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FRONT COVER: Rainbow lizard, Agama agama (M. Lewis)

REVIEW:

DISCREPANT USAGE OF THE TERM 'OVOVIVIPARITY' IN THE HERPETOLOGICAL LITERATURE

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A broad survey of the literature on reptiles and amphibians reveals that the ambiguous term 'ovoviviparity' has been applied to a variety of reproductive patterns that have little in common. Among these are patterns that can more clearly be referred to as aplacental viviparity, oviparous egg-retention, egg-tending, pseudoviviparity, and lecithotrophy. Some of the uses of 'ovoviviparity' are based on invalid assumptions, and some are mutually exclusive; thus, particular care must be used in interpreting literature reports that lack operative definitions. To minimize confusion, future reports and reviews should avoid 'ovoviviparity' in favour of unambiguous alternatives that explicitly distinguish patterns defined on the basis of reproductive products at deposition from patterns based on sources of nutrients for embryonic development.

INTRODUCTION

Comparative biology seeks to document organismal diversity by distinguishing recurrent themes and common patterns. Fundamental to attempts to recognize patterns that unify species is agreement on a common vocabulary that transcends boundaries of taxon, discipline, and language. Unfortunately, reproductive biology has long been handicapped by several terminological discrepancies, including the use of the term 'ovoviviparity' to describe very different patterns in separate species. Inattention to terminology coupled with the disinclination of many writers to explain their terms has given rise to conceptual confusion, as well as discrepant claims for many species (Blackburn, 1982a; 1993a). Although one's choice of words to represent reproductive modes is a trivial matter, contradictory terminologies, when overlooked, hinder attempts to recognize and communicate about significant biological phenomena (Packard et al., 1989).

This review has three goals: (1) to document the ambiguous and contradictory ways in which the term 'ovoviviparity' is used in the literature on reptiles and amphibians; (2) to alert researchers to use caution in interpreting reports that do not explain terminological usage; and (3) to encourage avoidance of 'ovoviviparity' and its cognates in favour of clearer alternatives that are now available.

PROVISIONAL TERMINOLOGY

Because this paper deals with the application of terms to reproductive concepts and patterns, a provisional vocabulary is necessary for the sake of communication. I will follow common usage in employing 'egg-laying reproduction' in its literal sense, i.e. in reference to species that reproduce by laying unhatched eggs that complete their development in the external environment. This category includes species with external fertilization, as well as those with internal fertilization in which some development precedes oviposition (e.g. Shine, 1983; Braña, Bea, & Arrayago, 1991), the reproductive product being an 'egg' rather than an autonomous, free-living offspring. 'Live-bearing' is used to describe species in which the female retains the eggs to term in her reproductive tract and bear fully-developed, autonomous offspring (neonates, larvae, or metamorphosed young). For most purposes, cases in which the young are still surrounded by an eggshell or jelly coat at birth but quickly break through these egg investments can also be considered 'livebearing'. The terms 'oviparous' and 'viviparous' frequently are applied to 'egg-laying' and 'live-bearing', respectively (e.g. Fitch, 1970; Tinkle & Gibbons, 1977). This terminology is discussed further below.

PAST USES OF 'OVOVIVIPARITY'

'Ovoviviparity', including its adjective form ('ovoviviparous') and non-English equivalents (e.g. 'ovoviviparité', 'ovoviviparie'), is a term with a long, varied history (Bertin, 1952; Bauchot, 1965; Luckett, 1977; Mackie, 1978; Wourms, 1981; Guillette, 1981, 1982; Blackburn, 1985a; Smith, 1986a). No form of the word appears in certain early, important works in zoology and embryology (Malpighi, 1672; Collins, 1685; Cuvier, 1802; Lamarck, 1809), and its origins appear not to have been traced. Bellairs (1970) speculated that the word was first used to describe reptiles that ovulated large yolks and gave birth to their offspring. Such may be the case, for by the 1830's, the term was being applied to lizards, snakes, and urodeles in which the young develop within the female reproductive tract and are born alive (Dumeril & Bibron, 1834; Owen, 1834*a*; Bonapart, 1837). Richard Owen (1834b) explicitly used the adjective 'ovoviviparous' to describe animals that give birth to their offspring yet never form a placenta, a pattern that he erroneously attributed to the monotreme and marsupial mammals. However, the term was by no means universally accepted, and some herpetological works of the 19th century noted difficulties in distinguishing 'ovoviviparity' from the commonly accepted categories of 'oviparity' and 'viviparity' (Haacke, 1885).

In the 20th century, the term 'ovoviviparity' has been used to represent a variety of reproductive patterns. The chief feature that these patterns have in common is that eggs begin to develop in some sort of association with one of the parents; however, the nature and duration of this association varies, as does the parent with which the association occurs. In the herpetological literature, the term 'ovoviviparity' has been used to represent at least five distinct reproductive patterns. Because formal definitions are seldom offered in sources using the word, these patterns are described below to provide operative meanings (i.e. usages) for the term.

Pattern 1 involves internal fertilization and partial development of the eggs within the maternal reproductive tract. The eggs at oviposition contain visible embryos, and continue their development in the external environment, where they eventually hatch. 'Ovoviviparity' has been applied to this pattern in egglaying squamates (e.g. McPhee, 1959; Brattstrom, 1964; Duda & Koul, 1977; Lemus, Illanes, Fuenzalida, Paz de la Vega & Garcia, 1981) as well as amphibians (e.g. von Wahlert, 1953; Grandison, 1978; Wake, 1980); some authors have applied additional criteria as well (see below). This usage has a long, respectable history; it extends to the early part of this century (Rollinat, 1904), and is found in both pioneering and modern works on development (Hrabowski, 1926; Weekes, 1930; Lemus, Martín, Blánquez, Fuenzalida & Illanes, 1984). In contrast, many recent authors have labelled this pattern as a form of 'oviparity' (e.g. Tinkle & Gibbons, 1977; Shine & Bull, 1979; Shine, 1985) and occasionally, 'viviparity' (Turner, 1947).

Pattern 2 is restricted to anamniotes, because the site of metamorphosis is central to its recognition. Fertilization is internal, and the eggs develop in the maternal reproductive tract no further than a premetamorphic stage; thus, the young at birth are larvae that metamorphose in the external environment. These larvae either hatch from the egg membranes before birth or immediately thereafter; thus, unlike Pattern 1, this pattern operationally can be considered a form of 'live-bearing' reproduction, as described above. Among amphibians, this form of reproduction is found in many populations of the urodele Salamandra salamandra (Joly, 1968; Fachbach, 1969, 1976). Although usually termed 'viviparity', this pattern often has been referred to as 'ovoviviparity' in literature on Salamandra, to distinguish it from maternal retention of the young through metamorphosis (Fachbach, 1969; Özeti, 1979; Warburg, Degani & Warburg, 1979).

Pattern 3 is a form of live-bearing reproduction in which nutrients for development are supplied by the yolk of the oocyte, rather than by an alternative source. Other potential sources of developmental nutrients in live-bearing amphibians and reptiles include placental membranes, oviductal secretions, and sibling yolks or embryos (Amoroso, 1952; Wake, 1977a, b, 1982, 1985, 1993; Xavier, 1977; Blackburn, 1985a, 1993b; Jones & Baxter, 1991). The term 'ovoviviparity' has been applied to live-bearing species in which the organic nutrients for development are of vitelline origin, in literature on both squamates (e.g. Domergue, 1959; Smith, Sinelnik, Fawcett, & Jones, 1972) and amphibians (Salthe & Mecham, 1974; Wake, 1978; Greven, 1980: Duellman, 1992). In other recent literature, livebearing species often have been termed 'viviparous' regardless of the sources of developmental nutrients (e.g. Fitch, 1970; Packard, Tracy & Roth, 1977; Marini, Trevisan & Benassi, 1983; Jones & Baxter, 1991).

Pattern 4 is similar to the previous pattern, but focuses on the presence or absence of anatomical features presumed to affect physiological exchange between foetal and maternal tissues. Thus, early sources often applied 'ovoviviparity' to live-bearing squamates in which any trace of an eggshell (shell membrane) is grossly visible around the oviductal egg during gestation (Harrison & Weekes, 1925; Kasturirangan, 1951*a*; Miller, 1959; Bellairs, 1970), the rationale being that even a thin shell would limit physiological exchange. According to this usage, truly 'viviparous' squamates are those live-bearing forms in which an eggshell disappears during gestation or is never deposited (Weekes, 1929; Kasturirangan, 1951*b*).

A similar approach has been to classify as 'ovoviviparous' any live-bearing squamates lacking placental organs that accomplish physiological exchange (Jacobi, 1936; Matthews, 1955; Smith *et al.*, 1972; Guillette, 1981). However, recent researchers have seriously questioned whether any live-bearing squamates lack functional placentas (Guillette, 1982; Smith, Preston & Smith, 1983; Blackburn, 1985*a*, 1992, 1993*b*), and this criterion seems to have fallen into disuse (Smith, 1986*a*,*b*; Guillette, 1987; Jones & Baxter, 1991).

Pattern 5 is represented by the unusual reproductive habits of certain egg-laying anurans. Eggs are fertilized externally, and subsequently are cared for by one of the parents. Eggs are brooded in the stomach, vocal sacs, or depressions in the dorsal integument, or carried on the back or legs. In some species, the parent carries hatched tadpoles on its dorsum. Such forms of parental care have been widely documented and described; for recent reviews see Salthe & Mecham (1974), Wake (1982), and Duellman (1992). These anuran patterns have been referred to as forms of 'ovoviviparity' by several authors (e.g. Amoroso, 1952; Salthe & Mecham, 1974), although others have described them as 'oviparity' (Webster & Webster, 1974) and 'viviparity' (Matthews, 1955, 1958; Jones & Baxter, 1991).

CONTRADICTORY CRITERIA AND THEIR CONSEQUENCES

Reproductive patterns that have been represented by the term 'ovoviviparity' have little in common. They include species with internal as well external fertilization, and species in which the reproductive product exiting the female is an immature, developing egg and those in which it is a fully-developed neonate. In some patterns, the eggs develop in the maternal oviducts whereas in another, eggs develop externally, but the hatchlings are carried on the back of the male parent. Some of the patterns have been defined with a focus on the reproductive product itself, whereas others are distinguished on the basis of the source of nutrients for development, two distinct parameters whose conflation arguably has obscured patterns of reproductive evolution (Blackburn, 1992).

Some criteria for application of the term are based on invalid assumptions. For example, in Pattern 4 above, one rationale for distinguishing live-bearing squamates as 'ovoviviparous' (as opposed to 'viviparous') when a shell membrane occurs is that this structure is presumed to limit or prevent significant physiological exchange beween maternal and foetal tissues. However, because a shell membrane is deposited around the egg in all live-bearing squamates that have been examined (Yaron, 1985; Blackburn, 1993b), no such distinction can be made. Moreover, although the shell membrane usually thins during gestation in live-bearing forms, it typically persists until birth (Heulin, 1990), including in certain species with significant physiological exchange between maternal and foetal tissues (Hoffman, 1970; Stewart, 1989, 1990; Stewart, Blackburn, Baxter, & Hoffman, 1990). The dubious assumption that some live-bearing squamates lack placental organs is discussed above.

Some of the criteria for 'ovoviviparity' apply to a limited range of species. Among these criteria is presence of an eggshell in a live-bearing form (Pattern 4), which is applicable to squamates and marsupials, but allows no discrimination among amphibian and osteichthyan species. Similarly, that pattern's focus on placental formation ignores the array of non-placental sources of extravitelline nutrients found among anamniotes (e.g. Wourms, 1981; Wake, 1982, 1993). Likewise, the focus on pre-metamorphic larvae in Pattern 2 may be of use in reference to Salamandra, but is of little value for amniotes, chondrichthyans, or even other amphibians. Other criteria for application of the term are so broad as to obscure important distinctions. For example, given that embryonated eggs are deposited by most or all egg-laying squamates (Shine, 1983) as well as by monotremes (Hughes & Carrick, 1978), these species would all be classified as 'ovoviviparous'

by the criteria of *Pattern 1*. In addition, by extension of the criteria of *Pattern 5*, 'ovoviviparity' would apply not only to the anurans described above, but also to monotremes, many egg-laying teleosts, and arguably, crocodilians and birds.

Given the varied, discrepant application of 'ovoviviparity', use of the term has the potential to produce much confusion. Nevertheless, few sources provide definitions or explanations (for exceptions, see Wake, 1982, 1993; Angelini & Ghiara, 1984; Mossman, 1987), perhaps under the assumption that widely-used terms are universally understood. Without detailed information, however, statements in the literature that a particular species is 'ovoviviparous' carry little meaning. Unfortunately, researchers who routinely apply the term to taxa that they study are not always aware of incongruent uses to which the same term has been put in other taxa. For example, although the adjective 'ovoviviparous' is often taken to be synonymous with 'live-bearing' in reference to squamates, in one of its common applications the former term is actually a variant of 'egg-laying' reproduction. One result has been contradictory claims about reproductive habits in various species of egg-laying squamates (Blackburn, 1985*a*,*b*). Similar terminological discrepancies are suspected in literature on other species for which contradictory reports are available (Blackburn, 1993a). Such terminological issues cannot be ignored if we wish to communicate accurately about reproductive patterns (Packard et al., 1989).

TERMINOLOGICAL ALTERNATIVES

Considering the contradictory usage of the word 'ovoviviparity', one possible solution might be to develop a new definition for the term and to encourage its careful application. However, such does not seem to be a viable option. The word has nearly lost the potential to impart meaning; confusion surrounding its use offers little prospect for establishing it as an unambiguous part of our biological vocabulary. Etymologically, the word lacks coherence; it seems to be a hybrid of the common terms 'oviparity' and 'viviparity', despite the fact that each of the patterns represented by 'ovoviviparity' is a subset either of egg-laying or of live-bearing reproduction. An additional problem is that most uses of the term attempt to distinguish a distinct category within biological continua, and therefore are difficult to apply to actual species. For these and other reasons, various reviewers (e.g. Bertin, 1952; Bauchot, 1965; Blackburn, 1982b, 1992; Smith, 1986a; also see Angelini & Ghiara, 1984) have recommended that the term be deleted from the modern biological lexicon. Such a course is consistent with the trend away from use of the term, especially evident in literature on squamates and fishes during the past decade.

How, then, can we represent and communicate about the reproductive patterns listed above? The most promising solution lies in the substitution of alternative

Pattern, description	Recommended term	Examples
(1) Oviposition of developing eggs	oviparity ^a	Liolaemus tenuis ^b , Nectophrynoides malcomi ^c
(2) Live-birth of pre- metamorphic larvae	viviparity	Salamandra salamandra ^d
(3) Live-bearing + mainly vitelline nutrients	lecithotrophic viviparity	Virginia striatula ^e , N. viviparus ^c , S. salamandra ^d
 (4) Live-bearing + no placenta; Live-bearing + shell membrane 	aplacental viviparity; viviparity	N. viviparus °, N. occidentalis ° Thamnophis sirtalis °
(5) Parental care of eggs	egg-brooding	oviparous Eumeces ^b ,
Parental care of eggs	pseudoviviparity ^f	Gastrotheca °, Rhinoderma darwini °, Rheobatrachus silus °

TABLE 1. Recommended alternatives to the term 'ovoviviparity' in descriptions of amphibians and reptiles. See the text for explanations of the numbered reproductive patterns. Reproductive data on the listed species are available in the following sources: Fachbach (1976), Wake (1980, 1982), Lemus *et al.* (1981), Duellman (1992), Stewart (1989), and Blackburn (1993*a*, 1994). The term 'matrotrophy' is recommended for substantial provision of extra-vitelline nutrients for development (e.g. *Nectophrynoides occidentalis, Salamandra atra*, and certain viviparous caecilians, squamates, and other vertebrates). ^a or 'oviparous egg-retention' when considerable development precedes oviposition; ^b Sauria; ^c Anura; ^d Urodela; ^e Serpentes; ^f see text for application of this term.

terms already in use in the herpetological literature. Most of the reproductive patterns described above are defined either by the type of reproductive product exiting the parent (*Patterns 1, 2, and 5*) or by the sources of nutrients for development (*Pattern 3*). The remaining pattern (*Pattern 4*) is indirectly based on nutritional sources, in that it focuses on features presumed to affect maternal-foetal exchange.

A bipartite terminology that has been introduced to the literature is adequate to represent both of these types of reproductive parameters. To describe the reproductive product deposited by the female (egg vs. neonate), the widely-used terms 'oviparity' and 'viviparity' are applied in their literal senses (e.g., Fitch, 1970; Marini et al., 1983; Braña, 1986; Heulin, 1988), that is, in reference to 'egg-laying' and 'livebearing' reproduction (as defined above) respectively. Accordingly, 'oviparous' species include those that deposit unfertilized eggs as well as those that lay developing eggs that complete their development in the external environment. By the same token, 'viviparous' species give birth to autonomous, free-living offspring, whatever their state of maturity. In reference to egg-laying squamates in which an unusual amount of embryonic development precedes oviposition, the term 'oviparous egg-retention' can be useful (Shine, 1983; Blackburn, 1985b). In addition, the term 'ovuliparity' is available to describe a type of oviparous reproduction involving maternal deposition of unfertilized eggs (Bertin, 1952; Blackburn, 1985a), as occurs in agnathans, many anurans and osteichthyans, and certain urodeles.

To represent the sources of nutrients for development, two other terms that were first introduced to the piscine literature (Wourms, 1981) have proven applicable to vertebrates in general (Blackburn, 1982b, 1992, 1994; Stewart, 1989, 1992). 'Lecithotrophy' describes provision of nutrients via the yolk, and 'matrotrophy' describes maternal provision of nutrients during gestation by some other means (e.g. oviductal secretions, placental tissues). These terms are derived from the words lekithos (Greek for egg-yolk), mater (Latin for mother), and trophe (Greek for nourishment). Because lecithotrophic and matrotrophic nutrition represent extremes of a continuum, species can be described according to the relative contributions of nutrient sources. Furthermore, specific types of matrotrophy can be recognized according to whether nutrients are supplied by placental membranes ('placentotrophy'), sibling sibling ('oophagy'), foetuses yolks

('adelphophagy') or maternal secretions that are absorbed ('histotrophy') or ingested ('histophagy') (Wourms, 1981; Blackburn *et al.*, 1985).

The above terminology offers several advantages. First, the adoption of terms that translate literally into their intended meanings minimizes the chances for semantic confusion. Second, because each of the two sets of terms is applied to a different parameter (reproductive product vs. nutrient sources), the important distinction between these parameters is made explicit. Thus, species can be described as exhibiting lecithotrophic oviparity, lecithotrophic viviparity (e.g., Stewart et al., 1990; Stewart, 1992), matrotrophic viviparity (Blackburn & Vitt, 1992), and in rare cases, matrotrophic oviparity (Blackburn, 1992). Third, the existence of two sets of terms allows investigators to focus on the feature of interest, while allowing a more complete description as information becomes available. Fourth, unlike other systems, the bipartite system does not presuppose a particular evolutionary sequence, but is flexible enough to recognize that matrotrophy and viviparity can evolve according to different pathways in different taxa. The common assumption that strictly lecithotrophic viviparity (one of the variants of 'ovoviviparity') evolves prior to matrotrophic viviparity appears to be unjustified for both reptiles and mammals, if not other vertebrates (Blackburn, 1985a, 1992). Finally, application to all amphibians and reptiles, of a vocabulary that is achieving acceptance in literature on squamates and the three piscine classes, facilitates species comparisons across the broad spectrum of vertebrate diversity. Detailed criteria for inferring parity modes and sources of developmental nutrients in reptiles, and thereby for applying the proposed terminology to particular species, are explored elsewhere (Blackburn, 1993a, 1994).

In Table 1, application of the recommended terminology is illustrated by reference to the reproductive patterns that previously have been labelled as 'ovoviviparity'. As indicated, each of the five patterns can be described by a different set of terms, allowing fine distinctions to be drawn between the species listed.

Two other points deserve mention. The proposed terminology does not explicitly distinguish between populations of Salamandra that give birth to larvae and those that give birth to metamorphosed young (see Pattern 2 above). In the mammalian literature, the terms 'altricial' and 'precocial' describe the state of development of the young at emergence; perhaps application of analogous terms might be useful in descriptions of this urodele genus. The proposed terminology also offers no special recognition of the anuran specializations of Pattern 5, which would be classified here as variants of oviparous lecithotrophy. The common term 'brooding' (see Somma, 1988) should be adequate in most cases, with 'egg-tending' and 'parental care' as further alternatives. For anurans in which the eggs are brooded until hatching in the stomach or vocal sacs, the term

'viviparity', by definition, would be deemed inappropriate. However, such species might be referred to as 'pseudoviviparous' (Table 1; Blackburn, 1985*a*), in recognition of their superficial similarity to species in which the eggs develop to term in the female reproductive tract.

Individual researchers should continue to enjoy the freedom to employ terminologies and classification systems that prove most useful for their own purposes. Nevertheless, conceptual clarity requires explicit attention to the tools of communication, particularly in view of the potential for confusion that has long been presented by use of 'ovoviviparity' and its cognates in the zoological literature.

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THE EMBRYO AND HATCHLING MORTALITY OF THE GREEN TURTLE (CHELONIA MYDAS) AND OLIVE RIDLEY (LEPIDOCHELYS OLIVACEA) IN RELATION TO CLUTCH SIZE

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This study was carried out at the Kosgoda Victor Hasselblad Turtle hatchery in Sri Lanka, between December 1988 and November 1989. A total of 64 nests of *Chelonia mydas* and 32 nests of *Lepidochelys olivacea* were examined after the emergence of hatchlings. The percentage late mortality (late embryonic stages and early hatchlings) of *C. mydas* and *L. olivacea* showed a positive relationship with the increasing number of eggs in a clutch. There was no significant relationship between the percentage early embryonic mortality and the clutch size. The percentage of live hatchlings of both species showed a negative relationship with the increasing clutch size. Dividing and transplanting *C. mydas* clutches with more than 120 eggs and *L. olivacea* clutches with more than 110 eggs may result in higher percentages of live hatchlings. Investigations on the incubation temperature of small egg clutches need to be made in order to find the effect on the sex ratio of hatchlings.

INTRODUCTION

Five species of marine turtles nest on the beaches of Sri Lanka. They are the green turtle (*Chelonia mydas*), olive ridley (*Lepidochelys olivacea*), leatherback (*Dermochelys coriacea*), hawksbill (*Erythmochelys imbricata*), and the loggerhead (*Caretta caretta*). Artificial hatchery programs are implemented as a means of conserving turtles. Unlike in other countries there are no trained personnel to collect eggs. Instead eggs are collected by villagers and are brought to the hatcheries. It was observed that the mortality of hatchlings in large egg clutches of *L. olivacea* in hatcheries was high. The objective of this study was to find whether there is a relationship between hatchling mortality and clutch size of marine turtles in hatcheries.

METHODS

The standard practice in hatcheries is to transplant egg clutches of all five species of turtles within the same enclosure. Each clutch is transplanted in a separate nest in the same sequence as they are brought to the hatchery. The dimensions of the transplanted nests are similar to those of the natural nests, and the spacing between nests is 50 cm. This study was carried out with such transplanted nests of *C. mydas* (n=64) and *L. olivacea* (n=32) at the Kosgoda, Victor Hasselblad turtle hatchery from December 1988 to November 1989. Nests of the other three species of marine turtles were rare.

In order to examine the contents, the transplanted nests were excavated 4-6 days after the emergence of hatchlings (C. mydas nests about 52 days and L. olivacea about 58 days after burying). The contents at the bottom of some of the nests were found lumped together (especially those of L. olivacea during the rainy season) at the time of excavation. This made it difficult to distinguish the individuals that had died at various

stages of the hatching process (i.e. individuals that were completely out of the shell, those that were half-way out, and those that had pipped the egg shell). Under these circumstances, the contents of the excavated nests were categorized as follows:

(1) *Infertile eggs.* Eggs without embryos and without evidence of development visible to the naked eye. Spoilt eggs (blackened eggs, eggs infested with maggots, eggs in which the yolk and the albumin were mixed in a liquid consistency or like a solid yellow mass) were also included in this category.

(2) Early embryonic mortality (Early mortality). Eggs with evidence of development such as blood vessels and pre-carapace stage embryos.

(3) Late embryonic and early hatchling mortality (Late mortality). Dead embryos with carapaces, dead hatchlings that had pipped the egg, those that were half-way out of the shell and those that had completely come out of the shell.

(4) *Total mortality*. Early mortality and late mortality together was considered as the total mortality.

(5) *Live hatchlings.* Live hatchlings that were assisted out of the nest at the time of excavation were categorized together with the hatchlings that had already emerged.

RESULTS

The categories 2, 3, 4 and 5 were presented as percentages of the total number of eggs in a clutch (known prior to transplanting) and also as percentages of fertile eggs in a clutch (fertile eggs = total number of eggs – infertile eggs). The mean percentages of these categories are given in Table 1.

Correlation analysis revealed (Table 2, Figs. 1 & 2) that there is a positive relationship between the total number of eggs (clutch size) and the percentage late mortality of *C. mydas* and *L. olivacea*. A similar rela-

	%Live Hat.	%Early M.	%Late M.	%Total M.	
Me	an 71.97	7.29	11.03	18.32	
	(79.03)	(8.25)	(12.52)	(20.77)	
SD	17.02	7.05	10.43	12.10	
	(14.63)	(10.39)	(12.74)	(14.82)	
n	64	64	64	64	
L. olivace	ea				
Me	an 61.82	11.66	14.09	25.75	
	(69.74)	(14.35)	(15.91)	(30.26)	
SD	23.16	12.22	18.13	17.19	
	(19.33)	(15.26)	(19.78)	(19.33)	
n	32	32	32	32	

TABLE 1. Live hatchlings, early, late, and total mortalities of *C. mydas* and *L. olivacea* as a percentage of the total number of eggs and as a percentage of fertile eggs (figures given in parentheses) in a clutch. n = number of clutches, Hat. = hatchlings, M. = mortality.

	%	Total no. of eggs vs 6Live Hat.	Total no. of eggs <i>vs</i> %Early M.	Total no. of eggs <i>vs</i> %Late M.	Total no. of eggs <i>vs</i> %Total M.
С. ти	das	5			
	n	64	64	64	64
	r	-0.32	0.1	0.33	0.36
	Р	< 0.05	NS	< 0.01	<0.01
	df	62	62	62	62
L. oliv	vac	еа			
	n	32	32	32	32
	r	-0.58	0.14	0.58	0.52
	Р	< 0.01	NS	< 0.01	< 0.01
	df	30	30	30	30

	ľ	lo. fertile	No. fertile	No. fertile	No. fertile
		eggs	eggs	eggs	eggs
		vs	vs	vs	vs
	%	Live Hat.	%Early M.	%Late M.	%Total M.
C my	ła				
C. 11.90	1	, 64	64	64	64
		0.36	01	0.35	0.37
	_	-0.30	0.1	0.55	0.57
F)	< 0.01	NS	< 0.01	< 0.01
d	f	62	62	62	62
L. oliv	ac	еа			
n	1	32	32	32	32
r		-0.57	0.06	0.61	0.57
F)	< 0.01	NS	< 0.01	< 0.01
d	f	30	30	30	30

TABLE 3. Correlation coefficients for the number of fertile eggs and live hatchlings, early, late and total mortalities of C. mydas and L. olivacea. NS= not significant, Hat. = hatchling, M. = mortality.

			Corre	elation coef	fficient
i e	No. eggs	df	%Live Hat.	%Late M.	%Total M.
C. myda	S				
	170	62	-0.32*	0.33**	0.36**
<	(160	61	-0.24NS	0.36**	0.28*
<	130	54	0.24NS	0.30*	0.24NS
<	120	43	0.36*	0.40**	0.28NS
<	110	27	-0.20NS	0.30NS	0.22NS
<	100	16	-0.14NS	0.00	0.00
L. olivac	cea				
<	150	30	-0.58**	0.58**	0.51**
<	130	27	-0.45*	0.37NS	0.24NS
<	120	25	-0.49*	0.39*	0.26NS
<	110	23	0.40*	0.00	0.00
<	100	20	-0.37NS	0.00	0.00
<	<90	15	0.1NS	0.00	0.00

TABLE 2. Correlation coefficients for the total number of eggs and live hatchlings, early, late and total mortalities of C. mydas and L. olivacea. NS= not significant, Hat. = hatchling, M. = mortality.

TABLE 4. Significant cut off points in the total number of eggs that reduce the percentage live hatchlings of *C. mydas* and *L. olivacea.* (*P < 0.05; ** P < 0.01). Hat. = hatchling, M. = mortality.



FIG. 1. Relationship between the total number of C. mydas eggs and (a) live hatchings (b) early mortality (c) late mortality (d) total mortality.

tionship existed between the clutch size and the percentage total mortality for both species, while there was no significant relationship between the clutch size and the percentage early mortality. The percentage of live hatchlings of both species showed a negative relationship with the increasing clutch size.

Similar relationships were found when only the fertile eggs in a clutch were considered (Table 3).

The following method was adapted to find the maximum number of eggs in a nest that will not reduce the percentage of live hatchlings. The clutches with a total number of eggs above a certain value were progressively reduced and the correlation analyses were repeated on these reduced samples (Table 4). The results indicated a significant reduction in the percentage of live hatchlings of *C. mydas* and *L. olivacea* when the total number of eggs are around or more than 120 and 110, respectively.

DISCUSSION

It was not possible to find out the percentage hatching success because of the difficulty in distinguishing the individuals that had died at different stages of the hatching process. Percentage hatching success is generally defined as follows:

		Total no. of hatched individuals	
Percentage		(dead and alive)	
hatching	=		x100
success		Total no. of eggs buried	
		(clutch size)	



FIG. 2. Relationship between the total number of *L. olivacea* eggs and (a) live hatchlings (b) early mortality (c) late mortality (d) total mortality.

Therefore, it is not possible to conclude in this study whether the hatching success is affected by the increase in the number of eggs in a clutch. However, Fowler (1979) observed high hatching success rates (70-80%) in some of the smallest green turtle clutches (natural) in Tortuguero, Costa Rica. Similar studies on natural nests in Sri Lanka have not been carried out.

As mentioned earlier, eggs that are transplanted in Sri Lankan hatcheries are not collected by trained people. Therefore, these eggs may have been subjected to rough handling by the villagers, who sell them to the hatcheries. In spite of this, a mean of 71.98% live hatchlings of C. mydas and 61.82% live hatchlings of L. olivacea is impressive.

Signs of very early embryonic death are difficult to detect when the nest is excavated at the end of the incubation period. Therefore, it is likely that such eggs were mistaken for infertile eggs. There is also the possibility that the spoilt eggs that were categorized as infertile eggs, contained early embryonic stages. As a result, the calculated percentage early mortality stages would be underestimated.

However, if all the spoilt eggs in the nests were considered to be infertile (*C. mydas* 9.70%; *L. olivacea* 12.16%) the fertility rates of *C. mydas* and *L. olivacea* would be 90.3% and 87.8%, respectively. According to Ehrhart (1982), fertility rates of the loggerhead, leatherback and the green turtle vary between 80-90% but these could be higher. The fertility rates obtained in this study are within this range.

The percentage of live hatchlings, early, late and total mortalities showed similar relationships with the 76

total number of eggs in a clutch as well as with the number of fertile eggs. This may be due to the fact that the fertility of the eggs was high.

Increase in the late mortality, which includes the late embryonic and early hatchling stages, may be due to several factors. A possible cause would be due to the reduced gas exchange within the nests. According to Ackerman (1980), mortality increases in environments in which gas exchange is reduced below "naturally occurring levels". The oxygen consumption of turtle eggs appears to increase "sigmoidally" throughout incubation becoming relatively constant just prior to hatching (Ackerman 1980). This probably results in reducing the available oxygen within the nests of large egg clutches during the late stages of development.

Carr & Ogren (1960), observed that during emergence, hatchlings show periodic bursts of activity. Prange & Ackerman (1974), suggested that periodicity of activity in a clutch of hatchlings is related to the demand and supply of oxygen in the active nest. During sustained activity the hatchlings exhaust the supply of oxygen in the nest and build up an oxygen debt which halts their efforts. Hatchlings resume their digging efforts after recovering from the oxygen debt following the diffusion of oxygen into the nest. In an artificial hatchery where a number of clutches are buried, the diffusion of oxygen may be slow and may even be insufficient. It is possible that this causes the death of emerging hatchlings and embryos within the nest.

The results of this study suggest that the percentage of live hatchlings in large egg clutches of *C. mydas* and *L. olivacea* are less than those with small ones. Thus dividing and transplanting *C. mydas* clutches with more than 120 eggs (in this study maximum was 164) and *L. olivacea* clutches with more than 110 eggs (in this study maximum was 140) into two batches, may result in higher percentages of live hatchlings.

However, it has been found that the sex ratio of hatchlings is dependent on the incubation temperature (Mrosovsky, 1982). It is possible that the reduced clutch size will have an effect on the incubation temperature which will in turn affect the sex ratio of hatchlings. Investigations should be made on the incubation temperature of small clutches in order to find out the effect on sex ratios.

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FIELD STUDIES ON REPRODUCTIVE BEHAVIOUR IN TWO DART-POISON FROG SPECIES (*EPIPEDOBATES FEMORALIS, EPIPEDOBATES TRIVITTATUS*) IN AMAZONIAN PERU

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The reproductive behaviour of two syntopic dendrobatid species, *Epipedobates femoralis* and *Epipedobates trivittatus* were studied in two separate field studies in Amazonian Peru. Males of both species defended territories against calling conspecifics; females were not territorial. Pair-formation, courtship, and mating took place in the territories of the males. Females entered territories and approached calling males who attempted to lead females to oviposition sites. Females rejected males during courtship by leaving males or refusing nest sites. Males never rejected females. Brood care was performed by males only. The reproductive behaviour of the two species varied in length and pattern of courtship, oviposition behaviour and male brood-care behaviour.

INTRODUCTION

Neotropical frogs of the family Dendrobatidae are particularly promising subjects for ethological studies because of their conspicuous appearence, diurnal habits, and their complex reproductive and social behaviour. Dendrobatids deposit their eggs on land and the tadpoles are transported to aquatic sites on the back of a parent, except in *Colostethus stepheni* where the tadpoles develop in terrestrial nests (Junca, 1989; Lima, 1992). All species studied to date demonstrate elaborate courtship behaviour and parental care (e.g. Crump, 1972; Weygoldt, 1980*a*,*b*; Zimmermann, 1990). Male and/or female territoriality and aggressive behaviour have been reported in several species (e.g. McVey, Zahary, Perry & Mcdougal, 1981; Wells, 1980*a*,*b*; Summers, 1989; Roithmair, 1992, 1994).

Courtship may be viewed as an exchange of signals to provide information about the potential mate (Wells, 1977), possibly including cues for the females' mate selection and information for males about the receptivity of females. In frogs, courtship usually starts with the male advertising his position and trying to persuade a female to mate with him rather than with his neighbouring competitor. Advertisement calls of male frogs are reported to serve as a cue for female mate choice (e.g. Gerhardt, 1991). Information for males about the receptivity of females could be especially important in dendrobatids because of their terrestrial and prolonged breeding habits with receptive as well as non-receptive females entering the territories of males (Wells, 1977). In dendrobatids, brood care is assumed to be the "initial condition" (Maynard Smith, 1977), because larval transport is essential for the survival of the offspring, which would die if they were not carried to a suitable environment for development (Weygoldt,

1987). This situation creates perhaps a more sophisticated courtship and mating system in which both partners attempt to obtain as much information about the other as possible.

In previous papers, I have described my field observations on the social behaviour of the dart-poison frogs, *Epipedobates femoralis* and *Epipedobates trivittatus*. Main emphasis centered on male territorial behaviour and the interrelation between territoriality and mating success in males (Roithmair, 1992, 1994). In this paper I report on my results concerning the reproductive behaviour of these two dart-poison frogs. The main objective is to describe in detail the behavioural patterns of courtship, mating and brood care as shown by the two species in the field, thus providing a basis for comparison.

MATERIALS AND METHODS

STUDY AREA

Both species were studied at the Panguana biological station located on the lower Rio Llullapichis (approx. 9°35' S, 74°48' W) in lowland Amazonian Peru. The site is situated in a primary rain forest at an elevation of 260 m. The wet season ran from September/October to March/April. Annual precipitation ranged from 2000 to 2600 mm, and the mean annual temperature was 25°C.

STUDY SUBJECTS

Epipedobates femoralis (Boulenger, 1883) and *Epipedobates trivittatus* [Spix, 1824; taxonomy of both species following Myers (1987)] are diurnal inhabitants of the forest leaf litter. Calling activity of the frogs began about August/September (i.e. before the onset of the wet season) and ended about February/ March. Males of both species have two types of calls:

an advertisement call for long-range communication (Schlüter, 1980; Hödl, 1983) and a courtship call of a lower sound intensity for short-range communication (Weygoldt, 1980a; Roithmair, 1994). There is no significant difference in the sound pressure levels of calls of conspecific males in either species (Hödl, 1983; Roithmair, 1994). During these studies, E. trivittatus males mostly called in the mornings between 04.40 and 09.00 hr, and E. femoralis males called the most in the afternoons between 14.00 and 18.00 hr. In both species females are slightly larger than males. In E. femoralis, the snout-vent length (SVL) of adult males averaged 26.8 mm (n = 55); average SVL of females measured 27.5 mm (n = 33). The skin of *E. femoralis* is non-toxic (Daly, Myers & Whittaker, 1987). Epipedobates femoralis is a feeding generalist, taking as prey mainly ants and termites in proportions not significantly different from those in the leaf litter (Toft, 1980). In E. femoralis, activity ceased completely during the dry season and adult frogs could neither be seen nor heard. In E. trivittatus, average SVL of adult males measured 39.3 mm (n = 27); SVL of adult females measured 43.6 mm (n = 65). The skin of *E. trivittatus* is toxic (Daly et al., 1987). This frog is considered to be a feeding specialist actively searching for prey, mostly ants (Toft, 1980). Epipedobates trivittatus could be seen throughout the entire year, however, only very few frogs could be heard in the dry season; one unmarked male was found carring tadpoles in July. None of the frogs in the study group showed reproductive activity during the dry season.

FIELD METHODS

Observations were made on both species for the duration of two reproductive periods. *Epipedobates femoralis* was studied from August 1985 to February 1987, and *E. trivittatus* from October 1988 to January 1990.

Two study areas were marked off in separate and undisturbed forest patches: one was $625-720 \text{ m}^2$ (E. femoralis), and the other about 3300 m^2 (E. trivittatus). I visited the study area mornings and afternoons for a total of 300 observation days for the E. femoralis study, and for a total of 407 observation days for the E. trivittatus study. The surface structures of the two different study sites did not change in the two years of the respective study. All frogs captured in the study area were measured to the nearest 0.1 mm (snout-vent length). A detailed sketch of the dorsal and lateral pattern of each frog was made to enable individual recognition without handling. In E. trivittatus, toeclipping was not necessary for marking since the highly variable dorsal pattern (see Silverstone, 1976) permitted reliable individual recognition up to three meters distance without handling. In E. femoralis, all adult frogs were toe-clipped to ensure identification since the individual pattern (i.e. dorsolateral stripes) was not as easily distinguishable as in E. trivittatus.

Toe-clipping did not appear to impede the frogs' normal behaviour and toe regeneration was never detected. In both species frogs were sexed by the presence of two vocal slits (longitudinal folds) on the throat of males. Frogs smaller than 24.5 mm (in E. femoralis) or smaller than 33.0 mm (in E. trivittatus) and lacking vocal slits could not be sexed externally and were regarded as juveniles. My usual procedure was to locate all calling males within the study area during the first hour of observation. Then I slowly moved through the area or sat quietly in one spot and observed interactions between individuals. Locations of all individuals were marked with small, numbered plastic tags attached to the vegetation and recorded on a map. For methods of estimating territory size and maps of territories see my previous papers (Roithmair, 1992, 1994).

DATA ANALYSES

The data were analyzed with product-moment correlation using the STATS 2.1 software program. The distribution of the data was checked for normality by applying the Kolmogoroff-Smirnov test using the STATGRAPHICS 2.0 software program. The 10% level was used to determine difference from normality; \bar{x} and SD are given for normally distributed samples, otherwise median and interquartile ranges (*iqr*) are used.

RESULTS

BRIEF DESCRIPTION OF SOCIAL BEHAVIOUR

In E. femoralis, 81 adult frogs (55 males, 26 females) were marked. In E. trivittatus, 92 adult frogs (27 males, 65 females) were recorded in total. Males of both species established territories using the advertisement call as a cue for spacing and marking their territories. Females, juveniles and non-calling males were not territorial and could enter a territory and move around freely without evoking aggression. In both species, territory sites could be occupied by different males in succession. Size and shape of the territories varied for each resident. A total of 44 E. femoralis males were regarded as territorial. They established 18 territories during the first wet season and 16 territories during the second wet season. In E. trivittatus, 20 territorial males established 14 territories during the first wet season and seven during the second wet season.

In *E. femoralis*, territory sizes ranged from 0.25 to 26 m², and in *E. trivittatus*, territory sizes varied between 4 and 156 m². In both species territory size was positively correlated with the number of days with calling activity of the owner and length of residence. Successful males (i.e. males mating at least once) maintained significantly larger territories and called on more days than unsuccessful males (i.e. no mating). SVL of males did not influence mating success (for details see Roithmair 1992, 1994).

REPRODUCTIVE BEHAVIOUR

Courtship. In *E. femoralis*, I observed six complete courtships and one only partially, and in *E. trivittatus* I observed 17 courtships leading to oviposition. In both species, pair formation, courtship, and mating always took place within the territories of the males. Females entered the territories and approached calling males. I observed a few females of *E. trivittatus* nudging the male to initiate courtship. Vocally inactive males were ignored by conspecific females in both species. As soon as a male detected the presence of a female he started to lead her to an oviposition site. In most cases, the nest

site was hidden under dead leaves on the forest floor or, occasionally in *E. trivittatus*, in fallen palm-leaf sheaths (Table 1). In neither species was the length of the courtship march influenced by the size of the resident's territory (product-moment correlation: r = -0.58, t = 1.43, NS in *E. femoralis*; r = -0.19, t = 0.76, NS in *E. trivittatus*).

Males of both species were not able to distinguish between a non-calling conspecific male or female. In either case the territorial males commenced courtship by emitting courtship calls and their subsequent activity was determined by the behavioural response of the other frog. Males never rejected a female. In unsuc-



FIG.1. Courtship behaviour of *Epipedobates femoralis* (A) and *Epipedobates trivittatus* (B) in the field. The males led the females to oviposition sites within their territories. A: Arrows = start of courtship march; dots = males; small circles = females; large circles = symbol of nest sites. B: In the case of multiple matings of the resident only the male is drawn, symbols of courtship marches are different. Full squares = first courtship march; full circles = second one; open circles = third one. Large arrow = start of courtship march; full squares = males; open squares = females; points of small arrow(s) indicate location of nest sites.

cessful courtships (i.e. courtships not leading to mating) females either did not follow at all (but orientated themselves towards the courting male), or stopped following the courting male, or refused the male-chosen nest site. After a female had rejected a resident she left the territory. If a courtship sequence was disturbed by me the pair hid under leaves starting again with courtship after a few minutes. In E. femoralis 89 unsuccessful courtships were observed (59 in the first season and 30 in the second). When faced with unwilling partners, males of E. femoralis immediately switched from courtship calls to normal advertisement calls and ceased attempting to lead females to a nest site. The average duration of these unsuccessful courtships was 20 min (range: 4 - 47 min, SD = 10.58; n =25). In E. trivittatus, nine out of a total of 48 unsuccessful courtships (= 19 %) were due to the female refusing the nest site. The females (n = 8; one femalerefused the nest sites of two males) followed the male to the nest site, entered the nest and left it almost immediately. Duration of these mating attempts ranged from 23 - 96 min ($\bar{x} = 61.33$, SD = 25.75; n = 9). Except for one case where the male-chosen palm-leaf sheath was full of water, the refused nest sites seemed (to the observer) as suitable for egg development as the sites accepted by females. On two occasions, the male tried without success to clasp the female leaving the nest.

In E. femoralis, females stayed at least two days before mating in the male's territory where they were courted daily by the residents. During this premating presence, however, the female followed the courting male only over a short distance and disappeared under leaves within the territory. Epipedobates femoralis females, who signaled their interest in mating by constant phonotactic approach, were led by the male in a roundabout trip throughout the territory. The males hopped along the boundaries of their territories, but never left them. Regularly they gave courtship calls. The females followed quickly behind, sometimes hopping to the same spot, which the male occupied a few minutes before. The distance between the two mates never exceeded 50 cm, and averaged 20-40 cm (Fig. 1A). If a female lost sight of her mate, she was unable to locate him without the help of advertisement calls. Towards the end of the courtship march the male started to crawl under leaves and into rolled leaves. The entire courtship lasted from 95 to 225 min, beginning with the first reaction of the male (i.e. turning towards the female, emitting courtship calls) and ending when both partners entered the nest and the male stopped calling (Table 1).

In *E. trivittatus*, only two of 17 paired females stayed one or two days in the territory prior to mating. Fifteen females were never seen in their mating territory before courtship and mating. *Epipedobates trivittatus* courtships never lasted longer than 68 min, the shortest was 12 min (Table 1). The males led the

		E. femoralis	E. trivittatus
No. matings		7	17
Premating pre of female in male territory	esence	2.4(2-4)days	0*(0-2)days
Courtship dur (min)	ation	157.2(95-225)	32.7(12-68)
Stay in nest (min)	male female	13(7-18) 37.8(18-60)	79(29-187) 133.5 ⁺ (43-1440*)
Total duration of courtship and mating(m	in)	170.2(111-232)	113.1(58-209)
Nest site		old leaves	old leaves:n=14 palm-leaf sheath n=3

TABLE 1. Description of courtship and mating behaviour of *Epipedobates femoralis* and *Epipedobates trivittatus*. Mean or median (+) values and ranges (in parentheses) are given. Courtship start = the first reaction of male to female and vice versa; courtship end = male and female stay in the nest and male stops calling; mating end = male leaves the nest. *= the overnight stay of females in the nest is estimated at 1440 min (=24hr)

females in a roughly straight line to an oviposition site (Fig. 1B). Courtship calls were not given regularly. In one case they were entirely absent and in two cases given only once. Females of *E. trivittatus* were able to locate mates by courtship calls. *Epipedobates trivittatus* males did not demonstrate nest-searching behaviour, such as looking under leaves or crawling inside palm-leaf sheaths. During one courtship, a male was interrupted twice in his attempts to lead a female to an oviposition site. Finally, on the third attempt he led her to a nest site which was nearly at the same distance and in the same direction as the former trials.

Mating. Matings occurred in the early morning starting between 06.05 and 06.35 hours in *E. femoralis* and between 04.57 and 06.30 hours in *E. trivittatus.* In both species more than 60% of all recorded matings occurred in November and December.

In *E. femoralis*, seven matings were recorded. No male or female was observed to mate twice. In *E. trivittatus* 17 matings were performed by 11 males: eight males mated once, two males mated twice. One male defended a territory in both rainy seasons and mated three times in the first season and twice in the second. The clutches were always laid in different nest sites (Fig. 1B). No *E. trivittatus* females were observed to mate more than once. In both species, the males en-

tered the nest site first and the females always left after the males.

Epipedobates femoralis males continued to give courtship calls; when both partners were in the nest and in the proper position the males ceased calling. The male sat behind the female without body contact (number of observations = 4). I could not determine the exact moment of fertilization, but I presume it took place almost simultaneously with egg deposition, since males left the clutch two to four minutes after egg deposition staying in the nest for seven to 18 min. The average number of eggs laid was 26. Afterwards, the females slowly turned around one or two times, than sat motionless on the clutch and finally left after approximately 40 min (Table 1).

In *E. trivittatus* cephalic amplexus was performed. Fertilization occurred after egg deposition. Accidentally, I disturbed a pair during mating. Both frogs left the nest, and since the laid eggs neither changed their colour nor developed, I assume, they were not fertilized. The average number of eggs per clutch was 40. The pairs stayed much longer in the nests than *E. femoralis*: the males remained up to three hours in the nests and 30% of the females stayed with their clutches the whole day and the following morning, thus very probably stayed overnight (Table 1).

In neither of the two species were correlations found between clutch size and (a) female body size (productmoment correlation: r = 0.26, t = 0.61, NS in *E. femoralis*; r = 0.58, t = 1.01, NS in *E. trivittatus*) or (b) male body size (product-moment correlation: r = 0.48, t = 1.23, NS in *E. femoralis*; r = 0.03, t = 0.13, NS in *E. trivittatus* or (c) size of male territory (product-moment correlation r = -0.18, t = 0.40, NS in *E. femoralis*; r = -0.05, t = 0.19, NS in *E. trivittatus*). *Brood Care.* In both species, brood care was performed

by males only. After mating, females left the territories and neither visited their clutches nor carried tadpoles.

Males of E. femoralis were never found within their nests attending their clutches. They either returned only to transport their offspring or for very short visits unnoticed by the observer (see also Weygoldt, 1980a). Males continued to call during embryonic development, except for the first two days after mating (Table 2). They did not use calling sites next to their clutches. Males of E. trivittatus attended their clutches three to nine times during embryonic development (Table 2). I did not observe them moistening or cleaning their clutches; they just sat quietly beside the clutches, touching them with one side of the body. One E. trivittatus male had two clutches at the same time in his territory for two days, until one clutch was destroyed by fungus. After mating, males stopped calling for 1 - 22 days (median = 4, iqr = 5, n = 17). Throughout the period of embryonic development, six males continued to call and three males ceased calling entirely. Males never called while in the nest. No correlation was found between the number of surviving

No. clutches	7	17
Eggs/clutch	26.3(24-30)	39.7(26-51)
Tadpoles/clutch	13.86(0-23)	17.71(0-41)
Tadpoles transported	19.4(16-23)	33.4(22-41)
Behaviour of the resident (=father) Days calling Days courting other females	8.4(5-10) 0 ⁺ (0-2)	3 ⁺ (1-11) 0 ⁺ (0-2)
transport Days attendin	g 0	5.78(3-9)
Transport of clutches on x th day after mating	14.2 (12-16; <i>n</i> =5)	17.9 (15-18; <i>n</i> =9)
Destruction of clutches on x th day after mating	1 st,2nd (<i>n</i> =2)	3.6 (2-7; <i>n</i> =8)

TABLE 2. Description of clutches and brood-care behaviour of *Epidedobates femoralis* and *Epipedobates trivittatus*. Mean or median (+) values and ranges (in parentheses) are given. *calculated from data of successful development only:=number of tadpoles moving in the clutch, counted one day before transport.

larvae and number of paternal visits to the clutch in *E. trivittatus* (product-moment correlation: r = -0.19, t = 0.51, NS).

In *E. femoralis*, development until larval uptake lasted 12 to 16 days, averaging 14 days. In *E. trivittatus*, eggs developed into larvae in 15 to 22 days, averaging 18 days. Two of seven *E. femoralis* clutches and eight of 17 clutches of *E. trivittatus* were destroyed by fungus, fly larvae or heavy rainfalls within one to seven days (Table 2).

I never was able to observe individually marked males taking up tadpoles nor did I capture marked males while carrying tadpoles. Outside the study area, all frogs captured with tadpoles on their back were males (n = 9 in *E. femoralis*; n = 11 in *E. trivittatus*). Usually, after rainfall the clutches were missing in the study area. No water sites were recorded inside of male territories. *Epipedobates femoralis* males required no more than half a day to transport their offspring to water sites, since they were found calling in the morning and the afternoon of the day of transport. *Epipedobates trivittatus* males stopped calling for at least one day and up to 40 days after clutch transport. However, one male transported 24 tadpoles on one day and returned

E. femoralis E .trivittatus

to carry the remaining eight tadpoles of the same clutch on the following day.

Except for this one case in *E. trivittatus*, tadpoles of one clutch were transported all at once. In *E. femoralis*, the average number of tadpoles transported by individually known males was 19 (range = 16 - 23, SD = 2.42, n = 5); in *E. trivittatus* the number of tadpoles (carried at once) averaged 33 (range = 22 - 41, SD = 5.31, n = 8; calculated from the number of tadpoles moving before the clutch was transported). When the transport of the last clutch was completed or if the clutch was destroyed, residents either deserted their territories immediately (n = 1 in *E. femoralis*; n = 3 in *E. trivittatus*) or maintained residency calling for up to 24 days (n = 6 in *E. femoralis*; n = 9 in *E. trivittatus*).

DISCUSSION

The basic patterns of courtship are similar in both dart-poison frog species studied: males tried to attract females by advertisement calling, the females approached residents and were courted by the males who attempted to lead the females to oviposition sites. While advertisement calls are directed towards all females as a signal of male sexual activity (besides the function of a signal to competitors of male readiness to defend a specific territory), courtship calls rather are signals to an individual female in the vicinity. Males of E. femoralis or E. trivittatus were not immediately able to distinguish the sex of a silent conspecific, or differentiate a non-receptive or unwilling female from a female ready to mate. After courting for a short time, however, males were able to decide if it paid continuing with courtship or stopping it. Thus, courtship behavioural patterns of females appear to provide males with sufficient valuable information about female physiological conditions.

Epipedobates femoralis courtships lasted, on average, nearly five times longer than those of E. trivittatus. However, if the duration of courtship and time spent in the nest by both partners were added, then courtship lasted only one and half times longer in E. femoralis (Table 1). In E. trivittatus, females entering a male-chosen nest site sometimes decided not to mate at all and left the nest immediately. Presumably, females ovulated in the nest during cephalic amplexus considering the prolonged stay of both partners in the nest. Perhaps the tactile stimulus is necessary for ovulation. I suppose that female E. femoralis needed the time of courtship to reach the proper physiological condition. To deposit a clutch and to fertilize it, the mating pair required only a few minutes in the nest (Table 1). The courtship calls regularly given by E. femoralis males may serve as acoustic stimuli to females, since the calls did not provide cues for orientation. Since females E. femoralis never refused a nest site, a male followed continously and without hesitation by a female could be "confident" that this female would mate with him and be ready for oviposition. Thus, the long courtship duration in *E. femoralis* is suggested to serve as well to assure the male of the female's willingness and readiness to mate. Furthermore, *E. femoralis* males spent additional time searching for a nest site during courtship. The absence of this searching behaviour in *E. trivittatus* and the observation that one *E. trivittatus* male repeatedly led a female to the same spot in the same direction, suggest that males have chosen a nest site previous to the start of a single courtship trip.

Although females of both species were observed to sample males and males mating successfully defended larger territories and called on more days than unsuccessful males (Roithmair, 1992, 1994), the time of the female's decision with which male to mate was different in the two species. E. femoralis females "had made up their minds" when engaging in the final prolonged courtship in the morning, whereas E. trivittatus females were able to delay their decision until the last possible moment, i.e. before performing cephalic amplexus. I suppose neither the duration nor the performance of courtship (e.g. number of courtship calls, shape of trip through the territory) were decisive factors in the female mate choice in either species. Although it seems possible that E. trivittatus females could use nest site quality to choose mates, one would expect a higher percentage of unsuccesssful courtships due to nest site refusal (see also Roithmair, 1992, 1994).

In dendrobatid species where both sexes defend territories, courtship may serve to overcome the aggressive tendencies of the partner (Dole & Durant, 1974; Wells, 1980a, b). In the two species studied here, however, any non-calling frog could enter a territory without evoking aggression. In Dendrobates auratus and D. leucomelas, active courtship of males by females may be performed in order to prevent males from multiple mating, which infers costs to the female in terms of reduced offspring survival (Summers, 1989, 1990, 1992). Although E. trivittatus males mated polygynously, no male attended two clutches or carried tadpoles of two clutches simultaneously to the same water site. Courtship initiation by some E. trivittatus females was rather to draw the male's attention to the female's presence and females never remained in their mates' territory after mating. Intra-female aggression was observed only once (Roithmair, in press).

Brood care behaviour in anurans may increase their reproductive success (e.g. McDiarmid, 1978; Townsend, Stewart & Pough, 1984) and may involve defence against other frogs and egg predators, provision of skin secretions to inhibit fungal growth, and provision of moisture to prevent desiccation (Salthe & Mecham, 1974; Wells, 1981). In *E. trivittatus*, paternal care did not involve special behavioural patterns such as cleaning or watering the eggs and defence of clutches against egg predators never was observed. Although cannibalism never was observed and causes of clutch destruction were always explicable, males never called in or near their nests, as if to avoid attraction of predators to the nest site. The most essential contribution to the survival of the offspring in dendrobatids is to transport the larvae to a water site, requiring the presence of at least one parent to perform it. The behaviour of staying with or repeatedly returning to the clutch, as demonstrated by *E. trivittatus*, may be regarded as the easiest way to ensure that the parent can relocate the nest site (Weygoldt, 1987). Consequently, *E. femoralis*' behaviour of returning to the clutch only for larval transport, would be a derived behavioural pattern of brood care that requires site memory.

In both species studied here, only males carried tadpoles (see also Aichinger, 1991). Since females always left the nest after males, they would be pre-disposed to care for the offspring, i.e. to engage in egg attendance and larval transport (Dawkins & Carlisle, 1976). Presumably, the possibility of the male to desert first is outweighed by the association with the offspring by the territorial sex (i.e. the male) and the combination of territoriality and external fertilization, enabling assurance of paternity (Trivers, 1972; Ridley, 1978; Gross & Shine, 1981). In addition, remains the simple question, of whether a female would be able to find her clutch again. Although females may have information about the extensions of various territories (Roithmair, 1992, 1994), the degree of their familiarity with their mate's territory remains unknown and presumably is less than that of the resident male.

A summary of the behavioural patterns of *Epipedobates femoralis* and *Epipedobates trivittatus* demonstrated in courtship, mating, and brood care (for detailed explanation see results), are shown in Tables 3-5.

E. femoralis

E. trivittatus

Chisaccessian courisinp	U	nsuccess	ful	cour	tsł	ip	s
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no nest-site	19% due to nest-
refusal	site refusal
Successful of	courtships
females in territory at least 2 days before mating	rare premating presence
males lead females	a roughly straight line
around territory	to nest site
never shorter than	never longer than
95min ($\bar{x} = 157$ min)	68min ($\bar{x} = 32min$)
regular courtship calls	courtship calls not
	obligatory
nest-site searched	nest-site chosen
towards end of courtship	previously?

TABLE 3. Summary of courtship behaviour.

E. femoralis E. trivittatus				
males $(n = 7)$ stayedmales $(n = 11)$ stayedin nests 13min $(= \bar{x})$ in nests 79min $(= \bar{x})$				
simultaneous fertilization fertilization after e and egg deposition deposition				
females left nests after $38\min(=\bar{x})$ 70% females left nests after 110min $(=\bar{x})$ and 30% stayed overnight				
females always left nests after males				
\overline{x} number of eggs:26 \overline{x} number of eggs:40				
no correlation between male or female body size and clutch size				
no males mated twice 27% males mated more than once				

females never mated twice

TABLE 4. Summary of nesting and mating.				
E. femoralis E. trivittatus				
males did not attend clutches	males attended clutches			
no correlation between territory (= clutch) defence and hatching success	no correlation between clutch attendance (= no of visits) and hatching success			
53% hatching success (= eggs laid/transported tadpoles)	45% hatching success			
tadpoles were ca	arried to water by males			
females neither visited their clutches				

TABLE 5. Summary of brood care.

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HAEMATOLOGICAL VALUES OF THE RAINBOW LIZARD AGAMA AGAMA L.

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Haematological values of blood of 82 rainbow lizards, *Agama agama* L. collected during the rainy season in Ago-Iwoye, Nigeria were determined and the influence of age, sex and reproductive condition were investigated. Red blood cells were oval and nucleated and had a mean size of 18.6 by 13.0μ . Means of parameters commonly used in patho-physiological investigations were: red blood cell (RBC) - 0.78×10^{12} /l, white blood cell (WBC) - 0.46×10^{11} /l, packed cell volume (PCV) - 28.9% and haemoglobin (Hb) - 6.1g/dl. There were differences in blood parameters between males and females (thrombocyte, WBC, Hb and red cell indices), adult and subadult males (Hb and PVC) and non-breeding, vitellogenic and ovigerous females (WBC, RBC and PCV).

INTRODUCTION

Studies on the blood of reptiles have been reviewed by Pienaar (1962) Dessauer (1970), Saint Girons (1970) and Duguy (1970). Data from these studies show that reptilian blood plasma makes up 60 to 80% of the blood volume and contains inorganic and organic substances such as sodium, haemoglobin and urea, most of which occur in trace quantities. The pH ranges between 7.2 and 7.8, the haematocrit or packed cell volume between 20 and 35% and the blood contains 6 to 12g/dl haemoglobin. Erythrocytes or red blood cells are oval, 14-23 μ by 7-14 μ in size, each with an oval nucleus which is centrally located such that its long axes lie parallel to those of the red blood cells. The number of erythrocytes in the reptilian peripheral blood ranges between 0.15 and 2.05 x $10^{10}/l$. White blood cells are highly variable in number.

Pienaar (1962) and Duguy (1970) identified age, sex, reproductive state, disease, nutrition, season and environment as factors which influence haematological values in reptiles and Newlin & Ballinger (1976) reported differences in haemoglobin content of lizards living at different altitudes.

In the studies on reptilian blood, only few species were investigated (Saint Girons, 1970) and detailed studies on African reptiles were few (Pienaar, 1962; Otis, 1973; Simbotwe, Ghosh & Grimms, 1983) despite the need to establish normal haematological baseline values for healthy animals relating to pathological and physiological condition (Duguy, 1970; Kocan & Pitts, 1976; Brown, 1980; Gee, Carpenter & Hensler, 1981; Needham, 1981; Ugochukwu, 1982).

In this paper, we present haematological values for apparently healthy rainbow lizards, *Agama agama* L. a species which is widespread in Africa (Harris, 1964) and which is used in epidemilogical studies of malaria infections (Dipeolu & Nutinga, 1989). We have also examined the influence of age, sex and reproductive state on haematological values of rainbow lizards. For comparison, haematological data for other vertebrate classes are presented.

MATERIALS AND METHODS

Lizards were obtained from Ago-Iwoye, Nigeria 6°58'N, 3°54'E) during rainy season months (May and June 1991) when the lizards enjoy their best health status (Harris, 1964; Ekundayo & Otusanya, 1969). Subsequently, each lizard was anaesthetized with chloroform for 10 to 25 minutes depending on the size and was bled thereafter from the severed jugular vein directly into small EDTA-coated bottles.

Lizard body weight (nearest g) before blood was taken, and snout-vent length (SVL) (nearest mm) were recorded. Reproductive condition of females whether breeding or non-breeding based on the criteria reported by Sodeinde & Kuku (1989), and relative age of males were determined. Breeding females were either vitellogenic having yolked ovarian follicles, or they were ovigerous with shelled oviductal eggs. Nonbreeding females had undeveloped, milky-white ovarian follicles and no oviductal eggs. Subadult males were non-rainbow coloured and had SVLs of 75-125 mm while adults were rainbow-coloured and longer than 125 mm SVL (Harris, 1964).

Blood values were determined using methods described in Brown (1980) and which have been used in the study of reptilian blood (see Pienaar, 1962; Engbretson & Hutzlison, 1976; Needham, 1981; Simbotwe et al., 1983). The packed cell volume was measured by a microhaematocrit technique. Whole blood drawn to marked levels in two haematocrit tubes was centrifuged for 5 min. in a microcapillary centrifuge and the space occupied by the packed red cells, the haematocrit reading, was taken. White blood cells, red blood cells and thrombocytes were counted in the Improved Neubauer haemocytometer after 1:200 dilution of uncentrifuged whole blood with the appropriate diluents (0.85% sodium chloride - RBC, Turk's solution -WBC, platelet diluting fluid - thrombocytes). The number of cells of each type in undiluted whole blood (N) was calculated using the formula, N = n.DF.VCF, where n is the number of cells in diluted blood, DF is the dilution factor (200 for the 3 cells types) and VCF,

the volume correction factor (50 for RBC, 2.5 for WBC and thrombocytes).

Haemoglobin content was determined colorimetrically by the cyanomethaemoglobin method. For each parameter duplicate samples were run for each lizard and the values were averaged. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin content (MCHM) were calculated from RBC, PCV and Hb values using standard formulae (Thompson, 1969).

Blood films were made from blood collected directly from each lizard and were stained with Giemsa stain. On each film, length and breadth measurements of five non-parasitized red blood cells and their nuclei were recorded using an ocular micrometer. A measure of the shape of red blood cells was obtained based on the ratio: RBC length/RBC breadth (Simbotwe *et al.*, 1983). Values greater than 1.0 indicated oval shapes where 1.0 represents round cells.

Means were expressed \pm SD, pair-wise and multiple comparisons were made using the *t*-test and one-way analysis of variance (ANOVA) and multiple means were separated using the New Duncan's Multiple Range Test.

Sex	RBC Measurement	Mean	SD	Range
Male (5)	length breadth L/B ratio nucleus length nucleus breadth	18.9 13.1 1.48 9.2 5.0	3.5 2.5 0.16 1.3 1.3	12.6-25.2 8.4-16.8 1.25-1.75 8.4 -12.6 4.2-8.4
Female (5)	length breadth L/B ratio nucleus length nucleus breadth	18.3 12.8 1.46 9.3 5.4	2.0 2.3 0.21 1.7 2.0	16.8-21.0 8.4-16.8 1.25-2.0 8.4-12.7 3.8-10.5
Male & female pooled (10)	length breadth L/B ratio nucleus length nucleus breadth	18.6(NS) 13.0(NS) 1.47(NS) 9.3(NS) 5.2(NS)	2.8 2.4 0.19 1.5 1.7	12.6-25.2 8.4-16.8 1.25-2.0 8.4-12.7 3.8-10.5

TABLE 1. Means, SDs and ranges of red blood cell sizes (in μ) of male and female rainbow lizards. Number of lizards examined in each category is shown in parentheses. NS, male and female lizards means not significantly different (P > 0.10).



FIG. 1. Ranges, means, standard deviations and coefficients of variation of blood values of rainbow lizards (n = 82). The unit of measure for each blood parameter is given on top of the bar drawing and coefficients of variation are given at the base.

RESULTS

MORPHOMETRY OF RED CELLS

Dimensions of the RBC of rainbow lizards are presented in Table 1. Red blood cell length (t = 1.20, df = 24, P > 0.30) and RBC breadth (t = 0.62, df = 24, P >0.50) were not significantly different in male and female lizards. The oval appearance of the cells each of which had a centrally-placed nucleus was confirmed by the RBC length/RBC breadth ratios which were greater than 1.25.

HAEMATOLOGICAL VALUES

Values of the blood parameters (Fig. 1) showed that thrombocytes and WBC had the greatest variances around the mean. They had coefficients of variation of 44% and 66%, respectively. The MCHC had the lowest coefficient of variation.

Male lizards had higher body weights and SVL (both non-haematological attributes), WBC, Hb, MCHC and MCH than females (*t*-test, df 80, P < 0.05) while thrombocytes were more numerous in females (Table 2). Differences between sexes in other blood values were not significant (P > 0.10).

Analysis of blood values of males by relative age showed that adults that were bigger and heavier, had higher Hb and PCV values than did subadults (*t*-test, df 39, P < 0.05) but did not differ from subadults in other values (Table 2).

There were no size and weight differences between non-breeding (non-gravid), vitellogenic and ovigerous female lizards (*F*-tests; df = 2,38; P > 0.10) (Table 2). Lizards in these groups differed in WBC, RBC and PCV only. Ovigerous and non-gravid females had the highest RBC and PCV values.

	А		I	3		С	
Characteristic	Male (41)	Female (41)	Sub-adult (21)	Adult (20)	Non-gravid (6)	Vitellogenic (31)	Ovigerous (4)
Body wt (g)	52.1±14.7*	34.8±8.3	39.7±13.4*	63.0±7.7	35.5±8.2	34.5±8.8	36.1±4.9NS
SVL (mm)	118±12.0*	104±7.0	109±10.0*	128±4.0	105±8.0	104±8.0	100±3.0NS
WBC (x10 ¹¹ /l)	0.54±0.26**	0.40±0.16	0.48±0.20NS	0.58±0.31	$0.35 {\pm} 0.02^{b}$	0.38 ± 0.16^{b}	$0.60 {\pm} 0.09^{a}$
RBC (x10 ¹² /l)	0.80±0.19NS	0.76±0.15	0.77±0.22NS	0.83±0.17	0.83 ± 0.10^{ab}	$0.73 {\pm} 0.16^{b}$	$0.90{\pm}0.03^{a}$
Thrombocytes (x10 ¹⁰ /1)	0.56±0.30**	0.79±0.29	0.51±0.14NS	0.62±0.40	0.88±0.53	0.77±0.24	0.83±0.08
PCV (%)	29.3±4.6NS	28.5±4.9	26.7±3.7**	32.0±3.8	32.3±5.7 ^a	27.2±4.5 ^b	32.1±1.7 ^a
Hb(g/dl)	6.6±1.2**	5.7±0.7	6.2±1.1*	7.0±1.2	5.6±0.6	5.6±0.7	6.2±0.6
MCHC (g/dl)	22.9±3.1**	20.8±3.6	23.3±3.1NS	21.8±2.4	'17.6±1.9	20.8±3.1	19.4±0.8
MCH (pg)	88.1±27.7*	76.7±16.8	86.3±31.2NS	87.5±22.5	68.2±8.5	79.4±18.3	69.0±4.4NS
MCV (fl)	388.8±103.9NS	381.6±66.9	378.8±121.8NS	399.1±82.9	392.3±83.0	382.7±68.6	358.0±9.9NS

TABLE 2. Mean body weight, SVL and haematological values (\pm SD) of rainbow lizards by sex (A), male relative age (B) and female reproductive condition (C). Number of lizards in each category is shown in parentheses. NS, non-significant; * P < 0.05; ** P < 0.01; multiple means along rows with the same superscripted letter are not different (P > 0.05).

DISCUSSION

The red blood cells of rainbow lizards and those of other reptiles are oval and have centrally-placed nuclei (Pienaar, 1962; Saint Girons, 1970; Simbotwe *et al.*, 1983). The mean RBC size of rainbow lizards falls within the range for reptiles (Saint Girons, 1970) but is less than the 23.8 by 16.2 μ given for the frog *Rana pipiens* (Rouf, 1969), and greater than the 13.1 by 6.6 μ described for the duck, *Aythya valisineria* (Kocan, 1972) and the RBC diameter of 7.2 μ for man (Brown, 1980).

The PCV for rainbow lizards falls within the 20% to 35% range reported for reptiles (Table 3, Pienaar, 1962; Duguy, 1970). The PCV can be an index of health status; a reduced value for a species can indicate anaemia while a raised value can indicate decreased plasma volume or dehydration (Pienaar, 1962; Dessauer, 1970; Thompson, 1969). Comparison with other vertebrates shows that PCV, RBC and Hb are lower in ectotherms than endotherms (Table 3). Emery (1986) ascribed this to different oxygen requirements by the animal groups. Sheeler & Barber (1965) obtained higher values of these parameters in the rabbit and rat than in the turtle.

Although values of MCHC, MCV and MCH were given in some reports on reptiles (Wintrobe, 1933;

Pienaar, 1962) their significance was not discussed. These red cell indices are measures of blood iron content and are usually low in iron-deficiency anaemia (Thompson, 1969). For ectothermic vertebrates such as rainbow lizards which have larger-sized but fewer red blood cells compared with endotherms, MCV and MCH values are higher (Table 3). This is because the RBC is a denominator term in the formulae for calculating the parameters; $MCV = (PCV/RBC) \times 10$ and MCH= $(Hb/RBC) \times 10$ (units of expression as in Table 2). The relatively low RBC count in rainbow lizards is, therefore, responsible for the high MCV and MCH. The MCHC is not affected by RBC size. According to Thompson (1969) MCHC is the most accurate of the three red cell indices because it is estimated from the most accurately determined parameters, the PCV and Hb. These parameters also had the lowest coefficients of variation in this study.

The large individual variation in WBC and thrombocytes observed here has been reported for other ectothermic species and is considered normal (Pienaar, 1962; Rouf, 1969; Duguy, 1970).

Whereas male and female rainbow lizards differed in many haematological vlaues (Table 2), males and females of the lizard *Cordylus vittifer* (Pienaar, 1962) and

Species & Class	PCV	Hb	RBC	МСНС	МСН	MVC	Source
PISCES Sandbar shark Carcharhinus plumbeus	14.9	5.1	+NG	35.0	+NG	+NG	Emery (1986)
AMPHIBIA Leopard frog Rana pipens	24.7	6.8	0.31	27.4	211	772	Rouf (1969)
REPTILIA Boomslang Dispholidus typus	27.8	7.4	1.42	26.5	52	196	Simbotwe et al. (1983)
Lizard Agama atra	32.5	7.8	1.25	24.5	64	260	Pienaar (1962)
Lizard Cordylus vittifer	32.0	8.4	0.83	23.5	95	415	Pienaar (1962)
Chameleon Chamaeleo dilepsis	36.5	10.8	1.18	29.5	94	300	Pienaar (1962)
Rainbow lizard Agama agama	28.9	6.1	0.78	21.9	82	385	This study
AVES Canvasback duck Aythya valisineria	56.5	16.6	2.75	29.0	60	206	Kocan & Pitts (1976)
MAMMALIA Vampire bat Desmodus rotundus murinus	54.2	19.4	10.72	35.8	18	51	Krutzsch & Wimsatt (1963)
Man Male	40	13.5	4.5	++32.0	++27	++76	Thompson (1969)
Female	- 54 36	18.0 11.5	- 6.5 3.9	36.0	32	- 79	
	47	16.4	5.6				

TABLE 3. Comparative red cell values for ectothermic and endothermic species belonging to the same or other vertebrate classes as the rainbow lizard. Except man all values are means. Units of measurement are the same as in Table 2. ^{+NG}, values not given; ⁺⁺, pooled male and female values.

the snakes *Vipera aspis* and *Natrix maura* (Duguy, 1970) differed only in RBC and WBC. Engbretson & Hutchinson (1976) found no difference in PCV, Hb and RBC between males and females of the lizard, *Liolaemus multiformis*. The implication of observed gender-related differences in blood values where such exist, is that the values for each sex should be used for patho-physiological comparison between the same sex only as is done for humans (Thompson, 1969; Brown, 1980). Such differences in values due to gender have been attributed to hormonal influence (Duguy, 1970; Nirmalan & Robinson, 1971; Gee *et al.*, 1981).

Higher PCV values are frequently reported in adults than subadult and juvenile ectothermic vertebrates (Duguy, 1970; Nirmalan & Robinson, 1971; Kocan & Pitts, 1976). Differences in other values are not as frequent. Nirmalan & Robinson (1971) reported that young quail differed from adults in RBC, Hb, MCHC and thrombocytes. We also observed a difference in Hb between adult and subadult male rainbow lizards.

In birds, egg production (vitellogenesis) decreases PCV, Hb (Nirmalan & Robinson, 1971) and RBC (Ronald, Foster & Dyer, 1968) and it probably does likewise in female rainbow lizards. Among female rainbow lizards, only vitellogenic ones commonly have developing yolked ovarian follicles (Sodeinde & Kuku, 1989). These had the lowest RBC and PCV values. Duguy (1970) also reported a decrease in the number of RBCs in pregnant females of the snake *V. aspis*.

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NEW OBSERVATIONS ON THE *ELAPHE* SNAKES FROM AMORGOS (CYCLADES, GREECE) AND THE VALIDITY OF *ELAPHE RECHINGERI* WERNER AS AN ENDEMIC SPECIES

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Three specimens of *Elaphe quatuorlineata* (Lacépède) and three referable to the disputed taxon *Elaphe rechingeri* Werner were caught on the Cycladean island of Amorgos in April 1993. Analysis of this and earlier material collected by the author reveals the taxa are non-synonymous and separable chromatically, morphologically and in head scalation. A new definition of *E. rechingeri* is given. The status of *E. quatuorlineata* is discussed but no decision taken pending the need for further research. The study is presented against past debate on the Amorgos *Elaphe* snakes, island physiography, sympatric herpetofauna and the urgency for conservation measures.

INTRODUCTION

The type specimen of the Amorgos rat snake *Elaphe* rechingeri, described in 1932 by Werner as a new species endemic to the island of Amorgos, remained the only documented record of a snake of the genus *Elaphe* from this island until 1966. In that year I collected two adult snakes of this genus on Amorgos, one agreeing with Werner's description of *E. rechingeri* the other being unmistakeably *Elaphe quatuorlineata*, the fourlined snake. A juvenile example found at the same location as *E. rechingeri* was assigned to this species (Clark, 1971). This was followed by a return trip to Amorgos in April 1977, again finding one adult example of each taxon (Clark, 1990).

Comparing the two species, the most obvious distinguishing feature was the absence of any patterning in *E. rechingeri. Elaphe quatuorlineata* on the other hand had the four longitudinal dark stripes of exceptional boldness. Achilles Dimitropoulos of the Goulandris Museum of Natural History in Athens (Kifissia) has himself observed unstriped snakes on Amorgos and remarks that they are well known to the local people who readily distinguish between this "lafitis" and the striped one. In 1970 Erika Lotze found an adult four-lined snake (Lotze, 1970) but no *E. rechingeri* despite exhaustive investigations. In 1992 Buttle was also unsuccessful although he managed to capture an adult specimen of *E. quatuorlineata* (Buttle, pers. comm.).

The need for further evidence to ascertain the taxonomic position of the Amorgos *Elaphe* taxa seems to be a matter of priority. On the occasion of my third investigation in April 1993 I obtained three examples of each taxon. Examination of this material confirms my earlier belief that two *Elaphe* species are involved.

HISTORICAL DEBATE

The status of the unstriped *Elaphe* snake has occupied the attention of experts for some 60 years. Werner himself considered that it had affinities with *Elaphe*

longissima with which Wettstein (1953) was in agreement. Wettstein however relegated it to a subspecies of *E. longissima* and it is so listed in Mertens & Wermuth (1960). Buchholz (1961) examined the type and came to the conclusion that it stood nearer to *E. quatuorlineata* than *E. longissima*, a viewpoint with which Wettstein (1963) concurred after reappraisal.

The revelation in 1966 that there were two *Elaphe* taxa involved raised complications. Although the amount of material collected was admittedly small, amounting to Werner's "type" of E. rechingeri and my series of two of this taxon and one of E. quatuorlineata, I discounted the possibility of bimorphism within one species since the differences seemed too contrasted and proposed that recognition be given to E. rechingeri (Clark, 1971). Hans Lotze, who at the time was unaware of my work on Amorgos, came to the conclusion that E. rechingeri was nothing more that a patternless morph of the four-lined snake and described his single specimen as the topotype of E. quatuorlineata rechingeri on which the striped pattern was exceptionally well developed (Lotze, 1970). Kratzer (1973) remarks that on Amorgos the four-lined snake demonstrates a broad degree of variability with regard to patterning. This too is the conclusion of Gruber & Fuchs (1977), Arnold, Burton & Ovenden (1978), Gruber (1979), Beutler & Fror (1980) and Chondropoulos (1989). This opinion is not shared by Steward (1971) or Street (1979) who accept E. rechingeri as a species in its own right.

Lotze has argued that if the Milos grass snake, *Natrix n. schweizeri* and the leopard snake, *Elaphe situla*, on Milos show variability why not *E. quatuorlineata* on Amorgos? He is referring to melanism in the former and the occurrence of both barred and striped morphs in the latter species. I feel there is a flaw in the reasoning here since in the case of the two species mentioned both demonstrate bimorphic tendencies elsewhere: the situation is not exclusive to Milos. Extrapolation cannot be

used to account for the situation on Amorgos where, incidentally, E. situla, is absent. Dimitropoulos (1992) remarks that E. quatuorlineata is a constant species morphologically and in colouration. With the exception of the eastern subspecies sauromates, in which the juvenile livery is retained into adulthood and the striped pattern fails to develop, all other populations are similar to the nominate form. On the Cycladean islands the tendency is for the snakes to be paler in colour with narrower striping. The adult patterning develops at an earlier stage and adult size is smaller. The exception is Kea where the four-lined snake resembles those on the mainland but this island is not truly Cycladean in its herpetofauna as is proven by the presence of Chalcides o. ocellatus. On Amorgos E. quatuorlineata is characterized by darker rather than lighter ground colouration and has broader and more intense striping even when compared with the nominate form. It also grows larger than on the other islands.

The statement made by Böhme & Scerbak (1993) that a patternless population of the four-lined snake occurs in Bosnia needs to be considered. This implies that an exceptional situation could occur elsewhere. Is this perhaps the case on Amorgos? On the evidence available I feel that this is not so. Striped specimens have a consistent ground colour whereas in patternless individuals the ground colour is highly variable. Furthermore, as we shall see, there are distinct differences in head scaling. This implies either an abnormally plastic gene pool in one species or the existence of two species. I am inclined to accept the latter possibility.

PHYSIOGRAPHY

Amorgos belongs to the central group of the Cyclades lying some 35 km SE of Naxos. The principle islands in order of size are Naxos (448 km^2), Paros (209 km²), Amorgos (134 km^2) and Ios (105 km^2). Otherwise the land area of Amorgos is exceeded by Andros, Tinos and Milos making it the sixth largest island of the Cyclades.

The island itself is some 35 km long and 6 km across at the widest point with a NE/SW axis. A low mountain ridge runs the length with a mean altitude of some 400 m with peaks of 821 m in the north east, 698 m in the centre above Chora and 607 m in the south west. The hillsides are typically Cycladean, treeless and with phrygana scrub. There is extensive hillside terracing, regions of denser vegetation and pockets of cultivation in steep-to valleys with scattered hamlets and cottages. The coastal lowlands at Katapola and Aigali offer the greatest variety of habitat. At Katapola the plain has an area of roughly 2.25 km² within the 50 m contour. Towards the foothills it is dissected by a system of ravines and gullies which form seasonal water courses. The severe drought of recent years and the low rainfall of the winters of 1991/2 and 1992/3 meant that there was little surface water. An isolated spring was found near the head of a narrow gully and some water was present in

open cisterns and in two stream beds that had been dammed upstream from the dam wall. The area is extensively but thinly cultivated with olive plantations, vineyards, barley fields and scanty pasture as well as fallow, stony fields. There are innumerable tracks, paths, stone walls and embankments.

At Aigali the coastal plain is narrower with an area of about 1 km² within the 50 m contour. The extremities are flatter and rockier, there are no water courses and much of the region is given over to olive production and olive groves are more extensive than at Katapola.

STUDY AREA

The coastal regions and hinterland at Katapola and Aigali had formed the main areas for previous field work. *E. rechingeri* had been found in a harvested field at Katapola and in an olive grove at Aigali. At both locations *E. quatuorlineata* was caught on paths near stone walls.

It was decided to follow-up this work in 1993. Since the Katapola region offered a richer spectrum of biotopes, activities were concentrated here during the time spent on the island (April 15-21) with one day at Aigali. Particular attention was paid to deserted buildings, abandoned gardens and orchards, rocks in open fields by overturning, stone piles and discarded litter.

Weather conditions were favourable being dry apart from a few showers on April 17. On April 18 strong north winds caused the build up of heavy low cloud to windward and over high ground, clearing abruptly to leeward. Otherwise there was a good deal of sunshine. Early morning temperatures (07.30 hr) in the range of 9°C to 13°C, maxima 18°C to 24°C and evening values (17.30 hr) 15°C to 22°C.

FIELD RESULTS

ELAPHE QUATUORLINEATA

Katapola, April 17: Young female under flat rock near base of stone wall in field on margin of olive grove in hiding with an adult *Eryx jaculus*. Adult male coiled in spiny bush at base of dry stone wall on west-facing, sloping open field, 14.00 hr. April 18: juvenile freshly killed on tarmac section of road bordering olive groves before ascent to Chora, 14.00 hr. No further sightings were made and no sloughs found.

ELAPHE RECHINGERI

Aigali, April 19: adult male basking on margin of vineyard/stony path close to thickets, 15.30 hr. Katapola, April 21: adult female on open hillside amongst phrygana, 11.00 hr; adult male on rough path nearby, 11.15 hr. No further sightings were made and no sloughs found.

REMARKS

The two *E. rechingeri*, from Katapola, were taken in a habitat not previously associated with this species, namely in the dry scrub. This raises the question of differing ecological preferences between the Amorgos rat snake and the four-lined snake. Although *E. rechingeri* certainly enters cultivated areas, it may do so whilst foraging for food. Crop growing is non-intensive with little effort to claim unprofitable land. Fields and scrub are in juxtaposition and the peripheral zones often illdefined. This hypothesis needs testing by further field work.

SYMPATRIC HERPETOFAUNA

The Amorgos herpetofauna is depauperate compared with the neighbouring islands of Naxos, Paros and Ios. This, combined with the fact that Amorgos is as rich in habitats as the other islands, might well have been a factor in the evolution and maintenance of an endemic species. The following amphibians and reptiles exist on Amorgos, all of which were found in 1993, subspecific status omitted: Rana ridibunda, Bufo viridis; Hemidactylus turcicus, Tenuidactylus kotschyi, Ablepharus kitaibelli, Lacerta erhardii; Eryx jaculus, E. quatuorlineata, E. rechingeri. Most conspicuously absent are Lacerta trilineata and Vipera ammodytes. These are both found on the other islands and the latter is present on the small islands of Ano Kufonissi and Iraklea in the Erimonissia group situated between Naxos and Amorgos (Lotze, 1973; Dimitropoulos, 1992). Reptile species that might be anticipated but which are lacking are: Mauremys caspica, Natrix natrix, Agama stellio (Naxos, Paros), E. situla, Telescopus fallax (Paros), Typhlops vermicularis (Naxos). The most interesting apparent absentee is T. fallax which is one of the most successful of the Aegean snakes with regard to distributional potential. This species is in fact listed from Amorgos (Chondropoulos, 1989; Dimitropoulos, 1992). These inclusions are based on Werner (1938) and Wettstein (1953). In the absence of more recent confirmation it must be assumed that this snake is either rare or, as is more likely, the original listing was in error. Eryx jaculus reaches high population densities on Amorgos. I disclosed 13 in hiding in 1993 and Buttle (pers. comm.) 20 in 1992. This may be correlated with the absence of V. ammodytes. On islands where the two snakes occur V. ammodytes always occurs in larger numbers than Eryx jaculus.

Eryx jaculus cannot be considered a serious competitor with any European *Elaphe* species either in its dietary requirements or ecology. The absence of *E. situla*, *N. natrix* and *T. fallax* could have been instrumental in providing a niche for the Amorgos rat snake unless one reasons that this species itself led to the extermination of one or more of the above mentioned species.

SYSTEMATIC ACCOUNT

Elaphe rechingeri and E. quatuorlineata have been synonymised. It is thus necessary to present a revised diagnosis of the former and a comparison with the latter. Amongst the 1993 collection was a male E. rechingeri, SVL 109.5 cm, and a male E. quatuorlineata, SVL 104 cm. Since both were of the same sex and practically identical in body length it was decided to use these to assess differences in appearance, proportions and pholidosis. Additional material was utilised to determine range of characters and variability and thus build up a description of each taxon. Only one juvenile of each has been available for study: *E. rechingeri* SVL 46.2 cm, tail 8.7 cm; *E. quatuorlineata* SVL 29 cm, tail 5.8 cm. The subadult female *E. quatuorlineata*, SVL 58.5, cm tail 12.3 cm, had the stripes present but not completely formed and the juvenile cross barrings still plainly evident.

ELAPHE RECHINGERI WERNER

Colouration. Olive-green, grey-green or brown but never dark brown. Head, dorsal and lateral zones uniform. Flank scales inconspicuously light tipped anteriorly. Sometimes a suspicion of faint, shadowy dorso-lateral and/or lateral lines. A dark mid-dorsal narrow line may be present (Dimitropoulos, 1992). Indefinite dark "smudge" occasionally determinable from eye to jaw angle. Eye pale with a well defined ring round pupil. Venter white, cream or less often pale yellow. Belly nearly immaculate or lightly mottled or flecked with grey, more pronounced along ventral margins. Underside of tail dark marked in equal proportion to ground. Throat lightly marked with grey or fawn, sometimes with pinkish flush extending onto head and neck. No sexual dimorphism.

Morphology. A robust snake especially large males which are markedly thick along first third of body displaying scale interstices. Body weakly compressed with vertebral ridge. Head narrow, not distinct from neck. Temporal region "taut" and does not flatten. Neck and anterior one-fifth of body can "spread" on nervous and newly captured individuals. Eye moderately large, round, angled inward anteriorly and not obviously protruding laterally when viewed from above. Forwardmost point of eye does not attain first third of length of frontal shield. Supralabials only narrowly visible viewed from above (Fig. 1).



FIG. 1. Elaphe rechingeri - lateral view

Size. Large for a Cycladean snake, to at least 130 cm total length (SVL 109.5 cm, tail 23.5 cm) though usually less. SVL on five examples 74.5 - 89 cm. Damaged tails frequent at around 67%.

Pholidosis. Dorsals 25 at midbody, ventrals 203-205 (males) 214-219 (females). Subcaudals 65 (one male) 60 (one female). Dorsals lightly keeled except three lower rows. Scales on back of head granular, rather smaller than on neck. Supralabials 8 with 4th and 5th bordering eye. Sublabials 10 or 11. Surpraocular long, anterior point nearly reaches in line with the junction of the 2nd and 3rd supralabial, posterior point in line with junction of 5th and 6th supralabial; supraocular markedly "jutting" laterally giving frowning appearance, straight margined over eye. Loreal trapezoid with upper side shorter than lower, higher than long. Two preoculars, lower small and in infra-ocular position. Parietals large occupying not less than one-third of head area (ratio 2.7 in named specimen), sharp-edged, angular. Temporals straight-edged, about 6 large and 4 smaller. The latter twice the size of neck scales; border between posterior temporal region and neck sharply defined. Rostral viewed from below broad, widely grooved projecting forward. Posterior postmentals meet along midline or if not only narrowly separated by less than one gular scale.

ELAPHE QUATUORLINEATA (LACÉPÈDE) SUBSP.?

Colouration. Vertebral and dorso-lateral zones darkish brown, flanks lighter and greyish. Head above not as dark as top of body. Four intense black lines down body continuing onto tail, the upper pair three scales in width the lower one-and-a half scales wide. Dark line from orbit to angle of jaw. Eye dark, almost black with the pupil ill-defined. Anterior face of lateral body scales cream or white, more pronounced below mid-lateral stripe. Venter white or cream marked with grey flecking and powdering extending onto throat and chin. Tail below more or less dark grey. No sexual dimorphism. Buttle (pers. comm.) states that on his example the body striping was of equal width occupying two scale rows.

Morphology. Body rather slender, cylindrical not compressed. Head well distinct from neck, markedly broad in temporal region which is "flaccid" and can flatten. Eye prominent, large, somewhat broader than deep and in same plane as side of head; eye obviously protruding laterally when viewed from above with supralabials plainly in view (Fig. 2).

Size. Larger than from other Cycladean islands except Kea (nominate form) and possibly Ios. SVL on largest individual 104.3 cm, tail 18.2 cm incomplete (Lotze, 1970). Otherwise SVL in range 92.5-104 cm. Damaged tails in 80% of cases. SVL/tail ratio in only complete specimen (male) 4.2. This indicates a maximum length of at least 130 cm.

Pholidosis. Dorsals 25 at midbody, but 23 in one example; ventrals 206-210 in males, in single female 216; subcaudals 70 in one male, 68 in one female, 64 in one



FIG. 2. Elaphe quatuorlineata - lateral view

juvenile. Dorsals keeled except for the lowest row and rather more strongly than in E. rechingeri. Scales on back of head no smaller than on neck. Supralabials 8 with 4th and 5th bordering the eye. Sublabials 9, less often 10. Supraocular not long, anterior point in line with the junction of the 3rd and 4th supralabials, curved over eye and thin edged. Loreal almost square, slightly trapezoid seldom higher than long, if so markedly oblique. Two preoculars, the lower small and in infraocular position. Parietals medium occupying not more than one-quarter of head area (ratio 4.2 in named specimen). Two or three long narrow and obtuse temporal scutes with several smaller scales; boundary with neck poorly defined. Rostral viewed from below narrow, slightly grooved not projecting forward. Posterior postmentals clearly separated by one or two gular rows.

SYNOPSIS

The two taxa are separable on a number of features amongst which can be named:

(1) *Elaphe rechingeri* patternless, coloration variable. *Elaphe quatuorlineata* strongly marked black striping, coloration consistent.

(2) *Elaphe rechingeri* strongly built, robust, body obviously thickened anteriorly. Head slim, narrow across temporals. *Elaphe quatuorlineata* more slender, no thickening of the body anteriorly. Head markedly broad across temporals.

(3) Elaphe rechingeri eye not large, non-protruding viewed from above; eye pale, pupil well-defined with darker surrounding ring. Elaphe quatuorlineata eye large, protruding laterally viewed from above; eye dark, pupil ill-defined with no darker surrounding ring. (4) Elaphe rechingeri with weakly keeled dorsals, lowermost three rows unkeeled. Snake smooth to the touch. Elaphe quatuorlineata with more strongly keeled dorsals, lowermost row unkeeled. Snake rather rough to the touch.

(5) Head scalation, the most obvious points being: character of the supraocular, size and shape of the loreal and parietals, number and form of the temporals, appearance of the rostral and condition of the postmentals.

DISCUSSION

On the limited juvenile material available there seems little to differentiate the two species. Seen in the wider context of the Elaphe genus as a whole it is questionable how much difference one would expect. A characteristic juvenile pattern is common to very many species; basically dark bands or saddles on a lighter ground and this is found across the whole range of the genus from Europe to the Far East as well as the New World. In E. rechingeri the single juvenile had a brown/ grey ground with darker narrow cross barrings; in E. quatuorlineata the ground was more dull silver grey and the narrow bars fawn/grey. A point of difference was that the scales forming the cross bars had a light central streak in the latter species. The change from juvenile to adult livery takes several forms in the genus and it is worth mentioning that the four striped pattern is not peculiar to the four-lined snake but is parallelled in E. obsoleta quadrivittata and E. bairdi (New World), and E. quadrivirgata and E. climacophora (Far East).

With regard to scalation the condition of the preocular(s) varies between species. Some have only one but a number have a large upper and a smaller lower which can be defined as a subocular. Elaphe rechingeri and E. quatuorlineata fall into the latter category. Scale series in which one might anticipate differences are the dorsals, ventrals and subcaudals. Both taxa have 25 mid-dorsal rows, 23 in one E. auatuorlineata. There is no indication of dissimilarity between ventral counts, these being within the general range for not only E. quatuorlineata (195-234) but also E. longissima (205-248) with one E. rechingeri having only 203. The overlap in ventral counts for E. quatuorlineata and E. longissima is broad but exceeded by the subcaudals which are almost the same: 56-90, 60-91, respectively. The number of damaged tails in Amorgos Elaphes does not permit an evaluation but again the implication is that both species are similar in this respect.

DECISION

Although it is undeniable that *E. rechingeri* and *E. quatuorlineata* have characteristics in common these are shared by other species of the genus. There appear to be sufficient distinguishing features to separate the two taxa under consideration at the specific level. It is therefore proposed that *E. rechingeri* Werner be reinstated as a species distinct from *E. quatuorlineata* (Lacépède).

DISTRIBUTION AND SUBSPECIATION

ELAPHE RECHINGERI

This snake appears to be endemic to Amorgos. Since the smaller islands and islets off the coast remain uninvestigated the possibility must remain that *E. rechingeri* could exist on one or more of these. The most likely, on account of size and physiography, is Nikuria off the north west coast line from which it is separated by a narrow and shallow channel. Nikuria has an area of about 4 km² and a maximum elevation of 350 m. There is no known subspeciation.

ELAPHE QUATUORLINEATA SUBSP.

The four-lined snake has a broad distribution in Greece. On the Cycladean islands it is known from Kea, Andros (Clark, unpublished record from July 1966), Tinos, Mykonos, Paros, Naxos, Ios, Iraklea, Schinoussa, Amorgos, Santorini and Antimilos. It is also present on Pholegandros (Underwood, pers. comm.). This is not listed in Böhme & Scerbak, (1993). This specimen is in the British Natural History Museum collection no. 1938.8.5.1 and is an adult male. Snakes from Kea belong to the nominate form *quatuorlineata*. In the case of Andros and Santorini the evidence is from cast skins making subspecific designation problematic. Otherwise on most of the above named islands the fourlined snake belongs to the subspecies *muenteri*, the characteristics of which have been given.

Juveniles of this subspecies differ from the nominate form in having less contrast between the ground colour and patterning which is more in the nature of narrow bars instead of wide spots of bands. In this respect the Amorgos four-lined snake is similar to *muenteri* although differing in the adult phase. Adults from the neighbouring island of Ios seem to be closer to Amorgos than to the other islands in having wider striping and attaining larger size. On Amorgos and Ios SVL in excess of 100 cm seems not uncommon with a total length of around 130 cm. On the other Cyclades SVL seldom reaches 90 cm and a total length of a little over 100 cm can be considered "large". My biggest, a female from Naxos caught in March 1971, had SVL 90.3 cm, total length 109.8 cm.

In order to place the Amorgos four-lined snake in a subspecific category a thorough analysis and possible revision of other Cycladean populations is required. This is beyond the scope of the present paper.

CONSERVATION

It is important to secure the future of both the Amorgos rat snake and the four-lined snake on Amorgos. On several Greek islands snake populations have been reduced due to illegal collecting, often on a large scale for commercial gain, as for example on Milos where Macrovipera schweizeri is threatened. Just as serious is the destruction of natural habitats as the result of massive tourist expansion over the last 20 years or so. The building of hotel complexes and apartment blocks on vital habitats, especially the coastal plains and wetlands, has claimed the habitat of many reptile species. Natural scrub has disappeared owing to private building often some distance from the coast itself and the landscape has been radically altered. Traffic is heavy on many islands and with a wider road network claims substantial mortalities amongst snakes.

There is no indication that Amorgos is vulnerable at the present time. Tourist development has been minimal even compared with 30 years ago but it would be unwise to assume that this benign situation will continue. Neither has the island become a collector's target though there was a case recently. Thanks to the vigilance of the local police who had been alerted the person was apprehended and found to have a number of snakes in his possession which were released.

The need for a conservation initiative is vital. The question of setting up protection involves engaging local support. That such a scheme is feasible is demonstrated by the success the SEH (Societas Europaea Herpetologica) has achieved on Milos. This has involved the setting up of notices on the island in Greek, German and English "All snakes on this island are protected by law" and explanatory information. Educational talks are given to the school children and the police force are active in implementing control. This was in 1985 some time after the commercial exploitation of the viper population on the island had begun. Clearly a similar project needs to be put into operation on Amorgos before the snake population becomes decimated by potential bounty hunters.

FUTURE INTENTIONS

In addition to conservation the need to know more about the life style of *E. rechingeri* is paramount and to ascertain population densities and abundance. This must be the objective of further field work. However the study of this snake in captivity is also necessary in order to compare it with *E. quatuorlineata*. A captivebreeding programme will be initiated in 1994. If this is successful it will provide valuable information on the juvenile phases of the two taxa and their subsequent development.

HYBRIDIZATION

The possibility of hybridization between E. rechingeri and E. quatuorlineata needs considering. This occurs infrequently in European snakes under natural conditions but is known viz: Vipera berus x Vipera ammodytes, Vipera aspis x Vipera ammodytes (Street, 1979) and Dimitropoulos (pers. comm.) has evidence that Vipera berus bosnica and Vipera ursinii may hybridize in parts of the Pindos mountains in Greece. In captivity species that have absolutely no possibility of mating in the wild due to wide geographical separation may do so readily and produce offspring. This is strikingly illustrated by a case quoted by Haagner (1992) where a Burmese python, Python molurus bivittatus, mated with a Southern rock python, Python sebae natalensis. It would be very natural therefore if E. rechingeri and E. quatuorlineata did hybridize under captive conditions and not too surprising if they did so in the wild. The case quoted in Böhme & Scerbak (1993) of both morphs resulting from a single clutch of eggs laid by a female E. rechingeri might be the result of such a situation. Fertile progeny resulting from such crosses would not help to support the case for *E. rechingeri* as a good species distinct from *E.* quatuorlineata but neither would this in itself invalidate it, especially if it could be shown that sterile hybrids also occurred: it could be reasoned that E. rechingeri is a relatively young species perhaps not fully stabilized. What is of interest is that on present evidence there is no suggestion of adult snakes found in the field showing mixed characters. The presence of faint body striping on some individuals is akin to the situation sometimes found in E. longissima and does presume necessarily affiliation with not Ε. quatuorlineata. I have indicated that there seems to be a habitat difference between the two taxa and there might be other exclusion factors, such as a staggered breeding season, that would help to minimise potential hybridization.

CONCLUSIONS

I believe there is strong evidence to regard E. rechingeri and E. quatuorlineata as separate species. Apart from colouration and appearance there are undeniable points of distinction in head scalation and morphology. The depauparate reptile fauna on Amorgos may have allowed sympatry between these two large species, although E. quatuorlineata is found more often in cultivated areas. Future areas of study are (i) careful and controlled captive breeding experiments to establish phylogeny and (ii) organized and detailed field work over a long period to determine population densities, habitat and dietary needs as well as observations on courtship and mating. There is the need to ascertain whether E. rechingeri is extra-limital on the smaller islands round Amorgos. Lastly it is imperative that conservation measures are put into operation to ensure the future survival of both species.

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DIET OF THE FALSE SMOOTH SNAKE, *MACROPROTODON CUCULLATUS* (SERPENTES, COLUBRIDAE) IN THE WESTERN MEDITERRANEAN AREA

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Based on the analysis of the digestive tracts of 158 specimens, the diet has been established for *Macroprotodon cucullatus*, one of the European colubrid species with poorly-known biology. The specimens belong to three subspecies, *M. c. ibericus* (Iberian Peninsula), *M. c. brevis* (Morocco) and *M. c. mauritanicus* (Balearic Islands). The species feeds exclusively on vertebrates, mainly reptiles, and mostly long-bodied prey which are burrowers or live under rocks (*Blanus, Chalcides*). Some aspects of its morphology and feeding habits, in addition to our general field observations, suggest that it is not nocturnal, but rather lives under rocks. In comparison with other Mediterranean colubrids, this species feeds with very low frequency, taking relatively long prey with great biomass. In the specimens from the Balearic Islands the body length and feeding habits appear to have changed in a brief period, now being the largest of the distribution area of the species and basing its diet on small mammals. In the Iberian Peninsula and in northwestern Africa, there is no appreciable ontogenetic or sexual variation in the diet, since the different ages and sexes feed principally on amphisbaenians.

INTRODUCTION

The false smooth snake, Macroprotodon cucullatus, inhabits the Mediterranean Basin from southern Palestine to western Morocco, including relict populations in the Hoggar (Algeria) and Rio de Oro (Morocco). It also inhabits the southern half of the Iberian Peninsula and some islands in the Western Mediterranean, such as Mallorca, Menorca, Lampedusa and the Galita Islands (Busack & McCoy, 1990). It is the smallest Mediterranean colubrid and, until some years ago, one of the least known species, probably because of its secretive habits. Recently Wade (1988) has studied the scalation and pattern of the species, and has differentiated the northern Algerian populations to the subspecies level. Busack & McCoy (1990) have studied this species morphologically throughout its range, differentiating the population from the Iberian Peninsula to the subspecies level. There are also some works dealing with its distribution and scalation (Almeida & Almeida, 1986; Gisbert & García-Perea, 1986; Malkmus, 1992).

Nevertheless, there is still little information about its biology, particularly its feeding and reproductive habits. Most information on diet is non-quantitative data taken from few individuals, either in nature (Dumeril, Bibron & Dumeril, 1854; Bons & Bons, 1959; Bons, 1960; Lanza & Bruzzone, 1960; Bons & Saint-Girons, 1963; Hiraldo, 1974; Vericad & Escarré, 1976; Almeida & Almeida, 1986; González de la Vega, 1988; Wade, 1988; Busack & McCoy, 1990), or in captivity (Wade, 1988). Only Bons (1967) offers a rather extensive list of prey in Morocco, although this author includes no quantitative data. The general opinion is that the false smooth snake feeds almost exclusively on long-bodied reptiles, mainly amphisbaenians (Bons & Saint-Girons, 1963; Almeida & Almeida, 1986; Wade, 1988).

Here we report the diet of the species as judged from a broad sample of specimens from northwestern Africa, the Iberian Peninsula, and the Balearic Islands. The diet has been studied in relation to the size and sex of the specimens. In addition, attention is given to the geographical variation in the diet. The inclusion of the Balearic population offers information on the biology of a continental species introduced in historical times into an island environment. That indigenous lizards are still common on the snake-free Balearic Islands, and absent from the snake-present islands, suggests that the indigenous species, Podarcis lilfordi, was extirpated from Mallorca and Menorca Islands by the false smooth snake (Eisentraut, 1949; Mayol, 1985). Regardless of the accuracy of the latter comment, if continental populations of M. cucullatus feed mainly on reptiles, and if P. lilfordi was the only indigenous lizard, the question remains as to what path the false smooth snake followed in the Balearic Islands to adapt and survive.

MATERIALS AND METHODS

The gut contents of 171 *M. cucullatus* specimens from the western Mediterranean area have been analyzed. The specimens belonged to three of the four subspecies recognized for this species: the entire range of *M. c. ibericus* (116 individuals, Iberian Peninsula), the entire range of *M. c. brevis* (32 individuals, Morocco) and island populations of *M. c. mauritanicus* (23 individuals, Balearic Islands).

The specimens examined were obtained from following institutions: Museo Nacional de Ciencias Naturales (Madrid); Estación Biológica de Doñana (Seville); Instituto Pirenaico de Ecología (Zaragoza); Mus. Bocage (Lisbon, Portugal); Mus. de la Naturalesa de les Illes Balears (Majorca); Mus. de Zoologia (Barcelona); Dep. Biología Animal (Univ. of Granada); Dep. Biol. Animal (Univ. of Málaga); Dep. Biol. Animal (Univ. of Badajoz); Dep. Biol. Animal (Univ. of Balearic I.); Dep. Biol. Animal (Univ. of Salamanca); Dép. Biol. Animale (Tetuan, Morocco).

We have examined the gut contents of specimens in alcohol, considering for each specimen: size, body mass, sex, prey type, body size and body mass of the prey. For body size, snout-vent length (SVL) was recorded in *M. cucullatus* and its prey; in predators and prey, SVL has been calculated to the nearest mm and biomass to the nearest 0.1 g. Because of the lack of complete data of some museum specimens before fixation, SVL and biomass were measured from preserved ones, and only well-preserved specimens were considered. Specimens with a difference in body mass exceeding 5% before fixation and when studied, were rejected. Also, we avoided all specimens suspected to have died by starvation before fixation. Then, 72 museum specimens studied were rejected in the diet analysis, but some of them used in the morphological analysis. Prey were identified to species. Nevertheless, the tables indicate the genus level, and comparisons also list only the genera involved; in this way, we have tried to avoid the appearance of important geographical differences in diet due simply to prey species which were vicariant to both sides of the Strait of Gibraltar, and thus of similar sizes and habits. Specimens were sexed by examination of gonads after dissection.

For the analysis of ontogenetic shifts in diet, we have considered three artificial SVL classes. Diet-niche breadth was calculated using Levins's index (Levins, 1968) and overlap between sexes in diet composition by the Pianka's symmetrical index (Pianka, 1973). To calculate these indices, we used prey frequency. To calculate the diet niche breadth and overlap, the specimens without gut contents or with unidentified prey were not considered.

For comparing populations in terms of size, we have used mean SVL in each population and mean SVL for the 10 largest specimens in each population. The latter method is less sensitive to differences in sample size and change in age structure when comparing populations (Schwaner & Sarre, 1990). All variables compared were normally distributed.

RESULTS

TAXONOMIC COMPOSITION AND GENERAL DIET ANALYSIS

Of the 171 guts examined, only 42 had prey; that is, less than 25% of the sample contributed to the diet information of this species (Table 1). Among the specimens with prey, most had a single item (95.2%), only one had two (two *Psammodromus algirus*), and another had three (three *Chalcides mionecton*).

The populations of *M. cucullatus* from the western Mediterranean fed almost exclusively on reptiles, while small mammals represented only a small portion of the

Dress	Fre	quency	Biomass				
Prey	n	%	g.	%			
Blanus	15	33.3	47.1	29.6			
Psammodromus	10	22.2	28.0	17.6			
Poaarcis	2	4.4	10.6	0.0			
Mesalina	l	2.2	1.5	0.9			
Chalcides	8	17.8	39.6	24.9			
Malpolon	1	2.2	5.0	3.1			
Unid. reptiles	5	11.1					
Mus	3	6.7	27.5	17.3			
Prey number	ė		45				
Snakes with prey		4	42(24.6%)				
Prey class		7					
Diet niche breadth			3.96				

TABLE 1. Diet composition and basic data of trophic analysis of 171 specimens of M. cucullatus in the western Mediterranean area. Unid. reptiles, unidentified.

diet (Table 1). *Blanus* was the most consumed prey; other numerically important prey were *Psammodromus* and *Chalcides*. Long-bodied prey and prey that burrow or live under rocks (*Blanus* and *Chalcides*) represented more than 50% of the diet. Analysis of the biomass which each prey type contributed to the diet, shows the importance of the classes of prey (Table 1).

The false smooth snake consumes prey ranging in sizes between 24 and 211 mm SVL; the extremes are represented by *Psammodromus hispanicus* and *Malpolon monspessulanus*, respectively. The mean prey size was 92.7 mm SVL (SD = 52.0, n = 38); the relative prey size (prey/predator SVL ratios, SVLR) was very high, SVLR = 0.30 (SD = 0.18, n = 38).

The prey biomass ranged between 0.3 and 13.0 g, the extremes represented by *Psammodromus hispanicus* and *Mus spretus*, respectively. The mean prey biomass was 4.1 g (SD = 2.8, n = 37). The relative biomass of the prey (prey/predator weight ratios, WR) was also high, WR = 0.21 (SD = 0.11, n = 37), although less than the SVLR. This indicates that *M. cucullatus* feeds primarily on prey with large SVL, although not a commensurate biomass, i.e. long-bodied prey. In the 19 cases where we could determine the position of the prey (most were *Blanus*), the majority (18) were swallowed head-first.

GEOGRAPHICAL VARIATIONS IN DIET

There was a little difference in diet between the two continental populations, *M. c. ibericus* and *M. c. brevis* (*G* test = 14.09, df = 5, P < 0.05). The population from

Ssp.	<i>ibericus</i> (Iber. Pen.) (<i>n</i> =116)		brevis (Morocco) (n=32)		mauritanicus (Balearic I.) (n=23)		
Prey	n	%	n	%	n	%	
Blanus	13	50.0	2	14.3			
Psammodromus	7	26.9	3	21.4			
Podarcis			1	7.1	1	20.0	
Mesalina			1	7.1			
Chalcides	2	7.6	6	42.9			
Malpolon	1	3.8					
Unid. Reptiles	3	11.5	1	7.1	1	20.0	
Mus					3	60.0	
Prey number Snakes with	26]	14		5	
prey	24 (20.7)	12 (37.5)	5 (21.7)	
Diet niche bread	th 2	.37	3	.31		-	

TABLE 2. Diet composition of the three subspecies of *M. cucullatus* present in the study area. The prey species are: *Blanus cinereus* (Ibe. Pen.), *B. tingitanus* (Mor.); *Psammodromus algirus* (Ibe. Pen. and Mor.), *P. hispanicus* (Ibe. Pen.); *Podarcis hispanica* (Mor.), *P. sicula* (Bal. I.); *Mesalina olivieri* (Mor.); *Chalcides bedriagai* (Ibe. Pen.), *Ch. mionecton* and *Ch. polylepis* (Mor.); *Malpolon monspessulanus*; *Mus spretus*.

Morocco had more specimens with prey in their guts (G test = 5.58, df = 1, P < 0.03), and the diet-niche breadth was higher than in the sample from the Iberian Peninsula (despite a smaller sample size, Table 2). In *M. c. brevis, Blanus* loses importance as a prey item with respect to the Iberian specimens (G test = 6.28, df = 1, P < 0.02), while the importance of lizards increases (G test = 7.52, df = 1, P < 0.01; Table 2).

More differences exist between the diet of the population in the Balearic Islands and the continental populations: *M. c. mauritanicus/M. c. ibericus* (*G* test = 22.90, df = 6, P < 0.01), *M. c. mauritanicus/M. c. brevis* (*G* test = 15.80, df = 6, P < 0.05). The fundamental difference is that while the continental populations feed exclusively on reptiles, in the island population more than half of the diet was composed of small mammals.

There were differences in SVL among the populations studied (two-tailed ANOVA, F = 4.08, df = 2,204, P < 0.018, males and females pooled; Table 3); the Balearic specimens, had a longer SVL than the Iberian and Moroccan specimens when the 10 largest individuals from each population were considered (Iberian versus Balearic, Scheffé *F*-test *F* = 3.33, P < 0.05; Moroccan versus Balearic, F = 7.10, P < 0.01). The results from biomass comparisons are even clearer

	To	tal samp	ole	10 largest specimens		
	n	Mean	SD	Mean	SD	
SVL (mm)						
Iber. Penin.	111	285.7	81.4	429.2	45.7	
Morocco	69	314.9	68.2	407.0	16.4	
Balear. I.	27	328.9	133.6	477.2	53.3	
Biomass (g)						
Iber. Penin.	68	14.6	9.3			
Morocco	13	18.3	12.2			
Balear. I.	13	33.5	27.9			

TABLE 3. Body size (SVL) and biomass of *M. cucullatus* in the populations studied. Note: here we use a greater sample size than in the diet analysis.

(ANOVA, F = 10.40, df = 2,111, P < 0.001, males and females pooled; Table 3); the Balearic specimens are heavier than the Iberian and Moroccan specimens (Iberian versus Balearic, Scheffé *F*-test F = 10.40, P < 0.01; Moroccan versus Balearic, F = 4.02, P < 0.05). There were no differences between Iberian and Moroccan populations, either in SVL (Scheffé *F*-test = 2.46, NS), or biomass (F = 0.395, NS; in both comparisons, the total sample has been considered; Table 3).

ONTOGENETIC AND SEXUAL SHIFT IN DIET

To compare these dietary aspects of *M. cucullatus*, we have pooled the specimens of the three populations studied. We obtained no discordant results when the population from the Balearic Islands was removed from the calculations.

When we analyzed the diet of *M. cucullatus* in the three classes of size separately, no marked trends appeared between body size and the percentage of specimens with prey or the different types of prey consumed (Table 4). Nor are there any differences in the frequency of the prey consumed (small/medium, *G* test = 12.38, df = 6, P > 0.05; medium/large, G = 8.06, df = 6, P > 0.05; small/large, G = 5.74, df = 4, P > 0.05). *Blanus* proved to be the most frequent prey among the three groups. In this sense, both in the Iberian Peninsula and in Morocco, the smallest specimens of *M. cucullatus* invariably consumed *Blanus*. Prey of greater size, such as *Chalcides* and *Malpolon*, were consumed only by the medium or large groups.

There was no relationship between the size (SVL) of *M. cucullatus* and that of its prey (r = 0.18, n = 36, P > 0.26), nor between the absolute SVL of the snake and the relative prey size (SVLR, r = -0.23, n = 36, P > 0.16). Nevertheless, if instead of comparing linear di-

Body-size	Small		Me	edium	Large	
SVL (mm)	12 (7	23-250 n=50)	25 (n	1-340 =66)	341-524 (<i>n</i> =55)	
Prey	n	%	n	%	n	%
Blanus	6	42.9	3	25.0	6	31.6
Psammodromus	4	28.6	1	8.3	5	26.3
Podarcis	1	7.1			1	5.3
Mesalina			1	8.3		
Chalcides			5	41.7	3	15.8
Malpolon			1	8.3		
Unid. reptiles	3	21.4			2	10.5
Mus			1	8.3	2	10.5
Prey number Snakes	ļ	14		12	1	9
with prey	14	(28.0)	12	(18.2)	16	(29.1)
Prey class		3		6		5

	Males (<i>n</i> =92)		Fe (n	males =79)
Prey	n	%	n	%
Blanus	9	52.9	6	22.2
Psammodromus	4	23.5	6	22.2
Podarcis	1	5.8	1	3.7
Mesalina			1	3.7
Chalcides			7	26.0
Malpolon			1	3.7
Unid. reptiles	2	11.7	3	11.1
Mus	1	5.8	2	7.4
Prey number Snakes		17		27
with prey	16	(17.4)	26	(32.9)
Prey class		4		7
Diet niche breadth		2.28	4	.51

TABLE 4. Diet composition of *M. cucullatus* according to body size (three subspecies pooled).

mensions, we compare biomass, we do find a significant and positive relationship between the biomass of the colubrid and its prey (r = 0.68, n = 36, P < 0.001).

We detected no differences in prey frequency (G test = 11.72, df = 6, P > 0.05), prey size (*t*-test = 0.97, df = 36, P > 0.1) or biomass of the prey (t = 0.50, df = 36, P > 0.5) between the sexes, and overlap of the trophic niche between the sexes being high, (0.72). Table 5 indicates that the percentage of specimens with prey is less in the males (G test = 5.54, df = 1, P < 0.05). Females consumed less *Blanus* than did males (G test = 4.80, df = 1, P < 0.05), this prey being substituted for others also of long bodies, such as Chalcides. This, together with the consumption of a greater number of prey types in the females, caused the breadth of the female diet niche to be clearly greater (Table 5). Because in this species the ontogenetic shift in diet is of little importance, in the sexual analysis of the diet we have considered all the specimens, even the immature individuals. By comparing the 10 largest individuals for each sex, we have detected no sexual differences in SVL (*t*-test = 0.59, df = 18, P > 0.1) and biomass (*t* = 1.56, df = 18, *P* > 0.05).

DISCUSSION

The previous dietary information (but not quantitative data) indicates that *M. c. ibericus* feeds on *Blanus cinereus* (Hiraldo, 1974), *Anguis fragilis* (Almeida & Almeida, 1986), *Psammodromus algirus* (Busack & TABLE 5. Diet composition of *M. cucullatus* according to sex.

McCoy, 1990), undetermined small mammals (Vericad & Escarré, 1976), orthopterans, slugs, spiders and colubrids (González de la Vega, 1988). *M. c. brevis* feeds on *Blanus mettetali*, *Trogonophis wiegmanni*, *Tarentola mauritanica*, *Agama bibroni*, *Chalcides mionecton*, *Acanthodactylus erythrurus*, *A. pardalis*, *Mesalina olivieri*, *Lacerta pater* and *Psammodromus algirus* (Bons & Bons, 1959; Bons & Saint-Girons, 1963; Bons, 1967; Wade, 1988; Busack & McCoy, 1990). Except for the case of a cockroach regurgitated by a young specimen (Mayol, 1985), there is no previous information on the diet of wild *M. c. mauritanicus* in the Balearic Islands.

Qualitatively, our results on the diet in the specimens from the western Mediterranean substantiate previous information. The only difference is that we have found no invertebrates in the diet. It is clear that *M. cucullatus* is an almost exclusively reptile feeder, at least in the western part of its distribution area. In addition, our results confirm the preference that this species shows for amphisbaenians (Bons & Saint-Girons, 1963; Almeida & Almeida, 1986; Wade, 1988).

Almeida & Almeida (1986) stress the dependence of this snake largely on amphisbaenians because of their sharing of crepuscular and nocturnal habits. Wade (1988) concludes that the false smooth snake is nocturnal in Morocco on finding three ingested diurnal lizard species, one being *Lacerta pater*, a relatively powerful animal, unlikely to be captured when active during the day by the snake. In general, many authors concur that *M. cucullatus* is a nocturnal snake (e.g. Mertens, 1926; Lanza & Bruzzone, 1960; Bons & Saint-Girons, 1963).

However, the genus Blanus is not nocturnal, but principally diurnal, with individuals which burrow or dwell beneath rocks (Bons & Saint-Girons, 1963; Martín, López & Salvador, 1990, 1991; López, 1993); in a recent study carried out in central Iberian Peninsula on the diel daily activity of B. cinereus, Gil, Guerrero & Pérez-Mellado (1993) never encountered individuals active on the surface during the night (see also Busack (1978) for similar findings in the southern part of the Iberian Peninsula). We believe that the false smooth snake, rather than being nocturnal, also dwells under rocks and burrows to some extent. Of the 46 field observations we have recorded for this species in the Iberian Peninsula and North Africa, only three showed crepuscular or nocturnal activity, and 84.8% were under rocks (mostly in galleries). The pure cone retina discovered by Rasmussen (1985:535, Fig. 3) in a specimen from Morocco, indicates that this animal may be diurnal (see also Wade, 1988). Some skeletal aspects of this species, such as the great reduction of the postorbital bone, relatively short anterior portion of the skull, and vertebral morphology, are correlated with fossorial habits and the secretive habits of M. cucullatus (Rasmussen, 1979).

Since *M. cucullatus* shelters beneath stones most of the time, and *Blanus* (at least *B. cinereus*) is always beneath stones or tree trunks (Martín *et al.*, 1991; Gil *et al.*, 1993; López, 1993), we suspect that the false smooth snake eats mostly *Blanus* because they share habitat, not nocturnal activity. The importance of other long-bodied prey in the diet, such as *Chalcides* (Sura, 1983) which also adapted to burrowing habits, support this hypothesis. In Australia, Shine (1984) found that some burrowing snakes (Elapidae) ingested primarily burrowing prey (Typhlopidae). *Macroprotodon cucullatus* is probably an ambush hunter which waits beneath stones for passing prey, either from the exterior (small lizards), or from the ground under the stone (amphisbaenians).

Although in its main prey, *Blanus*, the head and tail extremes of the body are very similar, and there are no sharply pointed imbricated scales on the body, *M. cucullatus* looks actively for the head tip to swallow the amphisbaenian. *Macroprotodon cucullatus* is a rearfanged and poisonous snake, and probably injects the secretion of its Duvernoy's gland into a vital area to kill the prey as soon as possible; to swallow a living *Blanus*, an animal with strong body musculature, would be dangerous for the snake. In the few *Blanus* specimens found in the interior of the digestive tract of *M. cucullatus* in which the head was well preserved, there were signs of the fangs of the snake.

The false smooth snake feeds on higher SVLR and WR prey than do other Mediterranean colubrids (Pleguezuelos, unpub. dat.); high values for the SVLR or WR for the prey favour special immobilization techniques (Greene & Burghardt, 1978; Rodríguez-Robles & Leal, 1993). Thus, the consumption of large prey by *M. cucullatus* is similar to that shown by highly venomous snakes such as elapids and viperids (Greene, 1983).

This study revealed a low frequency of *M. cucullatus* with prey, lower than any Mediterranean snake with diet information (Valverde, 1967; Bruno & Hotz, 1976; Díaz-Paniagua, 1976; Vericad & Escarré, 1976; Bruno, 1977; Cattaneo, 1979; Bea & Braña, 1988; Braña, Bea & Saint-Girons, 1988; Pleguezuelos & Moreno, 1988, 1990), and among the lowest in snakes (see Shine [1986] for a review). Is this low frequency due to its tranquil habits and low activity? Alternatively is M. cucullatus prey limited? The very low percentage of specimens found with gut contents during the inactive seasons (autumn-winter, Pleguezuelos, unpub. data), seems to answer the first question; the ambush-hunter habits of the snake (according to our field observations), seems to answer the second. Both hypotheses appear to be true, but we must await further works for more information on the habits and biology of this rare and secretive species. At least, it seems that the false smooth snake has low energy requirements, as is normal in reptiles which live under rocks or lead a fossorial life style. (Kamel & Gatten, 1983).

The false smooth snake is a north African species that reached the Iberian Peninsula probably before the formation of the Strait of Gibraltar (see Bailón, 1991). In Morocco the diet is more diversified, which agrees with the greater number of potential reptile prey on the southern side of the Strait of Gibraltar (more than twice that on the northern side). In the Iberian Peninsula the false smooth snake appears to be more specialized for preying on another north African species, such as B. cinereus. Now, the current distribution of this snake in the Iberian Peninsula is close to that of B. cinereus (Salvador, 1981), and does not exceed the latter towards the north. We might pose the question of whether the Iberian distribution of the snake is determined by its main prey, or whether the similar range for both species simply reflects similar environmental requirements for two species with the same biogeographical origin.

Moreover, the diet of the few specimens from the Balearic Islands studied here indicates that this species would have plasticity in its feeding habits. The population which inhabits the Balearic Islands was probably introduced during historical time, as is true for many colubrid species in different Mediterranean islands (see Bruno & Hotz, 1976; Lanza, 1983, 1988; Mateo, 1990); this is consistent with the fact that the snake is absent from the fossil record for previous periods (J. A. Alcover, pers. comm.). The Balearic snakes were apparently introduced by people travelling from the nearby coasts of Africa (Busack & McCoy, 1990). At first, following the lizard-eating habits of the North Africa populations (Bons, 1960), the snake could have preyed on the only indigenous lizard species inhabiting the islands, Podarcis lilfordi, and was therefore considered responsible for the extirpation of this species from the two larger islands, Mallorca and Menorca (Eisentraut, 1949; Mayol, 1985).

However, the identification of the causes of the extirpation of *P. lilfordi* is not straightforward. Another lizard-eating animal, the weasel, was introduced around the same time (Vigne & Alcover, 1985; Alcover, 1987), and the effects of competition between the native lizard and the introduced lizard, *P. sicula*, may also be significant (J.A. Alcover, pers. com.). Too few data exist to evaluate this loss with rigour.

In any case, the false smooth snake continued to prey upon other species in the islands, which were also introduced by man. According to our limited results and those of Wade (1988), animals in captivity readily accept mice, which are very rare prey in the diet of *M. c. ibericus* and *M. c. brevis* (only one record, Vericad & Escarré [1976]). Island populations of *Thamnophis sirtalis* have also shown phenotypic plasticity of food habits with respect to mainland populations (Greenwell, Hall & Sexton, 1984).

Another biological trait that has changed in the Balearic population of M. cucullatus is the body size. As a result of the lack of potential predators (e.g. Malpolon monspessulanus and Herpestes ichneumon) or because they feed mainly on mice - large prey with normally more biomass than small reptiles - the populations currently inhabiting Mallorca and Menorca islands are the longest and heaviest in the distribution area of the species. This shift is common in island species (Fretwell, 1978) and has been also observed in some colubrids inhabiting other islands in the Mediterranean area, where predator and/or competitors are lacking, such as Elaphe scalaris (Hyeres Islands, Salgues, 1937), Coluber hippocrepis (Pantellaria Island, Cattaneo, 1985), and in other snakes inhabiting mainland and islands (Shine, 1987). Working on the tiger snake (*Notechis scutatus*) Schawaner & Sarre (1988) observed that the largest snakes were on islands where large muttonbird chicks were the principal prey.

Our study failed to find major differences in diet selection based on size or sex classes. The absence of correlation between prey size and body size of *M. cucullatus* can be explained because the young also feed on long-bodied prey (*Blanus*) as do the adults. Even so, *Blanus* appears to constitute the first prey consumed by the newborn snakes. The lack of differences in diet between males and females agree with similar SVL and body mass in both sexes (Feriche, Pleguezuelos & Cerro, 1993).

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SHORT NOTES

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THE EFFECTS OF NITRATE ON TADPOLES OF THE TREE FROG (LITORIA CAERULEA)

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Nitrate is relatively harmless to animal life, and is generally not considered to present a toxic hazard in surface waters (Russo, 1985). However, there is documented evidence of toxicity to fish (Russo, 1985; Tomasso & Carmichael, 1986) and freshwater invertebrates (Camargo & Ward, 1992). A comparison of the median lethal toxicity levels in these species suggests that fish are relatively tolerant of nitrate. For example, the 96 hr median lethal toxicity levels in fish (Ictalurus punctatus, Lepomis macrochirus, Oncorhynchus tshawytscha, Poecilia reticulata, Salmo gairdneri [review in Russo, 1985] and Micropterus treculi [Tomasso & Carmichael, 1986]), range from 800-8860 mg/l nitrate. However, some freshwater species may be more sensitive; for example, the equivalent toxicity levels for caddis larvae (Cheumatopsyche pettiti and Hydropsyche occidentalis) are 430-730 mg/l nitrate (Camargo & Ward, 1992). Hence, there is a need to establish the effects of nitrate on other freshwater species.

Preliminary work has shown that larvae of the common toad (*Bufo bufo*) subjected to sodium nitrate, in a laboratory situation, exhibit reduced growth and increased mortality (Baker & Waights, 1993). Such effects could be attributable to the presence of either sodium or nitrate ions, or a combination of both. The present experiment was designed to investigate the separate effects of sodium and nitrate ions on the larvae of an amphibian.

Larvae used in the experiment were taken from a single spawning of Litoria caerulea. When the external gills became covered and the larvae had begun to feed (stage 25 of Gosner, 1960), individuals were taken randomly from the spawning tank and measured, until 72 larvae measuring 7 mm had been obtained. These larvae were assigned to one of six treatments on a random basis. The six treatments consisted of concentrations of 40 mg/l and 100 mg/l of nitrate ion (made up by dissolving sodium nitrate in distilled water) and four controls. The lower concentration of nitrate was chosen because it is similar to the highest nitrate levels recorded from ponds on farm land in the eastern Midlands, England (n = 78, median = 6.6, maximum = 42). The high nitrate level was chosen arbitrarily, to magnify any effects too subtle to detect at a concentra-

tion of 40 mg/l nitrate. The four control treatments consisted of distilled water only, reconstituted soft water, and two concentrations of sodium chloride. The distilled water control was used to quantify larval growth in the absence of the sodium nitrate. Reconstituted soft water contains ions found in soft water and was used to test the validity of using distilled water as a control and as a 'dilution water'. The reconstituted soft water was made up by dissolving 48 mg NaHCO₂, 38 mg MgCl₂, 46.5 mg CaCl, and 2 mg KCl in each litre of distilled water, following Ireland's (1991) modification of Stephen's (1975) solution for toxicity testing. Sodium ion concentrations in the sodium chloride controls matched those of the two sodium nitrate treatments, and chloride ion concentrations did not exceed those of the reconstituted soft water.

Each larva was grown in a clear, food-quality, plastic beaker containing 250 ml of distilled water or solution, according to treatment. The beakers were arranged in 12 blocks on a laboratory bench. Indirect sunlight and a natural photoperiod was obtained via the laboratory window. Over the course of the experiment water temperature ranged from 22.5-26°C. Larvae were fed on JMC Aquatics cat fish pellet food; 0.015 g was provided on day 0, 0.02 g on day 5 and 0.035 g on day 9. On day 13 feeding was switched to an ad libitum regime, because by this stage the variation in larval food requirements was so great that a uniform *per capita* feeding rate would result in either pollution of the water of the smaller larvae, or prove to be insufficient to feed the larger larvae. The solutions were replaced every four days. pH was measured on three occasions during the course of the experiment. Nitrate levels in the nitrate treatments were recorded at the outset of the experiment and also immediately before and after the solutions were replaced.

Each larva was measured to the nearest 0.5 mm every four days, by placing the larva in a 'v'-shaped trough, marked with a mm scale. Growth of larvae in all six treatments is shown in Fig. 1. Any mortalities were noted each day, and, to record developmental rates, larvae were staged, without magnification, on day 16.

ANOVA of the size of larvae at 16 days was used to assess the effects of treatment and block on larval growth. There was no effect of block on larval growth, but there was a significant treatment effect, F_{532} = 8.187, P < 0.001. Tukey multiple range tests showed that both of the nitrate treatments were significantly different from all four controls (P < 0.05), but not from each other, and that none of the controls differed from each other. By the end of the experiment, significantly more larvae had died in the nitrate solutions (13/24)than in the four controls (11/48), χ^2 (Yates' correction) = 5.70, df = 1, P < 0.05. There were also evident differences in development rates. Of the surviving larvae, significantly fewer had attained Gosner stage 27, in the nitrate treatments (1/11) than in the controls (28/37), χ^2 (Yates' correction) = 13.09, df = 1, P < 0.001.



FIG. 1. Growth of larvae in experimental and control solutions. DW = distilled water, RSW = reconstituted soft water, LC & HC = low and high concentrations of sodium chloride, LN & HN = low and high concentrations of sodium nitrate. The data for the sodium chloride controls and sodium nitrate treatments have been combined, since growth curves for the individual treatments are indistinguishable.

pH varied between treatments. Lowest values were recorded in distilled water (pH 5.6-7.1) and highest values in nitrate solutions (pH 5.8-7.6) and reconstituted soft water (pH 7.0-7.3). Nitrate levels tended to decrease with time, falling from 40 to as low as 20 mg/l and from 100 to as low as 60 mg/l.

The presence of sodium nitrate in the growth media of the larvae of Litoria caerulea decreased rates of growth and differentiation and increased the rate of mortality. These adverse effects appear to be due to the presence of nitrate ions rather than sodium ions because the same concentrations of sodium ions were present in the sodium chloride controls, which had no statistically significant effect on the growth or survival of the larvae. Other studies have found sodium chloride to be toxic to anuran larvae (Beebee 1985; Padhye & Ghate, 1992), but this only occurs at much higher concentrations than those used in the present experiment. The higher of the two concentrations of sodium chloride used in the present experiment was 0.0094% (w/v) whereas Beebee (1985) found that mortality in Bufo calamita larvae occurred at approximately 0.8%, and Padhye & Ghate (1992) recorded mortality in Microhyla ornata at 0.4%. Sodium chloride may have adverse effects on Litoria caerulea, but these may be detectable only at concentrations much greater than those of the present experiment.

The adverse effects of nitrate seemed to be similar at both of the concentrations used in this experiment. Mortality was similar at both levels of nitrate (six of the twelve larvae died in the low nitrate treatment, and seven died in the high nitrate treatment). However, because of the small sample sizes it is difficult to draw conclusions concerning differences in mortality between these two nitrate concentrations. The growth trajectories of the larvae under the two different nitrate concentrations are similar, so it appears that the concentrations of nitrate used in this experiment had a similar adverse effect on the growth of larvae. It would be useful to examine the effects of lower nitrate concentrations on amphibian larvae to ascertain how much nitrate can be tolerated without affecting growth, development and survival.

There were pH differences between treatments, and it is known that pH can affect amphibian larvae. For example, low pH can cause mortalities in amphibian embryos and larvae (review in Freda, 1986) and, in addition, may decrease growth rates (review in Böhmer & Rahman, 1990). However, pH differences are unlikely to explain the mortality and growth differences observed between treatments in the present experiment, because lethal pH levels for amphibians are much lower (pH 3-4.5 [Freda, 1986]) than those recorded here, and because, although the nitrate solutions were the most inimical to the *Litoria caerulea* larvae, these solutions were less acidic than distilled water and no more basic than reconstituted soft water. Both of the latter supported healthy larvae.

Although the present study did not seek to establish median lethal toxicity concentrations of nitrate, it appears that Litoria caerulea may be more sensitive to nitrate than are fish. In fish the median lethal tolerance levels over a short time period (96 hr) were between 800-8860 mg/l nitrate (Russo, 1985; Tomasso & Carmichael, 1986). In the present study half of the larvae in the nitrate groups had died by day eight. This is a longer time scale than observed in the fish studies, but it should be noted that most larvae died within the first four days, and that the nitrate levels of the present study are one or two orders of magnitude smaller than those that resulted in deaths of half of the fish samples. If nitrate acts on amphibian larvae in nature in a similar way to that described here, in the laboratory, then the adverse effects on growth and development will have important consequences. Small larvae suffer a greater risk of predation (Travis, Keen & Julianna, 1985) and so poor larval growth rates presumably reduce an individual's probability of survival. Poor growth during the larval stage can also result in failure to escape from a deteriorating aquatic environment (Savage, 1961), prolongation of the time taken to attain maturity (Smith, 1987) and reduced body size at maturity (Berven & Gill, 1983; Semlitsch, 1987; Smith, 1987), a factor associated with low reproductive potential in amphibians (Halliday & Verrell, 1986).

The sensitivity of amphibian larvae to nitrate in the laboratory makes it desirable to assess this effect in the field. Effects observed in the laboratory may not be representative of events in the field, since toxicity to aquatic organisms varies with water quality and temperature (Mason, 1991). Particular attention needs to be paid to sub-lethal effects, such as reduced growth rates, which can be harmful to individuals: it is not sufficient to record data concerning mortality alone.

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NOTES ON THE REPRODUCTION OF ELAPHE SCALARIS (SCHINZ, 1822) IN SOUTHERN SPAIN

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Reproductive aspects of the genus *Elaphe* have been studied in North American species (Gillingham, 1974, 1979, 1980; Seigel & Ford, 1987), but information on European species is scarce and lacking in quantitative data. References to the ladder snake *Elaphe scalaris* can be found in Butler (1991), Dupres (1991), Cheylan & Guillaume (1993) and Pleguezuelos (in press).

In this paper we present information on the reproduction of *E. scalaris*, as a part of a more extensive study on the ecology of the species which was carried out in Doñana National Park (Huelva). The study area is situated in the SW of the Iberian Peninsula (37° 7', 36° 48' N; 6° 12', 6° 30' W). The climate is Mediterranean with Atlantic influence, with mild and wet winters and hot and dry summers. Average annual rainfall is 560 mm. The mean monthly temperature during the study varied between 10.5°C in January and 24.5°C in July-August. The substratum is sandy with shrub-like vegetation and isolated *Quercus suber* trees. Descriptions of the study area may be found in Rivas Martínez, Costa, Castroviejo & Valdés (1980) and Blázquez (1993).

During 1988 and 1989, 14 adult individuals (six males and eight females) were force fed radio-transmitters (Blázquez, 1993). Prior to their release, the snakes were retained in the terrarium for one day for transmitter calibration and to ensure that ingestion had been successful. None of the snakes subsequently released were detected mating or became gravid. The reproductive behaviour described here was observed in the terrarium before the transmitters were inserted.

Each terrarium was a glass cage 1.5 m length, 75 cm wide and 0.5 cm high with a movable wood and screen cover at top. The cages were provided with sand and grass on the floor, a bowl of water, and a piece of cork bark as refuge. They were placed outside the laboratory, in the field at ambient humidity, temperature, and light/dark diel rhythm.

Mating was observed on two occasions; one mating involved a male of 864 mm in snout-vent length (SVL), and 295 g weight, and a female of 1070 mm in SVL and 593 g. The pair was captured by hand on 25 and 26 May respectively, and kept in a separate terrarium until 8 June. The final stage of another mating between a male of 995 mm in SVL and 419 g captured on 17 June and a female of 920 mm in SVL and 340 g captured on 15 June also was observed.

The mating season in captivity lasts from April to the beginning of June in Southern France (Cheylan & Guillaume, 1993). González de la Vega (1988) refers to mating dates of ladder snakes in captivity in Southern Spain as between May and July. In captivity with temperature and light/dark conditions controlled, mating occurred from mid April to early May (Butler, 1991; Dupres, 1991). Observed mating in Doñana occurred on 9 and 22 June, respectively. Moreover, on 24 and 25 June we found two ladder snake pairs in the field, suggesting they were in courtship.

The behaviour of both sexes during mating on 9 June followed the three stages that Gillingham (1979) defined in other species of the *Elaphe* genus. The male was put inside the female cage on 9 June at 10.00 hr, and apparently they showed no immediate interest in each other. At 13.00 hr the male moved close to the female and continuously touched her with his chin and tongue. The female responded to this stimulation by uncoiling and stretching out completely. The duration of this stage is unknown (in other species of the genus it lasts between 1 and 23 min.; Gillingham, 1979) as we were not able to observe the beginning, but after two minutes the individuals went to the second stage -"copulatory attempts" or TSCA - (Gillingham, 1979). This stage began with a coital bite from the male in the nape of the female, and a more intense rubbing with the whole body. During this stage the animals were stretched out (the male on top of the female), while both experienced rapid epidermic caudocephallic waves. The duration of this stage was 4 min. (between 1 and 43 min. in other species of *Elaphe*; Gillingham, 1979) and resulted in the insertion of the hemipenis in the cloaca of the female. The third stage or copulation was exceptionally long (40 min.) compared with other species of the genus (between 11 and 31 min.; Gillingham, 1979), finishing with the separation of the cloaca and releasing the female from the coital bite. During this stage the epidermic waves continued, while the individuals spun around raising their tails at an angle of 45° from the substratum. Later they remained almost motionless, with the epidermic waves coming slower. Finally, just before separation they experienced spasmodic shocks again from tail to head.

The copulatory behaviour of *E. scalaris* described here is similar to that noted by Dupres (1991) and that of *E. guttata* in the length of the first two stages and the existence of a "coital bite" (Butler, 1991). However, the length of the third stage in the Iberian species exceeded that of any of the species studied by Gillingham (1979) by almost ten min.

Three ovipositions occurred in the terrarium; one from the female of the first described courtship (female

1, hereafter) and kept in captivity since then; another, from a female captured on 11 July (female 2); and the last one from a female captured on 9 July (female 3). All the ovipositions lasted between two and four days.

Egg laying by female 1 took place on 25-28 July, starting 46 days aftermating. According to information on specimens in captivity, the gravid period was considered to be between 41-71 days (Butler, 1991) and between 20 and 44 days (González de la Vega, 1988; Cheylan & Guillaume, 1993). The laying period in other areas was June-July in the centre of Spain (Pleguezuelos, in press), May-June and July in Southem France (Cheylan & Guillaume, 1993), and mid June to early July in strict captivity (Butler, 1991; Dupres, 1991).

During pregnancy, female 1 ate normally until 20 days before laying, when it began to shed. This pre-laying shedding was also recorded in strictly captive females (Butler, 1991; Dupres, 1991). During the week between shedding and starting to lay it did not feed, probably due to the physical impediment of the eggs on the digestive tract (Gregory & Stewart, 1975). From the moment laying began to two days later, the female remained hidden and coiled up next to the eggs, without eating. On leaving the nest it appeared very active, accepting food immediately.

Although the three clutches were maintained in the terrarium under the same "natural" conditions described above, only one hatching was observed, because the other two clutches failed. The average air temperature throughout incubation period inside the terrarium was 25.5° C \pm 0.6; (mean \pm SD, range 22- 36° C). The oviposition dates and the sequence of laying from the terrarium clutches are showed in Table 1.

Clutch size data come from ovipositions observed in captivity and in the field; four clutches of ladder snakes were found inside abandoned rabbit warrens, hatched and presumably complete (Blázquez & Villafuerte, 1990).

Measurements of eggs were taken from 19 newlylaid eggs and from 23 well-preserved hatched eggshells from the field. The eggshell were humidified with a blend of water (70%) and alcohol (30%) to soften them before taking their measures.

Clutch size observed in the terrarium (Table 1) is rather constant around 12, perhaps because the females were similar in size, but the clutches found in the field, on 12 and 23 September 1988, and 19 September and 1 October 1989, were of 4, 5, 6 and 12 eggs, respectively. Total clutch size ranged between 4 and 13 eggs (n = 7), which is consistent with previous observations (Butler, 1991; Dupres, 1991; Cheylan & Guillaume, 1993; Pleguezuelos, in press).

The eggs of the ladder snake have a parchment-like appearance and are rounded and yellowish, with a delicate shell. Sizes of 19 just-laid eggs were (mean \pm SD) 50.9 mm \pm 2.7 (range 45.9-55.6 mm) long and 30.3 mm \pm 1.5 (range 26.8-33 mm) wide. The hatched shells

Female number	1		2		3	
SVL (mm)	1070		1060)	1050	
Weight preoviposition	693		845		710	
Date (July)	25	28	16	17	12	13
No. of eggs	10	1	10	2	11	2
Total clutch size	11		12		13	

TABLE 1. Clutch size and sequence of laying in three *Elaphe* scalaris females.

were 44.2 mm \pm 4.9 (range 35-50.7 mm) long and 28.9 m \pm 2.4 (range 22.7-33.1 mm) wide. The difference was significant in length (t = 5.3, P < 0.001) but not in width (t = 1.9, P > 0.05), probably because the newly laid eggs are more turgid. Other authors generally refer only to ranges, which usually correspond to eggs longer and thinner than those we found (González de la Vega, 1988; Cheylan & Guillaume, 1993).

The hatching of the clutch of 12 eggs occurred on 20-23 September, between 66 and 69 days after laying. The length of the incubation period, which is dependent on the temperature (Lillywhite, 1987; Burger, 1991), ranged between 51 and 55 days in Southern France (in nests maintained at temperature of 28°C; Cheylan & Guillaume, 1993); and between 52 and 63 days in captivity in Southern Spain (González de la Vega, 1988). Butler (1991) refers to a 50-day incubation period. The relatively longer incubation period observed in Doñana was probably due to daily fluctuating temperatures in the terraria.

The hatching began on 20 September when cracks appeared on two eggs; on 21 September eight had cracks, and in the two earlier the young could be seen beginning to emerge; on 22 September three young left their eggshell and on 23 September ten young were outside. One of them died that afternoon and the two remaining eggs failed to hatch. The healthy nine newborn spent the first few days together inside the nest, almost motionless and curled up against each other.

This situation changed after the first slough, which occurred in all the young between 9 and 13 days after hatching (Butler, 1991). The young left the nest to facilitate sloughing by rubbing against the ground, and finally scattered. This behaviour is similar to that observed in *Malpolon monspessulanus* (Hailey, 1982; Blázquez, 1993), but it had never been described for *E. scalaris*.

The average size of the nine young (75% of the clutch) at birth was 290 mm \pm 12.5 (range 280-315 mm) in SVL and 19.6 g \pm 2.2 (16-23 g) in weight. After the first slough, their weight was 19.4 g \pm 1.7 (range 17-22 g). The dead hatch ling was smaller than the others at birth (SVL = 265 mm, weight = 9.5 g). The sizes of newborn snakes described here agree with those noted by Cheylan & Guillaume (1993) and Pleguezuelos (in press). Additional data were obtained from two juveniles captured in the field in April, after their first winter; they measured 320 and 340 mm in SVL, respectively.

Out of a total of 10 adult females captured during the potential gravid period (last week of May-first week of July) only four contained eggs, which implies that the proportion of females reproducing each year is low (approximately 40%) compared to other oviparous species of colubridae: 76.97% according to Seigel & Ford (1987), with only 20% of species showing less than 50% of females with oviductal eggs.

The number of gravid females and clutches of *E.* scalaris discovered during the favourable period of this study was rather low, in spite of the considerable number of traps set and active searches in the field. A small density of ladder snakes, their low reproductive rate in the area, and/or the fact that the species selects oviposition sites that we were unable to discover, may all explain the situation. In Doñana *M. monspessulanus* (Blázquez and Villafuerte, 1990) and other terrestrial colubrids (Blázquez, pers. obs.) usually select abandoned rabbit warrens to oviposit because there is a scarcity of suitable places, but surprisingly during the study we found few clutches of *E. scalaris* there. Probably the burrowing abilities of this species enables it to make holes under shrubs in less evident places.

As for the effort that breeding represents in terms of weight loss, the only information available refers to a female (female 1) of 1070 mm in SVL, weighing 693 g (100 g more than at mating time) a week before laying and 480 g just after laying (30.7% weight loss). Eleven eggs were laid weighing 223 g just after laying. The only other known reference to this subject comes from Cheylan & Guillaume (1993), which refers to a female 950 mm in SVL that after laying 10 eggs (260 g) had lost 46.6% of its weight.

Other parameters (clutch size, size of eggs and offspring) are within the normal variation range in oviparous terrestrial snakes of temperate zones (Seigel & Ford, 1987).

ACKNOWLEDGEMENTS

I would like to thank J. Juste and M. Delibes for their continuous interest and helpful suggestions during the work. R. Rodríguez-Estrella, R. Clark and two anonymous referees constructively criticized a previous version of the manuscript. N. Bustamante and L. Miller revised the English version. The work has been supported by an FPI grant of MEC.

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BOOK REVIEWS

Amphibians and Reptiles of Connecticut and Adjacent Regions. Michael W. Klemens. (1993). 318 pp. State Geological and Natural History Survey of Connecticut, Bulletin No. 12.

The great hue and cry over the paradoxical loss of systematics expertise while the importance of biodiversity achieves increasing political recognition has attracted much attention of late. However, biological diversity also has the dimension of space, through geographic distribution, as well as taxonomy. Researchers interested in the analysis of geographic distribution are frequently confronted by two types of information. One commonly finds either a solidlyshaped range map or a note on range extensions. Why do we never see a paper describing a marsh or pond which should contain a particular turtle species but does not? The answer is all too obvious, as is the loss to our understanding of the real factors underlying population distributions.

Part of the problem is the detail required. It is in the details that Amphibians and Reptiles of Connecticut and Adjacent Regions excels. Assembled over 17 years, this volume treats each of the 22 amphibians and 29 reptiles of this area of New England with attention to their biology, distribution, and conservation status. With each species account comes a township-by-township map of the state of Connecticut, with records from particular townships being represented by a red square within the township boundaries, or a red dot for current known locations. Although each is rendered as a black-and-white map showing township boundaries and river courses, the inclusion of two transparent colour overlays, one of topographic relief and one of specific geological features, printed to the same size as all of the range maps, provides an effective way of comparing the ranges to the physical features.

The book itself is a delight, with over 140 colour photographs, excellently reproduced on 30 plates. The layout and typography add to a sense of unhurried detail. Although the detail and standards of Dr Klemens' research and reporting is at a very professional level, this book is useful for anyone who wishes a solid reference work on the natural history of amphibians and reptiles in eastern North America.

For all of its positive characteristics there are a few difficulties with this work. The sections entitled *Conservation Status* vary in their content, Some are terse descriptions of the economic utility of a species as a food source for humans (in the entry for *Chelydra serpentina*) or a few lines indicating that there is no problem now (for *Coluber constrictor*). Others are longer narratives which detail threats to specific populations in nearby New York City or even two pages of detailed notes on the status of threatened species (such as *Crotalus horridus*). Of course, such variation is a natural consequence of a survey of species which vary from the common to the severely endangered.

Museum environments are ideally suited to supporting the detailed, painstaking work required for a study of this type. If the 4,872 square miles of Connecticut required 17 years and 318 pages to treat in this detail, how much more will the rest of North America - or the world - require? Simple extrapolation would suggest that the remaining 8,222,532 square miles of North America is due for about another 28,000 man-years and 537,000 pages of documentation at this resolution (of course about 2,000,000 square miles in the north are reptile and amphibian free) - roughly equivalent to the careers of all herpetologists of any sort in North America at present. Given the tendency of museums to self-eviscerate by losing their scientific core (witness the 'reorganisation' of both the National Museum of Natural Sciences in Canada and the Natural History Museum in Britain), it is unlikely that many more studies of this calibre will be forthcoming. We are all the losers.

David A. Galbraith

Centre for Endangered Reptiles, Canada

Environmental Physiology of the Amphibians. Martin E. Feder & Warren W. Burggren (Eds.). (1992). 654 pp. The University of Chicago Press. Chicago and London. £37.95 (paper); £107.95 (cloth).

Until fairly recently, succinct reviews of particular areas of amphibian biology appeared at irregular and infrequent intervals. This meant that the newcomer to the field would be faced with a fragmented, and often out of date body of literature. The three volume Physiology of the Amphibia, published between 1964 and 1976, was the starting point for many a graduate student (including the reviewer!). It has taken some sixteen years for a successor to this series to emerge. Although contained within a single volume, the 654 pages of Environmental Physiology of the Amphibians really consists of several books within a book. Not only does this volume illustrate the enormous advances made in amphibian physiology over the past twentyfive years or so, it underlines how research priorities have also shifted over the same period.

This central theme is emphasised in the opening chapter, A Perspective on Environmental Physiology of the Amphibians by Martin Feder. This introductory chapter is short, sharp and thought-provoking, as Feder systematically debunks the traditional view of the "typical" amphibian lifestyle. Feder's central thesis is that the vast majority of the research on amphibian environmental physiology has focused on four genera -Ambystoma, Bufo, Rana and Xenopus. He points out that all of these are extremely specialised forms, no more "typical amphibians" than are hummingbirds "typical birds". Feder goes on to dismantle several further misconceptions, all linked to the idea of amphibians as an evolutionary intermediate group representing a transitional stage between water and land. He rightly points out that the diverse life-styles observed within the class include both completely terrestrial and completely aquatic forms. Using amphibian physiology as a model system to illustrate incomplete adaption to life on land may therefore be misleading.

With the stage so set the rest of the volume is divided into four sections, covering Control Systems; Exchange of Gases, Osmolytes, Water and Heat; Energetics and Locomotion and Development and Reproduction. The number of chapters within each of these sections varies between two and seven, but each section, and indeed some individual chapters could have been published separately in their own right. Bearing in mind the sheer volume of information contained within some of the sections the short essays which precede each one provide a useful synthesis of what is to come. The two chapters comprising Part 1 cover the nervous system and endocrinology respectively. Both of these chapters highlight how these areas have gone through a complete revolution in recent years, and, consistent with Feder's thesis, have shifted the emphasis to the diversity, rather than the typicality, of the systems involved. Part 2 is the largest section of the book. The first five chapters of this section cover the mechanisms underlying temperature and water balance, ion regulation and respiration, while the last two chapters emphasise the role of such mechanisms in the day-to-day lives of individuals. Certainly, topics such as thermoregulation, estivation and hibernation have received disproportionate attention in the reptile literature and it is refreshing to see the balance being redressed here. Part 3 commences with a chapter reviewing the physiology and functional morphology of striated muscle. The three following chapters go on to discuss how muscular activity and energetics relate to studies of locomotion, feeding, and behaviour in both laboratory and field studies. Growth, development and reproduction are reviewed in the two concluding chapters comprising Part 4. These topics have long been studied, largely from a non-physiological angle, by ecologists and evolutionary biologists. Such researchers seeking to broaden their outlook can do no better than to consult these two chapters.

All citations within the volume are gathered into a single bibliography at the end of the book. The bibliography lists some papers which are relevant but not mentioned within the text, and very conveniently, provides for each reference the chapters in which that paper is cited. With over 4000 references listed, this section will relieve many a graduate student of long hours sifting the literature.

The production of this book must have been a formidable task for the forty contributors. Although there are many very active amphibian physiologists in the Old World, the list of authors is dominated by scientists from North American institutions. Perhaps this is merely a reflection of the Atlantic Ocean as a barrier to collaboration. The editors have been rigorous in ensuring that continuity in style and subject areas is maintained throughout the volume, and although some topics crop up in more than one chapter, they are always treated in a complementary rather than a duplicated fashion. The appeal of the book will extend beyond herpetologists - all comparative physiologists will at some stage need to consult this book, and ecologists and ethologists would be strongly advised to do so. Environmental Physiology of the Amphibians is no light bedtime read, but it is likely to remain the unchallenged successor to Physiology of the Amphibia for many years to come.

Richard Griffiths

University of Kent

Natural History, Ecology and Conservation of the Italian Spadefoot Toad, Pelobates fuscus insubricus. Franco Andreone, Riccardo Fortina, Alessandro Chiminello. (1993). 93 pp. Società Zoologica La Torbiera, Scientific Reports 2, Agrate Conturbia (Novara).

The Italian spadefoot toad, *Pelobates fuscus insubricus* is an endemic subspecies to the Po valley, and is probably the most endangered amphibian in Italy. Its decline is due to human development and habitat alteration. Because of its rarity and restricted distribution, the Italian spadefoot has received much attention by Italian herpetologists during recent years, and some conservation measures (including, for example, the creation of new breeding areas) have begun.

The present book, the second of a monographic series of contributions by the "Società Zoologica La Torbiera" on rare organisms (the first contribution dealt with the herpetological fauna of Madagascar, and was also written by F. Andreone), is an up-to-date review of the knowledge of the biology of the Italian spadefoot toad.

The book consists of 93 acid-free, well illustrated pages, with the main text written in both English and Italian. The authors review morphology and colouration, historical and present distribution, ecology, bioacoustics, embryonic development and conservation measures concerning *Pelobates fuscus insubricus*. They go on to briefly summarize worldwide distribution of either Pelobatidae or genus *Pelobates* and suggest some areas for future research.

Nearly all the topics examined were well written and rich in information, and on the whole the book is

to be considered useful not only for amphibian biologists involved in the study of Pelobates and similar species, but also for herpetologists interested in the management of rare taxa. Most of the information given here came from original scientific papers by the authors, but there is also some valuable and original data. Some of the figures were taken from previous publications by the authors, e.g. sonograms of the Italian spadefoot, which have already been published in Bollettino di Zoologia (the Italian Journal of Zoology). The section dedicated to "Ecology" is detailed and interesting, but I think that important fields such as dietary habits and ecological relationships with coexisting species, are too superficially commented upon. For instance, there is no table that summarizes diet composition, nor comparisons with populations of Pelobates fuscus fuscus from other European countries.

However, this is generally a very good book that should be a must for all European amphibian biologists involved in the management of rare taxa. The authors (one of whom, A. Chiminello, died at a tragically young age) should be complemented for their valuable efforts.

Luca Luiselli University of Rome 'La Sapienza'

BOOKS RECEIVED

Ecology of Red Maple Swamps in the Glaciated Northeast: A Community Profile. Golet, F. C., Calhoun, A. J. K., DeRagon, W. R., Lowry, D. J. & Gold, A. J. (1993). 151 pp. U.S. Dept. of the Interior, Biological Report No. 12. Washington.

Famphur Hazards to Fish, Wildlife, and Invertebrates: A Synoptic Review. R. Eisler. (1994). 23 pp. U.S. Dept. of the Interior, Biological Report No. 20. Washington.

World Checklist of Threatened Amphibians and Reptiles. World Conservation Monitoring Centre. (1993). 99 pp. Joint Nature Conservation Committee, Peterborough.

Objetivos y métodos biogeográficos. Aplicaciones en Herpetologia. Edited by Vargas, J. M., Real, R., y Antunez, A. Asociación Herpetológica Española. Monogr. Herpetol. Vol. 2. Madrid

Modern Parasitology (Second Edition). F. E. G. Cox (Ed.). (1993). Blackwell, Oxford. £18.95 (paper).

OBITUARY

DR STEPHEN PAUL GITTINS

1951-1994



Paul Gittins will be remembered, not only as a fine scientist, but also as a man of great courage with an indefatigable spirit of adventure. After graduating in zoology from University College, London, Paul studied for a doctorate in primatology at St John's College, Cambridge. This took him to the forests of Malaysia where his research focused on the behaviour of the agile gibbon. It was probably here that he got his first taste of herpetology by checking out his forest hut for snakes before settling down for the night!

After the heat of the jungle he returned to his native Pontypridd where his true patriotism showed especially when Wales were playing at Cardiff Arms Park. It was from here that he came to mid-Wales in the late 1970's and discovered the toads of Llandrindod Wells Lake, which were to mould much of his later research. After a break from toads to study the wildlife of the Sundarbans in the Ganges Delta on a United Nations contract, he took up a University of Wales Postdoctoral Fellowship through which he put Welsh amphibians well and truly on the map. His detailed study of the common toads of Llandrindod shed new light on those factors which influence the breeding migrations and population biology of these amphibians. In more recent years 'toads on roads campaigns' have gained nationwide publicity, but Paul Gittins' work remains the only study to have integrated mortality on roads into a model of toad population dynamics.

Paul Gittins' involvement with the British Herpetological Society began with a lecture to the Society on 25 November 1981. Soon after this he was instrumental in instigating an annual meeting of amphibian ecologists in Britain, one of the first of which was hosted at his research base, Llysdinam Field Centre in mid-Wales. Collaborations and partnerships forged at those early meetings are still bearing fruit today. His first herpetological research paper was published in the *British Journal of Herpetology* in 1979, and for the next few years Paul was a prolific contributor to this, and other scientific journals. In 1983 Paul was elected to the BHS Council, on which he served for two years. Illness curtailed his further involvement with the Society's activities, although his influence lives on: two of his protegés are currently serving on the BHS Council.

By the early 1980's Paul's research group was growing rapidly, and his interests had expanded to encompass studies of the population ecology of frogs and newts. Then, towards the end of his fellowship, tragedy struck and Paul contracted multiple sclerosis. He fought this increasingly disabling disease with a courage few could match, on one occasion attending a meeting and presenting a paper from his wheelchair. His courage took him as far afield as Australia, and gained him the honour of 'Man of the Year' in his hometown.

Sadly, on 10 January 1994, Paul died at the early age of forty-two. Although the great scientific potential of the man was never fulfilled, through his research and publications he laid the foundations of work which still continues to this day.

He will be remembered by those who knew him with affection, admiration and gratitude as well as with a sense of loss, heightened by a productive life prematurely curtailed.

F. M. Slater

University of Wales College of Cardiff

ANNOUNCEMENTS

ERRATUM

Wood, F. & Wood, J. (1993) Release and recapture of captive-reared green sea turtles, *Chelonia mydas*, in the waters surrounding the Cayman Islands. *Herpetological Journal* **3**, 84-89.

Table 6 should have been as follows:

2	Release weight (kg)	Release age (months)	Recapture weight (kg)	Recapture age (months)	Weight gain (kg/yr)
n	60	60	60	60	60
mear	n 3.6	13.6	8.7	32.4	3.04
SD	0.2	0.7	0.6	12.5	1.11
Max	9.2	15.0	24.5	83.0	6.15
Min	1.4	11.0	2.8	15.0	0.86

TABLE 6. Release and recapture weight and age for 60 green sea turtles.

EDITOR'S NOTE

The Editor is grateful to the following for refereeing manuscripts submitted to the *Herpetological Journal*:

R. Ackerman, R. Andrews, J. Arntzen, R. Avery, J. Baker, D. Bauwens, T. Beebee, V. Bels, D. Bennett, M. Bobyn, W. Böhme, D. Broadley, R. Bruce, J. Burger, S. Busack, J. Castanet, A. Castilla, K. Christian, B. Clarke, J. Cloudsley-Thompson, W. Cooper, M. Crump, C. Cummins, R. van Damme, G. Degani, J. Diaz, S. Evans, F. Frye, D. Galbraith, A. Gent, M. Gil, L. Gillett, P. Gregory, T. Halliday, C. Hawkey, J. Jeffers, U. Joger, P. Joly, D. King, K. Lawrence, M. Massot, R. Meek, R. McNeil-Alexander, C. McCarthy, A. Milner, H. Mushinsky, G. Nilson, M. Peaker, B. Pierce, C. Reading, R. Shine, A. Smart, I. Spellerberg, M. Swan, I. Swingland, B. Thiesmeier, M. Thompson, M. Tyler, G. Underwood, M. Veith, K. Wells, Y. Werner, F. Wood, P. Zwart

INSTRUCTIONS TO AUTHORS

(revised January 1992)

- 1. 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 illustrations. 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, Acknowledgements, 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 l; they should be typed double spaced on separate sheets with a title/short explanatory paragraph underneath.
- 7. Line drawings and photographs are numbered in sequence in arabic numerals, e.g. Fig. 1. Colour photographs can

only be included at cost to the author. If an illustration has more than one part each should be 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. and 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 J. Physiol. 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 off prints and one complimentary copy of the Journal are provided free of charge. Further copies (minimum of twenty-five) 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.

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