insemination. In *T. alpestris* spermatozoa travel for at least 24 hours through the main duct of the spermatheca and during this time the spermatozoa must free themselves from the mucopolysaccharide substrate, subsequently travelling towards dorsal tubules in the seminal receptacle (Pecio, unpublished observation).

Smith (1954) suggested that "a female once inseminated will not take up any more spermatophores until those eggs ready to be fertilized have been laid", and states that a second mating would seem unnecessary since "the number of spermatozoa contained in the spermatophore is far in excess of the number produced by the female". Verrell (1984) also noted that the majority of females remain unresponsive to male courtship for 20 days after the first insemination. This study shows that 44% of noninseminated females eagerly followed a courting male until the end of sexual sequences.

The prolonged receptiveness of females and the successive taking up of spermatophores might have both proximate, physiological and ultimate, adaptive reasons. When the tubules are tilled with a great number of spermatozoa from several spermatophores (or from one large spermatophore) the functioning of the sperm distribution might be more efficient.

It seems that the amount of sperm reduces quickly in females which lay eggs each day, as some time after insemination the number of unfertilized eggs increases. This might explain the phenomenon, that the multiple inseminated females produce on average 11.9% unfertilized eggs, while once inseminated as much as 31.8%.

In this experiment some of the females after insemination with one spermatophore only produced a large number of eggs which is close to a measured value of the complete clutch size for a season (Hagström, 1980). A similar phenomenon was observed in *Ambystoma tigrinum* and *Desmognathus orhrophaeus* (Halliday and Verrell, 1984). The size of a spermatophore is highly variable and most probably depends on the level of male sexual activity. It might be that a large spermatophore may contain as may sperms as several smaller ones.

Multiple insemination can significantly increase genetic variation of the progeny produced by a female. This might be especially significant for a species which colonises transient environments and experiences sharp reductions of a population size as is the case of many *Triturus* species.

ACKNOWLEDGEMENTS

I would like to thank Dr Jan Rafiński for his guidance throughout this study and helpful comments in the preparation of this manuscript. I would also like to thank Dr Neil Sanderson for his helpful comments on the manuscript.

REFERENCES

- Baylis, H.A. (1939). Delayed reproduction in the spotted salamander. *Proc. Zool. Soc. Lond.* 109A, 243-246.
- Bell, G. (1977). The life of the smooth newt (*Triturus vulgaris*) after metamorphosis. *Ecol. Monogr.* **47**, 279-299.
- Hagström, T. (1980). Egg production of newt (*Triturus vulgaris* and *T. cristatus*) in southwestern Sweden. ASRA Journal 1, 1-8.
- Halliday, T.R. and Verrell, P.A. (1984). Sperm competition in amphibians. In: Smith, R.L. (ed.). Sperm competition and the Evolution of Animal Mating Systems, Academic Press, New York, pp. 487-508.
- Joly, J. (1966). Ecologie et cycle sexuelle de Salamandra salamandra (L). These, CNRS.
- Rafiński, J.N. (1981). Multiple paternity in a natural population of the alpine newt. *Triturus alpestris* (Laur.). *Amphibia-Reptilia* 2, 282.
- Smith, M. (1954). The British Amphibian and Reptiles. Collins. London.
- Verrell, P.A. (1984). The responses of inseminated female smooth newts, *Triturus vulgaris*, to further exposure to males. *British Journal of Herpetology* 6, 414-415.
- Verrell, P.A. and Halliday, T. (1985). Reproductive dynamics of a population of smooth newts, *Triturus vulgaris*. in southern England. *Herpetologica* 41, 386-395.

HERPETOLOGICAL JOURNAL, Vol. 2, pp. 7-18 (1992)

EGG, CLUTCH AND MATERNAL SIZES IN LIZARDS: INTRA- AND INTERSPECIFIC RELATIONS IN NEAR-EASTERN AGAMIDAE AND LACERTIDAE

ELIEZER FRANKENBERG^{1 2} AND YEHUDAH L. WERNER² *

¹Nature Reserves Authority, 78 Yirmeyahu St., 94467 Jerusalem, Israel

²Department of Evolution. Systematics and Ecology, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel *Author for correspondence

(Accepted 18.5.90)

ABSTRACT

We provide data on the fecundity of locally common Israeli reptiles, and use these data to examine current ideas on the reproductive ecology of lizards. Our methodology was selected in consideration of the acute problems of nature conservation in Israel. In the museum collections of the Hebrew University of Jerusalem and Tel Aviv University we used radiography to locate the shelled oviductal eggs of 164 female lizards, belonging to eleven species (Agamidae and Lacertidae). Each sample sums the species' variation over its range and over different years. Female body size, egg number and egg volume were determined. Specific clutch volumes, relative to maternal body lengths, resembled those reported in iguanid lizards from tropical America. Clutch size varied intraspecifically and, in most species, correlated to maternal size. In others, egg size was more influenced by maternal size. We argue that the latter species oviposit in more stable environments than do the majority.

INTRODUCTION

The amount of energy available to an organism at any given time is finite; the amount expended may be partitioned into maintenance, growth and reproduction. In lizards the allocation of energy to reproduction has been reviewed by several investigators. The main areas of concern have been, first, whether and what differences in parental investment exist between species differing in body size, body shape, environment or behaviour, and how such differences may be explained.



Fig. 1. A typical small desert lacertid lizard, *Mesalina guttulata*, and her freshly laid (modal) clutch of four eggs (female collected in the Judean Desert on 5.IV.1981, oviposited and photographed on 12.IV.81; from Kodachrome diapositive).

For example, species of lacertid lizards (Fig. 1) have been characterized by clutches which range from 14 to 40% of the pregnant female's weight. And second, how a female with limited energy available for reproduction reaches a compromise between emphasizing either egg number or energy content per egg. The latter value is roughly, though not precisely, paralleled by egg mass and size (Tinkle, 1969; Ballinger and Clark, 1973; Emlen, 1973; Ballinger, 1978; Vitt, 1978; Huey and Pianka, 1981; Vitt and Price, 1982; Fitch, 1985; Pianka, 1986).

Several factors affect the apportionment of energy in egg production, since natural selection should result in an energetic compromise that maximizes the parent's total (long-range) contribution to future generations. Larger eggs may be more resistant (especially to drought) during incubation (Ackerman, Seagrave, Dmi'el and Ar, 1985). They give rise to larger young (Ferguson, Brown and DeMarco, 1982; Werner, 1986a, 1988), which are able to utilize a broader range of food items, more able to withstand a shortage of food, and compete better in social encounters (Fitch, 1970; Ferguson et al., 1982; Rand, 1982). On the other hand, a low level of competition, or a high level of predation of a type not affected by juvenile size, may press for a strategy of producing many small eggs. The production of large numbers of small eggs may be adaptive also in coarse-grained patchy environments (Emlen, 1973) or in those changing in time. As the environment becomes less stable, selection favours greater fecundity rather than survivorship (Cody, 1966).

The aspect of reproductive ecology most commonly studied in reptiles is the number of eggs in a clutch (Turner. 1977; Fitch. 1985). From studies elsewhere this number is known to vary intraspecifically between populations (Kramer, 1946; Fitch, 1985). In some species, clutches are larger in warmer parts of the specific range; this results in part from the variation in female size. larger females producing larger clutches (Oliver, 1955; Fitch, 1970). But in a majority of wide-ranging new-world species clutches are larger in cold latitudes (and high altitudes), allegedly in compensation for the smaller number of clutches in the shorter season (Turner, 1977).

Nevertheless Vitt and Price (1982) listed several reports in which also the size of the eggs had been considered. These authors calculated the relation of clutch mass to parent mass, and concluded that this relation differs between ecological types and between families. The accuracy of some of the data and hence the validity of some of the conclusions have since been questioned by Werner (1988). Dunham, Miles and Reznick (1988) analyzed the relations among clutch size, relative clutch mass and foraging mode in reptiles and found that active foragers produce significantly smaller clutches than sit and wait foragers, with significantly smaller relative clutch mass. They also found a relation between clutch size and habitat: fossorial (burrowing) lizards produced the smallest clutches, arboreal and arenicolous (sand-dwelling) lizards produced larger clutches and terrestrial and saxicolous (rock-dwelling) lizards produced the largest clutches.

Little is known of the reproductive ecology of Israeli reptiles, as, with the exception of a few species, mostly venomous snakes and gekkonid lizards (Mendelssohn, 1963, 1965; Werner, 1965, 1966a, 1986a, 1988; Dmi'el, 1967; Orr, Shachak and Steinberger, 1979), knowledge is limited to the scanty information in local general texts (Margolin, 1959; Werner, 1966b, 1973; Arbel, 1984; Dor, 1987).

In this paper we examine the relationships among clutch size, egg volume, clutch volume and maternal size, in eleven common oviparous Israeli lizards in which clutch size is variable. Our aims are, first, to provide basic data about the fecundity of Israeli reptiles. Such information is necessary (though insufficient) for the planning of nature conservation. Second, to examine some of the currently prevailing generalizations (quoted above) concerning the reproductive ecology of lizards.

MATERIALS AND METHODS

SPECIES

Gravid females of eleven lizard species (agamids and lacertids) were examined. These are listed in Table 1, which details their full specific, and, where applicable, subspecific, names. Most of these species have been beautifully described and depicted in Anderson (1898). For the others (and some of the same) the reader is referred to Barash and Hoofien (1956), Başoğlu and Baran (1977), Arnold, Burton and Ovendon (1978) and Arbel (1984). The taxonomy of the Agamidae has been reviewed by Wermuth (1967) and that of the genus *Acanthodactylus* by Salvador (1982) and Arnold (1983).

EGG RELATIONSHIPS OF LIZARDS

Species and subspecies sampled	World Distribution of the species	Distribution in Israel	Annual rainfall (mm) (Isohyet ranges of drought year/wet year)	Average monthly August temperature (°C) (isotherm range)	Simplified summary of habitat
Agamidae					
Agama pallida pallida Reuss, 1833	E Egypt & SW Asia	Whole Negev	0-150/0-400	24-32	Desert and Steppe: except sand dunes
Agama savignii Duméril & Bibron, 1837	E Egypt, N Sinai & S Israel	N Negev	0-100/100-250	26-28	Semi-desert; sands
<i>Agama sinaita</i> Heyden, 1827	NE Africa & SW Asia	S Negev	0-100/0-250	28-34	Extreme desert; rocks
Agama stellio (Linnaeus, 1758) sspp.	SE Europe, N Egypt & SW Asia	Countrywide except most of S Negev	50-900/200-1400	20-28 (Isr)	Mediterranean and semi-deserts: rocks, tree trunks and buildings
Lacertidae					
Acanthodactylus boskianus asper (Audouin, 1829)	N Africa & SW Asia	Whole Negev	0-100/0-300	26-34	Desert and Steppe; sands, shingle etc
Acanthodactylus pardalis (Lichtenstein, 1823)	N Africa & S Israel	N Negev	0-150/150-400	26-28	Steppe; loess soils
Acanthodactylus schreiberi syriacus Boettger, 1879	S Turkey to N Israel	Mediterranean coastal plain	100-600/500-1000	24-28	Meditteranean; sands and light soils
Acanthodactylus scutellatus scutellatus (Audouin, 1829)	N Africa & SW Asia	N Negev & S Mediterranean coastal plain	0-300/250-800	26/28	Semi-desert and Mediterranean; sand dunes
Lacerta laevis Gray 1838	Turkey to N Israel	Countrywide N of Hebron	300-900/700-1400	20-28	Mediterranean; scrub, gardens etc
Mesalina guttulata guttulata (Lichtenstein, 1823)	N Africa & SW Asia	Whole Negev & S Jordan Valley	0-100/0-400	26-34	Desert and Steppe; except sand dunes
Ophisops elegans (Ménétriés, 1832) sspp.	SE Europe & W Asia	N Negev & northwards	50-900/200-1400	20-30	Mediterranean and Steppe; open habitats except sand dunes & sheer rocks

TABLE 1. Habitats of species studied (sources explained in the text).

We emphasize that our material of *A. boskianus* excludes the sibling species *A. opheodurus* Arnold, 1980 (Werner, 1986b).

Specific distributional and environmental data are condensed in Table 1 from the following sources: World distribution - Flower (1933) and Werner (1966b), and, for Agama pallida, Haas and Werner (1969); Distribution in Israel - Werner (1966b) and, especially, Wahrman (1970) who presents approximate distribution maps for all the species except Lacerta laevis; Annual rainfall relevant isohyet ranges between rainfall maps (Atlas of Israel 1970) for the drought year 1946/47 and the wet year 1944/45 (the potentially rainy season extends from September through May); and average monthly August temperatures - relevant isotherm ranges in an Atlas of Israel (1970) map averaging the ten years 1940-1949. Finally, the simplified summary of the habitats is based on Wahrman's (1970) classification of types of animal distribution in Israel and data in Werner (1966b) augmented by our personal knowledge.

SAMPLES

Lizard eggs are not truely cleidoic (closed to water flux). Depending on the conditions they may decrease or increase in mass and dimensions fairly rapidly after oviposition (Fitch and Fitch, 1967; Packard, Tracy and Roth, 1977). Therefore it is difficult to base a comparative study of egg size on laid eggs (although dry weights and caloric values could be substituted). We preferred to use eggs inside oviducts of females already preserved in collections. a policy which also appeared particularly desirable from the point of view of nature conservation, an acute problem in the small natural areas of Israel (Ashkenazi, 1987; Frankenberg, 1989).

		га	N	v	
Species	n	x CV	x CV	x CV	RP
Agamidae					
A. pallida	17	78.8 8 67.5-85	9.4 34 1-12	0.63 30 0.36-1.06	II-VIII
A. savignii	2	70.5 15 63-78	4.0 35 3-5	0.86 5 0.89-1.01	?
A. sinaita	4	76.4 3 73-79	5.3 9 5-6	0.86 51 0.42-1.46	V-VIII
A. stellio	18	111.1 11 92-130	7.7 23 5-12	1.29 37 0.80-2.68	I-VII
Lacertidae					
A. boskianus	. 15	60.1 7 54.5-67	4.1 27 2-7	0.32 31 0.18-0.49	Ιν-νι
A. pardalis	23	66.0 7 59-74	4.8 25 3-7	0.36 25 0.20-0.53	III-VII
4. schreiberi	10	69.0 7 58-75	2.6 31 1-4	0.53 21 0.32-0.69	IV-IX
A. scutellatus	13	52.2 9 40-58	2.7 30 2-4	0.32 19 0.23-0.43	111-X
L laevis	16	61.9 8 55.5-71	4.5 33 2-7	0.28 29 0.16-0.41	II-VII
M. guttulata	38	46.8 7 40-54.5	4.4 27 2-7	0.14 21 0.09-0.20	II-IV
O. elegans	8	48.1 6 45-52	4.0 53 2-8	0.09 33 0.05-0.11	V-VIII

TABLE 2. Basic reproductive data for eleven lizard species. Averages (\bar{x}), coefficients of variation (CV) and (underneath) ranges, of body length (ra), number of oviductal shelled eggs (N) and egg volume in cm³ (V), for each of the species. RP, reproductive (oviposition) period of the species in months; and n is the number of females yielding data.

All the preserved lizards of relevant species in the National Collections (Hebrew University of Jerusalem and Tel-Aviv University) were inspected. These had been collected during many years and over all seasons. Females apparently gravid were radiographed for verification as explained below. The ensuing sample sizes (numbers of females yielding data) varied from 2 to 32 per species (total 164) and are given in Table 2. These individuals originated, in principle, from throughout the specific ranges in Israel and Sinai, with emphasis on the former. However, of three species material from neighbouring countries was available and included: *Agama pallida*, Syria; *L. laevis*, Lebanon (Bayrut only); *O. elegans*, Cyprus and Turkey.

DATA COLLECTION AND ANALYSIS

Our technique involved the calculation of egg volumes from linear measurements of preserved shelled eggs.

Various distorting effects of fixation and storage in formalin and alcohol on the relative weight of lizard eggs have been described (Martin, 1978; Vitt, Howland and Durham, 1985). Guillette, Rand, DeMarco and Etheridge (1988) even found that the weight of eggs preserved after removal from their mother by dissection, is reduced during fixation by a third as a stable factor. Our material had been preserved fairly uniformly for all species (mothers fixed in 10% formalin, stored in 70% ethanol for periods usually >1 yr) and our study involved mainly the interspecific comparison of intraspecific traits; hence we deem the technique adequate. We observed no obvious collapse of the eggshells as noted by Rand (1982).

Each suspected gravid female was radiographed (Softex type E X-ray machine and Ilford Industrial G film) to find out whether it contained shelled (or other oviductal) eggs. We initially tested whether measurements of eggs on the X-ray plate were reliable, by comparing a sample to measurements of the same eggs after dissection. Fig. 2 shows that in the case of radiographs, considering the egg as representing an ellipsoid, as done hereinafter with the direct measurements of the eggs, would yield a somewhat poorer approximation than regarding the egg as an irregular sphere, with a diameter averaged between length (L) and width (W), using the equation V = 4/3 π [(L+W)/4]³. Although approximations of egg sizes could be obtained radiographically in this way if dissection were precluded, we considered the inaccuracy excessive and employed only direct measurement of oviductal eggs in opened bodies.



Fig. 2. The correlation of egg volumes (cm³) based on the measurement of radiographs (R) and calculated in either of two manners, to the volumes of the same eggs derived from measurements in dissections (D). In the first regression, R₁ is calculated from the formula for an irregular sphere, R₁=4/ 3π [(L+W)/4]³=1.37D-71.4; in the second, R₂ is calculated from that of an ellipsoid, R₂=4/ 3π (LW²/8)=0.71D+12.25. D is based on the latter for both regressions.

For each lizard the following data were noted: body length (ra = rostrum-anus = snout-vent, following Werner, 1971); number of eggs in each oviduct; whether these were shelled; length and width of all eggs in lizards carrying up to four, and of four eggs in lizards carrying a greater number; and the date and location of capture.

The volume of each egg was calculated by using the equation $V = 4/3 \pi$ (LW²/8), where V is egg volume in mm³, L is egg length in mm, and W is egg width in mm. The average volume of all eggs measured of one female gave the characteristic egg volume for that particular specimen. These individual averages were then averaged for each species to yield the specific egg volume.

Linear and partial correlations and linear and multiple regressions (Steel and Torrie, 1960) between the three variables, body size (ra) in mm (Rand, 1982), egg number (N) and egg volume (V) (average per female) were calculated to test the relationships between them. In all these calculations only mature, shelled, eggs were used. The length of the reproductive period and the number of clutches were roughly estimated for each species by relating egg volumes to the dates of collection. For this purpose, immature oviductal eggs were measured as well.

RESULTS

The data obtained from the eleven species are summarized in Table 2. It will be remembered that in our samples, we pooled for each species, quite randomly, females collected from different areas of the range of distribution, in different years (including rainy ones and drought ones) and at different times during the year. Hence, for the time being, we ignored any geographical and temporal effects (Van Devender, 1982; Ballinger, 1983: Fitch. 1985), including those of enriched food supply through above-average rainfall in xeric habitats (Turner, Lannom, Medica and Hoddenbach, 1969). Rather, by combining all data, we derived the average characterizations of the specific clutch parameters, and approximate limits of their extreme variation in the area concerned (constrained, as ranges are, by sample size). Geographical variation remains to be treated.

The body space a lizard can devote to carrying eggs is limited. Species-specific clutch volume (species-specific V x N from Table 2) was significantly correlated (interspecifically) to body size (Fig. 3). Viewing clutch volume as if it were packed in a sphere, its diameter maintained a quite constant proportion to specific body length: 23.5 ± 3.2 percra (percents of ra — Werner, 1971) with no significant deviations from the mean (Chi square test) (Fig. 4). Therefore, on spatial grounds, any attempt by one of these species to increase egg volume would have to be compensated by a decrease in egg number (per clutch), and vice versa.



Fig. 3A. Mean clutch volume (cm³) as a function of mean body size (mm,ra) among eleven lizard species.



Fig. 3B. Mean clutch diameter derived from a clutch volume assumed spherical, as a function of mean body size (mm, ra), among eleven lizard species studied herein (solid symbols) and among thirteen iguanid species studied by Rand (1982) (hollow symbols).

It is apparent from Table 2 that the species could be classified into two categories regarding egg number and egg size. Lizards with relatively large but few eggs were *Agama savignii*, *A. sinaita, Acanthodactylus schreiberi* and *A. scutellatus*; whereas all the others had relatively small and numerous eggs (Table 2).

The relation between the three variables, maternal body size, egg volume and the number of eggs, is presented three-dimensionally for each species in Fig. 4. (Excluded is Agama savignii of which the sample size was too small.) From these figures and from Table 3 it is apparent that in the terms of this relation each lizard belonged to one of four categories: (1) Lizards which, as stated above, basically have relatively numerous small eggs, and which in response to increased body size strictly increase the number of eggs; Agama pallida, A. stellio, Acanthodactylus pardalis, L. laevis, M. guttulata, and O. elegans. (2) A lizard which also basically has numerous eggs but which with increased maternal size increases egg size rather than egg number is Acanthodactylus boskianus. (3) A. schreiberi, which basically has few large eggs, further increases egg size with increasing maternal size. (4) Three other lizards which basically have large and few eggs are Agama sinaita which with increasing maternal size increases both egg number and egg size (as far as known), Acanthodactylus scutellatus which revealed no significant response to increasing maternal size, and Agama savignii where sample size enabled no conclusions.













H Lacerta laevis



Fig. 4A-J. Number of eggs (N) as a multiple function of maternal body size (ra) in mm and mean egg volume (V) in cm³, for each of ten species. Each vertical bar represents the clutch of one female. The top of each bar represents the multiple function: the base of each bar, the relation between egg volume and maternal body length. The multiple regression function for these graphs. expressing the number of eggs in a clutch (N) as a function of maternal body size (ra) and egg volume (V), is respectively: A. N=0.32ra-4.65V-12.6; B, N=0.22ra-0.92V-10.38; C, N=0.09ra-0.83V-1.34; D. N=0.52ra-31.75V-17.27; E, N=0.19ra-1.31V-7.03; F, N=0.05ra-0.87-0.39; G, N=4.92ra-6.76V-0.002; H, N=0.26ra-2.72V-10.84; I, N=0.18ra-10.13V-2.67; J, N=0.4ra-16.3V-15.2.

		r			R	Т. Т
Species	ra-N	ra-V	N-V	ra-N(V)	ra-V(N)	N-V(ra)
Agamidae						
A. pallida	0.60**	0.17	-0.16	0.65*	0.34	-0.95
A. sinaita	0.68	0.52	-0.24	0.97	0.96	-0.31
A. stellio	0.62**	0.09	-0.19	0.65*	0.27	-0.31
Lacertidae						
A. boskianus	0.42	0.52*	0.20	0.37	0.50	-0.03
A. pardalis	0.71***	0.19	0.04	0.72**	0.23	-0.14
A. schreiberi	0.18	0.76**	0.09	0.18	0.76**	-0.07
A. scutellatus	0.26	-0.44	-0.61*	-0.01	-0.37	-0.57
L. laevis	0.76***	0.40	0.19	0.76**	0.39	-0.19
M. guttulata	0.46**	0.23	-0.12	0.51**	0.32	-0.26
O. elegans	0.52	0.26	-0.06	0.55	0.34	-0.23

TABLE 3. Correlations between reproductive data in each of ten lizard species. Coefficients of correlation, r; and of partial correlations, R; between the three variables N, ra and V, explained and reduced in Table 2. In the partial correlations the variable in parantheses is the one held constant. Significance levels indicated thus: *, P < 0.05; **, P < 0.01; ***, P < 0.001; otherwise, the correlation is not significant.

The averages of all the species are plotted together along the three variables (ra, N and V) in Fig. 5, which demonstrates that their mathematical interspecific relation in Israeli agamid and lacertid lizards, as a group, is as follows. Any additional reproductive effort enabled through an (interspecific) increase in body size, is directed so that it is invested in enlarging the egg rather than increasing the number of eggs per clutch (ra-V(N) = 0.92). This accords with the widespread observation, that larger species produce larger offspring.

Table 2 also indicates the reproductive months (when females contained shelled oviductal eggs) per species.



Fig. 5. Mean number of eggs in each species, as a multiple function of specific (mean) body length and of specific (mean) egg volume (as in Fig. 4). The circles represent for each species an average SD, calculated from the mean CV values of the three variables.

DISCUSSION

In order to reproduce, each species of lizard has evolved so as to devote a certain proportion of the female mass (or energy) to produce eggs, and so as to divide this biomass into either many or few eggs (Smith and Fretwell, 1974). These two aspects represent different selection pressures, the first primarily affecting the survival of the mother and by this, of the eggs she carries; the second, only the survival of her offspring, since there is no parental care (Pianka, 1986). Both contribute to increasing the fitness (long-term reproduction success) of each individual in the species.

PARENTAL INVESTMENT

The clutch mass of an individual female represents a compromise between several selection forces. Increased reproductive effort by investing in, and carrying, a larger clutch mass may lead to increased probability of the mother falling victim to predation (Shine, 1980). Vitt and Congdon (1978) demonstrated that in lizards the specific clutch mass is related to body shape and habitual mode of escape from predators. Nevertheless, it seems that lizards have attained optimization regarding the proportion of clutch mass to mother mass, values which we represent, respectively, by their correlates, clutch volume and body length. Rand (1982) furnished comparable data for 13 species of iguanid lizards in tropical America. We calculate from his data a strong interspecific correlation of clutch volume to body length $(r = 0.94, P \le 0.001)$, with a regression slope of 0.22, not significantly different from ours (Fig. 3). Calculation of the diameters of the average specific clutches found by Rand (from total clutch masses conceived of as sphereshaped) yields a mean diameter of 25.0 \pm 1.7 percra, again not significantly different from our result of 23.5 + 3.2 percra (t-test) (Fig. 4). Thus, clutch diameter observes a fairly constant proportion of body length in these lizard groups. Interestingly, Vitt and Price (1982) too have found that the mean relative clutch mass of lizards of the families Agamidae, Iguanidae, and Lacertidae (also Scincidae) is of a uniform order of magnitude, about 25%. Lizard species apparently invest a certain amount in each clutch, regardless of how many clutches are produced during the year. This conclusion accords with those of Vitt and Congdon (1978) and Rand (1982). On the other hand, Barbault (1975) suggested that lizards in a fairly stable but predationheavy environment are selected to increase fecundity, with an increased clutch volume, even at the expense of parental survivorship.

The reproductive investment per clutch seems to be uniform (by one yardstick) for Mediterranean, desert, subtropical and tropical lizards. A clutch diameter of about 25 percra represents a widespread value for Agamidae, Iguanidae and Lacertidae, presumably as an optimal compromise between retaining maternal survival and increasing reproductive success. In view of the near-uniform relative clutch volume, the number of clutches during a year seems to depend on the length of the available reproductive season rather than on the investment already made by the lizard at a given time. The longer the reproductive season, the more clutches are produced. In tropical zones with faint seasonality, lizards produce numerous clutches during the year (Barbault, 1975).

The reproductive period of the Israeli lizards considered here is restricted to spring and early summer, due to the pronounced climatic difference between winter and summer. It has often been suggested, that starting the breeding season relatively early in the year enables lizards to produce an additional clutch (thus Goldberg, 1975, 1977). But in our observations lizard species which are geographically (and climatically) sympatric nevertheless begin their breeding at different specific times in the season. Moreover, the number of reproductive months (when oviducts contain shelled eggs) is not affected by the seasonal timing of the onset of reproduction in itself (Table 2): M. guttulata which begins to oviposit early in the year had only 4 reproductive months, the same as *O. elegans* and *Agama* sinaita which begin to reproduce later in spring. Agama stellio, Agama pallida and L. laevis, which are earlyseason reproducers had 7, 6 and 6 months, respectively, whereas the late-season reproducers Acanthodactylus schreiberi and Acanthodactylus scutellatus, have an even longer reproductive season of 8 months each. Despite its long reproductive season, Lehman (1980) found in the latter only 1-4 clutches per year, with 2-6 eggs per clutch. No sacrifice at the expense of the first clutch occurs in favour of increasing the chance of reproducing a later perhaps Constraints and clutch. uncertainties concerning the length of the reproductive period lead to a maximization of each clutch; a smaller clutch mass (volume) seems not to be compensated for by the production of more clutches. By the same token, in a lizard population reproducing biennially (e.g., Anguis fragilis - Patterson, 1983), a female would not be expected to carry a clutch mass double that which accords with her survival. Thus when we deal with the relationships between the number of eggs in a clutch and the volume of each egg, each clutch may be regarded independently of others produced by the lizard.

NUMBER VERSUS SIZE OF EGGS

Since parental investment (per clutch) in all lizards considered here is similar (proportional to maternal body size), selective forces on reproductive success operate along one dimension — increasing either egg volume or egg number, each at the expense of the other. (This phenomenon is significant in our sample of *Acanthodactylus scutellatus*, as shown in Table 3.) The relative importance of each trend for offspring survival determines the relationship between egg volume and number within a clutch. Optimization is reached by a combination of maximizing both the number of eggs and the probability of each offspring to survive to maturity, which is believed to significantly depend on egg size (Ferguson et al., 1982; Vitt and Price, 1982).

Dunham and Miles (1985) reported a significant interspecific correlation of clutch size to maternal body length but did not consider egg size or clutch volume.

In tropical forests (and on islands — Fitch, 1985) larger young are selected for. In other environments a relatively greater nonselective mortality presses more strongly for large clutches (Tinkle, Wilbur and Tilley, 1970; Barbault, 1975; Rand, 1982). It is generally contended, though not proven, that tropical forests are stable over time, and that this characteristic accounts for the particular apportionment of clutch mass in this environment.

We may summarize the ecological and reproductive adaptations in the lizard species studied here. Whereas both Mediterranean and desert species mostly have relatively numerous eggs, most species which deviate and have large eggs live on sand. The only exception is Agama sinaita which has few, large eggs and lives on rocks in extreme desert. Initially this seems hard to reconcile with the prevalent theory: Both the Mediterranean and desert habitats in Israel are unstable and unpredictable, as may be seen in Table 1, and as indicated by the extreme fluctuations in rainfall and in the average temperatures of the warmest month between drought and wet years (Atlas of Israel, 1970). Such environments, as suggested by Rand (1982), impose nonselective mortality of eggs and young, with a selection pressure towards increasing the number of eggs per clutch.

Sands, however, seem to have a contrary effect on eggs. The lizards which live on sands bury their eggs in the ground. Due to the special character of the soil, arenicolous lizards are better able to dig, for oviposition, down to a level of appropriate moisture. Thus they secure for the eggs a stable environment (Ackerman, in press; Ratterman and Ackerman, 1989) conducive to high hatching success, enabling the evolution of fewer and larger eggs.

The large eggs of *Agama sinaita* may also be explicable by the predictable stability of the conditions in which they develop. Of all the species investigated here, this rupicolous desert lizard is the one whose occurrence is most strictly restricted to the extreme desert. Conceivably the correct oviposition sites may be relatively dry, or rare and hard to locate, or both, but, once located and employed, the conditions there remain stable.

EFFECT OF MATERNAL SIZE

In three Australian agamids of the genus Amphibolurus, both clutch size and the number of clutches per year increase with the age of the female (Bradshaw, 1981). Certainly, lizard species which are. selected for a relatively large number of eggs in their clutch, would be expected to further increase clutch size with increasing maternal body size; indeed so do six of the species described here. These include Agama stellio, for which the same phenomenon has been verified in a Greek population (Loumbourdis, 1987). Acanthodactylus boskianus is an exception: This species retains the character of laying relatively numerous eggs in its clutch, although it lives also on sands; perhaps because sand is not its sole habitat. But with increasing body size it tends to increase egg volume, perhaps as an adaptation to its partial occupation of sandy habitats.

Acanthodactylus scutellatus which is an extreme sanddweller, would seem to have reached optimum egg volume and clutch size, showing no increase in egg volume with the increase of body size. It is an individual forager, unlike Acanthodactylus schreiberi which is more territorial and has a rigid social structure (Avital, 1981). For this latter species, selection may be stronger for egg size, not merely to improve survival but also for attaining better social status. Young of lizards have been observed to establish social hierarchies depending on size (Fox and Rostker, 1982). Agama sinaita, which lives in extreme arid southern Israel (Hertz and Nevo, 1981) exhibits strong individual territoriality (Arbel, 1981). In such a case of extreme environmental conditions and strong intraspecific competition, selection seems to be compromising, both egg number and egg volume increasing with body size.

CONCLUSIONS

The lizards treated here, as a group, basically tend to maximize the number of eggs, presumably because nonselective or random mortality favour an increased number of eggs. On the other hand, selection may favour large eggs when the conditions for their development are stable and when a larger offspring has a better chance to survive (Congdon, Vitt and Hadley 1978; Rand, 1982). Desert and Mediterranean environments seem to be alike in being unpredictable and unstable, selecting for an increase in clutch size, whereas extreme deserts and sandy environments appear to be predictable, though harsh, habitats, selecting for large eggs.

ACKNOWLEDGEMENTS

This project was enabled by the support of The Fund for Basic Research administered by The Israel Academy of Sciences and Humanities (1980-82). We are also obliged to Prof. H. Mendelssohn and Dr. M. Goren for free use of the Tel Aviv University collection; to all persons who brought specimens to the Hebrew University and Tel Aviv University collections, and especially to the late Dr. Hermann Zinner for material collected in 1965/66 in Lebanon and Syria; to Orit Greenbaum and Merav Frid for most of the measurements and radiography; to Miriam Chertkow for graphic and A. Niv for photographic assistance; to R.A. Ackerman for an enlightening discussion; and particularly to Y. Yom-Tov and several anonymous reviewers for comments on the manuscript.

REFERENCES

- Ackerman, R.A. (in press). Physical factors affecting the water exchange of buried eggs. In: *Physical Influences on Embryonic Development in Birds and Reptiles,* Deeming, D.C. and Ferguson, M.W.J. (Eds.) Cambridge University Press.
- Ackerman, R.A., Seagrave, R.C., Dmi'el, R. and Ar, A. (1985). Water and heat exchange between parchment-shelled reptile eggs and their surroundings. *Copeia* 1985, 703-711.
- Anderson, J. (1898). Zoology of Egypt: Vol. 1, Reptilia and Batrachia. London: Quaritch.
- Arbel, A. (1981). Social organization of the common hardun. Teva Va-Aretz 23, 103-108. (In Hebrew).
- Arbel, A.(ed) (1984). Plants and Animals of the Land of Israel. Vol.
 5, Reptiles and Amphibians. Tel Aviv: Ministry of Defence and Society for the Protection of Nature in Israel.
- Arnold, E.N. (1983). Osteology, genitalia and the relationships of Acanthodactylus (Reptilia: Lacertidae). Bull. Br. Mus nat. Hist. (Zool.) 44, 291-339.
- Arnold, E.N., Burton, J.A. and Ovenden, D.W. (1978). A Field Guide to the Reptiles and Amphibians of Britain and Europe. London: Collins.
- Ashkenazi, S. (1987). The nature reserves of Israel. *Eretz Haya'el* 1(3), 44-48. (In Hebrew).

- Anonymous (1970). *Atlas of Israel.* Ministry of Labour, Jerusalem, and Elsevier, Anisterdam.
- Avital, E. (1981). Resource partitioning between two lizard species of the genus *Acanthodactylus* living in the same sand area. Unpublished M.Sc. Thesis, Department of Zoology, the Hebrew University of Jerusalem, Jerusalem. (In Hebrew).
- Ballinger, R.E. (1978). Variation in and evolution of clutch and litter size. In: *The Vertebrate Ovary*. Jones, R.E. (Ed). Plenum, New York. pp 789-825.
- Ballinger, R.E. (1983). Life history variations. In: *Lizard Ecology.* Studies of a Model Organism, Huey, R.B., Pianka, E.R. and Schoener, T.W. (Eds.). Harvard UP, Cambridge. pp 241-260, 465-473.
- Ballinger, R.E. and Clark, Jr., D.R. (1973). Energy content of lizard eggs and the measurement of reproductive effort. J. Herpet. 7, 129-132.
- Barash, Al. and Hoofien, J.H. (1956). *Reptiles of Israel*. Hakibutz Hameuchad, Tel Aviv (In Hebrew).
- Barbault, R. (1975). Observations écologiques sur la reproduction des lézards tropicaux: les stratégies de ponte en forêt et en savane. Bull. Soc. Zool. Fr. 100, 153-168.
- Başoğlu, M. and Baran, I. (1977). Türkiye Sürüngenleri. Kisim I Kaplumbaga ve Kertenkeleler. Bornova-Izmir: Ege Üniversitesi Fen Fakültesi Kitaplar Serisi No 76 Ilker Matbaasi. (In Turkish with English Summary). pp. 191-236.
- Bradshaw, S.D. (1981). Ecophysiology of Australian desert lizards: Studies on the genus Amphibolurus. In: Ecological Biogeography of Australia, Keast, A. (Ed.). Junk, The Hague. pp 1395-1434.
- Cody, M.L. (1966). A general theory of clutch size. *Evolution* 20, 174-184.
- Congdon, J.D., Vitt, L.J. and Hadley, N.F. (1978). Parental investment: Comparative reproductive energetics in bisexual and unisexual lizards, genus *Cnemidophorus*. *Am. Nat.* 112, 509-521.
- Dmi'el, R. (1967). Studies on the reproduction, growth and feeding in the snake Spalerosophis cliffordi (Colubridae). Copeia 1967, 332-346.
- Dor, M. (1987). Zoological Lexicon, Vertebrata. 2 vols (ed 3) Dvir, Tel Aviv. (In Hebrew).
- Dunham, A.E. and Miles, D.B. (1985). Patterns of covariation in life history traits of squamate reptiles: The effects of size and phylogeny reconsidered. Am. Nat. 126, 231-257.
- Dunham, A.E., Miles, D.B. and Reznick, D.N. (1988). Life history patterns in squamate reptiles. In: *Biology of the Reptilia* 16, Gans, C. and Huey, R.B. (Eds). Alan R. Liss Inc., New York. pp 441-522.
- Emlen, J.M. (1973). *Ecology: An Evolutionary Approach*. Addison-Wesley, Reading, Massachusetts.
- Ferguson, G.W., Brown, K.L. and DeMarco, V.G. (1982). Selective basis for the evolution of variable egg and hatchling size in some iguanid lizards. *Herpetologica* 38, 178-188.
- Fitch, H.S. (1970). Reproductive cycles in lizards and snakes. Univ. Kans. Mus. Nat. Hist. Misc. Pub. 52, 1-247.
- Fitch, H.S. (1985). Variation in clutch and litter size in New World reptiles. Univ. Kansas. Mus. Nat. Hist. Misc .Publ. 76, 1-76.
- Fitch, H.S. and Fitch, A.V. (1967). Preliminary experiments on physical tolerances of eggs of lizards and snakes. *Ecology* 48, 160-165.
- Flower, S.S. (1933). Notes on the recent reptiles and amphibians of Egypt, with a list of the species recorded from that Kingdom. *Proc. Zool. Soc. London.* 1933, 735-851.
- Fox, S.F. and Rostker, M.A. (1982). Social cost of tail loss in Uta stansburiana. Science 218, 692-693.
- Frankenberg, E. (1989). The importance of conserving natural areas. *Eretz Haya'el* 2(4), 44-45. (In Hebrew).

- Goldberg, S.R. (1975). Reproduction in the Arizona alligator lizard. Gerrhonotus kingi. Southwestern Naturalist 20, 409-420.
- Goldberg, S.R. (1977). Reproduction in a mountain population of the side-blotched lizard, *Uta stansburiana* (Reptilia, Lacertilia, Iguanidae). J. Herpet. 11, 31-35.
- Guillettes, Jr., L.J., Rand, M.S., DeMarco, V. and Etheridge, K. (1988). Fixative and alcohol-induced weight change in eggs from the lizard, *Sceloporus undulatus*. J. Herpet. 22, 115-118.
- Haas, G. and Werner, Y.L. (1969). Lizards and snakes from south-western Asia, collected by Henry Field. Bull. Mus. Comp. Zool.(Harvard) 138, 327-406.
- Hertz, P.E. and Nevo, E. (1981). Thermal biology of four Israeli agamid lizards in early summer. Isr. J. Zool. 30, 190-210.
- Huey, R.B. and Pianka, E.R. (1981). Ecological consequences of foraging mode. *Ecology* 62, 991-999.
- Kramer, G. (1946). Veraenderungen von Nachkommenziffer und Nachkommen Groesse sowie der Altersverteilung von Inseleidechsen. Z. Naturforsch. 1, 700-710.
- Lehman, T. (1980). Observations on the biology and behavior of *Acanthodactylus scuttelatus*. Unpublished Highschool Graduation Project, Tel-Aviv. (In Hebrew).
- Loumbourdis, N.S. (1987). ("1 984/85") Energy content of eggs and bodies and the measurement of relative clutch mass in the lizard *Agama stellio stellio*. *Isr. J. Zool.* 33, 85-89.
- Margolin, Y. (1959). *Zoology, vol. 2 Vertebrata.* Ed. 2, revised by Merom, H. and Dor, M. Hakibbutz Hameuchad, Tel Aviv. (In Hebrew).
- Martin, R.F. (1978). Clutch weight/total body weight ratios of lizards (Reptilia, Lacertilia, Iguanidae): preservative induced variation. J. Herpet. 12, 248-251.
- Mendelssohn, H. (1963). On the biology of the venomous snakes of Israel. Part I. Israel. J. Zool. 12, 143-170.
- Mendelssohn, H. (1965). On the biology of the venomous snakes of Israel. II. Jsr. J. Zool. 14, 185-212.
- Oliver, J.A. (1955). The Natural History of North American Amphibians and Reptiles. Van Nostrand, Princeton, NJ.
- Orr, Y. Shachak, M. and Steinberger, Y. (1979). Ecology of the small spotted lizard *Eremias guttulata* in the Negev desert (Israel). J. Arid. Env. 2, 151-161.
- Packard, G.C., Tracy, C.R. and Roth, J.J. (1977). The physiological ecology of reptilian eggs and embryos, and the evolution of viviparity within the class Reptilia. *Biol. Rev* 52, 71-105.
- Patterson, J.W. (1983). Frequency of reproduction, clutch size and clutch energy in the lizard Anguis fragilis. Amphibia-Reptilia 4, 195-203.
- Pianka, E.R. (1986). Ecology and Natural History of Desert Lizards. Princeton University Press, Princeton, NJ.
- Rand, A.S. (1982). Clutch and egg size in Brazilian iguanid lizards. *Herpetologica* 38, 171-178.
- Ratterman, R.J. and Ackerman, R.A. (1989). The water exchange and hydric microclimate of painted turtle (*Chrysemys picta*) eggs incubating in field nests. *Physiol. Zool.* 62, 1059-1079.
- Salvador, A. (1982). A revision of the lizards of the genus Acanthodactylus (Sauria: Lacertidae). Bonner Zool. Monogr. 16, 1-167.
- Shine, R. (1980). "Costs" of reproduction in reptiles. *Oecologia* (Berl.) 46, 92-100.
- Smith, C.C. and Fretwell, S.D. (1974). The optimal balance between size and number of offspring. Am. Nat. 108, 499-506.
- Steel, R.G.D. and Torrie, J.H. (1960). Principles and Procedures of Statistics with Special Reference to the Biological Sciences. McGraw-Hill, New York.
- Tinkle. D.W. (1969). The concept of reproductive effort and its relation to the evolution of life histories of lizards. *Am.* Nat. 103, 501-516.

- Tinkle, D.W., Wilbur, H.M. and Tilley, S.E. (1970). Evolutionary strategies in lizard reproduction. *Evolution* 24, 55-74.
- Turner, F.B. (1977). The dynamics of populations of squamates. crocodilians and rhynchocephalians. In: *Biology of the Reptilia* Vol. 7, 157-264. Gans. C. and Tinkle, D.W. (Eds.). Academic Press, London.
- Turner, F.B., Lannom, Jr., J.R., Medica, P.A. and Hoddenbach, G.A. (1969). Density and composition of fenced populations of leopard lizards (*Crotaphytus wislizeni*) in southern Nevada. *Herpetologica* 25, 247-257.
- Van Devender, R.W. (1982). Comparative demography of Basiliscus basiliscus. Herpetologica 38, 189-208.
- Vitt, L.J. (1978). Caloric content of lizard and snake (Reptilia) eggs and bodies and the conversion of weight to caloric data. J. Herpet. 12, 65-72.
- Vitt, L.J. and Congdon, J.D. (1978). Body shape, reproductive effort, and relative clutch mass in lizards: resolution of a paradox. Am. Nat. 112, 595-608.
- Vitt, L.J., Howland, J.M. and Dunham, A.E. (1985). The effect of formalin fixation on weight of lizard eggs. J. Herpet. 19, 298-299.
- Vitt, L.J. and Price, H.J. (1982). Ecological and evolutionary determinants of relative clutch mass in lizards. *Herpetologica* 38, 237-255.
- Wahrman, J. (1970). Distribution maps. Pls VII/1-2. In: Atlas of Israel. Ministry of Labour, Jerusalem, and Elsevier, Amsterdam.

- Wermuth, H. (1967). Liste der rezenten Amphibien und Reptilien, Agamidae. Das Tierreich, Lfg 86. Walter de Gruyter, Berlin.
- Werner, Y.L. (1965). Ueber die israelischen Geckos der Gattung Ptyodactylus und ihre Biologie. Salamandra 1, 15-25.
- Werner, Y.L. (1966a). Cyrtodactylus kotschyi orientalis in Israel. Lacerta 24, 94-96.
- Werner, Y.L. (1966b). The Reptiles of Israel. Department of Zoology, The Hebrew University of Jerusalem, Jerusalem. (In Hebrew).
- Werner, Y.L. (1971). Some suggestions on the standard expression of measurements. *Syst. Zool.* 20, 249-252.
- Werner, Y.L. (1973). The Repuiles of the Sinai Peninsula. Department of Zoology, The Hebrew University of Jerusalem, Jerusalem. (Text in Hebrew; abstract, key to Eremias and map legends in English).
- Werner, Y.L. (1986a). Ecology of eggs and laying sites of *Ptyodactylus geckos*. 441-444. In: *Studies in Herpetology* (Proc. Europ. Herp. Meeting, Prague, 1985), Rocek, Z. (Ed.). Charles University, Prague.
- Werner, Y.L. (1986b). Geographic sympatry of Acanthodactylus opheodurus with A. boskianus in the Levant. Zoology in the Middle East 1, 92-95.
- Werner, Y.L. (1988). Egg size and egg shape in Near-eastern gekkonid lizards *Isr. J. Zool.* 35, 199-213.

HERPETOLOGICAL JOURNAL, Vol. 2, pp. 18-23 (1992)

SODIUM CHLORIDE AND POTASSIUM CHLORIDE TOLERANCE OF DIFFERENT STAGES OF THE FROG, MICROHYLA ORNATA

A.D. PADHYE AND H.V. GHATE

Post-Graduate Research Centre, Department of Zoology, Modern College, Pune 411 005 India.

(Accepted 18.6.90)

ABSTRACT

Short term effects of different concentrations of NaCl and KCl on embryos and tadpoles of the frog *Microhyla omata* were studied. Both NaCl and KCl caused significant reduction in swelling of the perivitelline space (PVS), an effect very similar to that reported for acidic pH. Tadpoles were observed to be somewhat more resistant to both NaCl as well as KCl, as compared to the embryos. KCl was found to be more toxic than NaCl. A typical teratogenic effect was observed in KCl treated embryos which showed swollen head coelom, whereas NaCl caused incomplete closure of the neural tube.

INTRODUCTION

Amphibian embryos may be exposed to different salinities during the period of their embryonic development. Later the tadpoles also have to face varying environmental conditions. The reasons for variation in salinity are many. Intermittent rainfall often leads to drying of temporary rain-water pools thereby increasing the salinity (Munsey, 1972). It is also likely that the breeding sites on the coastline may be affected by tidal inundation. Thus, the salinity of the medium is an important factor in the developmental ecology of amphibians. Some work has been done regarding the effects of salinity on breeding and development of a few amphibians (Ely, 1944; Ruibal, 1959; Beebee, 1985). Considerable work has been done on salt tolerance of the embryos, tadpoles and adults of the Indonesian frog *Rana cancrivora* (Gordan et al., 1961; Gordan and Tucker, 1965; Dunson, 1977). This frog is known to tolerate high salt levels in the ambient medium. However, no information is available regarding any of the Indian species of frog. In this study we estimated both the NaCl and the KCl tolerance limits at different stages of development of the frog *Microhyla ornata*.

MATERIALS AND METHODS

Spawn was collected from natural ponds/temporary rain-water pools during the early morning hours (07.00-09.00hr), brought to the laboratory, manually "dejellied" using forceps, and exposed to the various concentrations of NaCl and KCl at late gastrula stage (Gosner stage 11/ 12, Gosner, 1960).

Eight day old (stage 24), and hind-limb stage (stage 39) tadpoles collected from the field were also exposed in a similar manner. The media were prepared by adding 10% NaCl or KCl stock solution to aged tapwater to obtain the desired concentration of NaCl or KCl. The physico-chemical parameters of the aged tap-water were: pH 7.5-7.8, total hardness <75 ppm (as CaCO3), total alkalinity <60 ppm (as CaCO3). The experiments were run at room temperature which varied between 23 and 27°C. The Na and K content of this tapwater were negligible. Ten embryos or tadpoles were picked up randomly and released in a 250 ml capacity glass bowl containing 100 or 200 ml of medium. Larger tadpoles were exposed in 31 capacity glass troughs with 1 1 of medium. At least 30 embryos or tadpoles were exposed to each concentration to study the effects of the two salts. Aged tap-water served as a control medium. This water was kept in a large polyester container for 15 to 30 days and aerated using an electrical aerator pump.

The embryos and tadpoles were observed every 24 hours for a total period of 96 hours from the time of exposure. Mortality data of the embryos and tadpoles was recorded. Morphometric measurements were taken as described earlier (Padhye and Ghate, 1988). Embryos were fixed in Bouin's fluid, embedded in BDH ceresin wax and 7 μ m sections produced using routine techniques. Stains were haemaetoxylin and eosin. LC 50 values were calculated by Reed-Muench method (Woolf, 1968).

RESULTS

The control embryos showed normal development and differentiation of head, trunk and tail region at 24 hours. The embryonic perivitelline space (PVS) was swollen and the egg diameter with vitelline membrane (VM) increased by about three times to that of the egg at the late gastrula stage. At 48 hours all embryos hatched normally and were found attached to the inside of the vessel with the help of the suckers. At 96 hours the tadpoles were actively swimming and showed well developed head with a typical pigment pattern on the dorsal side.

TOXIC AND TERATOGENIC EFFECTS ON THE EMBRYOS

As far as the toxic effects were concerned the experimental embryos exposed to 0.2% NaCl did not show any mortality during 96 hours of exposure. In 0.3% NaCl some mortality occurred at 96 hours, however, at 0.4 and 0.5% NaCl mortality was observed at 72 hours. The embryos exposed to 0.6% NaCl died within 48 hours, while those in 0.7% NaCl died within a few hours showing total mortality in 24 hr.

Compared to NaCl, KCl appeared to be more toxic. E mbryos exposed to 0.1% KCl showed slightly stunted growth while there was total mortality of the embryos within 96 hours at 0.2% and higher concentrations. At 0.4%, KCl was totally lethal within 72 hours while 0.5% KCl was lethal to all the exposed embryos within 48 hours. The LC 50 values at different periods of exposure for both NaCl and KCl are given in Table 1.

Hours of	LC50 values						
exposure	in % NaCl	in % KCl					
24	0.6482 (0.6226-0.6748)	>0.5					
48	0.5604 (0.5335-0.5887)	0.3548 (0.3233-0.3894)					
72	0.4222 (0.3931-0.4535)	0.2732 (0.2497-0.2989)					
96	0.2711 (0.2493-0.2947)	0.1414 (0.1312-0.1524)					

TABLE 1. Toxicity of NaCl and KCl to late-gastrula stage embryos of *M. ornata*. Figures in parentheses indicate 95% confidence limits.

The prominent teratogenic effect, observed in the embryos treated with the higher concentration of NaCl was incomplete closure of the neural tube at 48 hours. At 0.5% some embryos (>50%) showed anteriorly open neural tube while all the embryos exposed to 0.6% NaCl showed almost completely open neural tube (Fig. 1). Histological observations revealed that in these cases the organisation of the neural cells within the neural tube was totally disturbed. The neural folds were formed, however, but they failed to close. These embryos did not survive. At lower concetrations the neural tube closed normally. A photographic record of the neural tube of the control and experimental embryos is presented in Figs. 2-6. No such effect was seen with KCl treatment.



Fig. 1 Control embryo (at the right) showing closed neural tube. Two curved embryos (NaCl treated) at the left showing incompletely closed neural tube (arrows).



Fig. 2 T.S. of control embryo passing through the sucker region (S). See the well-developed neural tube (NT). Differentiation of eyes (E) has also started.



Fig. 5 T.S. of another NaCl treated embryo with abnormal neural tube. Note highly disorganised mass of embryonic cells. It is apparent that the non-neural ectoderm (NE) has failed to expand and enclose the neural cells.



Fig. 3 T.S. of an experimental embryo (NaCl-treated) through the sucker region. Note abnormal formation of the neural tube (NT) and eyes (E).



Fig. 6 Close-up of the above experimental embryo showing disorganised neural cells with no evidence of tube formation.



Fig. 4 Close-up of the neural tube showing its organisation in a control embryo.



Fig. 7 Control (right) and experimental tadpoles at 96 hours. Note swollen head region in tadpoles due to continuous treatment of KCl from gastrula stage onwards.

The pigmentation in embryos was reduced gradually with the increase in the concentration of both NaCl and KCl, however, KCl was more potent in its effect in this regard. At the highest tolerated concentration of NaCl the embryos were almost pale white and the pigment was present only in eyes and suckers. However, at and beyond 0.2% KCl, even the eyes and suckers did not show much pigmentation in the surviving tadpoles.

The general development of the embryos was almost normal up to a concentration of 0.2% NaCl. At the higher concentrations the embryos showed distinct microcephaly, retarded growth as evident from total length and "swollen belly" due to reduced yolk absorption. KCl-treated embryos showed only slightly stunted growth up to 0.1% concentration. At 0.2% KCl there was retardation of growth and the entire head coelom of the tadpole was swollen (Fig. 7). This gave unusual buoyancy to the head region of the tadpole and the tadpoles then floated with their ventral side upwards. This effect was not observed in embryos treated with NaCl, at least during 96 hours of exposure.

Tail-fin erosion was a distinct feature of the tadpoles surviving after embryonic exposure to 0.3% NaCl and above. The reduced tail-fin had a wavy margin.

The experimental embryos, both KCl and NaCltreated, showed prominent reduction in swelling of the PVS after 24 hr exposure as compared with the controls (Fig. 8). In 0.6% NaCl and 0.5% KCl the egg diameter with VM remained almost the same as that of the egg at the late gastrula stage. This caused curving of the body axis of the embryos within the intact VM, due largely to inadequate space. Even at lower concentrations both NaCl and KCl caused significant reduction in normal swelling of the PVS. After hatching such tadpoles showed curved body axis and abnormal swimming. At lower concentrations (up to 0.2%) these embryos showed only partial curvature of the body axis, the abnormality which was not apparent after hatching. No significant effect on hatching was observed, at least at lower concentrations, although hatching was invariably delayed by a few hours to one day.



Fig. 8 Bar diagram showing diameter of PVS of the control and experimental embryos at 24 hours exposure to different concentrations of NaCl and KCl. The mark inside the control (C) bar, indicated by an arrow, shows the diameter at the beginning of the experiment. The values listed are mean and standard deviations of at least 20 observations.

TOXIC EFFECTS ON THE TADPOLES

Under similar treatment conditions 8 day old tadpoles tolerated up to 0.3% NaCl and did not show mortality in 96 hours. At a concentration of 0.4% and above, however, mortality was observed. The hind-limb as well as fore-limb stage tadpoles tolerated up to 0.6% NaCl without any mortality up to at least 48 hr.

KCl was found to be toxic at and above 0.2% to 8 day old tadpoles. Total mortality was observed at 0.3% KCl in 96 hours, while 0.4% KCl and above caused immediate mortality. Hind-limb as well as fore-limb stage tadpoles showed slight increase in the tolerance. They could tolerate 0.2% KCl without any mortality while 0.4% KCl was lethal in 96 hours. The 96 hour LC 50 values of these salts for different stages of the tadpoles are given in Table 2.

Stage	96 hour LC 50 values					
-	in % NaCl	in % KCl				
Late gastrula stage	0.2711 (0.2493-0.2947)	0.1414 (0.1312-0.1524)				
8 day old tadpoles	0.5027 (0.4623-0.5466)	0.1593 (0.1397-0.1817)				
Hind-limb stage tadpoles	0.6929 (0.6014-0.7985)	0.2539 (0.2325-0.2773)				

TABLE 2. Comparative toxicity of NaCl and KCl to different developmental stages of *M. ormata*. Figures in parentheses indicate 95% confidence limits.

DISCUSSION

Experimental exposure of the embryos of *Microhyla* to NaCl and KCl solutions revealed that the potassium salt is more toxic than the sodium salt. It is possible that potassium ions interfere with a large number of biochemical reactions and hence are more toxic. There is no comparable data regarding the effects of KCl on amphibian embryos, however, it is known that KCl is more toxic than NaCl even to freshwater fish and molluscs (McKee and Wolf, 1963).

A very prominent effect of treatment of NaCl and KCl was prevention of swelling of the PVS of the embryo. It is known that the PVS, a space between the embryo proper and the VM, enlarges as development proceeds. This enlargement, or swelling, is due to slow flow of water into the PVS and it is necessary for normal development of the embryo (Krogh, 1939). The size of the PVS is said to be determined by osmotic gradients across the egg membrane (Holtfreter, 1943). It is possible therefore that the reduction in the PVS in the embryos exposed to salt solutions is related to changes in these osmotic gradients, especially because the degree of reduction in size is proportional to salt concentration (Fig. 8). Even the so-called hatching enzyme is involved in altering the properties of the VM, the properties that control inflow of water into the PVS, and the salt solutions may affect the enzyme activity thereby reducing the inflow of water. Even altered pH of the surrounding medium has the same effect on the PVS of the amphibian embryo, as has been discussed earlier (Padhye and Ghate, 1988). The curling of the body axis is due to reduction in the PVS, as the space is inadequate for growth of the embryo.

Another important effect noted was on the neurulation process. The embryos exposed to near lethal concentration of NaCl showed an open neural tube. KCl did not induce this defect. The only other published report regarding effect of salt solution on the neurulation process in frogs is that of Ruibal (1959). In the case of *Rana pipiens*. Ruibal (1959) observed that the neural tube failed to close when the embryos were exposed to 0.5-0.6% salinity.

The process of neurulation is an extremely complex event in early development. The normal process of neurulation requires certain changes in the neural and non-neural ectodermal cells, especially changes in the volume and shape of certain cells (Karfunkel, 1974). It is also known that non-neural ectodermal cells (epidermis) help in raising and closing the neural folds during neurulation (Schroeder, 1970; Brun and Garson, 1983). It is likely that NaCl inhibited one of these processes because the cells involved did not undergo the required changes or that the cells were killed due to lethal salt concentration. A change in the behaviour of the cells such as cell-to-cell adhesion may also affect the neural tube formation. It is interesting to note here that concentrations greater than 90 mМ NaCl (approximately 0.53%) and 50 mM KCl (approximately 0.37% KCl), were shown to inhibit cell adhesion in amphibian (presumptive dissociated gastrula ectodermal) cells while lower concentrations actually promoted cell adhesion (Komazaki, 1989). This shows that ions in the external medium can affect cell adhesion. Why potassium did not have a similar effect on the formation of the neural tube is inexplicable at present.

Other effects of the treatment of salt solutions on the developing embryos, such as retardation of growth, reduction of pigmentation, microcephaly etc., were similar to those described by Ruibal (1959). These effects suggest that both salts severely interfere with the biochemical events of early development. Yolk absorption seems to be affected, leading to the formation of the embryos with "swollen belly".

The embryos that hatched as tadpoles in 0.2% and 0.3% KCl showed a swollen head region. This deformity indicates that osmoregulation in these tadpoles is affected. Again no such effect was observed with NaCl. There are no published reports of the effects of KCl on early development of frog embryos, however, mercuric chloride produces identical effects in *M. ornata* (Ghate and Mulherkar, 1980). Mercury is believed to be causing this defect through inhibition of enzymes like ATPase which are involved in osmoregulation. It is possible that K ions also inhibit ATPase, especially Na-K dependent ATPase that are known to occur in membranes.

As far as the exposure of the tadpoles to different salt concentrations was concerned, the tadpoles were found to be slightly more tolerant to low salt concentration. Similar observations were made by Beebee (1985) in the case of *Bufo calamita* embryos and tadpoles exposed to saline media. At this stage the tadpoles possess functional kidneys and skin, the organs that are involved in salt and water regulation. Such organs are not available to the developing embryos and hence the embryos are probably more sensitive to salt solutions than the tadpoles. What the foregoing discussion points out is that the embryos of *Microhyla* are sensitive to NaCl and KCl solutions and that increase in the salt concentration of the water bodies in which *Microhyla* breed may affect successful reproduction of this frog. The results also point out that K and Na ions are quite different in their action, some of the effects produced being very specific for a given ion. Normally Na and K concentration of temporary rain water pools (the favoured site for breeding in this frog) is below 5 to 10 ppm and desiccation may not always raise it to alarming levels. Tidal inundation of the breeding ponds is, however, possible at several places over the range of the species.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. S.N. Navalgundkar, Principal, Modern College, for encouragement and facilities. Thanks are due to Prof. M.R. Marathe, Head, Physics Department, for making available the word processing facility and to Mr. S. Kashikar for helping the authors during the final preparation of the manuscript. Mr. Narendra Naidu, who helped during preparation of the photographs also deserves thanks. We also gratefully acknowledge Prof. R. Ruibal for reading the preliminary draft and his suggestions. A.D.P. thanks U.G.C. New Delhi for awarding JRF.

REFERENCES

- Beebee, T.J.C. (1985). Salt tolerance of natterjack toad (Bufo calamita) eggs, and larvae from coastal and inland populations in Britain. Herpetological Journal 1, 14-16.
- Brun, R.B. and Garson, J.A. (1983). Neurulation in the Mexican salamander (Ambystoma mexicanum): a drug study and cell shape analysis of the epidermis and neural plate. Journal of Embryology and Experimental Morphology 74, 275-295.
- Dunson, W.A. (1977). Tolerance of high temperature and salinity by tadpoles of the Phillipine frog (Rana cancrivora). Copeia 1977, 375-378.
- Ely, C.A. (1944). Development of *Bufo marinus* larvae in dilute sea water. *Copeia* 1944, 256-260.
- Ghate, H.V. and Mulherkar, L. (1980). Effects of mercuric chloride on embryonic development of the frog *Microhyla* ornata. Indian Journal of Experimental Biology 18, 1094-1096.
- Gordan, M.S., Schmidt-Nielson K. and Kelly, H.M. (1961). Osmotic regulation in the crab eating frog (*Rana cancrivora*). Journal of Experimental Biology 38, 659-678.
- Gordan, M.S. and Tucker, V. (1965) Osmotic regulation in the tadpoles of crab eating frog (Rana cancrivora). Journal of Experimental Biology 42, 437-445.
- Gosner, K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16, 183-190.
- Holtfreter, J. (1943). Properties and functions of the surface coat in amphibian embryos. *Journal of Experimental Zoology* 93, 251-323.
- Karfunkel, P. (1974). The mechanism of neural tube formation. International Review of Cytology 38, 245-271.
- Komazaki, S. (1989). Effects of salts in promoting the adhesion of amphibian gastrula cells. *Journal of Experimental Zoology* 250, 40-48.
- Krogh, A. (1939). Osmotic regulation in animals. Cambridge University Press, U.K.
- Mckee, J.E. and Wolf, H.W. (1963). Water quality criteria. California State Water Resource Control Board, Publication No. 3-A. (1976 reprint).

- Munsey, L.D. (1972) Salinity tolerance of African pipid frog Xenopus laevis. Copeia 1972, 584-586.
- Padhye, A.D. and Ghate, H.V. (1988). Effect of altered pH on embryos and tadpoles of the frog *Microhyla ornata*. *Herpetological Journal* 1, 276-279.
- Ruibal, R. (1959). The ecology of the brackish water populations of *Rana pipiens*. *Copeia* 1959, 315-321.

Schroeder, T.E. (1970). Neurulation in *Xenopus laevis*, model based upon light and electron microscopy. *Journal of Embryology and Experimental Morphology* 23, 427-462.

Woolf, C.M. (1968). Principles of Biometry. D. van Nostrand Co. Inc., New Jersey, USA.

HERPETOLOGICAL JOURNAL, Vol. 2, pp. 23-26 (1992)

PLASMA CONCENTRATIONS OF ALDOSTERONE AND ELECTROLYTES IN GALLOTIA GALLOTI (SAURIA: LACERTIDAE)

MARIO DIAZ AND ANTONIO LORENZO

Laboratorio de Fisiología Animal, Departamento de Biología Animal, Universidad de La Laguna, Canary Islands, Spain.

(Accepted 29.7.90)

ABSTRACT

Plasma concentrations of aldosterone, sodium and potassium were measured in the lizard, Gallotia galloti. Aldosterone concentrations in control animals were 29.48 ± 8.65 ng/dl, which falls within the range reported for this hormone in mammals. Peripheral sodium and potassium concentrations were 132.81 ± 2.28 and 5.77 ± 0.32 meq/l, respectively. Plasma aldosterone and sodium were negatively correlated. A positive relationship could be established between potassium concentration and aldosterone levels. Acute or chronic administration of exogenous aldosterone increased the circulating levels of this hormone, being maximal in chronically treated animals. However, although plasma concentrations of aldosterone were augmented by acute administration, the sodium concentration in the plasma was only elevated by chronic treatment. No further changes to potassium concentration could be observed under primary hyperaldosteronism conditions. The extent to which aldosterone may be implicated in the regulation of sodium and potassium transport in reptiles and its possible action on postrenal structures of electrolyte transport are discussed.

INTRODUCTION

Adrenal corticosteroids have been implicated in the control of osmoregulation in reptiles (Bentley, 1976; Bradshaw, 1975; Callard & Callard, 1978), but their precise modes and loci of action await elucidation. Aldosterone has been isolated from reptilian adrenal tissue after in vitro incubation of this tissue, and it is also now known that this steroid is secreted into the plasma (Sandor, 1972; Vinson, Whitehouse, Goddard & Sibley, 1979). To date, only a few indications of the plasma level of this steroid hormone are available. Bradshaw & Grenot (1976) found that in a North African terrestrial agamid, Uromastix acanthinurus, and in the large omnivorous skink from Western Australia, Tiliqua rugosa, plasma levels were 36.04 and 31.74 ng/dl, respectively. However, Nothstine, Davis & DeRoos (1971) have reported a higher concentration of 760 ng/dl plasma in post-caval plasma from the caiman. The physiological significance of aldosterone in reptiles is not at all clear. Early studies in lizards, such as Amphibolorus ornatus and Dipsosaurus dorsalis, in which hypophysectomy and dexamethasone treatment were associated to an increased tubular reabsorption of sodium ions (Bradshaw, 1972; Bradshaw, Shoemaker and Nagy, 1972), are difficult to interpret because these treatments would also be expected to decrease plasma

aldosterone (Bradshaw, 1978). However, Rice, Bradshaw and Prendergast (1982) found that in adrenalectomized Varanus gouldii sodium and glucose concentrations fell after the operation, and potassium concentrations rose, as has been observed in mammals. The fall in aldosterone concentrations in Varanus gouldii as a result of salt-loading is associated with a marked decrease in fractional reabsorption of sodium and chloride ions by the kidney, and an increase in the rate of potassium secretion, suggesting an obvious mineralocorticoid effect of aldosterone. In an effort to elucidate the physiological role of aldosterone in reptiles, peripheral plasma concentrations were measured in relation to electrolyte concentrations under several treatments in an omnivorous lacertid endemic to the Canary Islands, Gallotia galloti.

MATERIAL AND METHODS

ANIMALS

A total of 18 adult male and female *Gallotia galloti* lizards were trapped between May and September 1989 in the zone of Tegueste (Tenerife, Canary Islands, Spain). Lizards were transported to the laboratory and acclimatized in a large indoor terrarium. Mean body weight of experimental animals was 39.34 ± 1.23 g. Food and water were provided *ad libitum*.

TREATMENTS

Lizards were ramdomly assigned to one of three groups: (1) untreated controls (UC), (2) acutely treated (AT) which received a single intraperitonel (i.p.) injection of 100 μ g/kg body weight d-aldosterone 4 hr prior to blood sampling, and (3) chronically treated (CT) which received i.p. injections 100 μ g/kg body weight, at 52, 42, 28, 18 and 4 hours prior to the experiment. Aldosterone (d-aldosterone, Sigma Chemical Company) was dissolved in a 50% solution of dimethylsulfoxide (DMSO) in water and immediately administered.

ANALYSIS

Blood samples were collected, following decapitation, in heparinized tubes and centrifuged inmediately at 3000g for 10 minutes, and the plasma was stored at -20°C until analysis. In these same animals, interrenal glands, urinary bladder and large intestine were removed and used for additional experiments regarding reptilian osmoregulation not included in the present study. Sodium and potassium concentrations were measured in 200 µl samples of plasma with an atomic absorption spectrophotometer (Perkin Elmer, Model No. 603). Plasma aldosterone concentrations were determined in 200 µl samples by a commercial radioimmunoassay (Diagnostic kit Products 125I-labelled Corporation, Los Angeles), using aldosterone and antibody-coated tube technology for the final separation of free from bound aldosterone. Since the existence of circadian oscillations of endogenous circulating aldosterone has been demonstrated (Clauss, 1984), blood samples were always collected at the same time (16.00hrs).

STATISTICS

The significance of differences between means was assessed by Student's *t*-Test. Treatment means were compared using Analysis of Variance coupled to Student-Newman-Keul's test. The relationship between electrolyte concentration and plasma aldosterone was assessed by means of regression analysis to a linear model followed by Analysis of Variance. Least-square linear regression equations are quoted in Table 2 with standard error of estimates of the coefficients, the correlation coefficient (r) and the significance levels of r (P). Values are quoted as means \pm S.E.M. A probability value (P) of less than 0.05 was considered to be significant.

RESULTS AND DISCUSSION

PLASMA ALDOSTERONE AND ELECTROLYTE CONCENTRATIONS

Results of measurements of plasma aldosterone, sodium and potassium concentrations in untreated control animals are shown in Table 1. As can be seen, both plasma concentrations of hormone and sodium fell within the range reported for mammals which, in general, for aldosterone is between 10 and 100 ng/dl depending on the sodium status of the animal. Moreover, observed concentrations in *Gallotia galloti* were very close to those reported for other terrestrial lizards, e.g. *Tiliqua rugosa* and *Uromastix acanthinurus* (Bradshaw & Grenot, 1976), and *Varanus gouldii* (Bradshaw & Rice, 1981). Reptiles, thus, in this respect

	Aldosterone (ng/dl)	Na+ (meq/l)	K+ (meq/l)	Na+/K+
UC	29.49 <u>+</u> 8.66 (6)	132.81 ± 2.28 (6)	5.77 ± 0.32 (6)	23.01
AT	$\begin{array}{c} 106.06 \pm 5.97 \\ (6) \\ P \ 0.005 \end{array}$	130.10 <u>+</u> 2.37 (6) NS	5.70 <u>+</u> 0.12 (6) NS	22.82
СТ	$190.14 \pm 6.29^{*}$ (6) P < 0.005	$145.18 \pm 2.42*$ (6) P < 0.05	6.07 <u>+</u> 0.17 (6) NS	23.91

TABLE 1. Plasma aldosterone and cation concentrations in *G. galloti* in control (UC), single-injected (AT) and chronically treated animals (CT). Values are means SEM. Numbers in parentheses are the sample size. *P* values are refered to UC. *: P < 0.05 between indicated value and AT animals.

appear to be closer to mammals than amphibians, the latter having very high circulating aldosterone concentrations of between 500 and 1000 ng/dl (Dupont, Leboulenger, Vaudry & Vaillant, 1976). A somewhat elevated plasma potassium was observed in relation to previous studies. This could be related to the feeding patterns of G. galloti since, although this species is considered to be omnivorous, in natural environments adults are mainly herbivorous, eating halophitic plants (Báez, 1987; Molina-Borja, in press) providing, possibly, a high concentration of dietary potassium. Related findings have been reported for Amblyrynchus cristatus, whose natural dietary items (macrophytic seaweeds) contain large quantities of this ion, with the consequence of very high concentrations of potassium in the plasma (Dunson, 1969).

Plasma aldosterone concentrations in untreated control animals appear to be a function of plasma sodium, since a significant negative correlation with r =-0.89 is evident for G. galloti (Fig. 1a, Table 2), suggesting that aldosterone is involved in the regulation of the sodium status of this species. Furthermore, a good positive relationship with r = 0.97, could also be observed for potassium (Fig. 1a). Other studies in reptiles have shown that aldosterone bears a negative correlation to plasma sodium concentration, e.g. Uromastix acanthinurus (Bradshaw & Grenot, 1976) and Varanus gouldii (Bradshaw & Rice, 1981). However, Bradshaw & Grenot (1976) have reported that such a relationship was doubtful for Tiliqua rugosa, in which it was observed that over a wide range of plasma sodium concentrations, there was very little alteration in the plasma aldosterone level.

*						
	ion	i	S	r	SE	Р
UC	Na ⁺ K ⁺	139.79 4.69	-0.023 ± 0.0058 0.003 ± 0.0004	-0.89 0.97	2.78 0.19	0.0157 0.0009
AT	Na+ K+	102.01 6.19	$\begin{array}{c} 0.026 \pm 0.0148 \\ \text{-}0.001 \pm 0.0001 \end{array}$	0.66 -0.22	4.85 0.32	0.1489 0.6713
СТ	Na+ K+	97.70 6.17	$\begin{array}{c} 0.034 \pm 0.0148 \\ \textbf{-0.000} \pm 0.0014 \end{array}$	0.73 -0.01	3.11 0.49	0.0172 0.9742

TABLE 2. Single regression equations with plasma aldosterone concentrations as the independent variable. Shown are estimates for intercepts (i), slopes (s), correlation coefficients (r) standard error of estimation (SE) and probability level from the analysis of variance (P) for each experimental group and electrolyte.

Recent evidence for ACTH-like immunoreactivity and angiotensin II in the *pars distalis* and *pars intermedia* of the adenohypophysis (Bello, Tramu, Pérez-Batista, Marti & Lancha, 1988), and, furthermore, the demonstration of increased unidirectional colonic sodium absorption tollowing exogenous aldosterone treatment (Diaz, Lorenzo, Badia & Gómez, 1988), suggest that the pituitary-adrenal axis is involved in osmoregulation in *Gallotia galloti*.









Fig I. Correlation between plasma aldosterone and sodium (crosses) and potassium (squares) concentrations in (a) control (UC), (b) single-injected (AT) and (c) chronically treated animals (CT). See Table 2 for regression equations.

EFFECTS OF EXOGENOUS ALDOSTERONE

Acute in jections of exogenous aldosterone significantly increased the fractional reabsorption of sodium ions and enhanced the tubular secretion of potassium in adrenalectomized V. gouldii (Rice et al., suggesting that aldosterone 1982). functions physiologically in this species. More recently, Bradshaw, Tom & Bunn (1984) have reported severe hyperkalemia in Uromastix acanthinurus following i.p. KCl injections which were associated with increased plasma aldosterone. In contrast. mineralocorticoid concentrations were reduced when NaCl was administered. Both the above studies suggest that aldosterone regulates sodium and potassium metabolism in terrestrial reptiles. The results presented in this paper show that primary hyperaldosteronism produced changes in plasma sodium but did not affect potassium concentration in either acutely or chronically treated animals (Table 1). Moreover, no relationship could be observed between potassium and aldosterone concentrations for any treatment (Fig. Ib and lc) in increased spite of the plasma aldosterone concentrations. These results contrast with the positive correlation observed in untreated control animals, and contrast with our previous findings that showed exogenous aldosterone to increase colonic potassium secretion by abolishing control absorptive processes (Diaz & Lorenzo, 1989). However, the fact that potassium concentrations were unaffected by aldosterone treatment suggests, and might indicate, the presence of effective additional osmoregulatory mechanisms whose loci require further investigation.

Minnich (1982) in a very complete review, has pointed out that in terrestrial reptiles, mechanisms for excreting potassium ions are usually more effective than those for excreting sodium ions. In fact, many species develop significant hypernatremia during drought. For example, Amphibolorus lizards (A.ornatus, A.minor, A. minimus, A. caudicinctus and A.maculosus) incur hypernatremia during the dry Australian summer (Bradshaw, 1970; Braysher, 1972). In the present paper, we could observe hypernatremia in animals in response to primary hyperaldosteronism. From Table 1, it is clear that the plasma cationic relationship (Na⁺/K⁺) was increased in chronically treated animals, which, additionally, indicates that elevations in plasma sodium are better tolerated than decreases in potassium concentration. In fact, our results indicated that chronic but not acute aldosterone treatment produced a significant rise in Na⁺ plasma concentration. Moreover, measurements of changes in circulating concentrations of aldosterone have shown that the negative correlation observed in untreated controls was reversed to a positive relationship in chronically treated animals, which indicate the presence of a regulatory mechanism for plasma sodium by aldosterone (Fig. 1b and 1c). These results are in good agreement with our previous results in which exogenous aldosterone increased colonic unidirectional sodium absorption in this species (Diaz et al., 1988; Diaz, 1990).

The changes in aldosterone concentrations in *G. galloti* with the various treatments utilized in this study are consistent with this hormone having an active role in the control of sodium metabolism in this species.

25

However, it is important to emphasise that these data in no way prove the existence of such a relationship.

The situation with potassium regulation on the other hand is far less clear. Both acute and chronic treatments augmented plasma aldosterone concentrations, but the correlation with potassium concentration was so marginal and the slope of the lines so little different from zero that it is difficult to envisage this hormone being involved in the intimate regulation of potassium metabolism in this species (Fig lb and lc). This was despite the existence of aldosterone-induced colonic K⁺ secretion. Nasal salt glands are absent in *G. galloti*, consequently K⁺ concentrations could not be controlled by this means, unlike in other species where the glands are present (e.g. *Tiliqua rugosa, Amblyrhynchus cristatus*).

Comparatively, the effects of the mineralocorticoids on potassium excretion in nonmammalian vertebrates are not very clear. In birds, as in mammals, these hormones tend to increase potassium excretion (Skadhauge, 1981), whereas the effects appear to be somewhat confused in reptiles. They appear to increase sodium reabsorption in salt-loaded snakes (LeBrie & Elizondo, 1969). decrease reabsorption in turtles (Brewer & Ensor, 1980) and either have no effect, or stimulate secretion in lizards (Bradshaw & Rice, 1981, Rice et al., 1982). Further investigations concerning renal and postrenal electrolyte metabolism will be neccesary to clarify the nature of potassium balance in reptiles and in particular in *Gallotia galloti*.

ACKNOWLEDGEMENTS

We express our gratitude to Dr. Cándido González (Laboratorio de endocrinologia analitica, Hospital General y Clinico) and Mr. Antonio Padrón Brito (Departamento de Edafologia, Universidad de La Laguna) for their expert assistance in the use of the \Im counter and the spectrophotometer, respectively. We would also like to thank Mr. Neil Abrey for his help with the English translation of the present manuscript.

REFERENCES

- Báez, M. (1987). Datos sobre la reproducción de Gallotia galloti en Tenerife. Rev. Esp. Herpetol. 2, 29-32.
- Bello, A.R., Tramu, G., Pérez-Batista, M.A., Marti, E. & Lancha, A. (1989). Coexistence of ACTH-Substance P and ACTH-Angiotensine II during the pituitary development in a lizard (*Gallotia galloti*). (Abstract) *Eur. J. Neurosci.* (suppl). 11.
- Bentley, P.J. (1976). Osmoregulation. In: *Biology of Reptilia*. Gans C. and Dawson W.R. (Eds). New York: Academic Press, pp 365-412.
- Bradshaw, S.D. (1970). Seasonal changes in the water and electrolyte metabolism of *Amphibolorus* lizards in the field. *Comp. Biochem. Physiol.* 36, 689-718.
- Bradshaw, S.D. (1972). The endocrine control of water and electrolyte metabolism in desert reptiles. *Gen. Comp. Endocr. Suppl.* 3, 360-373.
- Bradshaw, S. D. (1975). Osmoregulation and pituitary-adrenal function in desert reptiles. *Gen. Comp. Endocr.* 25, 230-248.
- Bradshaw, S.D. (1978). Volume regulation in desert reptiles and its control by pituitary and adrenal hormones. In: Osmotic and volume regulation. Jorgensen, C.B. and Skadhauge, E. (Eds). Alfred Benzon Symposium XI. Munksgaard, pp 38-53.
- Bradshaw, S.D. & Grenot, C.J (1976). Plasma aldosterone levels in two reptilian species, *Uromastix acanthinurus* and *Tiliqua rugosa*, and the effects of several experimental treatments. J. Comp. Physiol. 111, 71-76.

- Bradshaw, S.D & Rice, G.E. (1981). The effects of pituitary and adrenal hormones on renal and postrenal readbsorption of water and electrolytes in the lizard Varanus gouldii (Gray). Gen. Comp. Endocr. 44, 82-93.
- Bradshaw. S.D., Shoemaker, V. H. & Nagy, K.A. (1972). The role of adrenal corticosteroids in the regulation of kidney function in the desert lizard *Dipsosaurus dorsalis*. *Comp. Biochem. Physiol.* 43A, 21-635.
- Bradshaw, S.D., Tom, J.A. & Bunn, S.E. (1984). Corticosteroids and control of salt gland function in the lizard *Tiliqua* rugosa. Gen. Comp. Endocr. 54, 308-313.
- Braysher, M. (1972). Water and electrolyte balance in the agamid lizard. Amphibolorus maculosus (Mitchell). and the structure and function of the nasal salt gland of the sleepy lizard. Trachydosaurus rugosus (Gray). Ph.D. Univ. Adelaide. Australia.
- Brewer, K.J. & Ensor, D.M. (1980). Hormonal control of osmoregulation in chelonia. I. The effects of prolactin and interrenal steroids in freswwater chelonians. *Gen. Comp. Endocr.* 42, 304-309.
- Callard, I.P. & Callard G.V. (1978). The adrenal gland in reptilia: Physiology. In: General, comparative and clinical endocrinology of the adrenal cortex. Vol. 2. Chester Jones I. and Henderson I.W. (Eds). New York: Academic Press, pp 370-418.
- Clauss. W. (1984). Circadian rhythms in Na transport. In: Intestinal absorption and secretion. Skadhauge E. and Heintze K. (Eds). Lancaster: MTP Press, pp 273-283.
- Diaz, M., Lorenzo, A. Badia. A & Gómez, T. (1988). The role of aldosterone in water and electrolyte transport across the colonic epithelium of the lizard, *Gallotia galloti. Comp. Biochem. Physiol.* 91A, 71-77.
- Diaz, M. & Lorenzo, A. (1989). Modulation of active potassium transport by aldosterone. *Comp. Biochem. Physiol.* 95A, 79-85.
- Diaz, M. (1990). Modulación del transporte activo de electrolitos a través de la mucosa colónica de Gallotia galloti por la daldosterona. Tesis Doctoral. Universidad de La Laguna. Spain.
- Dunson, W.A. (1969). Reptilian salt glands. In: Exocrine glands. Botelho, S.Y., Brooks, F. P. and Shelley, W. B. (Eds). Philadelphia: University of Pennsylvania Press, pp 83-103.
- Dupont, W., Leboulenger, F., Vaudry, H. & Vaillant, R. (1976). Regulation of the aldosterone secretion in the frog Rana catesbiana L. Gen. Comp. Endocr. 29, 51-60.
- LeBrie, S.J. & Elizondo, R.S. (1969). Saline loading and aldosterone in water snakes *Natrix cyclo pion. Am. J. Physiol.* 217, 426-430.
- Minnich, J. E. (1982). The use of water. In: Biology of Reptilia. Physiological Ecology. Gans, C. and Harvey Pough, F. (Eds). London: Academic Press, pp 325-395.
- Molina-Borja, M. (1990). Alimentary habits and spatialtemporal distribution of eating behaviour patterns in a natural population of lizards (*Gallotia galloti*). Vieraea. (In press).
- Nothstine, S.A., Davis, J.O. & DeRoos, R.M. (1971). Kidney extracts and ACTH on adrenal steroid secretion in a turtle and a crocodilian. *Amer. J. Physiol.* 221, 726-732.
- Rice, G.E., Bradshaw, S.D. & Prendergast, P.J. (1982). The effects of bilateral adrenalectomy on renal function in the lizard Varanus gouldii (Gray). Gen. Comp. Endocr. 47, 182-189.
- Sandor, T. (1972). Corticosteroids in Amphibia, Reptilia and Aves. In: Steroids in non-mamalian vertebrates. Idler, D.R. (Ed). New York: Academic Press, pp 253-327.
- Skadhauge, E. (1981). Osmoregulation in birds. Berlin Heidelberg: Springer-Verlag.
- Vinson, G.P., Whitehouse, B.J., Goddard, C. & Sibley, C.P. (1979). Comparative and evolutionary aspects of aldosterone secretion and zona glomerulosa function. J. Endocrinol. 81, 5-24.

HABITAT SELECTION BY THE LIZARD LACERTA LEPIDA IN A MEDITERRANEAN OAK FOREST

AURORA M. CASTILLA^{1 3} AND DIRK BAUWENS²

¹ Museo Nacional de Ciencias Naturales, c/ J. Gutiérrez Abascal 2. E-28006 Madrid, Spain ² Institute of Nature Conservation, Kiewitdreef 3. B-3500 Hasselt, Belgium ³ present address: Dept. Biology, University of Antwerp (U.I.A.), Universiteitsplein 1. B-2610 Wilrijk, Belgium

(Accepted 21.8.90.)

ABSTRACT

We studied habitat selection by *Lacerta lepida* in an open, degraded Mediterranean *Quercus ilex*-forest using two different methods. First, the frequency of lizard observations in distinct habitat types was compared with habitat availability. Although lizards were seen in a wide range of habitats. a preference for sites with a complex vertical vegetation structure was evident. We also quantified structural features of the habitat at sighting spots of adult lizards. Both univariate and multivariate analyses indicated that lizards preferred sites with a relatively high coverage of high (>2 m) vegetation, but with some low (<50 cm) vegetation, and where some rocks were present.

INTRODUCTION

Lacerta lepida, the largest European lacertid lizard (adult SVL: 140-230 mm), is distributed throughout most of Spain and Portugal, southern France and extreme north-west Italy (Bischoff et al., 1984). It is a diurnal, heliothermic, ground-dwelling lizard that forages actively on a large variety of mainly arthropod prey (Castilla et al., 1991). This lizard occurs from sea-level up to ca. 2000 m (Seva, 1982; Filella, 1983), and is found in a wide variety of both uncultivated and manmade habitats, including coastal sand-plains and dunes, arable land, olive groves, orchards, vineyards, grassland, shrubland, etc. (Arnold & Burton, 1976; Seva, 1982; Bischoff et al., 1984; Barbadillo, 1987). Due to its seemingly ubiquitous presence in different habitats, it is generally considered as not exhibiting strong habitat preferences. However, quantitative data on habitat use are very scarce. Only Busack & Visnaw (1989) provide quantitative information on the presence of L. lepida in different habitats within the province of Cádiz (southern Spain). These authors concluded that lizards inhabited different habitat types in direct proportion to their availability (but see Discussion).

We here report on a study of the habitat selection patterns of *L. lepida* in an open Mediterranean oak woodland in central Spain. Very high densities are usually attained in this macrohabitat, which is often composed of distinct habitat types. We combine two complementary approaches to the study of habitat preference. First, we examine lizard sighting frequency in the different habitat categories in relation to their availability. Second, we attempt to identify the cues that guide habitat occupation through a comparison of quantitative features of the habitat structure between sites occupied by lizards and available habitats.

MATERIALS AND METHODS

The study was conducted between March and September 1985-1986 in an area of degraded oak woodland ("encinar adehesado") near Navas del Rey (40°23'N, 4°15'W, province of Madrid, Spain). The study site consists of several well-recognisable habitats, shaped mainly through varying degrees of past and current human exploitation (sheep and cattle grazing, burning).

HABITAT CATEGORIES

Observations on habitat utilisation were made while one of us walked along a fixed 3700 m transect, which crossed all habitat categories. The route was walked 125 times between 07.00-22.00 hr (Mean European Time), covering the entire daily activity period of lizards. Upon sighting of a lizard, we recorded: date, time, size (\gtrsim age) class [juvenile: \leq 70 mm snout-vent length (SVL); subadult: 71-139 mm SVL; adult: SVL \geq 140 mm], and habitat category.

We established 12 representative habitat categories: 1) Dry, homogeneous grassland; 2) low (<50 cm), mixed shrubland with young oak (Quercus ilex). dwarf-shrubs and grasses: 3) dry areas with a patchy covering of dwarf-shrubs (mainly Lavandula stoechas); 4) moist areas with abundant cover of rushes (Juncus sp.); 5) sites with a patchy covering of broom bushes (Lygos sphaerocarpa); 6) dense bushes of brambles (Rubus sp.); 7) almond (Prunus dulcis) orchard; 8) riverside areas with ashes (Fraxinus angustifolia); 9) riverside areas with willows (Salix atrocinerea); 10) isolated Juniperus oxycedrus trees with dense grassy undergrowth; 11) open, degraded oak forest (Quercus ilex) with grassy undergrowth; 12) open evergreen forest of mixed composition (Quercus ilex, Juniperus oxycedrus) and with a heterogeneous undergrowth of shrubs and grasses.

We estimated the availability of the distinct habitat categories by recording their presence at 25 m intervals along the transect line. These frequency counts were converted to the expected number of lizard observations under the null-hypothesis that sighting frequency in each habitat was directly proportional to its availability.

SELECTION OF HABITAT FEATURES

We quantified structural features of the habitat at sighting spots of 66 adult lizards. Habitat availability was estimated through sampling 51 sites that were situated at 70 m intervals along the transect line.

For each habitat sample we located 5 points: the spot where the lizard was first sighted, and the endpoints of two orthogonal 6 m lines that intersected at the sighting spot. The direction of these lines was determined haphazardly by throwing a pen on the ground. Each of these points served as the centre of a circle with I m radius. Within each circle we estimated the following 11 variables: 1-3) percentage cover at ground level of rock/ stone, sand and litter; 4-8) percentage cover of the vegetation layers with heights <25 cm, 25-50 cm, 50-100 cm, 100-200 cm and >200cm; 9) maximum height of the vegetation; 10) distance towards the nearest rock and 11) distance towards the nearest patch of vegetation that would potentially provide shelter to predators. Three additional variables were calculated; 12) total vegetation cover: summation of the vegetation cover over the 5 layers; 13) vertical diversity: calculated over the 5 vegetation layers with the Shannon-Wiener formula; 14) horizontal diversity: calculated over the habitat variables that cover the ground (rock/stone, sand, litter, vegetation <25 cm) with the Shannon-Wiener formula.

We calculated the mean value for each variable in the 5 sampling points. This value estimates the average habitat structure within a circular area with 4 m radius, surrounding the spot where the lizard was seen. Our data hence provide a description of habitat structure for an area, rather than for a particular point, that is a portion of the lizard's home range.

We performed a principal component analysis on the correlation matrix of the habitat variables to reduce the dimensionality of the habitat space. We use *F*-tests, and *t*-tests (equal variances) or Mann-Whitney *U*-tests (unequal variances) to evaluate the statistical significance of differences in variances and means among lizard observation sites and availability samples, both for the original variables and the scores on the principal components.

RESULTS

HABITAT CATEGORIES

Lizard size classes did not differ in sighting frequency in the distinct habitat categories ($X^2 = 14.99$, 22 df, P > 0.80). We did not detect seasonal differences in habitat occupation ($X^2 = 29.07$, 33 df, P > 0.10), and therefore lumped all data for further analysis.

Lizards were observed in all habitats. Sighting frequency in the distinct habitat categories was not directly proportional to their availability ($X^2 = 327.97$, 11 df, P < 0.001; Fig. 1). We saw more lizards than expected in the mixed forest, in mixed shrubland, and near bramble bushes. Lizards seemed to avoid grasslands and, to a lesser extent, vegetations dominated by rushes, patches of broom bushes, and oak forests (Fig. 1).



Fig. 1. Observed (N = 509) and expected sighting frequencies of *L. lepida* in distinct habitat categories. Gr: grassland; Sh: mixed shrubland; Lv: vegetations with *Lavandula stoechas*; Ru: moist areas with rushes; Ls: sites with *Lygos sphaerocarpa*; Br: dense bushes of brambles; Al: almond orchard; Fx: riverside areas with ashes; Sx: riverside areas with willows; Ju: isolated *Juniperus oxycedrus*-trees with dense grassy undergrowth; Qu: open oak forest; Mx: mixed evergreen forest.

SELECTION OF HABITAT FEATURES

Structural features of sites occupied by adult lizards and of available microhabitats are summarised in Table 1. Note that for many variables the observation sites had a higher variance, but a similar mean value than the availability samples. This indicates that lizards were seen more often than expected at sites with low values, and at places with high scores for these habitat characteristics.

		Observed	Available	Р	Р
				var	means
% cover sar	nd	21.1 <u>+</u> 15.2	27.5±15.4	NS	NS
roc	:k	21.1±16.8	15.5+12.6	**	NS
litt	er	8.7 <u>+</u> 15.1	5.8 ± 11.1	**	NS
% cover veg	g. <25cm	49.0 ± 21.3	50.9 ± 17.2	*	NS
	25-50cm	29.1 ± 18.1	30.8 ± 13.8	*	NS
	50-100cm	13.1 ± 11.1	11.8 ± 8.9	*	NS
	100-200cm	7.4+6.6	7.1 + 7.9	NS	NS
	>200cm	16.2 ± 21.6	8.5 ± 9.7	***	NS
tot. veg. cov	/er	114.9+43.4	109.2 + 39.4	NS	NS
max. veg. h	eight (cm)	246.2 ± 171.5	158.3 ± 90.0	***	**
distance ve	g. (cm)	146.1 +94.8	249.0 ± 172.4	***	***
distance ro	ck (cm)	238.3 ± 250.4	329.2 ± 241.9	NS	*
vertical div		0.84 ± 0.22	0.79 ± 0.21	NS	NS
horizontal	div.	0.59 <u>+</u> 0.18	0.61 ± 0.14	*	NS
PC1		-0.09+2.44	0.12+1.75	**	NS
PC2		0.48 ± 1.93	-0.63 ± 1.64	NS	**
PC3		0.19+1.27	-0.25 <u>+</u> 1.19	NS	=().()6

TABLE 1. Summary (mean \pm 1 SD) of structural habitat features at lizard observation sites and availability samples, and probability of differences between their variances (*F*-test) and means (*t*-test or Mann-Whitney *U*-test). N = 66 for lizard observation sites: N = 51 for availability samples. *: *P*<0.05; **: *P*<0.01: ***: *P*>0.001; NS: *P*>0.05.

A principal component analysis was used to obtain a more integrated picture of the habitat attributes. The first three principal components accounted respectively for 31, 24 and 10% of the total variance of the raw data. The first principal component (PCI) was strongly positively correlated with percent cover of the vegetation layers <25 cm, 25-50 cm and 50-100 cm, total vegetation cover, vertical diversity, and distance to the nearest rock.

	PC1	PC2	PC3
% cover sand	-0.052	-0.667	-0.314
rock	-0.566	0.303	0.605
litter	-0.514	0.627	-0.333
% cover veg. <25cm	0.845	-0.135	0.013
25-50cm	0.896	0.105	0.013
50-100cm	0.785	0.323	0.100
100-200cm	0.332	0.489	-0.084
>200cm	-0.357	0.795	-0.250
tot, veg. cover	0.844	0.480	-0.085
max, yeg, height (cm)	-0.331	0.821	-0.280
distance veg (cm)	-0.185	-0.489	-0.481
distance rock (cm)	0.507	-0.403	-0.438
vertical div	0.599	0.550	-0.185
horizontal div.	-0.465	0.001	-0.412

TABLE 2. Correlations between habitat characteristics and the first three principal components. Only correlation coefficients significant at $\alpha = 0.001$ and higher than 0.50 were considered in the interpretation of the principal axes, and are shown in boldface.

It was negatively correlated with percent cover by stones/rocks and by litter at ground level (Table 2). This represents a gradient from habitats with extensive cover by stones/rocks and litter, and sparse vegetation near the ground towards sites with abundant vegetation at <100 cm above ground level. The second principal axis was positively correlated with percent cover by vegetation >200 cm high, maximum vegetation height, vertical diversity and percent cover of litter, and exhibited a strong negative correlation with percent cover by sand at ground level. Thus, PC2 describes a gradient from open, sandy sites to locations with upright bushes or trees. The third principal component showed a strong positive correlation with percent cover by rocks (Table 2).

We examined habitat occupation by considering the projections (= scores) of the different samples on the component axes. The range of the scores for lizard sighting spots was similar to that of the available sites on the three first principal components. Comparison of the distribution of scores on PC1 reveals a higher variance, but a similar average, for lizard sighting spots than for available sites (Table I). This indicates that more lizards than expected were seen at both extremes of the gradient represented by PC1, i.e. at sites with few low vegetation and many rocks on the one hand, and at places with much low vegetation on the other hand. Scores on PC2 were significantly higher for observation sites (Table 1), indicating that lizards preferred habitats with upright trees or bushes, and that they avoided open, sandy areas. Lizard sighting spots had a marginally non-significant higher average score on PC3, suggesting a tendency for lizards to select sites with much cover by rocks at ground level.

DISCUSSION

Our data, collected within a restricted area of degraded Mediterranean woodland, indicate that L. lepida used the entire range of available habitats. Nevertheless, lizards exhibited a preference for certain habitat types. They were observed more often than expected in the mixed forest, in mixed shrubland and near bramble bushes, and they avoided grasslands. Busack & Visnaw (1989) reported data on habitat use collected in a large geographical area (province of Cádiz, Spain), and distinguished between several broad habitat categories, based on their type of land use. L. lepida was found in most of these biotopes and it was concluded that it did not exhibit any habitat preference (Busack & Visnaw, 1989). Reanalysis of their data, using appropriate frequency-dependent statistical an procedure, indicates that lizards do not inhabit the different habitat types proportionally to their abundance $(X^2 = 76.32, 12 \text{ df. } P < 0.001)$. More lizards than expected were seen in sclerophyllous shrub and grassland. whereas sighting frequency was lower than expected in agriculturally unproductive areas [see Table 4 in Busack & Visnaw (1989)]. Hence, although L. lepida should be considered as a habitat generalist, its preference for certain biotopes is evident both on a local and a regional scale.

Our study of the quantitative features of habitat structure indicates that lizards were seen throughout the range of available sites. This again shows that lizards occupied all structural habitats that were available in the study area. However, sighting frequency was higher than expected at sites with upright (>2 m) trees and bushes, at places with many stones or rocks and at habitats with much low (<1 m) vegetation. Lizards tended to avoid open, sandy areas with little vegetation. These results point to a preference for sites with a rather complex habitat structure, although lizards are definitely not restricted to such places.

Several factors might invoke a preference for habitats with a high structural diversity. The presence of trees or bushes creates a sun-shade gradient. This should facilitate the shuttling between sunlit and shaded sites, a conspicuous aspect of the thermoregulatory behaviour of Lacertid lizards (Avery, 1976; Arnold, 1987; Van Damme et al., 1987). Rocks and/or stones might also serve a thermoregulatory function. On repeated occasions we observed lizards basking on rocks or stones near abundant low vegetation. The variable orientation of stony surfaces should facilitate postural adjustments that increase net radiation intake, and a close contact with their sun-warmed substrates enhances heat gain through conduction. At the same time, rockcrevices and holes between piled stones provide shelter to predators. In addition, it might be hypothesised that the number of invertebrate prey might be highest in structurally complex habitats (Strijbosch, 1988).

REFERENCES

- Arnold, E. N. (1987). Resource partitioning among lacertid lizards in southern Europe. J. Zool. (Lond.) (B). 1, 739-782.
- Arnold, E. N. and Burton, J. A. (1978). *A field guide to the reptiles* and amphibians of Britain and Europe. Collins, London.
- Avery, R.A. (1976). Thermoregulation, metabolism and social behaviour in Lacertidae. In: *Morphology and biology of reptiles*. Bellairs, A.d'A. and Cox, C.B. (Eds.). Linnean Society Symposium Ser. no. 3, London, pp. 245-259.
- Barbadillo, L. J. (1987). La guia de Incafo de los anfibios y reptiles de la Peninsula Ibérica, Islas Baleares y Canarias. Incafo, Madrid.
- Bischoff, W., Cheylan, M. and Böhme, W. (1984). Lacerta lepida Daudin 1802 — Perleidechse. In: Handbuch der Reptilien und Amfibien Europas. Band 2/1. Böhme, W. (Eds.). AULA-Verlag, Wiesbaden, pp 181-210.
- Busack, S. D. and Visnaw, J. (1989). Observations on the natural history of *Lacerta lepida* in Cádiz Province, Spain. *Amphibia-Reptilia*, 10, 201-213.
- Castilla, A. M. (1989). Autoecología del Lagarto ocelado (Lacerta lepida). Ph. D. Thesis, Universidad Autónoma de Madrid, Madrid.
- Castilla, A. M., Bauwens, D. and Llorente, G. A. (1991). Diet composition of the lizard *Lacerta lepida* in central Spain. J. *Herpet.* 25, 30-36.

The broad range of habitat types occupied by L. lepida might, at least partly, reflect the large size of their home range and their high mobility. Average home range size for adult males and adult females in our study population was 1324 and 1699 m², respectively (Castilla, 1989). Corresponding estimates for home range size, obtained by the equations in Christian & Waldschmidt (1984) are 3200 m² for a 200 g male, and 2000 m² for a 160 g female. It is not unlikely that some areas within the home range are used more extensively that others (Stamps, 1977; Christian et al., 1986). Our samples might therefore include observations of lizards that were moving through "suboptimal" habitats that separate distinct core areas within the home range. An additional possibility is that lizards use distinct types of habitat for different activities. Such changes in habitat use might be the result of diel or spatial variation in the constraints imposed by the thermal environment (e.g. Huey & Pianka, 1977; Grant & Dunham, 1988), and/or by variation in food availability.

ACKNOWLEDGEMENTS

This study was supported by grants of the I.N.I.A. and the "Comunidad Autónoma de Madrid" (to AMC). Preparation of the manuscript was aided by a postdoctoral grant of the C.S.I.C. (to AMC), and by logistic support of IBERIA, National Spanish Airlines.

- Christian, K., Porter, W. P. and Tracy, C. R. (1986). Core areas within the home ranges of Galapagos land iguanas, *Conolophus pallidus. J. Herpet.*, **20**, 272-276.
- Christian. K. A. and Waldschmidt, S. (1984). The relationship between lizard home range and body size: a reanalysis of the data. *Herpetologica*, **40**, 68-75.
- Filella, E. (1983). Nota sobre *Lacerta lepida lepida*, Daudin 1802 (Sauria, Lacertidae). *Bull. Soc. Ictio. Herp.* **5**, 18-19.
- Grant, B. W. and Dunham, A. E. (1988). Thermally imposed time constraints on the activity of the desert lizard *Sceloporus merriami. Ecology*, 69, 167-176.
- Huey, R. B. and Pianka, E. R. (1977). Seasonal variation in thermoregulatory behaviour and body temperature of diurnal Kalahari lizards. *Ecology*, 58, 1066-1075.
- Seva. E. (1982). Taxocenosis de Lacertidos en un arenal costero alicantino. Publ. Universidad de Alicante, Alicante.
- Stamps, J. A. (1977). Social behaviour and spacing patterns in lizards. In: *Biology of the reptilia*. Vol 7. Gans, C. and Tinkle, D. W. (Eds.). Academic Press, London, pp 265-334.
- Strijbosch, H. (1988). Habitat selection of *Lacerta vivipara* in a lowland environment. *Herpet. J.*, 1, 207-210.
- Van Damme, R., Bauwens, D. and Verheyen, R. F. (1987). Thermoregulatory responses to environmental seasonality by the lizard *Lacerta vivipara*. *Herpetologica*, 43, 405-415.

THE EFFECT OF STOCKING DENSITY, ORIGIN OF EGGS AND WATER FLOW ON GROWTH, SURVIVAL AND BODY CONDITION OF NILE CROCODILES (CROCODILUS NILOTICUS)

A. ZILBER¹, D.N. POPPER² AND Y. YOM-TOV^{3*}

¹ Clal Crocodile Farm Ltd., Mamba Village, P.O. Box 85723, Mombasa, Kenya
 ² National Centre for Mariculture, P.O. Box 1212, Elat, Israel 88000
 ³ Dept. of Zoology, Tel Aviv University, Ramat Aviv, Israel 69978
 *Author for correspondence

(Accepted 30.8.90)

ABSTRACT

The effect of stocking density, origin of eggs and water flow regime on growth, survival and body condition of young Nile crocodiles was studied. No adverse effects of high densities, up to 15 animals/m² was found. Animals hatched from eggs collected in nature had a slight advantage in growth. All tested parameters had no effect on survival, apart from increased mortality of 4-8 month old crocodiles kept at high density during their first cold season. Continuous water flow did not improve growth. Practical implications of these results are discussed.

INTRODUCTION

The farming of crocodilians has gained interest during the last few decades. However, very little information has been published on rearing conditions. The few papers available deal mostly with alligators (e.g., Webb *et al.*, 1987).

Although Blake and Loveridge (1975), Chabreck and Joanen (1979) and Joanen *et al.* (1981) related growth and survival of crocodilians to temperature and diets, practically no solid data exist in the literature about the effect of other factors such as stocking densities and illumination.

The aim of the present study is to report results of experiments on the effect of stocking densities, sources of eggs and water flow on growth of young Nile crocodiles (*Crocodilus niloticus*).

MATERIALS AND METHODS

The study was carried out at the commercial farm of Clal Crocodile Farms Ltd., Mombasa, Kenya, during 1988 to 1990.

Two thousand three hundred and sixty-eight two month old crocodiles were incubated and hatched in the farm. The eggs were obtained from two sources: about half were collected from nests along the banks of the Tana River (250 km north of Mombasa) and the rest from nests of captive stock on the farm. The hatchlings were held in circular concrete ponds at a density of 10 individuals/m² until the start of the experiment.

The experiments were conducted in circular concrete ponds of 6 m radius. The pond walls were 1.2 m high and were covered by sloping conical asbestos roofs. Some sunlight entered the ponds through glass windows of 0.6 x 0.9 m located near the top. The floor of the ponds sloped gently (3%) towards a central drain and the water level was kept so that a radius of 5 m was water covered. Well water of $26^{\circ}-34^{\circ}C$ was supplied through taps at the centre and sprinklers at the water perimeter. Air temperature ranged from between 24° and 38° C and was equal in all treatments of the experiment. Each pond was divided by concrete walls to the level of the pond rim into eight equal sections of 14.2 m².

Food, a fresh mixture of fish, chicken and red meat, supplemented by fish concentrate (50% protein), vitamins and minerals was provided once daily *ad libitum* at sunset. The ponds were drained, washed and disinfected (Lisol solution) and refilled every morning.

The study included two consecutive experiments: one with 2-12 months old crocodiles (experiment 1), and the second with 12-22 months old animals (experiment 2).

EXPERIMENT 1

Two ponds were stocked, one with hatchlings obtained from eggs collected from nature ("wild") and the other with hatchlings from eggs of captive broodstock ("farm") which hatched two weeks before the "wild" crocodiles. Four sections in each pond had continuous water flow and four received water only when daily refilled. The experiment included four density treatments with two replicates for each pond. Initial densities were 6, 9, 12 and 15 animals/m². The numbers were reduced by 10% at the end of each two month interval. The experiment lasted 10 months and the final densities were 3.94, 5.91, 7.86 and 9.78 animals/m².

EXPERIMENT 2

One pond was stocked with the 1-year old crocodiles taken at the end of experiment 1. This experiment, too, included four initial densities of 7.5, 10, 12.5 and 15 animals/m². The numbers were again reduced by 10% at the end of each two month interval. The final densities after 10 months were 4.94, 6.57, 8.21 and 9.85 animals/m² respectively. This experiment had two repetitions for each density. During the experiment continuous water flow 1 m³/hr was provided in all treatments.

SAMPLING

In both experiments, the animals of each replicate were sampled bi-monthly. The sampling procedure was as follows. The water level was raised to a level which forced the animals to swim in order to prevent them from piling up due to stress. Twenty-five animals were randomly taken, weighed to the nearest gram, and measured for total length to the nearest centimetre. Dead animals were removed and recorded daily during the experiment, and replaced with similar size crocodiles held in a reserve pond.

RESULTS

EXPERIMENT 1

Weight and length were highly correlated (Ln weight = 0.0665 x length + 2.4569; r = 0.9815; P < 0.001; n = 96), with no significant differences between the replicates and treatments (Fig. 1).



Fig. 1 The relationship between weight and length of captive young crocodiles 2-12 months old. Data are means for samples of 25 animals taken from each experimental groups at 2-month intervals. The markings are for densities at the following declining order: dots (highest density), crosses, squares and triangles (lowest density).



Fig. 2 Growth curve of 2-22 months old captive crocodiles. Means, standard deviations and ranges are all for the samples of the experimental groups. Ambient mean monthly temperature is given. Note the inhibited growth during the cold months of July-November.

Comparison of growth and survival of crocodiles originating from eggs laid in nature ("wild") and those obtained from captive broodstock ("farm") yielded the results in Table 1. Although the respective "farm" animals were significantly heavier (means were 132 and 97 g; *t*-test, P < 0.01) and longer (38 and 35 cm, respectively; *t*-test, P < 0.01) than "wild" animals at the beginning of the experiment, their final weight and length were not significantly different. Total mean survival of "farm" and "wild" animals was equal (83 and 84%, respectively) for the entire 10 months.

Comparison of growth and survival between crocodiles reared in sections with continuously flowing ("flowing") water and once daily changed ("standing") water is shown in Table 2. There was no difference between the groups in either initial or final body weight and length. Mean survival rate was also similar — 86 and 83% in "flowing" and "standing" water groups, respectively.

	Two Mon	th Old G	Crocodiles		One Yea	r Old C	rocodiles		Grow	th Rate	
Hatched from eggs laid by	Average Length cm	S.E.	Average Weight g	S.E.	Average Length cm	S.E.	Average Weight g	S.E.	Change in Length cm	Change in Weight g	Mean Survival %
Farm Females	38	1.1	132	23.0	68	1.6	978	47.3	30	846	83
Wild Females	35	1.1	97	13.3	70	2.4	1031	122.6	35	934	84
t-tests	5.4	_	3.8	_	1.96	_	1.2	_		_	_
P	0.01	-	0.01	_	N.S.	-	N.S.	-		_	_

TABLE 1. Growth rate and survival of crocodiles that hatched from eggs laid by farm and wild (Tana River) females between the ages of 2-12 months. Means (\pm S.E.) are for 8 sections.

	Two Mon	One Year Old Crocodiles									
	Average Length cm	S.E.	Average Weight g	S.E.	Average Length cm	S.E.	Average Weight g	S.E.	Change in Length cm	Change in Weight g	Mean Survival %
Flowing	36	2.11	116	29.8	70	2.4	997	68.9	33.9	881.0	86
Standing	36	1.30	104	14.6	69	2.2	1025	129.1	32.82	920.5	83
t-value	0.46	_	1.0	_	1.22	_	0.52	-	_	_	_
P	N.S.	-	N.S.		N.S.	_	N.S.	_	_	_	

TABLE 2. Growth rate and mortality comparison between crocodiles that grew in ponds with flowing water and standing water between the ages of 2-12 months. Means (\pm S.E.) are for 8 sections.

Density animals/ m ²	BL, cm at age 2 mth	BL, cm at age 4 mth	Density animals/ m ²	BL, cm at age 6 mth	Density animals/ m ²	BL, cm at age 8 mth	Density animals/ m ²	BL, cm at age 10 mth	Density animals/ m ²	BL, cm at age 12 mth	Total mean growth (CM) from start
6	37 <u>+</u> 1.6	48 <u>+</u> 2.4	5.4	50 <u>+</u> 1.8	4.9	55 <u>±</u> 1.9	4.4	65 <u>+</u> 2.4	3.9	71±2.5	34
9	37 <u>+</u> 1.0	46 <u>+</u> 1.4	8.1	47 <u>±</u> 1.6	7.25	52 <u>+</u> 2	6.5	62 <u>+</u> 5.4	5.9	68 <u>+</u> 1.3	31
12	35 <u>+</u> 1.0	44 <u>+</u> 1.8	10.7	46 <u>+</u> 2.3	9.6	51 <u>+</u> 2	8.7	62 <u>+</u> 1.8	7.8	70 <u>±</u> 2.6	35
15	35±1.6	44 <u>+</u> 2.6	13.4	47 <u>+</u> 2.9	12.0	51±1	10.9	62 <u>+</u> 2.1	9.8	68 <u>+</u> 1.2	33

TABLE 3. The effect of stocking density on growth of 2-12 months old crocodiles, (*Crocodilus niloticus*). Body length (BL). Data are means (\pm S.E.) in cm for 50 animals in each group.

The above stocking densities had no effect on growth during any of the periods (ANOVA $F_{3,199} = 0.5578$, P = 0.6435, N.S.; Table 3 and Fig. 2).

Survival was lower during the first cold season (4 months, July-November, 1988), when the crocodiles were under 8 months old (Fig. 3), and was larger among 7-8 months old crocodiles at high densities, but this difference was not significantly affected by density (ANOVA $F_{3.199} = 0.13$, P = 0.9422, N.S.).



Fig. 3 Bi-monthly mortality percentage (\pm standard error) of 2-12 months old crocodiles arranged according to stocking density. Upper numbers are densities (animals/m²): lower numbers are the periods of growth (months) from the beginning of the experiments, and correspond to the ages of the animals.



Fig. 4 The relationship between weight and length of captive young crocodiles 12-22 months old. Data are means for samples of 25 animals taken from each experimental groups at 2-month intervals. The markings are for densities at the following declining order: dots (highest density), crosses, squares and triangles (lowest density).

EXPERIMENT 2

Weight and length were highly correlated (Ln weight = 0.040 x Length + 0.990; P < 0.001) without any significant differences between the replicates and

treatments (Fig. 4). No effect of density on either growth or survival was observed during this experiment (Table 4).

Density animals/ m²	BL, cm at age 12 mth	BL, cm at age 14 mth	Density animals/ m ²	BL. cm at age 16 mth	Density animals/ m²	BL, cm at age 18 mth	Density animals/ m ²	BL, cm at age 20 mth	Density animals/ m ²	BL. cm at age 22 mth	Total mean growth (CM) from start
7.5	67 <u>+</u> 0.2	74 <u>+</u> 0.4	6.7	81 ± 0.8	6.1	87±1.3	5.48	88 <u>+</u> 1.4	4.9	98±2.4	31
10	69±0.2	77±0.2	9	81±2.6	8.1	87±0.1	7.29	90 <u>+</u> 1.9	6.6	98±4.2	29
12.5	69 <u>±</u> 0.6	75 <u>+</u> 1.1	11.2	79±1.1	10.1	84 <u>+</u> 1	9.12	90±1	8.2	98±2.7	29
15	69±1.7	74±1.2	13.5	79±0.3	12.1	86. <u>+</u> 0.4	10.94	89 <u>+</u> 1.4	9.8	96±0.3	27

TABLE 4. The effect of stocking density on growth of 12-22 months old crocodiles, (*Crocodilus niloticus*). Body length (BL). Data are means (\pm S.E. in cm for 50 animals in each group.

DISCUSSION

Our results show that crocodiles which hatched from eggs collected in nature had an advantage in terms of growth over crocodiles originating from eggs laid in captivity. The "wild" crocodiles which started significantly smaller than the "farm" ones, reached mean weight and length after 10 months similar to those obtained by "farm" animals. Six of the 7 biggest groups of animals at the end of the experiment, were of groups originating from "wild" eggs. This was surprising since "wild" eggs suffered from rough treatment; they were removed from their nests, transported by boat to an artificial nest where they were kept for another month, and then transferred by car over 250 km of rough road to a room in which incubation conditions were far from ideal. The "farm" eggs remained in their original nests for 70 days and only then taken to the same incubation room. Possible explanations for the better growth of the "wild" crocodiles are, (a) "better" egg quality of "wild" crocodiles due to superior nutrition of the adults, and/or (b) only the "best" embryos survived the rough handling of the transport to the incubation room. We have no data to support or refute either of these explanations, but future experiments are planned to test them.

Although we reared our experimental animals at densities higher than normally practiced by alligator farmers (Chabreck, 1967), our high densities had no effect on growth. This is in contrast to the finding of Elsey *et al.* (1990) that growth of juvenile alligators was negatively affected by densities as low as $3/m^2$ and even lower. Further experiments are needed in order to find what density will negatively affect growth and survival of Nile crocodiles. We suggest that our results are due to better management which minimized stressing the animals. These results may be significant economically for crocodile farming due to savings in area, facilities and labour. However, increased densities may have negative consequences in terms of greater risks per unit.

Even though temperature was not a parameter dealt with in these experiments, we noticed it had dual negative effects; it inhibited growth during both cold seasons (Fig. 1), and increased mortality of 4-8 months old crocodiles during their first cold season. These two adverse effects may be overcome by increasing the temperature of the ponds during the cold seasons as suggested by Blake and Loveridge (1975), but further experiments are needed to test this.

ACKNOWLEDGEMENTS

Thanks are due to Clal Crocodile Farm Ltd., and particularly to Mr. S. Ranot and Mr. K. Kagan who made this study possible. Special thanks are due to Mrs. Amalia Zilber for her continuous help and support throughout the study. We are grateful to the Kenya Department of Wildlife and Management for allowing us to collect eggs along the Tana River and for the assistance they gave us. This study is part of an M.Sc. thesis of Mr. A. Zilber.

REFERENCES

- Blake, D.K. and Loveridge, J.P. (1975). The role of commercial crocodile farming in crocodile conservation. *Biol. Conserv.* 8, 261-272.
- Chabreck, R.H. (1967). *Alligator farming hints*. Int. Report, Louisiana Wildlife and Fisheries Commission, Grand Chenier, Louisiana, 9 pp.
- Chabreck, R.H. and Joanen, T. (1979). Growth rates of American alligator in Louisiana. *Herpetologica* 35, 51-57.
- Elsey, R.M., Joanen, T and McNease, L. (1990). Growth rate and plasma corticosterone levels in juvenile alligators maintained at different stocking densities. J. Exp. Zool. 255, 30-36.
- Joanen, T., McNease, L., Tarver, J. and Behler, J. (1981). Captive propagation of alligators in Louisiana. Int. Herp. Cong., Oxford England.
- Webb, G.J.W., Mandis, S.C. and Whitehead, P.J. (eds.) (1987). Wildlife Management: Crocodiles and alligators. Surrey Beatly & Sons. Ltd., Australia.

BOOK REVIEWS:

The Frogs of Zimbabwe. A.J.L. Lambiris. (1990). 247pp. Museo Regionale di Scienze Naturale, Torino. (Monographie X). 70 000 Lire.

This is the first book to cover the 65 species of Zimbabwean amphibians. Although several good books covering the amphibians of South Africa are available, many of the tropical species found in Zimbabwe do not occur south of the Limpopo.

The author was born in Zimbabwe and studied the local herpetofauna until he moved to Natal in 1983, where he joined the Natal Parks Board and in 1988 was awarded an M.Sc. degree (*cum laude*) by the University of Natal (Durban) for his thesis on the amphibians of Natal/Kwazulu. He is now working on his Ph.D. thesis.

The author states that the main purpose of the book is to provide a guide to the identification and general biology of the frogs of Zimbabwe for teachers, naturalists, conservationists, and biologists who, though not specialists in the subject, may have to work with and study these animals.

A brief outline of the origins and classification of frogs is followed by sections covering their biology and ecology, directions for collecting and preserving specimens, and illustrated descriptions of the morphological features used in the identification of amphibians.

The systematic account begins with an index of the taxa covered and a key to the 23 genera. The diagnosis of each genus is followed by a key to the local species. Each species account includes a diagnosis, description, and data on habitat and behaviour, call and call site, breeding and development, and range within Zimbabwe. The hand and foot of each species and lateral view and mouthparts of the tadpole (where known) are illustrated in the text, while the 24 colour plates and the distribution maps appear at the end of the book. The colour plates (227 watercolour paintings by the author) usually show the variation in colour pattern within each species, with the frog in a standard pose for ease of comparison: dorsolateral and ventral views are usually given.

This book admirably fills a serious gap in the literature covering the vertebrates of Zimbabwe. Not only are the adult frogs well illustrated, but in most cases the tadpoles as well.

In a few cases the colouration in the plates is inaccurate, e.g. adults of *Pyxicephalus adspersus edulis* are usually brown or olive rather than green; the dorsal ground colour of both species of *Strongylopus* is usually yellow rather than brown and the light gold dorsum of *Hylarana darlingi* is normally strongly contrasted with the dark brown flanks.

The ecological data presented is comprehensive, but a few additional points might be mentioned. *Schismaderma carens* is the toad normally encountered in caves and mine adits (where bat guano supports good populations of invertebrates). *Stephopaedes anotis* is usually found beneath or inside rotting logs, but may be seen hopping around in the leaf litter after rain. *Tomopterna marmorata* and *Hemisus marmoratus* aestivate beneath the sand in river beds.

D.G. Broadley

Checklist of the Amphibians and Reptiles of Connecticut with Notes on Uncommon Species. Michael W. Klemens. (1991). 24pp. Connecticut Department of Environmental Protection, Bulletin No. 14.

This useful little book provides a concise introduction to the Connecticut herpetofauna. Rather more than just a checklist, the book is largely a result of the author's own extensive field surveys carried out over a period of some fifteen years. All forty-five reptiles and amphibians occurring in the State are illustrated with colour photographs, and brief field notes are provided for twelve of the uncommon species. The checklist indicates the status of each species within Connecticut, and a list of species erroneously reported to be indigenous to the State is thoughtfully provided. The book will prove a valuable companion to anyone in this part of North America who is unfamiliar with the local herpetofauna.

Richard Griffiths



THE HERPETOLOGICAL JOURNAL

INSTRUCTIONS TO AUTHORS (revised January 1992)

- I. The Herpetological Journal publishes a range of features concerned with reptile and amphibian biology. These include: full papers (no length limit); reviews and mini-reviews (generally solicited by a member of the editorial board); short notes; controversies, under 'Forum' (details available from the Editor); and book reviews. Faunistic lists, letters, and results of general surveys are not published unless they shed light on herpetological problems of wider significance.
- 2. *Three* copies of all submissions, and illustrations, should be sent to the Editor. All papers will be subject to peer review by at least two referees.
- 3. Authors should consult a recent issue of the Journal regarding style. Papers should be concise with the minimum number of tables and figures. They should be written in English and spelling should be that of the *Oxford English Dictionary*. Papers should be typed or produced on a good-quality printer (at least near-letter quality, avoid worn ribbons), and double-spaced with wide margins all round. Typesetting is greatly assisted if accepted manuscripts can be supplied on microcomputer diskettes. Authors are therefore strongly encouraged to produce manuscripts using a wordprocessor (preferably on a PC-compatible microcomputer).
- 4. For all papers the title page should contain only the following: title of paper; name(s) of the author(s); address of the Institution where the work was done; a running title of 5 words or less. The text of the paper should begin on page 2 and be produced in the following order: Abstract, Text, Acknow-ledgements, References, Appendices. Full papers and reviews should have the main text divided into sections. Short notes (generally less than six manuscript pages and accompanied by a single data set) should be produced as continuous text. The first subhead will be centred in capitals, the second shouldered in lower case, and the third run on in italics. Footnotes are not permitted.
- 5. The usual rules of zoological nomenclature apply.
- 6. Tables are numbered in arabic numerals, e.g. Table 1; they should be typed double-spaced on separate sheets with a title/short explanatory paragraph underneath.
- 7. Line drawings and photographs are numbered in arabic numerals, eg. Fig. 1. Colour photographs can only be included at cost to the author. If an illustration has more than one part each should be clearly identified as (a), (b), etc. The orientation and name of the first author should be indicated on the back. They should be supplied camera-ready for

uniform reduction of one-half on A4 size paper. Line drawings should be drawn and fully labelled in Indian ink, dry-print lettering, or laser printed. A metric scale must be inserted in micrographs etc. Legends for illustrations should be typed on a separate sheet.

- 8. References in the text should be given as in the following examples: "Smith (1964) stated..."; "...as observed by Smith & Jones (1963)."; "...as previously observed (Smith, 1963; Jones, 1964; Smith & Jones, 1965)". For three or more authors the complete reference should be given at the first mention, e.g. (Smith, Jones & Brown, 1972), and *et al.* used thereafter (Smith *et al.*, 1972). For the list of references the full title or standard abbreviations of the journal should be given. The following examples will serve to illustrate the style and presentation used by the Journal:
 - Bellairs, A. d'A. (1957). *Reptiles*. London: Hutchinson.
 - Boycott, B.B. & Robins, M.W. (1961). The care of young-red-eared terrapins (*Pseudemys scripta* elegans) in the laboratory. *British Journal of Herpetology* 2, 206-210.
 - Dunson, W.A. (1969a). Reptilian salt glands. In Exocrine glands, 83-101. Botelho, S.Y., Brooks, F.P. & Shelley, W.B. (eds). Philadelphia: University of Pennsylvania Press.
 - Dunson, W.A. (1969b). Electrolyte excretion by the salt gland of the Galapagos marine iguana. *American Journal of Physiology* 216, 995-1002.
- 9. Final acceptance of a paper will depend upon the production by the author of a typescript and illustrations ready for the press. However, every assistance will be given to amateur herpetologists to prepare papers for publication.
- 10. Proofs should be returned to the Editor by return of post. Alterations should be kept to the correction of errors; more extensive alterations will be charged to the author.
- 11. Twenty-five offprints and one complimentary copy of the Journal containing the paper are provided free of charge. Further copies (minimum of twentyfive) may be purchased provided that they are ordered at the time the proofs are returned.
- 12. All submissions are liable to assessment by the editorial board for ethical considerations, and publication may be refused on the recommendation of this committee. Contributors may therefore need to justify killing or the use of other animal procedures, if these have been involved in the execution of the work.

THE HERPETOLOGICAL JOURNAL

Volume 2, Number 1 1992

CONTENTS

Aquatic oxygen conformity in the Mexican axolotl, Ambystoma mexicanum	H.I. Griffiths & D.H. Thomas	1
Insemination and egg laying dynamics in the smooth newt, <i>Triturus vulgaris</i> , in the laboratory	A. Pecio	5
Egg, clutch and maternal sizes in lizards: intra- and interspecific relations in near-Eastern Agamidae and Lacertidae	E. Frankenberg & Y.L. Werner	7
Sodium chloride and potassium chloride tolerance of different stages of the frog, <i>Microhyla omata</i>	A.D. PADHYE & H.V. GHATE	18
Plasma concentrations of aldosterone and electrolytes in Gallotia galloti (Sauria: Lacertidae)	M. Diaz & A. Lorenzo	23
Habitat selection by the lizard Lacerta lepida in a Mediterranean oak forest	A. M. Castilla & D. Bauwens	27
The effect of stocking density, origin of eggs and water flow on growth, survival and body condition of Nile crocodiles (<i>Crocodilus niloticus</i>)	A. Zilber. D.N. Popper & Y. Yom-tov	31
Baak Reviews		35