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REVIEW:

MULTIPLE PATERNITY AND SPERM STORAGE IN TURTLES

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ABSTRACT

Multiple paternity is the occurrence within a single clutch of offspring fathered by more than one male. This pattern may be an an adaptive feature of the mating system of turtles. Mating systems are the adaptive features of reproduction which contribute to variation in the probability of a particular egg being fertilized by a particular sperm. Turtle populations have rarely been studied specifically in terms of multiple paternity or mating systems. Five features related to multiple paternity are discussed: spacing behaviour prior to courtship, mate-seeking and mate choice, agonistic encounters between individuals, sperm storage, and sperm competition. Several studies have examined movements and spacing behaviours in turtles, and have drawn inferences about mate-seeking. At present, multiple paternity has been detected in clutches of loggerhead turtles (*Caretta caretta*), wood turtles (*Clemmys insculpta*), and snapping turtles (*Chelydra serpentina*). In wood turtles, a relationship between male dominance and paternity has been detected through DNA fingerprinting. Sperm storage has been demonstrated in several species, but studies of sperm competition are lacking. Directions for future research are considered.

INTRODUCTION

The reproductive biology and behaviour of turtles have rarely been considered from the perspective of mating systems: the "ensemble(s) of behaviours and physical adaptations specific to mating, as well as some of the social consequences of these behaviours" (p. 251, Vehrencamp & Bradbury, 1984). Mating systems consist of those factors influencing which pair of gametes fuse to form a zygote, ranging from social factors to the physiology of ovum/sperm interactions. In other words, a complete description of the mating system of a particular population would include all of the ways in which mate choice, copulation and fertilization differed from a random model of gamete assortment. As individuals may vary in the specifics of these factors, there is potential for both natural and sexual selection to produce adaptations. Some authors have approached adaptive explanations for specific features of reptilian life histories in terms of mating systems seen in other taxa (Kaufmann, 1992), or evolutionary expectations of features of the life histories, such as intense male-male competition for mating opportunities in species without significant paternal investment (Obbard, 1983, Trivers, 1972).

Within any mating system, conflicting strategies may evolve between males and females which result from differences in the costs of reproduction, either as differences in risks or in reproductive effort. The fundamental roles of male and female turtles are highly stereotyped. Pair-bonds are assumed not to form (but have they been looked for?), males contribute no parental care, and the investment of females ends with the completion and abandonment of the nest after egg laying.

One aspect of reproduction, the presence of multiple paternity within clutches, has recently been studied in many taxa using allozyme electrophoresis, and more recently DNA fingerprinting methods (Birkhead, 1989). It is possible that multiple paternity is a common feature of the reproductive biology of turtles. Only a few studies to date, however, have attempted to detect multiple paternity directly. These studies include those of nests of loggerhead turtles *(Caretta caretta)* from a beach in Australia (Harry & Briscoe, 1988); and nests of snapping turtles (*Chelydra serpentina*, Galbraith et al., 1993) and wood turtles (*Clemmys insculpta*) in North America (Galbraith, 1991).

Making use of limited genetic variation detected by allozyme electrophoresis, Harry & Briscoe (1988) were able to demonstrate significant deviations from expected Mendelian offspring genotype frequencies in clutches from eight of 21 female loggerhead turtles surveyed. These deviations were interpreted as evidence of multiple paternity. In addition, Harry & Briscoe (1988) found that in two cases, second nests from the same female in a single season appeared to have different paternal genome components, suggesting that the loggerheads mated between clutches but within the same year. This finding could also represent a case of sperm precedence in a species with very long-term sperm storage.

More recently, Galbraith et al. (1993) used DNA fingerprinting to indirectly detect multiple paternity in two of three clutches of snapping turtles examined, by detecting significant deviations from expected distributions of paternal minisatellite bands. In a study of wood turtles, Galbraith (1991) directly detected multiple paternity in two of six clutches by demonstrating that at least one offspring within each of the nests had a different father than the remaining offspring in that clutch.

Among other reptiles, multiple paternity has been demonstrated in four snake species. Snakes are relatively easy to breed in captivity, and several snake species exhibit interesting copulatory adaptations which may be related to sperm competition (see below). Multiple paternity has been demonstrated in natural nests of garter snakes (*Thamnophis sirtalis*)

using ratios of colour morphs (Gibson & Falls, 1975), and allozyme electrophoresis (Schwartz *et al.*, 1989). Multiple paternity has been demonstrated in three other species of snakes in captive breeding settings: copperheads (*Agkistrodon contortrix*) by the use of pigment patterns (Schuett & Gillingham, 1986), and in kingsnakes (*Lampropeltis getulus*, Zweifel & Dessauer, 1983), and adders (*Vipera berus*, Stille *et al.*, 1986) by protein electrophoresis.

In addition to the specific cases of multiple paternity detected in reptiles, it should be noted that the recent application of paternity analysis in populations of birds has revealed multiple paternity in many cases (Quinn *et al.*, 1987, Burke *et al.*, 1989, Gibbs *et al.*, 1990, and others).

The objective of this review is to provide a framework for the discussion of multiple paternity and sperm storage in turtles. Mating systems in turtles are therefore discussed, with occasional reference to other reptiles.

FACTORS AFFECTING MULTIPLE PATERNITY

Five groups of factors may be of particular relevance in explaining multiple paternity, and sperm storage, in turtles. The individual aspects of any mating system are, by definition, a suite of characters which are highly interdependent. As is the case with other biological systems, the mating system is composed of features whose state may be shared among the individual species or populations under study, and therefore assumed to be ancestral or 'primitive', or which may differ among populations or species ('derived', and usually assumed to be adaptive). Characters in the ancestral state are sometimes assumed to be limited by phylogenetic 'constraints', in which the species or populations under study do not vary in that character because natural selection has insufficient heritable variation upon which to operate.

First, turtles exhibit behaviour prior to courtship which will have a subsequent bearing on territoriality, spacing, or access to mates. In addition to such behaviour, population structure and sex ratios will influence how many males a female encounters.

Second, movements and activities by either sex may be directly associated with courtship and mate choice, and are of obvious importance to the number of times a female may be inseminated prior to clutch fertilization. As most publications concerning courtship and mate choice in turtles have concentrated on descriptions of mating behaviours this aspect of the mating system will not be discussed here in detail.

Third, aggressive encounters between individuals of the same sex, or between the sexes, may be very important for mate choice, the setting-up of dominance hierarchies, and mate access, and have been reported in some turtle species.

Fourth, the ability of females to store viable sperm for years (and perhaps the longevity of spermatozoa) will promote multiple paternity and sperm competition.

Finally, sperm competition, in which spermatozoa from different males present at the same time within a single female may compete for fertilization, and sperm precedence,

in which the order of insemination determines the order in which sperm from each male encounters ova, must be discussed relative to multiple paternity.

Specific characteristics, such as sexual size dimorphisms, may also have significant roles in several of these areas. Each of the five factors identified above are discussed below for their relevance to explaining multiple paternity in turtle clutches.

MOVEMENTS AND BEHAVIOURS PRIOR TO COURTSHIP

The social behaviours of turtles are complex and subtle, but few studies have investigated any of them in a systematic fashion (Harless, 1980). Movement patterns and spacing behaviours have received the most attention, largely from studies in which radiotelemetry has been used to follow individuals over periods of months or years (e.g. Obbard & Brooks, 1981, Morreale *et al.*, 1984, Galbraith *et al.*, 1987).

Home ranges may be a function of individual size, age, or experience, if the holding of home range is related to male-male or male-female agonistic encounters (Kaufmann, 1992). In the present context, home ranges are potentially important for mate acquisition in that home ranges may define areas in which males locate females, either during the course of daily activities or during the annual nesting migrations that females of some species exhibit (Obbard & Brooks, 1980). In yellow-bellied slider turtles (*Pseudemys scripta*), Morreale *et al.*, (1984) found that the movements of male turtles were earlier in the season, and of longer duration and distance than those of females. This suggests that males in this species may move in order to increase their chances of encountering potential mates. Such movements may therefore mean that females may encounter several males throughout the course of a season.

Movement patterns and home ranges have also been shown to be sexually dimorphic in snapping turtles (*Chelydra serpentina*; Obbard & Brooks, 1981), but follow a different pattern. In snapping turtles, a species in which males are larger than females (Obbard, 1983), male movements and home ranges were found to be smaller than those of females in a population in central Ontario, Canada. In addition, adult female snapping turtles in the same population undergo an annual nesting migration which may expose them to several of the more sessile males (Obbard & Brooks, 1980, see below).

AGONISTIC BEHAVIOURS

Another important component of the mating system in some turtle species is the behaviour which establishes dominance hierarchies (Harless, 1970, Schafer & Krekorian, 1983, Kaufmann, 1992). Among both wood turtles (*Clemmys insculpta*) and Galapagos tortoises (*Geochelone elephantopus*), dominance rank among adult individuals has been associated with physical attributes. Among adult wood turtles, dominant males tend to be larger, and older, than subordinate males (Harless, 1970, Kaufmann, 1992). Among Galapagos tortoises Schafer & Krekorian (1983) found that rank relationships varied between tortoises with different shell shapes. In tortoises with dome-shaped shells rank appeared to be correlated with the number of physical contacts between individuals. In those with saddle-shaped shells, however, rank was correlated with the height to which the neck could be extended.

To date a single study has demonstrated a relationship between male agonistic behaviour and subsequent reproductive success in a turtle population. In a population of wood turtles in central Pennsylvania, USA, dominance rank was shown to be significantly correlated with body mass and also with frequency of copulatory behaviour (Kaufmann, 1992). In order to test whether there was a relationship between observed dominance rank and reproductive success, DNA fingerprinting (Jeffreys et al., 1985a, 1985b) was used to carry out paternity analyses in the same population. Galbraith (1991) found that the males which were fathers of offspring from 10 nests laid in 1989 were significantly higher in the dominance hierarchy than expected by chance if there was no association between rank and paternity. Such hierarchies may be important in establishing access to females, or in influencing the chances of specific males completing copulations (Kaufmann, 1992).

COURTSHIP BEHAVIOURS AND COPULATION

Reproductive behaviour has been most often described in turtles from the point of view of courtship or mating behaviour as stereotypical acts (see an extensive review by Carpenter & Ferguson, 1977). Courtship displays and behaviour vary immensely among species of turtles. Some species exhibit a complex series of elaborate displays by the male, which includes "fanning" the foreclaws in front of and above the female's head, or while facing the female (Kramer & Fritz, 1989). Courtship of this sort has been documented from highly aquatic pond turtles in the family Emydidae (Berry & Shine, 1980). In contrast, some "bottom-walking" turtles such as snapping turtles appear to have a system of forced insemination (Obbard, 1983). Still other patterns of courtship have been demonstrated among tortoises, which include protracted periods of butting with the carapace, biting, and attempted mountings (Auffenberg, 1977). Among wood turtles, recent studies by Kaufmann (1992) have demonstrated complex courtship and mating sequences which include long periods of time in which males remain mounted on the females but do not initiate copulation.

The evolution of courtship behaviour and sexual dimorphisms in turtles are clearly related. Species in which males perform elaborate courtship displays also tend to be species with small males relative to females (Berry & Shine, 1980), and some species in which males are larger than females appear to have little or no courtship behaviour prior to insemination (Obbard, 1983).

Sexual dimorphism in adult size has been shown to vary between species and among populations in turtles (Iverson, 1985). Two broad patterns of sexual size dimorphism have been reported among turtles (Berry & Shine, 1980). Turtles in which locomotion is usually by swimming in open water generally display males smaller than females. Larger aquatic species which "bottom-walk", or large species such as tortoises, which are primarily terrestrial, display males which are the same size or more likely larger than females (Berry & Shine, 1980).

Several hypotheses have been proposed to account for the adaptive nature of sexual size dimorphisms in turtles. One theory is that the mating system in species with males much larger than females is a "forcible insemination" system, in which males must be above a certain size to subdue a female

and achieve mating opportunities (Berry & Shine, 1980, Obbard, 1983). Whether a male turtle can inseminate a female which is making all efforts to resist remains to be tested, but such a system is highly unlikely in those species which can close the carapace (e.g., the box turtle, Terrapene carolina). Although female mate choice has not been investigated in turtles, it is possible that females could use the force exerted by males prior to copulation as an indication of male fitness.

Intrasexual competition is another possible explanation for large males, if male size is advantageous in the acquisition of home ranges or in agonistic behaviours (Kaufmann, 1992). In addition, as intersexual agonistic behaviour and dominance hierarchies may also exist in turtle populations (Schafer & Krekorian, 1983), size-related dominance could establish a mating system based on the dimorphism in which forcible insemination was not necessary.

It is also possible that sexual differences in adult size are not related to sexual selection. Alternative hypotheses include ecological niche partitioning (Shine, 1980), in which intraspecific competition is reduced by each sex using a different resource base. It is also possible that a sexual size dimorphism is an emergent feature derived through energetic differences between males and females (Fitch, 1981): as one sex begins to divert energy from growth to reproduction, a disparity in size could result if the other sex continues to grow (Fitch, 1981). However, growth requires the investment of energy which could otherwise be diverted into reproductive activities, suggesting that such differences should be seen as a description of the mechanism, rather than the explanation of the phenomenon of a sexual size dimorphism.

The study of sexual size dimorphisms is difficult in long-lived species with indeterminant growth because body size varies with age. It is necessary to specify ages being compared, or to compare growth curves between the sexes. Unless differences due to age and experience are controlled for in the analyses, any differences observed between the sexes could be spurious.

Sexual dimorphisms other than size have been found in some turtle species. Among some pond turtles, for example, elongated claws on the forelimbs are used by the males to "titillate" the females during courtship (Kramer & Fritz, 1989). Perhaps the most spectacular sexual dimorphism in any turtle species, rivalling the colourful sexual displays of some lizards (Ferguson, 1977), are the seasonal colour changes in male painted terrapins (Callagur borneoensis), and related large Asian river turtles. Adult male painted terrapins change from a dark phase to a light phase through a complex series of changes in the characteristics of the epithelium, which can be triggered by testosterone administration (Moll et al., 1981). A reproductive function for this colour change has been suggested but not directly demonstrated (Moll et al., 1981). It is possible that the seasonal colour changes are behavioural status displays used by other males or by females, or possible as species-recognition features. Moll et al. (1981) observed that the species which display such colour changes are river-dwellers, for which visual signals would be more effective than olfactory signals, as olfactory cues would be swept away from individuals by flowing water.

The acquisition of mates by males is not a function of male behaviour alone, but is an interaction between males and fe-

males, and therefore female mate choice must be considered as well. No published research has yet been directed towards a quantitative assessment of female mate choice in a natural population of turtles.

SPERM STORAGE AND MULTIPLE INSEMINATIONS

Although relatively few studies have actually demonstrated multiple paternity in reptile populations, it is likely that multiple paternity is common (Saint-Girons, 1975, Devine, 1984). Several studies have demonstrated the storage of sperm within the reproductive tracts of female turtles which had been sequestered from males for periods longer than a year (reviewed by Saint-Girons, 1975, and Devine, 1984; see also Gist & Jones, 1987, 1989), and others have demonstrated the production of fertile eggs months or years after last contact with males (Schuett, 1982, Schuett & Gillingham, 1986).

Recently, Gist & Jones (1987, 1989) have undertaken detailed examinations of the reproductive tracts of female turtles, and have demonstrated the presence of sperm within tubules of the proximal oviducts, in representatives of seven taxonomic families of North American turtles. The sperm are stored within narrow tubules in the albumin-secreting region of the oviduct, which appear to be specialized for that function (Gist & Jones, 1989).

The storage of sperm within the oviducts of the females may present three reproductive advantages. First, as the first eggs which travel down the oviduct in each clutch will tend to "sweep" any contents of the oviduct downwards, sperm storage provides a reservoir of sperm for fertilization of subsequent eggs (Gist & Jones, 1989). Secondly, as many turtle species display asynchrony in the gonadal cycles of males and females, storage of viable sperm by the female may be required for successful fertilization (Gist & Jones, 1989). Thirdly, long term sperm storage may be an adaptive feature of the life-history of females in which individuals are very long-lived (Galbraith & Brooks, 1987), and hence may not have any contact with males in some years.

An important prediction arising from the demonstration of long-term sperm storage by female turtles is that mate-guarding behaviours by males may not be highly effective (Kaufmann, 1992). It would be informative to test for seasonal variation in the ability of the females to receive and retain sperm, but no studies of this, or of sperm precedence, have been attempted in turtles.

Among laboratory-mated adders (*Vipera berus*), females may mate with more than one male and produce bipaternal clutches (Stille *et al.*, 1986, 1987). Although specialized sperm storage structures have not been found in female vipers (Andren & Nilson, 1987), sperm storage for a year is possible (Stille *et al.*, 1986). A secretion from the male adder which induces contraction of a sphincter in the uterus of the female forms a copulatory plug (Nilson & Andren, 1982). In the case of the adder, the copulatory plug appears to retain sperm rather than to categorically prevent multiple insemination (Stille *et al.*, 1986, Stille & Niklasson, 1987, but see Andren

& Nilson, 1987, for an opposing view).

Evolutionary theory suggests that there is a fundamental conflict between males and females in the distribution of paternity within clutches. An individual male would maximize his reproductive success if he could father many or all of the offspring within the clutch of each female he mates with. In contrast, distributing paternity among more than one male within individual clutches may increase the reproductive success of individual females. If there are no advantages to having a particular male as sole father of a clutch, multiple paternity will decrease the expected relatedness among the offspring while retaining the expected relatedness of 0.5 between the female and each offspring. There is also direct evidence that multiple mating by female snakes may increase female reproductive success, possibly though sperm competition (Madsen *et al.*, 1992).

SPERM COMPETITION AND SPERM PRECEDENCE

Once copulation has been effected, there are additional opportunities for variation in male reproductive success in turtles. Prior to oviposition, sperm competition may take place if sperm from two or more males are present within the reproductive tract of a female simultaneously (Parker, 1970). Elegant mechanisms have been described which can evolve under conditions of sperm competition (reviewed in Smith, 1984, see also Birkhead *et al.*, 1987). In some taxa, copulatory plugs are deposited in the reproductive tract of the female by a male after insemination, apparently to thwart mating attempts by subsequent males (Devine, 1975). Some authors have even suggested that a proportion of the sperm in mammalian ejaculates has a "kamikaze" function, in that it may block or entangle sperm from subsequent inseminations (Baker & Bellis, 1988).

If polygynous species of birds are taken as a model for sperm competition in turtles, then males may be expected to attempt to copulate with many females, rather than concentrate on only a few females (Birkhead *et al.*, 1987). As male turtles provide no parental care, competition among males for mating opportunities is expected to be intense (Trivers, 1972).

Sperm competition has been inferred from the presence of multiple paternity. Two methods have been used to detect multiple paternity within clutches, or litters, of vertebrates. In the direct method, multiple paternity is demonstrated by paternity analysis for each offspring by which more than one male is assigned within the clutch (Zweifel & Dessauer, 1983, Stille *et al.*, 1986). Direct assignment is the most satisfactory method for detecting multiple paternity, and is required for the assessment of male reproductive success. However, if the males cannot be compared to the offspring, multiple paternity can be inferred from the distribution of paternal elements within the genotypes of the offspring sampled (Gibson & Falls, 1975, Schwartz *et al.*, 1989, Harry & Briscoe, 1988). A null hypothesis is constructed using the expected distribution of segregating genetic markers given a single father, and if the null hypothesis can be rejected on the basis of the observed distribution of the offspring's paternal markers, then more than one father is assumed to have contributed to the clutch (Westneat *et al.*, 1987).

Although Devine (1984) and others have predicted that sperm competition should take place in reptiles, no demonstration of relative competitive abilities of ejaculates of individual males has been made. Under conditions of sperm precedence, for example, mating order affects the success of males which sequentially inseminate females (Sims *et al.*, 1987). It is possible that the first male which achieves copulation with a given female in a receptive period would father her offspring, and perhaps have sperm stored as well. However, in some bird species, multiple layers of stored sperm result in a "first in-last out" pattern of sperm precedence (Compton *et al.*, 1978, Sims *et al.*, 1987). It is unlikely that a large volume of semen could remain within the uterus of a female turtle after the first few eggs have descended, and so sperm stored within the folds and glandular recesses of the oviduct may be important in fertilizing every clutch (Gist & Jones, 1989).

Recently, the demonstration that female adders which copulate more frequently than others also have a higher mean number of live offspring has led to the suggestion that the increase in viability of fertilized eggs is due to sperm competition, in which the 'best' sperm compete for the chance to effect fertilization (Madsen *et al.*, 1992). If this interpretation is correct, the study of vipers represents the first direct demonstration of a fitness advantage to females of sperm competition. The findings of Madsen *et al.* (1992) further supports the view that the copulatory plug does not prevent multiple insemination in adders.

PROSPECT

Although systematic research into the mating systems of turtles has been relatively sparse, several empirical studies have pointed to hypotheses which should be rigorously tested. Further research on the social behaviours of turtles in natural populations will fill out our understanding of the distribution of dominance hierarchies and agonistic behaviour. However, until genetic markers are used for determination of male reproductive success, quantitative assessment of the adaptive significance of turtle social behaviour, and the role of sexual selection, will remain untested.

The opportunities which are now available in this field are immense. The methodological limits of parentage analysis using allozymes (Hayasaka *et al.*, 1986, Westneat *et al.*, 1987, Wrege & Emlen, 1987) have receded as DNA fingerprinting has developed. DNA fingerprinting (Jeffreys *et al.*, 1985*a*, 1985*b*) and related methods (for example see Tautz, 1989) have been demonstrated in turtles (Galbraith, 1991) and in crocodilians (Demas & Wachtel, 1991), and have been used extensively for studies of behavioural ecology in birds (e.g. Quinn *et al.*, 1987, Wetton *et al.*, 1987, Burke *et al.*, 1989, Gibbs *et al.*, 1990).

We are therefore not faced with a lack of appropriate genetic markers for unambiguously determining parentage in reptiles. However, there remain few studies in which individual animals have been observed in both same-sex and intersexual behaviours in which the power of DNA profiling methods could then be used to demonstrate a connection between behaviour and male reproductive success, or between multiple paternity and the factors discussed above. Furthermore, relatively few studies of turtles have attempted to identify characteristics by which individual reproductive suc-

cess may vary in field populations.

Why is sperm stored at all in mobile, polygynous, long-lived species like turtles? Several adaptive hypotheses for sperm storage can be put forward at this time. Sperm storage is a feature of both the female and male reproductive systems. However, it may be argued that females stand to gain more than males from sperm storage.

First, storing sperm allows the male and female reproductive cycles in many turtle species to function asynchronously. Females must lay their eggs in the spring in temperate regions in order to allow for complete incubation. On the other hand, males inseminate females in the fall in some species, at the height of agonistic interactions and when the dominance hierarchy is well established. Perhaps the asynchrony in sexual cycles is an example of conflicting sexual strategies, and sperm storage is the evolved compromise (Gist & Jones, 1989).

Second, stored sperm provides a ready supply for fertilization in case the female is not inseminated in a particular year. Thus, stored sperm allows individual females to reproduce, and in some senses pre-adapts individual females to be colonizers (hence the propensity for tortoises to colonize oceanic islands) in situations of low population density.

Third, storing sperm will promote multiple paternity and sperm competition. Multiple paternity may be an advantage from the point of view of the female because it will lower genetic relatedness among offspring (Loman *et al.*, 1988). Thus, females can bring reduce the risk of inbred offspring by storing sperm from several males. If the female had to depend upon fresh sperm alone then multiple paternity would probably be much rarer. Interestingly, multiple paternity presents a conflict between male and female reproductive interests: males which fertilize entire clutches clearly would be at an advantage. Sperm competition may also be a feature of multiple paternity which presents an adaptive advantage to females, as suggested recently by Madsen *et al.* (1992), through the selection of most active sperm. This question of the consequences of multiple insemination on the rate of infertility among eggs within clutches should be reviewed and further investigated, as this may represent an important aspect of sperm competition.

Finally, there may be geometric constraints in the fertilization of a clutch of turtle eggs. If a female is inseminated after one clutch is shelled and before oviposition, the eggs will form an effective barrier to sperm reaching the storage sites for subsequent clutches, and the eggs may act to flush sperm out of the uterus upon oviposition. Perhaps females must store sperm so that later eggs in each clutch are fertilized: failure to fertilize the whole clutch would be a severe evolutionary penalty for females.

ACKNOWLEDGEMENTS

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SALMONELLOSIS DUE TO *SALMONELLA HOUTEN* IN CAPTIVE DAY GECKOS (GENUS: *PHELSUMA* GRAY)

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ABSTRACT

Two species of *Salmonella* are reported from captive *Phelsuma* spp., one of which (*Salmonella houten*), was pathogenic. Twelve geckos developed clinical signs of anorexia, diarrhoea, dehydration and cachexia. Ten died over a period of eight weeks and deaths occurred three to six weeks from the commencement of illness. Necropsy findings included dehydration, emaciation and liver necrosis. *Salmonella houten* was isolated. The response of sick geckos to antibiotics and supportive therapy is discussed.

INTRODUCTION

The normal flora of reptiles includes a wide range of Gram +VE and Gram -VE microorganisms especially of the family Enterobacteriaceae (*Escherichia coli*, *Proteus* spp., *Klebsiella* spp.), *Aeromonas* spp. and *Pseudomonas* spp. Bacteria also play an important role in reptilian diseases (Cooper, 1981). Potentially pathogenic organisms include *Salmonella* spp. of which over 1300 serotypes have been isolated from reptiles (Harvey & Price, 1983). According to Cooper (1981), however, the small number of *Salmonella* (< 4%) found in commensal reptilian flora are usually non-pathogenic. Salmonellosis was first reported in reptiles by Caldwell & Ryerson (1939) in wild horned lizards (*Phrynosoma solare*), chuckwallas (*Sauromalus ater*) and Gila monsters (*Heloderma suspectum*) in Arizona, Texas. They named the pathogen *S. arizona* and found it to be most similar to a *Salmonella* sp. initially isolated from a case of human pyrexia in Dar-es-Salaam, East Africa. Since then, and primarily because of the problem of zoonoses, salmonellosis has attracted considerable attention amongst herpetologists and veterinarians. Comparatively little is known about the treatment of the disease in reptiles since it is generally asymptomatic, especially in the early stages of infection and thus therapy is difficult.

The majority of work on *Salmonella* to date, in relation to reptiles, has been carried out with turtles and tortoises, since these come in contact with humans more frequently than do other reptiles, as a result of their popularity as pets. However, within the last 20 years an increasing number of studies have investigated zoonoses of snakes and lizards. In one study (Onderka & Finlayson, 1985) twenty-two (48%) out of forty-six lizards were infected with various *Salmonella* serotypes and five (11%) died from salmonellosis. The percentage of all reptiles harbouring *Salmonella* is estimated at 93.7% and may be as high as 77% in lizards (Chiodini & Sundberg, 1981). Because of the zoonotic implications of this carrier rate, reptiles have been the subject of extensive studies. Oboegbulem & Iseghohimhen (1985) reviewed the potential health risk from peridomestic Wall geckos (*Gecko gecko* and *Hemidactylus* sp.) and suggested that they may act as a primary reservoir or natural carrier of *Salmonella* for humans. Similar findings are outlined by Dhiraputra & Chavalittamrong (1979) in *Gecko fascicularis* in Bangkok. Kaura, Sharma & Singh (1970) found a 95.5% carrier-rate of *Salmonella* in the herbivorous lizard, *Uromastix hardwicki*. The occurrence of *Salmonella* in lizards has important epidemiological implications and an increasing number of studies

have considered the routes of transmission of infection (Hinshaw & MacNeil, 1947; Collard & Montefiore, 1957; Chambon, Le Minor & Martin, 1959; Kaura & Singh, 1968; Kaura *et al.*, 1970). Refai & Rohde (1969) and Sadek (1970) suggested transmission of *Salmonella* from mosquitoes and other flies to geckos and from humans to reptiles (Iveson, 1979). Fears of zoonoses are not unfounded. A retrospective survey of laboratory-confirmed cases of human clinical salmonellosis in the U. S. estimated that 14% of the approximately 2 million cases each year were turtle-associated (Lamm *et al.*, 1972). Whether a similar pattern occurs in other countries is unknown. Several workers have implicated reptiles as the source of human infections (e.g. Plows, Fretwell & Parry, 1968; deHamel & McInnes, 1971; Lamm *et al.*, 1972; Altmann *et al.*, 1972; Anon., 1992). Other workers have reported similarities between serotypes isolated from humans and those from reptiles in the same area (Mackey, 1955; Collard & Montefiore, 1957; Collard & Sen, 1960; Bockemühl & Moldenhauer, 1970; Kourany, Myers & Schneider, 1970; Baker, Anderson & Allard, 1972; Kaura *et al.*, 1972; Kumar & Sharma, 1978; Helm, 1981; Minette, 1984).

This paper records the clinical and necropsy findings of a *Salmonella houten* infection in a group of captive day geckos of the genus *Phelsuma* Gray. The efficacy of treatment is discussed.

MATERIALS AND METHODS

Twenty-four geckos (one *P. quadriocellata* (Peters), five *P. madagascariensis grandis* (Gray), ten *P. laticauda* Boettger, six *P. lineata chloroscelis* Mertens and two *P. guentheri* Boulenger) were maintained at the University facilities in glass or perspex vivaria within a constant temperature room (28°C). Relative humidity levels were maintained at 60-70% by daily misting. Geckos were maintained singly or in pairs, with the exception of three female and one male *P. lineata chloroscelis* maintained in a single vivarium. Individuals were fed twice weekly with crickets supplemented with "Cricket Plus", a mixture of puréed fruit and Heinz "fruit salad" baby food and provided with ground cuttlefish bone (calcium). All specimens were monitored on a daily basis as part of a programme of behavioural research on aspects of aggression and space utilisation. Thus detailed notes on the behaviour of each individual were available for a period of up to four months prior to the diagnosis of the disease. Emaciated individuals or individuals that refused to eat were force-fed a mixture of condensed milk, dextrose, vitamin sup-

plementation (especially D_3) and Heinz "fruit salad" with "Cricket Plus". A course of Dioralyte (an electrolyte balancing fluid) was also given to prevent dehydration. A control stock of specimens (*P. abbotti* Stejneger, *P. madagascariensis grandis*, *P. flavigularis* Mertens, *P. standingi* Metheun & Hewitt and *P. barbouri* Loveridge) were maintained at the junior author's premises.

Twelve geckos with clinical signs of salmonellosis were given antibiotics on the basis of antibiotic sensitivity tests (see results). Ten antibiotics were tested and three (Oxytetracyline, Ampicillin and Furazolidone) were given orally in water by pipette to *Phelsuma* specimens. Oxytetracyline (dosage 50 mg/kg/day^{-1}) was administered to one *P. lineata chloroscelis* and three *P. madagascariensis grandis* for a period of three days and then discontinued on the advice of the Dept. of Agriculture, Cork. Ampicillin (dosage 3.6 mg/kg/day^{-1}) was then administered for 12-14 days to four *P. laticauda*, two *P. madagascariensis grandis* and one *P. quadriocellata*. Furazolidone (dosage 0.025 mg/g/day^{-1}) was administered for nine days to one *P. quadriocellata*, one *P. laticauda* and two *P. madagascariensis grandis*. All disposable cage furnishings and food supplies were discarded and the room and all cages were disinfected using benzalkolium chloride (as Roccal D). Subsequently hands were disinfected with "Hibiscrub" before entering the room. The remaining stocks were monitored for clinical signs of illness.

Phelsuma faecal samples less than 4-5 hours old, were pre-enriched in Rappaport-Vassiliadis Broth (Oxoid) for 12-24 hours at 37°C and then plated on to MacConkey Agar No. 3 (Oxoid) and XLD medium (xylose-lysine-desoxycholate agar) (Oxoid) and incubated at 37°C. Biochemical analysis of colonies was carried out by a variety of tests including Gram stain, oxidase test, motility, Simmons Citrate Agar, Urea Agar and API 20E strips. Serotyping was performed by the Department of Agriculture in Cork and Colindale Laboratories in London. Faecal samples were collected from individual *Phelsuma* specimens to observe the frequency of *Salmonella* excretion. After death, samples from the liver, intestines and blood were cultured for *Salmonella* following the method described by Needham (1981, 1985) and Harvey & Price (1983).

Tissues were fixed either in Bouin's Fluid or in 70% ethanol, embedded in paraffin, sectioned at 7μm and stained with haematoxylin and eosin. Selected tissue sections were stained by the Gram method and examined using the Indirect Fluorescent Antibody Technique (IFAT).

Antibiotic senstivity tests were performed on the *Salmonella* isolates (Pat Sheehan, Dept. of Agriculture, Cork).

RESULTS

Clinical signs of illness in all cases, included apathy towards food, semi-solid faeces, raised frequency of ecdysis, dysecdysis, reduced response to external stimuli and dryness and looseness of skin, (an indication of dehydration, David Smyth, pers. comm.). Death generally occurred within three weeks of the first clinical signs, in the case of the smaller(< 140mm) species and up to six weeks in larger(> 170mm) species. Ten geckos died within eight weeks. These comprised one female *P. madagascariensis grandis*; one male and two female *P. lineata chloroscelis*; one female and four juvenile *P. laticauda* and one male *P. quadriocellata*. A further two individuals (one male *P. laticauda* and one male *P. madagascariensis grandis*) exhibited clinical signs of salmonellosis, were treated with antibiotics and after a period of 34 days and 46 days respectively, clinical signs ceased. Supportive therapy probably assisted in recovery and is strongly recommended in all cases of illness (Jackson, 1981; Lawrence, 1983).

The causative organism of the outbreak proved to be *Salmonella houten*, Subgenus IV 43: z4, z23: - (confirmed by the Dept. of Agriculture, Cork and Colindale Laboratories, London).

The gross necropsy findings were of cachexia, dehydration, pale swollen friable livers and marked congestion of the intestines. Liver samples in six cases showed abnormally large clumps of melanomacrophages (highly phagocytic tissue cells containing melanin). Large numbers of these cells had rup-

Fig. 1. Ruptured melanomacrophage cells in a liver section (stained with H & E) of a male *Phelsuma quadriocellata* (Peters), Scale bar = 50 μm

Sensitive		Resistant	
Bacteriocidal	*Bacteriostatic*	*Bacteriocidal*	*Bacteriostatic*
Amoxycillin/Clavulanate	Apramycin	Penicillin	Erythromycin
Ampicillin	Chloramphenicol	Streptomycin	Spectinomycin
Framycetin	Furazolidone		Sulphafurazole
Neomycin	Oxytetracycline		
	Trimethoprim/Sulphamethoxalole		

TABLE 1. Microbial sensitivity tests conducted on *Salmonella houten*, isolated from *Phelsuma lineata chloroscelis*.

Phelsuma species	Salmonella species
P. abbotti	S. houten[1]
P. barbouri	S. 45: g, z51:-[1]
P. laticauda	S. houten[1]
P. lineata chloroscelis	S. houten[1]
P. madagascariensis	S. enteritidis var chaco and S. soesterberg[2]
P. standingi	S. houten[1]

TABLE 2. *Salmonella* species isolated from *Phelsuma* species. 1, this study; 2, Zwart *et al.*, 1970.

tured causing local dispersion of the pigment granules (Fig. 1). The livers of three individuals contained granulomas, which appeared similiar to a heterophilic granuloma i.e. an abscess induced by masses of degenerated heterophils that are elicited by bacterial organisms (Montali, 1988; Montali *et al.*, 1989).

Rappaport-Vassiliadis, MacConkey Agar No.3 and XLD Medium proved effective for the isolation of *Salmonella* spp. and a number of other enteric organisms notably *Klebsiella oxytoca*, *Pseudomonas* spp., *Citrobacter freundi*, *C. diversus*, *Enterobacter cloacae*, *Proteus mirabilis* and *P. vulgaris*. *Salmonella* spp. appeared as small, dull colourless colonies on MacConkey Agar No. 3 and on XLD medium as distinctive orange and pink colonies with black centres resulting from hydrogen sulphide production. The *Salmonella* carrier-rate was 95.8% (23 out of 24 geckos) in treated stocks and no regular pattern of *Salmonella* excretion from the gut was detected.

Results of antibiotic sensitivity tests are given in Table 1. Ampicillin was the most effective antibiotic in the *in vivo* treatment of *Phelsuma* in this work based on the recovery of two individuals from clinical signs. Furazolidone, used on one individual, was not successful and once treatment ceased the clinical signs recurred and the individual died. Subsequent histological examination revealed necrosis of the liver and *Salmonella* was detected by IFAT.

Salmonella (including *S. houten*) were also isolated and serotyped from control specimens (*P. abbotti, P. laticauda, P. standingi* and *P. barbouri*) (Table 2).

DISCUSSION

The frequency of isolation of *Salmonella* from clinical pathology and necropsy specimens would indicate that this was the cause of the illness and death of the geckos. The necropsy findings of liver necrosis with heterophilic granulomas, in conjunction with the bacteriological findings, were supportive of a diagnosis of salmonellosis.

The pre-enrichment and selective enrichment procedures for *Salmonella* used in this study were chosen following preliminary studies with a wide range of growth substrates. Rappaport-Vassiliadis Broth was utilised in preference to Selenite Broth, which has teratogenic effects. The properties of pre-enrichment media are discussed in more detail by Harvey & Price (1983).

Kaura, Sharma & Chandiramani (1981) determined the

immunological response of five *Uromastix hardwicki* to *Salmonella* which they harboured and found an absence of haemagglutinins in the lizard sera (dilution 1:40). They concluded that this indicated a good host-parasite relationship.

Before treatment of a *Salmonella* infection is attempted, it is generally recommended that antibiotic sensitivity tests are performed and information on the properties of effective agents obtained (Watson, 1977; Cooper, 1981; Holt, 1981; Jackson, 1981). In general, narrow spectrum drugs are preferable since they conserve bacterial flora. Prophylactic drugs e.g. chloramphenicol should be avoided (Hamilton-Miller, 1975) and bacteriocidal drugs (penicillins, cephalosporins, aminoglycosides and polymyxins) are preferable to bacteriostatic ones (Watson, 1977). Antibiotics which are essentially bacteriostatic include tetracyclines, chloramphenicol, macrolides, lincomycin, sulphonamides and nitrofurans (Watson, 1977). The use of drug "cocktails" is sometimes considered inadvisable (Jawetz, 1975; Watson, 1977) as they are generally of little benefit and can be toxic. However, ampicillin and chloramphenicol used in combination eliminated *Salmonella* in turtles and tortoises (Koopman & Kennis, 1976) and neomycin and oxytetracycline suppressed but did not eliminate an infection in terrapins (*Pseudemys* sp.) (Siebeling, Neal & Granberry, 1975). The clinical condition of the animal and the appropriate dosage to administer are other parameters to consider, especially if utilising drugs with contra-indications. Tetracycline, erythromycin or chloramphenicol are not recommended for mammals with hepatic dysfunction (Watson, 1977), although these are often the antibiotics prescribed for reptiles (Murphy, 1975; Lawrence, 1983). The efficacy of the drug at different temperatures can also vary. Studies on gentamicin, for example, have shown it to be nephrotoxic at high temperatures (e.g. above 24°C in *Natrix fasciata confluens*) (Hodge, 1978). Lawrence (1983) stressed that all dose regimes for reptiles should be accompanied by recommended environmental temperatures, especially if utilising drugs with contra-indications. In the present work, therapy was not fully effective and did not prevent the progression of the condition in most cases. Siebeling *et al.*, (1975), found that antibiotics suppressed but did not completely eliminate the excretion of *Salmonella* from turtles. It is probable that antimicrobial treatment is most effective at the very early stages of infection when diagnosis is most difficult. For the treatment of *Salmonella* in reptiles, trimethoprim is a frequently recommended drug (Chris Marshall, pers. comm.). In the present work, ampicillin was used on the basis of sensitivity tests and recovery from clinical signs of disease, despite being a broad spectrum antibiotic. Lawrence *et al.*, (1983) found that of thirty-two *Salmonella* isolates from reptiles tested, only four were ampicillin resistant. Furazolidone has been used successfully in fish and poultry but no report of its administration to reptiles could be located in the literature. This antibiotic is a nitrofuran, bacteriostatic drug and in this study resulted in a cessation of clinical signs which recurred once treatment ended. Furazolidone is not therefore recommended for *Phelsuma*. According to Bullock, Conroy & Snieszko (1971) furazolidone leaves tissue residues in fish which can build up to toxic concentrations after prolonged treatment, especially in animals with renal failure.

In apparently clinically healthy reptiles, Kaura *et al.*, (1970), found that 16.4% (*n* = 134) of lizards examined car-

ried *Salmonella* in one or more internal organs such as the liver, spleen, gall bladder, ovary and testes. Gupta, Pal & Narula, (1980) isolated *Salmonella* from the liver/gall bladder of 6.5% ($n = 92$) *Hemidactylus flaviviridis*. In the present work post-mortem histological examination of *Salmonella* infected specimens revealed changes in the liver, characterised by necrotic foci and large numbers of phagocytic melanomacrophages. These cells had ruptured causing local dispersion of the pigment granules which, according to Roberts (1978), is indicative of toxaemic conditions in fish. Although little is known of these cells in reptiles, in fish they are thought to have a defensive function, acting as a source of quinone free-radicles and in association with peroxidase, as a bacteriocidal system (Ellis, 1977). Similiar histological changes have been reported in the experimental infection of *Python molurus* with *S. arizona* (von Schröder & Ippen, 1970). Gram staining histologically prepared sections of liver and intestinal tissue of *Phelsuma* proved particularly effective in the detection of bacteria and IFAT proved useful in verifying their identification as *Salmonella*.

A study by Habermalz & Pietzsch (1973) on *Salmonella*, isolated from 250 species of reptiles and amphibians in Berlin Aquarium, includes *Phelsuma madagascariensis* and an unspecified *Phelsuma* sp. Two serotypes (*S. nima* and *S. mosselbay*) were isolated in cages holding *Phelsuma* mixed with other gecko species (*Oedura tryoni*, *O. monilis* and *Gecko smithi*) so the exact origin of these serotypes is unknown. Mayer & Frank (1974) isolated *S. arizona* (*S. a.* 38: k: z35) from a gecko species and provide a plate of a paracolon infection in the liver of a *Phelsuma* sp. It is not clear however whether the gecko referred to in the text is the same as that illustrated. The *Salmonella* serotypes isolated from *Phelsuma* species in this study and two serotypes isolated by Zwart, Poelma & Strik (1970) from *P. madagascariensis* (subgenus I and subgenus IV strain), are given in Table 2. Subgenus II and subgenus IV salmonellae were isolated from *Phelsuma* in the present work. These are generally considered non-pathogenic or only midly pathogenic in humans.

In Denmark, Nielson & Clausen (1975), found that imported reptiles posed a potential risk to owners and handlers. Considering the high levels of *Salmonella* occurring as commensal bacterial flora in reptiles and the increasing popularity of these animals as pets, they may constitute a public health problem. The carrier rate, however, appears to be low in wild gekkonids e.g. from 2% in *Hoplodactylus pacificus* (deHamel & McInnes, 1971) to 33% in *Peropus mutilatus* (Bockemühl & Moldenhauer, 1970) compared with other lizards which may exceed 95% (Kaura *et al.*, 1970 - *Uromastix hardwicki*; Kourany *et al.*, 1970 - *Ameiva festiva* and Le Minor, Chambon, Bories, Marx & Charie-Marsaines, 1962 - *Leiolepis bellina guttata*).

The results of this study suggest that *Salmonella* bacteria are probably part of the normal commensal intestinal flora of *Phelsuma* species. However, stressed individuals (used for behavioural research), readily succumbed to an opportunistic invasion of *Salmonella* which resulted in the deaths of 42% of stocks ($n = 24$).

PRODUCTS LISTED IN TEXT

Cricket Plus, Monkfield Nutrition, Monkfield, Bourn, Cambridgeshire CB3 7TD, England; Dioralyte, Rhone-Poulenc Rorer Ltd., Eastbourne, BN21 3YG, England; Hibiscrub, ICI Pharmaceuticals (U.K.), Kingscourt, Waterlane, Wilmslow, Cheshire, SK9 5AZ, England; Roccal D, Sanofi Winthrop Ltd., 1 Onslow Street, Guildford, Surrey, GU9 4YS, England.

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DIET AND ACTIVITY OF *MABUYA ACUTILABRIS* (REPTILIA: SCINCIDAE) IN NAMIBIA

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ABSTRACT

Mabuya acutilabris is a terrestrial African scincid lizard distributed from Little Namaqualand to the mouth of the Zaire (Congo) River. In the central portion of its range (near Kamanjab, Namibia) the species is active in early winter from 09.50 hr to 17.10 hr. These skinks spend much time basking, and 73% of their surface activity occurs within 30 cm of clumps of vegetation where they construct shallow burrows. Single day movements of the skinks are short in both duration and distance, yet long-term movement may be considerable. Density of the species at the study site was at least 106/ha. Across its geographic range *M. acutilabris* is a generalist insectivore, with hemipterans and a variety of insect larvae constituting the most important prey classes.

INTRODUCTION

Scincid lizards of the genus *Mabuya* comprise one of the most diverse elements in the saurian fauna of the Pronamib and northern Namibian savanna. Eleven species have been recorded from the region of the Great Escarpment near the Damaraland/Outjo District boundary in northern Namibia (Bauer, Branch & Haacke, in press). In addition to many species of "typical" skinks, at least two highly morphologically-specialized species occur. One of these, *Mabuya acutilabris*, is a terrestrial psammophile and bears a striking resemblance to a number of the sympatric species of lacertids. Like many other psammophiles, *M. acutilabris* has a flattened snout with a sharp, shovel-like upper lip and a partially counter-sunk jaw, attributes typically associated with sand burrowing (Pough, 1970).

Mabuya acutilabris ranges from Little Namaqualand in the northern Cape Province, South Africa, through Namibia and Angola to southern Zaire (Fig. 1). Throughout most of its range it occupies sandy habitats in desert and semi-desert (Mertens, 1937, 1955; FitzSimons, 1943; Hellmich, 1957; Poynton & Broadley, 1978; Branch, 1988), although in Zaire it occupies beach habitats and perhaps suitably sandy alluvial deposits (Schmidt, 1919) and in parts of Angola it may be found under fallen needles in coniferous forest (Laurent, 1954). Although *M. acutilabris* is broadly distributed and occurs in high densities, natural history data for the species has been limited to only a few comments in more general works (Schmidt, 1919; FitzSimons, 1943; Mertens, 1955; Branch, 1988). The goal of the present study was to establish base-line information on diet and activity of *Mabuya acutilabris* which might be applicable to broader studies of Namibian lizard ecology. The community relationships of this skink to sympatric scincids and lacertids are addressed elsewhere (Castanzo, 1991).

MATERIALS AND METHODS

FIELD STUDY

Data were collected over a six-week period during the dry, winter season (May-June) of 1990 near the village of Kamanjab, in the western Outjo District of northwestern Namibia (Fig. 1). The study site consisted of a 220 m section of a dry river bed (a small tributary of the Okatembo River) on

Farm Franken. Lizard density appeared somewhat lower at this site than in surrounding areas, but the sparse vegetation of the river bed facilitated observation. Unusually heavy (405 mm vs. 300 mm rain/year average; Bauer *et al.*, in press) and late rainfall in 1989-90 supported relatively lush grass cover over much of the surrounding area well into the winter.
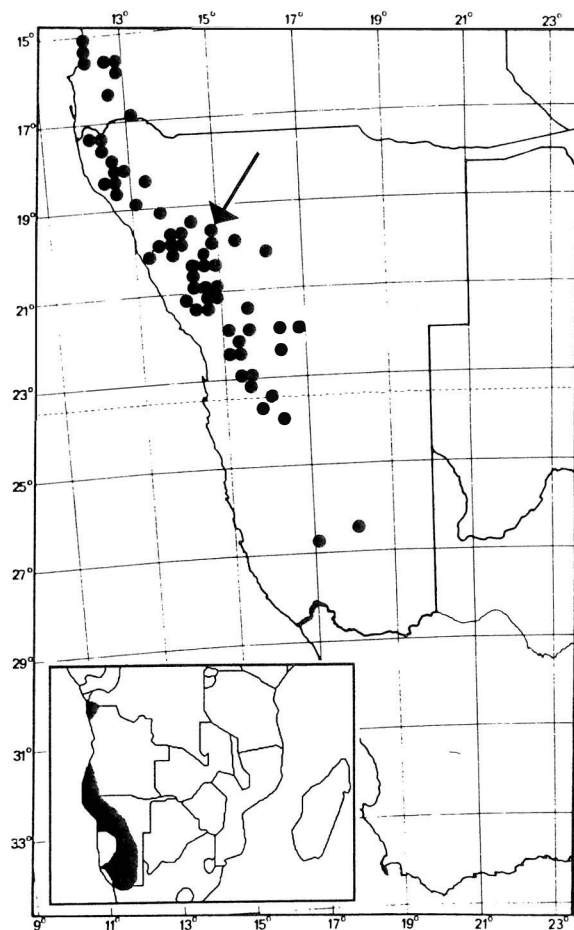

Fig. 1. Localities of specimens of *Mabuya acutilabris* examined in this study. The Kamanjab field site is indicated by the arrow. Inset map of Africa south of the equator shows the entire distributional range of the species. The disjunction between the northern and southern portions of the range may be a collecting artifact. If present in central Angola, *M. acutilabris* would be expected to occur in sandy coastal areas.

The river bed had dry, sandy soil that supported only a few plant species. The two dominant grass species were *Stipagrostis hirtigluma*, found in discrete clumps in the river bed, and *Cenchrus ciliaris*. Shrubs of several species of the Chenopodiaceae were also found in the river bed. The only reptiles other than *Mabuya acutilabris* that were observed in the study area were the lacertid species *Heliobolus lugubris* and *Pedioplanis undata*.

Other reptile species occurring in adjacent habitats on Farm Franken have been reported elsewhere (Bauer *et al.*, in press). Castanzo (1991) provided a list of potential predators present at the site. Environmental temperatures were measured hourly with a Fisher thermometer at 2 cm below the soil surface and 1 cm and 1.5 m above the substrate.

Lizards were observed for a total of 140 hr on 22 days during the study period. During this period 535 min of timed observations of behaviours exhibited and microhabitats utilized by individual lizards were recorded (mean $\pm$ SD = 11.6 $\pm$ 2.2 min, n = 46) using the focal-animal method (Altmann, 1974). Parameters recorded were: basking activity, distance moved, feeding behaviour, and escape behaviour (from humans, associated with collection for marking purposes). Microhabitat use data were placed into following categories: shrub/tuft, grass, and open. Time spent in each of these was further categorized into time spent in the sun and shade.

After data were collected for an individual for the first time, the lizard was captured, measured and marked uniquely by indelible paint pen markings and by toe clipping. Sighting and capture sites were determined relative to a marking grid of surveyors flags and plotted on a map of the study area. Lizards were always released at the point of the original sighting.

DIET

Specimens for dietary analyses were obtained from California Academy of Science, San Francisco (CAS), Field Museum of Natural History, Chicago (FMNH), Museum of Comparative Zoology, Cambridge (MCZ), Los Angeles County Museum of Natural History (LACM), Transvaal Museum, Pretoria (TM), and State Museum of Namibia, Windhoek (SMW). They included 174 individuals from 62 localities (Fig. 1), primarily from the central and southern portions of the species range. Specimens from the far north of the range, which seem to differ in at least some aspects of their ecology (Schmidt, 1919; Laurent, 1954), were not included in the study.

Stomach contents were removed and stored in 70% ethanol. Prey items were identified under a dissecting microscope and placed into food resource categories, chiefly those used by Pianka (1986). Percent of each prey type (by both item number and volume) were calculated. Volume was determined by measuring length (l) and width (w) of each item to the nearest 0.1 mm and approximating the prey body as a cylinder (Barbault & Maury, 1981). Body parts and partially digested material not associated with identifiable prey bodies (along with unidentified material) were recorded but excluded from dietary profiles (Huey *et al.*, 1974). Dietary niche breadth was determined using Shannon's Diversity Index (Pianka, 1966). Dietary niche overlap was calculated using Pianka's competition coefficient (Pianka, 1973). Be-

cause samples were obtained from many localities and during all seasons, no attempt was made to assess selectivity in diet. Statistical tests were performed primarily using Systat (Wilkinson, 1988).

RESULTS

DAILY TEMPERATURES AND ACTIVITY

Daily temperature changes of the soil and of the air at 1 cm and 1.5 m are shown in Fig. 2. No linear change in average daytime temperature occurred over the course of the study (Pearson correlation, $P \leq 0.05$). Although no recordings were made outside of the period of lizard activity, night temperatures appeared to be typical for early winter, and approached freezing on several occasions.

Mabuya acutilabris was active from 09.50 hr to 17.10 hr. Lizard surface activity commenced approximately 2 hours after sun-up. Although sundown was not until approximately 18.30 hr, the river bed was almost entirely shaded by 17.30 hr, at which time lizard activity ceased. Soil temperature averaged 20.0°C at 09.50 hr (time of emergence from burrows) and the air temperature at 1 cm was 29.1 °C at 17.10 hr (time of retreat to burrows).

Unless approached by the observer or otherwise threatened, lizards remained above ground for most of their activity period. While surface active, *Mabuya acutilabris* spent the majority of their time (73%) within 30 cm of shrubs or tufts of grass (44.2% in shade, 28.8% in sun). The burrows of this species are made in the roots of these plants and serve as retreats for the lizards when they are threatened or otherwise stressed. Tufts were also used as hiding places from which lizards darted to capture prey. Four predation attempts by the lizards, as well as numerous putative prey investigatory movements, were observed during the study. In all cases lizards moved < 1 m from vegetation to capture prey. Only 19.7% and 7.3% (5.9% in sun, 1.4% in shade) of monitored activity periods were spent in open areas and sparse (unclumped) grass, respectively.

Basking was frequently observed in all types of sites used by *M. acutilabris* and occurred at all times of day, but especially during the early morning and late afternoon. Basking lizards faced away from the sun, assuming a stereotyped partially-elevated posture. Morning basking, which began immediately following emergence, lasted as long as 43 min, although periods of complete immobility rarely exceeded 10 min. Afternoon basking lasted as long as 1.5 hr, and was often characterized by a raising of the forebody and tilting of the head, again with the dorsum exposed to the sun.

LIZARD MOVEMENT AND DENSITY

Quantification of short-term movements of skinks was based on 535 mins of focal-animal observation, representing 46 observational sessions. *Mabuya acutilabris* were found to travel 0.54 $\pm$ 0.13 m/movement at a rate of 0.29 $\pm$ 0.04 moves/min or 0.16 $\pm$ 0.05 m/min (means $\pm$ SD). Minimal estimates of movements of lizards over the study period were derived from multiple sightings of individuals. Twenty-five individuals were observed in more than one focal-animal session. The period of time over which any individual lizard was monitored varied from 1 to 22 days (Table 1). The greatest distance between any two sightings of the same individual varied between 2 and 80
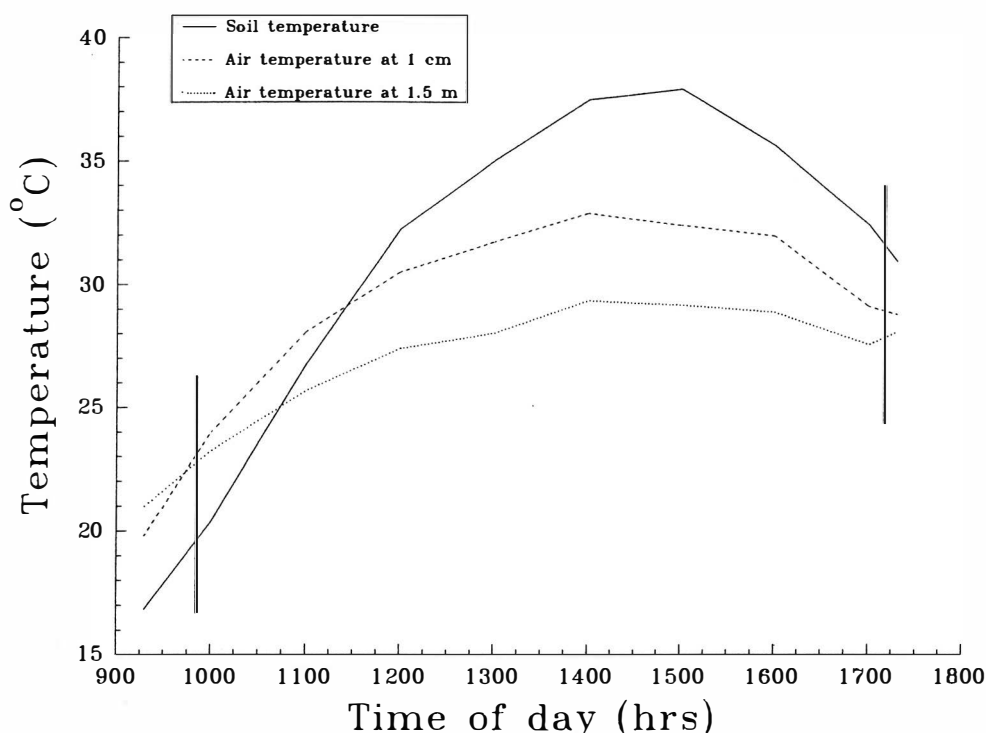
Fig. 2. Temperature (°C) vs. time of day (hrs) at the Kamanjab study site (hourly temperatures averaged over the period 30 May - 22 June, 1990). Mean soil temperature at a depth of 2 cm, air temperature at 1 cm, and air temperature at 1.5 m are given over the activity period of *Mabuya acutilabris* (bracketed with vertical bars).

No. observations	N	Max. distance between sightings (metres)	Time between first and last sightings (days)	Max. distance between two consecutive sightings (meters)	Time between maximally distant consecutive sightings (days)	Local lizard abundance (No./ 10 m radius)
8	1	22.0	22.0	12.0	6.0	2.0
6	4	6.0	20.0	3.0	3.0	6.0
4	6	17.2 (14.6)	17.5 (5.5)	14.8 (12.9)	5.5 (4.6)	5.2 (2.2)*
3	3	20.5 (10.9)	15.3 (9.8)	20.5 (10.9)	5.3 (5.1)	2.0 (1.0)
2	14	21.9 (19.8)	15.1 (8.3)	21.9 (19.8)	15.1 (8.3)	3.3 (2.1)**

TABLE 1. Movement and density of *Mabuya acutilabris* at the Kamanjab study site, based on multiple sightings of marked lizards.SD in parentheses for categories containing more than one individual. * sample size 5; not all individuals were recovered at end of study (see text). ** sample size 12 (see above).

Group	N	Total vol. (cm³)	Iso	Ort	Col	Hem	Hym	Lep	Dip	For	Lar	Egg	Ara	Cru	Pla	Ver
All individuals	174	10.3	11.1	12.4	14.1	24.8	7.2	1.4	0.6	1.7	18.2	0.0	7.0	0.2	1.2	0.0.
	(146)	(1009)	(21.8)	(3.7)	(11.4)	(17.4)	(9.3)	(0.8)	(1.0)	(8.7)	(16.4)	(0.3)	(5.7)	(0.6)	(2.7)	(0.3)
Adult males	67	5.3	14.9	16.8	17.1	17.6	4.0	1.7	0.4	1.6	16.7	0.0	9.0	0.0	0.2	0.0
	(54)	(510)	(23.7)	(3.3)	(11.4)	(21.2)	(9.2)	(0.6)	(0.4)	(8.0)	(16.9)	(0.0)	(2.9)	(0.0)	(2.2)	(0.2)
Adult females	63	4.1	8.5	5.3	10.3	38.3	11.2	1.0	0.8	1.7	18.0	0.1	1.7	0.6	2.6	0.0
	(50)	(395)	(24.8)	(3.0)	(11.4)	(14.4)	(10.6)	(1.0)	(1.3)	(10.4)	(10.4)	(0.8)	(6.6)	(1.5)	(3.3)	(0.5)

TABLE 2. Diet of *Mabuya acutilabris*, with prey categories presented by percentage of total volume consumed, and percentage of total no. items consumed (in parentheses). *N*, total no. lizards examined with total no. lizards that contained prey of any kind in parentheses. "All individuals" includes 36 juveniles plus 8 adults in which sex could not be determined due to specimen damage. Iso, Isopoda; Ort, Orthoptera; Col, Coleoptera; Hem, Hemiptera; Hym, Hymenoptera (not including ants); Lep, Lepidoptera; Dip, Diptera; For, Formicidae; Lar, insect larvae; Egg, arthropod eggs; Ara, Arachnida; Cru, Crustacea; Pla, plant matter; Ver, vertebrate material (lizard scales).

m, whereas the greatest distance between consecutive sightings (for animals observed three or more times) ranged from 3 to 38 m.

The last two days of the study period (June 20-21, 1990) were spent collecting all lizards seen on the study site. A total of 54 *Mabuya acutilabris*, four *Heliobolus lugubris*, and four *Pedioplanis undata* were captured, yielding a density of *M. acutilabris* of 106/ha and a total lizard density of 122/ha. These estimates are not based on standard density estimation techniques and are empirically-derived minimal estimates of density. For each site where a lizard was captured during this censusing period, local abundance was recorded as the total number of additional lizards within a 10 m radius (approximately one-half the average distance between consecutive sightings of the same lizard). This value varied from 1 to 8 (Table 1). Both the greatest distance between any two sightings and the distance between consecutive sightings of the same individual were negatively correlated with local lizard density, although not significantly so (Pearson correlations, $P=0.099$ and 0.114 respectively).

Throughout the study period, lizards entered the site from more heavily vegetated areas bordering the river bed. Other individuals disappeared from the site. A total of 41 *M. acutilabris* were marked during the study, of which 23 were recovered on the last two days of collecting. An additional 31 unmarked lizards were also collected during the removal census, yielding a minimum of 72 individuals present on the site sometime during the study period. Thirteen of the 18 marked individuals not recovered were only seen during the first five days of observation. One individual marked on day two of the study was not seen again for 21 days. Losses from the site probably involve both emigration and predation. No predation attempts on *M. acutilabris* were observed, but evidence of digging at likely burrow sites by nocturnal carnivores was seen on several occasions.

DIET

Mabuya acutilabris is predominantly insectivorous, and takes only small percentages of terrestrial isopods and arachnids. Measurable amounts of plant material appear to reflect accidental ingestion associated with insect prey capture, or in some cases may be derived from the rupture of the guts of ingested herbivorous insects. Vertebrate remains consisted solely of scales, probably the animal's own ecdysed skin, and constituted a non-measurable element of the total ingested material examined. For the adult skinks the five most important prey categories accounted for over 80% of the prey consumed by volume and over 70% by prey item number (Table 2). Hemipterans and homopterans as a single combined category comprised the largest single resource category and constituted 24.8% of prey by volume. Other important elements in the diet were termites, orthopterans and insect larvae (18.2% by volume).

Adult males and females consume similar prey (Table 2) and have similar dietary niche breadths (2.009 and 1.876, respectively). Some sexual differences in the relative volume of prey types occur. Males consume significantly greater percentages of termites, orthopterans, coleopterans, and arachnids, whereas females consume greater volumes of hemipterans and hymenopterans. Dietary overlap between the sexes was 0.802.

DISCUSSION

Mabuya acutilabris is an exclusively terrestrial, psammophilous skink (Horton, 1973; Poynton & Broadley, 1978; Visser, 1984; Branch, 1988). At the study site, *M. acutilabris* focused their activity around grass clumps or shrubs near the lizards' burrows, as it does elsewhere in the range of the species (Schmidt, 1919; FitzSimons, 1943). The size and extent of *M. acutilabris* burrows was not investigated, but they are probably comparable to those of *Heliobolus* and *Pedioplanis*, which are under 13 cm in depth (Nagy, Huey & Bennett, 1984).

At Kamanjab *Mabuya acutilabris* were active from 09.50 hr to 17.10 hr Comparable fall-winter activity times have been reported for other southern African diurnal lizards, including congeners (Huey & Pianka, 1977). Factors initiating daily activity in lizards have not been adequately addressed. It is generally assumed that activity is largely temperature dependent (Cowles & Bogert, 1944; Bradshaw, 1986), although endogenous circadian patterns may also be significant (Mitchell *et al.*, 1987; Seely *et al.*, 1988) and seasonally variable threshold activity temperatures may modulate the lizard's response to environmental cues (Porter & Tracy, 1983). At Kamanjab, *M. acutilabris* activity began at approximately the time that soil temperature reached 20°C and air temperature at 1 cm was 23°C. Soil temperature probably is of greater significance to the lizards at this time of day because lizard burrow temperature is largely dependent on soil rather than air temperature (van Wyk, 1992). The end of the activity period was more likely to be related to ground level air temperatures. By the time of last retreat to the burrows, air temperature had dropped to 29.1°C, 3.8°C below its highest point. In addition, large portions of river bed were shaded by this time and the loss of direct sunlight on the site at this time may have contributed to the cessation of surface activity. At the relatively low temperatures encountered, observed thermoregulatory behaviour consisted chiefly of postural adjustments during basking. Mertens (1955), however, reported that *Mabuya acutilabris* exhibits physiologically controlled colour-change in response to temperature, but only at ambient temperatures exceeding those encountered during the study.

The activity patterns of *Mabuya acutilabris* suggest that the species may be classed as a typical sit-and-wait predator (Pianka, 1966, 1971), in contrast to the majority of scincid lizards examined previously (Pianka, 1986). *M. acutilabris* spend the majority of their time near their burrows and only a small proportion of time in the open spaces between vegetation. Daily movements are few and both distance/min and distance/move are significantly lower than in the lacertids *Heliobolus lugubris* (a wide forager) and *Pedioplanis lineoocellata* (a nominal sit-and-wait predator) (Huey & Pianka, 1981), with which *M. acutilabris* is broadly sympatric. However, the lacertid data were collected in the Kalahari (Huey & Pianka, 1981), at a site with less vegetation than Kamanjab, and may not be strictly comparable. Movement may increase as the amount of abundant cover decreases. This appears to be the case for *H. lugubris*, which, at the Kamanjab site does not move as far or as often per unit time as in the Kalahari (Castanzo, 1991).

Although *M. acutilabris* remained stationary for most of each day, individuals moved as much as 80 m over the course of the study period. The total density of *M. acutilabris* on the

site (106/ha) is more than twice as high as the average value for other small lizards (Turner, 1977). Local abundance, however, varied greatly. Many lizards inhabited the eastern area of the site where grass tufts and shrubs were numerous, whereas few animals were observed in the sparsely vegetated western part of the study area.

Previous statements regarding the diet of the species reported that prey included beetles and ants (FitzSimons, 1943) and beetles, ant-lions and wasps (Branch, 1988). All of these prey items were taken by the animals examined, but only beetles accounted for more than 10% of the prey intake by volume and number. In this study hemipterans (+ homopterans), larvae (of all insect groups), coleopterans, isopterans and orthopterans were found to comprise the bulk of the diet, both with respect to volume and prey item number (Table 2). This is consistent with what is known of the foraging behaviour of *M. acutilabris*. This species' reliance primarily on relatively mobile prey (orthopterans, coleopterans, and hemipterans/homopterans) plus larvae (found in the same vegetation clumps as the lizards' burrows) seems to support its characterization as a sit-and-wait predator. The diets of Kalahari species of *Mabuya* contain high percentages of termites (up to 45% by volume in *M. striata*) (Huey & Pianka, 1977). Termites, a clumped resource, which typically predominate in the diets of widely foraging lizards (Pianka 1986), are present in the diet of *M. acutilabris*, but account for only 11.7% by volume of the prey consumed. The low level of utilization of this resource corroborates the aforementioned interpretation of the foraging mode of *M. acutilabris*.

Male and female *Mabuya acutilabris* consume similar types of prey and the relative importance of the prey types taken are in general agreement with the overall species dietary profile presented (Table 2). However, when volumes of prey are considered, greater sexual divergence is evident. The majority of female's diet is comprised of bugs, while males consumed considerably greater volumes of termites, spiders, orthopterans, and coleopterans. The significance of these difference, if any, is unclear, however, because the overall similarity of the diets, as measured by niche overlap (0.802), is high relative to interspecific overlap between sympatric congeners (Castanzo, 1991).

The ecology of *Mabuya acutilabris* would appear to be atypical in some regards with respect to that of other southern African skinks that have been examined (Huey & Pianka, 1977; Pianka, 1986). Both the limited daily movements and dietary profile of this lizard suggest that it is primarily a sit-and-wait predator. In this aspect the species may be convergent with syntopic lacertids (Castanzo, 1991). The differences observed between scincids from different arid regions, however, are likely to be influenced by both local prey availability and vegetational characteristics, and as such are not strictly comparable. In addition, the realized niche of each species will be heavily influenced by interactions with other taxa. While such interactions have been documented by community studies of Kalahari lizards (Pianka, 1986), these remain to be elucidated in the case of the lizard fauna of the northern Namibian savanna.

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THE RELATIONSHIP BETWEEN DISTURBANCE, RESPIRATION RATE AND FEEDING IN COMMON LIZARDS (*LACERTA VIVIPARA*)

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ABSTRACT

Slightly disturbing captive common lizards, *Lacerta vivipara*, by movement and noise while they are basking in laboratory arenas resulted in an increase in respiration frequency (R_f) and a decrease in the probability that a lizard would respond to the introduction of a potential prey item. Two categories of prey (crickets and mealworms) were presented at three locations defined in relation to the snout of a lizard; there were clear negative correlations between R_f and probability of feeding in all cases. Respiration frequency can thus be used as a means for determining whether a lizard in a feeding trial has been diverted by extraneous stimuli. This is important in investigations of foraging efficiency in relation to perceptual fields and movement patterns, in which it is necessary to know that experimental animals have not been diverted in this way.

INTRODUCTION

This paper is part of a programme designed to investigate pause-travel movement (Andersson, 1981) and its relation to perceptual fields in lizards (see Avery, Mueller, Jones, Smith & Bond, 1987 and Cowlishaw & Avery, 1991, for background information on pause-travel movement in Lacertidae). However, there are several factors which may potentially confound experimental hypothesis testing, especially on relationships between movement patterns and food distribution. One of the most important is the qualitative observation that lizards in both the field and the laboratory are easily diverted by extraneous stimuli, especially those caused by movements of the experimenter, reducing responsiveness to potential prey (Avery & Mynott, 1990). It is important to have some check that an animal in an experimental feeding trial is not diverted in this way. One potential method is to make use of the fact that the respiration frequency (R_f), which is readily observed from ventilatory movements of the anterior thorax (Milsom, 1984), often increases in response to external stimuli; the analysis of feeding trials can be confined to those trials in which R_f has not increased to a level above the undisturbed range. This requires an understanding of the relationship between R_f and the probability that responsiveness to prey will be reduced. The experiments described here investigate this relationship.

MATERIALS AND METHODS

LIZARDS AND FEEDING TRIALS

Fifteen adult male common lizards, *Lacerta vivipara* Jacquin (mass 3-4 g, snout-vent length (SVL) 46-49 mm), were housed individually in open-topped cages measuring 60 x 45 x 10 cm and maintained as described by Avery & Mynott (1990). The cages were floored with plywood. Each cage contained a scale ruled in cm along two edges and a protractor placed horizontally near one edge. Screens were placed in such a way that only a small part of the experimenter's face was visible to each lizard.

Feeding trials involved dropping an item of prey through a metal tube, only the lower tip of which, at a height of 30 cm, was visible to the lizard. The position at which the prey fell was recorded; the horizontal distance from the snout of the lizard was estimated using the scales at the edges of the cage, and the angle from the longitudinal axis of the lizard at the snout estimated by comparison with the protractor. Trials using a dummy lizard showed that distances could be estimated with an accuracy of ±4 mm, angle with an accuracy of ±8°. Because the lizards were not exactly the same size, distances were standardised for each lizard to SVL. Prey were presented on a schedule which was randomised for distance and angle (within the limits with which it could be placed accurately) to prevent a lizard associating any particular place with food. The reponse of each was recorded for 10 s after the presentation of an item of prey. All potential prey items were preweighed and only those whose mass relative to that of the lizard lay within the range 0.01-0.02 were used, since *L. vivipara* respond readily to prey in this size range, capturing and swallowing them rapidly (Avery & Mynott, 1990). If the prey were approached the trial was scored as a success, if not as a failure. No lizard was presented with more than two items of prey on any day. The capture probability for any kind of prey at any distance and orientation was defined as the proportion approached (it was only very rarely that a lizard approached an item of prey and then failed to capture it), summed for all lizards. The prey were either live crickets (*Acheta domestica*) or mealworms (larvae of the beetle *Tenebrio molitor*).

RESPIRATION FREQUENCY

Respiration frequency of lizards in the laboratory was determined by measuring the Doppler shift of a 25 kHz ultrasonic signal produced by movement of the body wall caused by the contractions of thoracic muscles and consequent movements of anterior ribs which power ventilation. The signal generator and receiver were placed diagonally above the basking site (Fig. 1). The technique can only be used successfully if the lizard is otherwise not moving (Avery & D'Eath, 1986; Avery & Mynott, 1990). If a lizard is disturbed, even only slightly, it becomes vigilant and tends to look upwards, and R_f usually rises; the probability that it will then respond to the presence of prey is reduced. A variable R_f was achieved by disturbing the lizard immediately prior to the presentation of prey. Slight disturbance involved slow movement of the experimenter's hands within sight of the lizard (by placing them above the screen used during food presentation),

sometimes accompanied by a soft noise. A greater degree of disturbance was achieved by more rapid movements, sometimes accompanied by louder noises; occasions involving this level of disturbance were separated by at least one week. Each prey item was introduced during the period between 5 and 10 s following the disturbance, and the R_f recorded as the number of ventilatory movements from 1 s following the disturbance to the time of food presentation (i.e. a period of 4-9 s; further measurement of R_f was impossible because of the extraneous signal due to movement by the experimenter, the prey and the lizard). The R_f of control lizards which were neither disturbed nor fed but in which the experimenter's hands were moved behind the screen was measured over periods of 9 s, and of totally undisturbed lizards over periods of 3 min. The R_f of three adult male lizards in the field was also determined over 3 min periods by observing lizards which had been basking on warm mornings for more than 15 min through a telescope from distances of more than 8 m, and counting the number of thoracic movements using a stopwatch to determine the overall time.

BODY TEMPERATURES

The cages containing experimental lizards were maintained in a room at 18-21°C. A 60 W tungsten bulb, oriented diagonally above the wooden floor of the cage and shone through a metal funnel with a cardboard tube so that a pool of light and heat radiation was confined to a small patch (Fig. 1), was switched on for 2 hr each morning to enable the animals to thermoregulate. Prey were presented at times when body temperatures were in the range 30.5-33.0°C, which is within the activity temperature range of the species (Jones & Avery, 1989). This was monitored by placing an infra-red video camera immediately above the basking site, enabling body temperatures to be recorded at regular intervals (Jones & Avery, 1989). Care was taken to ensure that the image of the lizard was not obscured by the cardboard tube (Fig. 1).

RESULTS

The R_f of undisturbed experimental lizards had a mean value of 30.2 min^{-1} (n=100, SD=4.46, range=21.3-40.2). The mean R_f values for control lizards (quiet movement of the experimenter behind the screen and with no presentation of food) and of basking lizards in the field were similar (controls, $\bar{x}$=29.1 min^{-1}, n=100, SD=4.81, t=1.69, P>0.5: field, $\bar{x}$=28.6, n=30, SD=5.86, t=1.39, P>0.1). Differences between individual lizards were not significant in either experimentals, controls, undisturbed lizards or the field (one-way ANOVA, P>0.1 in all four cases) and so data for individuals have been pooled in all subsequent analyses. Although this procedure may have violated the assumptions of independence of observations, the results are sufficiently clear cut to support the conclusions and the statistical tests are included to give an approximation of significance levels.

Capture probabilities were recorded for both crickets and mealworms when dropped 0.5 SVL (equivalent to 2-3 cm) and 2 SVL in front of the tip of the snout, and 0.5 SVL behind the snout on an imaginary line subtending an angle of 150° to the forward extrapolation of the longitudinal axis of the body (Fig. 1). The relationships between capture probabilities for these three positions and R_f grouped in increments of 5 min^{-1} are shown in Fig. 2. Each data point represents a capture probability determined from a total of between 20 and 45 presentations. In no case was there any significant difference between capture probabilities at R_f values in increments of 5 min^{-1} within the normal range for undisturbed lizards, i.e. 20-40 min^{-1} (Kruskal-Wallis tests, P>0.1 in all six cases). Above R_f=35-40 min^{-1}, however, there was for both prey types and in all three prey positions a progressive decrease in capture probabilities with increasing R_f (Kruskal-Wallis tests treating values for R_f=20-40 min^{-1} as single pooled subsamples and with probabilities for R_f>65 min^{-1} ignored since almost all were zero; P<0.001 in all six cases). with very high levels of disturbance
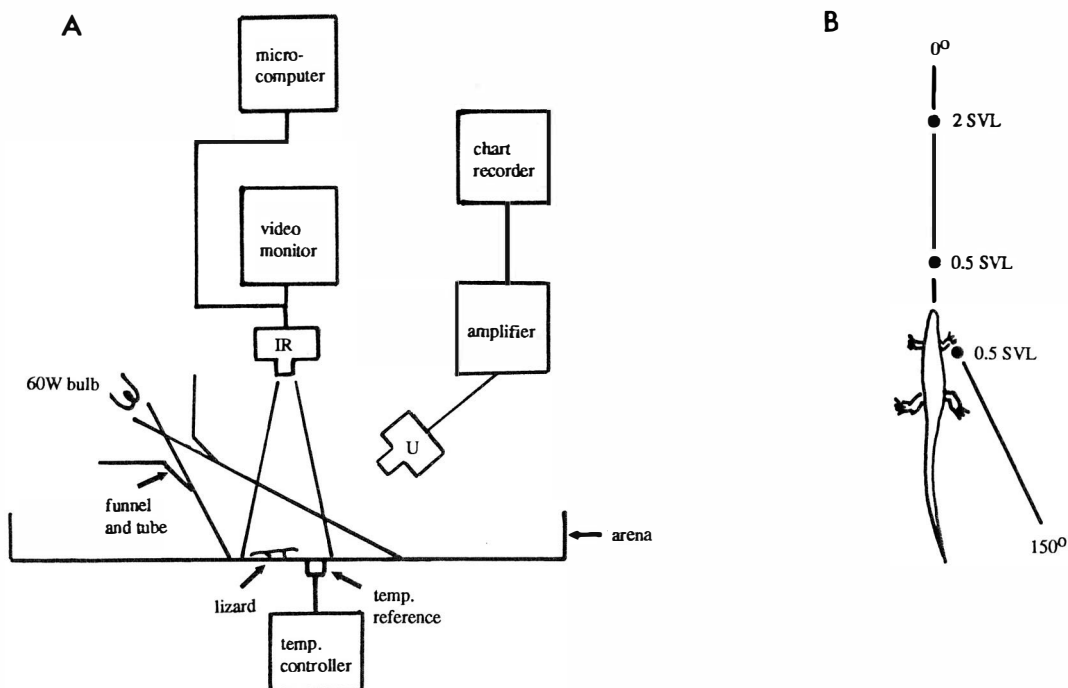

Fig. 1. A. Diagram of experimental apparatus. IR = infra-red camera, U = ultrasound generator and receiver. B. Positions at which food was presented in relation to the snout of an experimental animal.
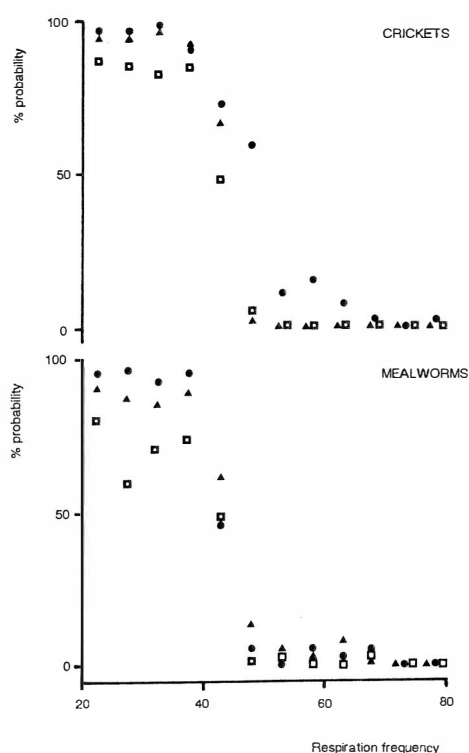
Fig. 2. Capture probabilities for crickets (upper graph) and mealworms (lower graph) presented 0.5 SVL in front of lizards (circles), 2 SVL in front of lizards (triangles) and 0.5 SVL along a line subtending an angle of 150° to the forward extrapolation of the longitudinal axis of the body (squares).

there was a change in the nature of respiratory movements, the pattern switching to rapid ($R_f > 100$ min^{-1}) shallow breaths; the lizards never fed when breathing in this way.

The capture probabilities for prey presented directly in front of lizards were in almost all cases lower at 2 SVL than at 0.5 SVL for both crickets and mealworms (Wilcoxon signed-rank tests, with the same conditions as for the Kruskal-Wallis tests above, $P < 0.01$ in both cases). There were no significant differences in capture probabilities for either kind of prey, however, between prey at 0.5 SVL directly in front of lizards and prey at 0.5 SVL at 150° (Wilcoxon signed-rank tests, with the same conditions as above, $P > 0.1$ in both cases).

DISCUSSION

The data show that disturbance by the experimenter which results in an increase in R_f decreases the probability that a *L. vivipara* will successfully capture a cricket or mealworm which has been placed at a distance and orientation at which it would normally be eaten. The mechanism for this effect is not known. It is likely, however, that the lizard is diverted from feeding by additional sensory input. For example, a hierarchy of tectal filters for different movement stimuli have been described for toads (Saton & Shiraishi, 1991), and a similar system may operate in lizards.

Respiration frequency can be used as a means for identifying occasions when an individual lizard has been diverted in this way; as noted in the Introduction, this can be of practical

importance in feeding trials. Such trials are used extensively in experiments on the relationships between the location of prey and the probability of feeding, which are important in analysing the significance of pauses in lizard locomotion (Avery, in preparation). An alternative solution to the problem of "diversion" would be to automate the food presentation. This would be feasible if the food were non-living, but *L. vivipara*, like many other lower vertebrates, will normally only respond to moving (which effectively means living) prey because the central neural mechanisms involved in feeding include movement receptors (Ewert, 1985; Saton & Shiraishi, 1991; data relating to toads). Presentation of living prey is difficult to automate.

Does the diverting effect wane with time, i.e. does a lizard habituate to the stimulus and the rise in R_f decrease with repeated exposure? It is a major criticism of this study that the possibility could not be investigated rigorously, because the disturbance could not be accurately quantified. Plotting the sequential R_f values for each individual lizard for stimuli that were judged subjectively to be equivalent, revealed no trend for the values to decrease with time. It is assumed, therefore, that habituation did not occur over the timescale of these experiments.

The experiments are artificial in the sense that they were carried out in the laboratory, but the observations of lizards in the field showed that values for resting R_f were the same in both. There is no reason to suppose that "diversion" does not also occur in the field. It is a common observation that the response of a wild *L. vivipara* to an approaching human is to watch him intently and to flee if the stimulus encroaches within the species-specific 'escape distance' (Bauwens & Thoen, 1981).

There are no previous studies with which the present data can be directly compared. The use of R_f to monitor any kind of stress in reptiles is novel, and the technique may also have other applications. The resting R_f levels recorded here are almost identical to those reported previously for *L. vivipara* at the activity temperature range (Avery & Mynott, 1990). This is the range of body temperatures within which the lizards usually feed in the field (Avery, 1971); feeding will take place at lower temperatures in the laboratory (Avery & Mynott, 1990; Van Damme, Bauwens & Verheyen, 1991), but this is facilitated by the relative ease of prey capture under captive conditions. It would have been possible to determine the effects of body temperature on the relationships reported in this paper; the primary reason that such studies were not carried out is that R_f falls rapidly with decreasing temperature and becomes so low and erratic that it is difficult to measure accurately over the short timespan between the disturbance and the presentation of food. It was necessary for this to be kept short (maximum 9 s in these experiments) because there is a gradual reduction in R_f towards resting levels after the rise induced by disturbance.

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MOVEMENT RATES OF THE SMOOTH SNAKE *CORONELLA AUSTRIACA* (COLUBRIDAE): A RADIO-TELEMETRIC STUDY

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ABSTRACT

The movement behaviour of free ranging smooth snakes (*Coronella austriaca*) was studied by radio-telemetry. The species exhibits a low median hourly-movement rate (0.54 m h^{-1}, range = 0.00 to 44.26 m h^{-1}, n = 1074) and a correspondingly low daily-movement rate (median = 13.30 m day^{-1}, range = 0.00 to 166.81 m day^{-1}, n = 138). There were no significant differences in movement rates between the sexes. Smooth snakes do not generally occupy specific features (such as dens) and move through parts of sites at differing rates; some animals will remain within small areas for some while whilst others move through an area quite quickly. The effect of attachment of radio-transmitters on the behaviour of the snakes was investigated via a short laboratory study using time-lapse video recording. Only minor differences were observed between movement rates and behaviours of tagged and untagged animals and no differences were detected for changes in body weight over the period. These observations indicated that the attachment of a radio-transmitter did not significantly affect the behaviour of smooth snakes.

INTRODUCTION

An understanding of movement behaviour is important when studying the ecology of an animal. This allows an assessment of the dispersal potential of the species. Studies of movement ecology also provide information about foraging behaviour and site tenacity. These, in turn, can be related to energy expenditure and all other aspects of the species' ecology. Such knowledge is valuable when considering conservation management; this is particularly so where habitats of reptiles are becoming fragmented.

Many studies of movement behaviour of snakes have been published (e.g. Freedman & Catling, 1979; Tiebout & Cary, 1987). Indeed movement behaviour of the smooth snake *Coronella austriaca* has previously been studied (Breeds, 1973; Spellerberg & Phelps, 1977; Goddard, 1981). These studies suggested that the species is relatively sedentary and therefore has only a limited potential for dispersal and for colonising new areas. In addition, smooth snakes do not appear to occupy separate summer and winter ranges (Phelps, 1978) and hence would not be expected to show seasonal migration.

These earlier workers, however, relied upon recapture studies. Due to the secretive nature of the species and the difficulty in obtaining regular observations, detailed studies of movement rate in this species have not been possible. Furthermore, recapture studies will be subject to observer bias (Tiebout & Cary, 1987). During the present study radio-transmitters were attached to *C. austriaca*. This enabled regular and frequent locations and thus permitted a more detailed investigation of smooth snake movement behaviours than had previously been possible.

With a view to evaluating the effect of the methodology on the behaviour of the snakes, a short behavioural study was undertaken in the laboratory using time-lapse video-recording.

MATERIALS AND METHODS

FIELD STUDY

Smooth snakes were studied at two sites in the south west of the New Forest (southern England). Site 1 was a forestry inclosure planted mostly with coniferous species (including *Pinus* spp. and *Picea sitchensis*) and incorporated the immediately surrounding heathland and adjacent grass 'grazing area'. The site had formerly been heathland and considerable areas within the inclosure had been left unplanted. These unplanted areas were predominantly heathland of ling *Calluna vulgaris*, bell-heather *Erica cinerea* and cross-leaved heath *E. tetralix*, grasses e.g. *Molinia caerulea* and *Agrostis curtisii*, bracken *Pteridium aquilinum* and gorse *Ulex europaeus* and *U. minor*.

Site 2 was located approximately 2 km south-west of Site 1. The site was bisected by the course of a dismantled railway line which provided two steep embankments. The southern embankment and the north facing hill behind was heathland that had been burnt some five years before the start of the study. The vegetation here was quite short and sparse as a consequence. Gorse species were abundant over much of the embankments. The area to the north of the railway cutting was a heathland dominated by heather species which gradually merged into a wet bog system predominantly of purple moor grass *Molinia caerulea* and bog myrtle *Myrica gale* over a distance of approximately 300 m. To the north of the bog was a southerly slope of mature humid and dry heathland.

Snakes were fitted with small radio-transmitters (173.20 to 173.35 MHz range), weighing approximately 2.5 g. These were attached externally to the base of the tail using surgical adhesive tape (Fig. 1). Once released, snakes were located at intervals of approximately two hours during daylight (typically 0800 to 2000 hrs BST). This interval was chosen to give regular observations throughout the day whilst minimising disturbance. The time of each location was recorded and the
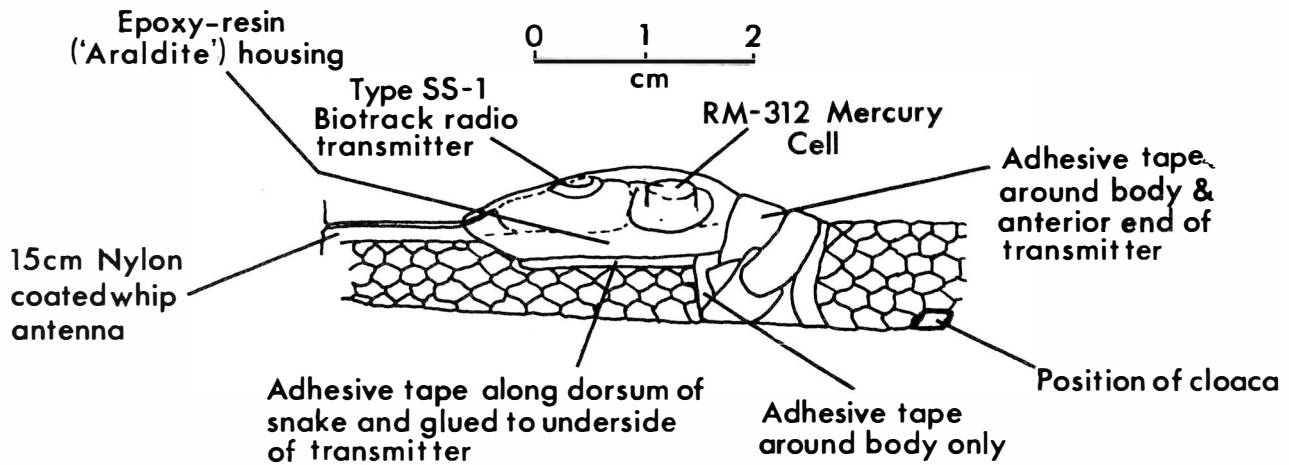
Fig. 1. Design and attachment of radio transmitter.

exact interval calculated. The position of the snake was described relative to pre-positioned markers in the field and this allowed subsequent calculation of the straight line distance between locations. Hourly movement rates were calculated by dividing this distance by the time interval and daily movement rates determined by summing all monitored distances recorded during a day.

The interval between sightings may influence estimates of movement rates. With a view to ensuring consistency, hourly movement rates were only calculated where the sampling interval was between 1 and 3 hr. Estimates of daily movement rate are affected by the number of observations used in the calculation (Fig. 2). Although no clear asymptote was observed in the relationship between daily movement rate and number of monitored distances, a division was made between those estimates where the movement rate was based on five or less distances and those where six or more had been summed. Consequently, only those daily movement rates where six or more distances were used in their calculation were considered for subsequent analysis.

The direction of movement was determined by relating the straight line distance between successive locations to a compass bearing. Each measured distance was assigned to one of eight 45° bearing classes. Directional movement was analyzed in three ways. Firstly, where total distances moved were in excess of 40 m (smaller values were excluded as they were too small to allow statistical analysis), the observed pattern of dispersal was compared against a theoretical equal movement in all eight defined directions. Secondly, as an indication of net dispersion over short time periods, a ratio was calculated by dividing the distance between first and last locations (net dispersion) by the sum of all monitored movement distances (total movement). This Net movement/Total movement ratio allowed an assessment of dispersal that was independent of the variation in periods of time over which animals were studied and the amount of movement shown in those periods. However, to provide a degree of standardisation, periods of observation that yielded total movements less than 20 m were (arbitrarily) excluded from analysis. Thirdly, the rate of net dispersion was also calculated, by dividing the net distance moved by the time period (in days). This value gives a comparable measure of site tenacity during the short term. With a view to removing likely inherent errors within very small data

sets, these rates were only calculated for periods of observation in excess of two days (i.e. in excess of 48 hr).

Radio-transmitters were attached to snakes for a total of ·236.05 days (5665.1 hr) during 50 separate periods each of which lasted between 0.07 and 9.71 days (mean=4.72±2.81 days) [i.e. 1.73 to 232.9 hr (mean=113.3±67.4 hr)] in three consecutive years (April 1984 to July 1986) (Fig. 3). Thirty-nine different animals were studied (24 males and 15 females); most animals were studied only on one occasion, although seven were radio-tracked twice (four males, three females) and two were radio-tracked on three occasions (both males).
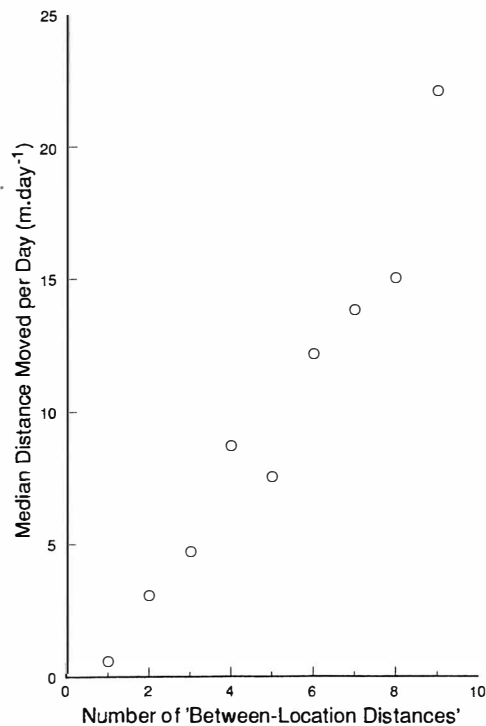

Fig. 2. Median distances moved during a day (m day^{-1}) and the number of between locations distances used in the calculation of daily movement rates (distances moved during a day were calculated from the sum of all straight line distances measured between subsequent sightings during each day [n= 8, 37, 12, 23, 44, 61, 69, 6 and 2 for 1 to 9 measured distances in any day, respectively]).
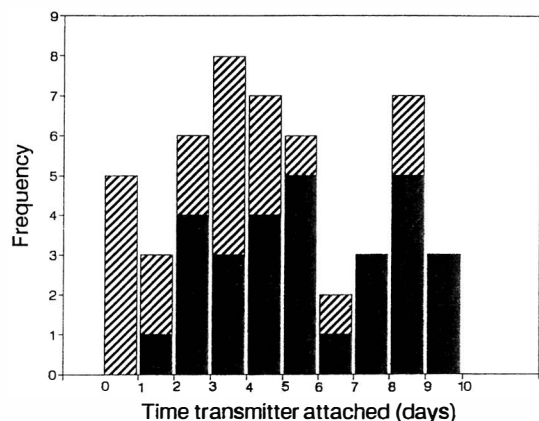
Fig. 3. Duration of attachment of radio-transmitters to *Coronella austriaca* showing those occasions where the transmitter was removed as planned (solid shading) and those cases where the radio-tracking period was terminated early due to experimental difficulties (hatched shading) [see text for details].

Radio-tracking periods were intentionally kept short with transmitters being deliberately removed between 1.34 and 9.71 days after attachment. In the majority of cases transmitters were left on for more than 4 days before removal. The short tracking periods were considered desirable due to the short life expectancy of the radio-transmitter batteries (maximum of 21 days) and with a view to minimising the possibility of harm to the animal. Seventeen experiments were terminated early due to experimental difficulties (and on five of these occasions transmitters were detached less than one day after attachment); on seven occasions the transmitter was removed from the animal when it was found to be snagged on vegetation or below ground, on six occasions the transmitter was shed (twice in connection due to the snake shedding its skin) and in three cases the transmitters malfunctioned. There was a single fatality of a radio-tagged snake; this, however, appeared to be as a result of predation rather than as a direct result of the attachment of the radio-transmitter. On five occasions the transmitter on the snake became snagged early during a radio-tracking period and the animal could be freed and subsequently followed. In all cases where the transmitter was not shed, the animal was recaptured and the radio-transmitter removed.

For the purpose of analysis three seasons were identified (which included all observations). These were defined as: spring = 1 March to 31 May; summer = 1 June to 31 August and autumn = 1 September to 31 October. Eight radio-tracking periods were exclusively or mostly in the spring, 29 in summer and 13 in autumn. These periods ranged between 1.84 and 6.17 days (mean=4.07±1.44 days), 0.07 and 9.71 days (mean=4.31±2.96 days) and 0.76 and 9.03 days (mean=6.03±2.85 days) for spring, summer and autumn respectively.

ASSESSMENT OF METHODOLOGY

The effect of attachment of a radio-transmitter on the movement and activity behaviours of smooth snakes was investigated in the laboratory using time-lapse video recording.

Two smooth snakes (three males, one female) were observed during each of two experiments. A metal and brick indoor vivarium was divided centrally into two identical arenas. Each measured 168 cm by 109 cm with walls 61 cm high and an overhanging lip to prevent escape. The floors were concrete and covered with a generous layer of sand. Each arena had a 275 W heat lamp positioned at the centre of one side (and 30 cm above the ground), a centrally positioned wooden board providing cover and four petri dishes of water. A video camera was positioned such that the floor of both arenas could be filmed at the same time. The heat lamp and overhead fluorescent lighting in the room were controlled via time switches to simulate approximately current day lengths (Heat lamp on 0745 to 1645 hr; overhead lighting on 0500 to 2100 hr). Two red 60 W bulbs were used to allow filming at night.

One snake was introduced to each of the arenas and left to become accustomed to the surroundings for at least 24 hr. Each snake was then removed from the arenas and handled as if being first captured in the field (i.e. weighed and measured). A radio-transmitter was then attached to one of the snakes and then both animals were released back to the their respective sides of the vivarium. The time-lapse recording was then started.

Between two and three days later, both snakes were removed from the arenas, 'processed' as before and the transmitter removed from the first animal and attached to the second. On release the trial continued for two to three days. The two trials were necessarily short; the smooth snake is a protected species and the licensing conditions restricted keeping animals in captivity to a total period of seven days.

Behaviour was assigned to five different categories: "Below cover", "Inactive in open", "Active", "Movement thermoregulation" (the animals moving such that all or part of the body was in a circle where the ground temperature was raised above normal due to the influence of the heat lamp [this area had been determined prior to the experiments]) and "Non-movement thermoregulation" (stationary within the area warmed by the heat lamp). Hourly movement rates were determined by tracing the movement of the animals from the video screen using a map measurer. Analysis of both activity and movement behaviour was performed on data for the whole day and where the data were divided into six 4-hr time periods (0100-0459 hr, 0500-0859 hr, 0900-1259 hr, 1300-1659 hr, 1700-2059 hr and 2100-0059 hr).

Changes in weight were described as rates per day (g day^{-1}) and compared between radio-tagged and untagged animals.

RESULTS

HOURLY MOVEMENT RATES

Hourly movement rates showed a strong positively skewed distribution. No difference was detected in hourly movement rates between sexes (Mann-Whitney U-test; $U = 128498.5$, $n_1 = 652$, $n_2 = 422$, $P > 0.05$) and thus data were pooled for further analysis.

The frequency distribution of hourly movement rates for both sexes (with 0.5 m h^{-1} intervals) is presented in Fig. 4. Movement rates varied between 0.00 m h^{-1} and 44.26 m h^{-1} ($n = 1074$). A median movement rate of 0.54 m h^{-1} was obtained. Notably 672 observations (62.6%) were of movements under 1.00 m h^{-1} and only 41 observations (3.8%) were of movements over 10 m h^{-1}.
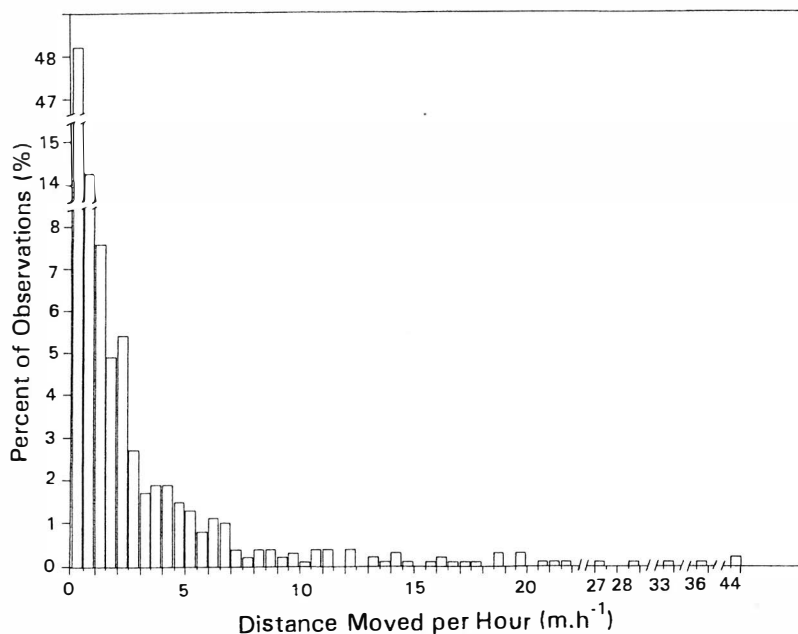
Fig. 4. Hourly movement rates (m h^{-1}) of *Coronella austriaca* as determined by radio-tracking ($n=1074$); intervals between successive locations were between 1 and 3 hr. Data are presented as classes with increments of 0.5 m h^{-1}.

Fig. 5. Daily movement rates (m day^{-1}) of *Coronella austriaca* as determined by radio-tracking ($n=138$); daily movement rates were calculated only where six or more 'between locations distances' were record. Data are presented as classes with increments of 5 m day^{-1}.

DAILY MOVEMENT RATES

No difference was detected between daily movement rates of males and females (Mann-Whitney U-test; $U=1890.0$, $n_1=80$, $n_2=58$, $P>0.05$); subsequent analysis was therefore restricted to combined data.

A strong positively skewed distribution was obtained for daily movement rate (Fig. 5, where data are presented using 5 m day^{-1} intervals) with values ranging between 0.00 and 166.81 m h^{-1}. From 138 estimates of daily movement rate, a median value of 13.30 m day^{-1} was obtained. Twenty-four records (17.4%) were less than 5 m day^{-1} and 53 (38.4%) were less than 10 m day^{-1}. Only 5 observations (2.9%) were of movement rates greater than 100 m day^{-1} and 18 (12.3%) were in excess of 50 m day^{-1}.

SEASONAL VARIATION OF MOVEMENT RATE

No differences were detected for either hourly or daily rates of movement between the three seasons (Hourly rate : Kruskal-Wallis test, $\chi^2=2.765$, $n=1074$, $P>0.05$; Daily rate : Kruskal-Wallis test, $\chi^2=0.206$, $n=138$, $P>0.05$).

DIRECTION OF MOVEMENT

Total distances recorded during all 50 radio-tracking periods varied between 0.3 m and 317.0 m (this included four cases where only two consecutive observations were made which accounted for the lowest four readings (all below 2 m); the next lowest value was 4.7 m). In all cases where total distances moved exceeded 40 m, thus allowing statistical analysis ($n=30$), a significant deviation from even dispersion was detected (G-test; $P<0.001$ in each case).

Excluding the four values obtained from only two consecutive readings, net movements (distances between first and final observations) ranged from 1.6 m to 203.4 m ($n=46$). Where values of the ratio of Net movement/Total movement were calculated, i.e. where Total movement exceeded 20 m ($n=39$), values ranged between 0.03 and 0.90. No difference
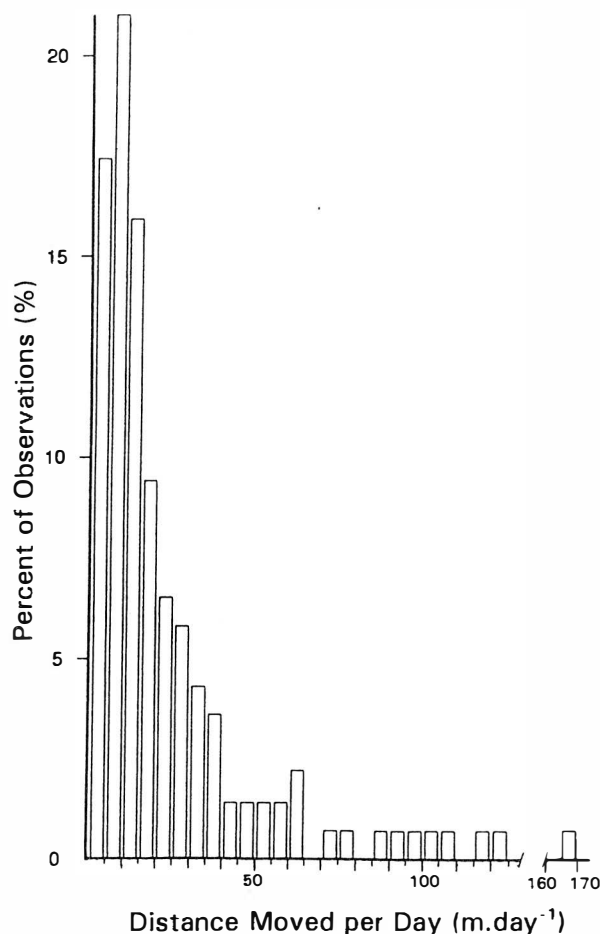
was detected between the values of the ratio between males and females (Wilcoxon pairs test; $Z=1.603$, $n_1=15$, $n_2=24$, $P>0.05$) nor between any of the seasons when analyzed pair-wise (Wilcoxon pairs test; spring and summer: $Z=1.343$, $n_1=22$, $n_2=5$; spring and autumn: $Z=1.637$, $n_1=12$, $n_2=5$; summer and autumn: $Z=0.252$, $n_1=12$, $n_2=22$; $P>0.05$ in all cases).

These data can be divided into two categories; those in which the value of the ratio is less than 0.50 (such that under half of the total movement contributes to net dispersion) and those with a ratio value of 0.50 or more (such that half or more of the total movement contributes to net dispersion). This division shows that on 20 occasions (51.3%) a ratio of less than 0.50 was obtained and in 19 cases (48.7%) the ratio was 0.50 or more. The majority (74.4%) of these values lie between a value of 0.25 and 0.75 (29 cases) with 5 observations each being below 0.25 and above 0.75 (12.8%).

Where greater than two days data were available, net dispersion rates varied between 0.19 and 52.9 m day^{-1} ($n=40$). No differences were detected in this rate between the sexes (Wilcoxon pairs test; $Z=1.311$, $n_1=16$, $n_2=24$, $P>0.05$) nor between any of the seasons when analyzed pair-wise (Wilcoxon pairs test; spring and summer: $Z=1.371$, $n_1=22$, $n_2=6$; spring and autumn: $Z=0.983$, $n_1=12$, $n_2=6$; summer and autumn: $Z=0.523$, $n_1=12$, $n_2=22$; $P>0.05$ in all cases). The data were highly skewed, with 12 observations (30.0%) being of rates below 5 m day^{-1}, 15 (37.5%) between 5 and 10 m day^{-1} and four (10.0%) being in the range 10 to 15 m day^{-1}. The remaining nine records (22.5%) were fairly evenly spread between values of 15 and the upper value of 52.9 m day^{-1}.

ASSESSMENT OF METHODOLOGY

As with data recorded in the field, the frequency distribu-

tions of hourly movement rates were highly positively skewed. No significant differences were observed between the hourly movement rates of those animals with radio-transmitters attached and those without when looking at pooled data for observations over the whole 24 hr period (Wilcoxon pairs test; $Z=0.520$, $n_1=237$, $n_2=239$, $P>0.05$). A significant difference in movement rate was observed during only one of the six 4-hourly periods, 0500-0859 hr, during which snakes without transmitters moved a greater distance than those with transmitters attached (Wilcoxon pairs test; $Z=2.082$, $n_1=40$, $n_2=40$, $P<0.05$). During the other five periods no differences were detected in movement rate (Wilcoxon pairs test; 0100-0459 hr: $Z=0.138$, $n_1=42$, $n_2=40$, $P>0.05$; 0900-1259 hr: $Z=0.306$, $n_1=37$, $n_2=37$, $P>0.05$; 1300-1659 hr: $Z=1.613$, $n_1=40$, $n_2=40$, $P>0.05$; 1700-2059 hr: $Z=0.712$, $n_1=40$, $n_2=40$, $P>0.05$; 2100-0059 hr: $Z=0.363$, $n_1=40$, $n_2=40$, $P>0.05$).

The proportions of time spent in each activity are summarised in Fig. 6. No differences were detected in pair-wise comparisons of behaviours using Mann-Whitney U-tests ($P>0.05$) for the whole day nor for any behaviours in any time period except for 'Active' (05-0859 hr) and 'Below cover' (05-0859 hr). During this period snakes fitted with radio-transmitters were under cover more often (29.7% of time cf. 15%: $U=584$, $n_1=40$, $n_2=40$, $P<0.05$) and were active for a smaller proportion of time than were those that were not radio-tagged (32.8% cf. 49.1%: $U=570$, $n_1=40$, $n_2=40$, $P<0.05$).

Mean rates of change of body weights of captive animals during these experiments were -0.325 g day^{-1} $\pm$ 0.442 ($n=4$) for animals with radio-transmitters attached and -0.578 g day^{-1} $\pm$ 0.889 ($n=4$) for snakes without transmitters. These values are not significantly different (Student's t-test; $t=0.513$, df=6, $P>0.05$).
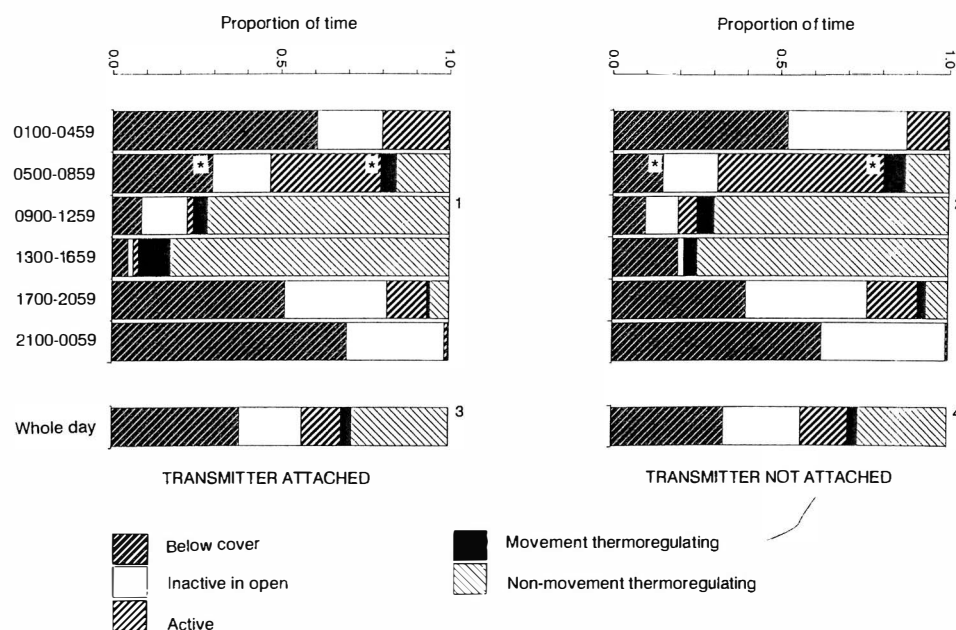

Fig. 6. Proportion of time assigned to each of five defined behaviours by snakes with radio transmitters attached and those without radio transmitters attached during six 4 hr periods and over the whole day ($n=40$ hourly observations in all cases except 1. $n=37$; 2. $n=36$; 3. $n=237$ and 4. $n=236$.) * denotes a significant difference between proportions of time spent on each behaviour by snakes with and without transmitters attached (at 5% level).

DISCUSSION

Radio-telemetry proved to be a very valuable technique for studying the movement behaviour of smooth snakes, since this allowed a reliable and consistent sampling regime to be followed. The laboratory study, although necessarily short in duration, corroborated the subjective observations made in the field that the attachment of radio-transmitters did not affect the behaviour of the snakes. However the physical bulk of the transmitters did occasionally impede movement of animals particularly when they were travelling through vegetation or moving below ground. Notwithstanding this, most snakes were able to free themselves quickly, or where this was not the case data were discarded, and it is the view of the experimenters that the movement data obtained by this method are representative of the species.

The external attachment of transmitters was not considered an ideal solution; however it was one borne of necessity. Force feeding transmitters has been used on this species (de Bont, van Gelder & Olders, 1986) but was not considered during this study for two reasons. Practically there was concern about being able to develop a transmitter package of dimensions that allowed it to be force fed to a small snake like *C. austriaca* (and have an aerial that gave adequate range). In addition, behaviour may be affected by the presence of food (or similar) in the gut which has reportedly induced thermophilic responses (Regal, 1966; Lutterschmidt & Reinert, 1990). Surgical implantation could not be entertained since at the time of the study such methods were not permitted in British law; further such a method would be impractical given the size of the available transmitters (and their short battery life) relative to the size of the animal and would provide an unacceptable risk to an endangered species.

The external attachment of transmitters together with a short study period, during which the animal would be regularly located, meant that welfare of the animal could be closely monitored and the radio-transmitter removed if problems occurred. Accepted consequences of this approach were the need to have only short tracking periods and the fact that transmitters would be shed when the animal sloughed its skin. Regular location allowed detailed study of short term movements which compensated for the short period over which transmitters were fitted. Thus movement behaviours were 'sampled' during intensive study of different animals. Extended study periods where transmitters are left attached for greater lengths of time (which are possible using internally fixed transmitters) would have provided greater information about seasonal movements and range use. Frequently though, during such studies, the detailed observation of animals during each day is neglected.

The absence of detectable differences between the sexes in either hourly or daily movement rate, or in the degree of directional movement, indicated that both sexes have similar dispersal potentials during the short term. Thus, for further discussion, comments will be restricted to dealing with the two sexes together.

Radio-tracking data from the present study showed that movement rates of *C. austriaca* are small, with 62.6% of hourly movement rates being less than 1 m h^{-1} and 18.7% showing no movement at all. These observations were restricted to day time (and hence the active period of this diurnal species), and thus excluded the period of over night inactivity. This therefore corroborated the generally reported thesis that this species is relatively immobile (Breeds, 1973; Spellerberg & Phelps, 1977; Goddard, 1981; Nature Conservancy Council, 1983). A comparison with studies of the movement rates of the two other species of snake found in Britain (*Vipera berus* (family Viperidae) and *Natrix natrix* (family Colubridae)) indicates that *C. austriaca* is the least mobile of the three (Prestt, 1971; Madsen, 1984; Brown, 1991).

A habitually low movement rate will confer several advantages to an animal. In an ectotherm, such as *C. austriaca*, movement will greatly increase metabolic rate, typically up to ten fold (Bennett, 1982). In addition, rapid movements in reptiles generally require anaerobiosis. Thus it is energetically and metabolically beneficial for a reptile to minimise its movements. Lowered energy expenditure, in turn, results in a decreased need to actively forage for food.

It is worth considering the need for movement at all during much of the day. One of the primary concerns of an ectotherm is the maintenance of body temperature. Selection of a thermally heterogenous environment may allow behavioural thermoregulation, and avoidance of extremes of temperature, through only small movements. In addition the smooth snake is cryptically coloured and seemingly relies on this to avoid detection by both predators and prey. Crypsis may be enhanced through long periods of immobility.

A consideration of maximum movement rates will give an indication of the species' dispersal potential. Hourly movement rates were based on approximately two hourly intervals; thus the minimum straight line distance used in the calculation of hourly rate were sometimes quite large. The four highest movement rates (32.99, 35.72, 44.07 and 44.26 m h^{-1}) were obtained from measured distances of 62.7, 81.55, 94.02 and 98.11 m. It is likely that a greater estimate of hourly movement rate would have been obtained if shorter time intervals had been used.

Only five observations (2.9%) of daily movement rates exceeded 100 m day^{-1}, with these data being obtained from both male ($n=3$) and female ($n=2$) snakes. (It is worth noting that there was one additional case where movement during one day was in excess of 100 m, and in fact was the largest observed movement during any day. However this record was excluded from the above analysis since it was based on the summation of only four straight line distances after which the transmitter was removed; this was a female that moved 170.92 m during its final period of radio-tracking).

Similar maximum movement rates were reported by Breeds (1973) who, in his recapture studies, observed movement rates of 20, 36 and 46 m h^{-1} for two individuals (recapture intervals of between 1.75 and 5.0 h); he also reported a further movement of 180 m between two successive afternoons by one animal. The absence of clearly distinguishable differences in movement rates between seasons could indicate that there are not distinct 'migration movements' at either end of the activity period. This is consistent with Phelp's (1978) observations of range use in which he suggested that *C. austriaca* do not use separate summer and winter ranges. Dispersion is a product of net directional movement and not simply a reflection of the total amount of movement that occurred. In over half the cases studied, un-

der 50% of the measured movement contributed to overall dispersion and in over 12% of the cases this figure was under 25%. Thus much movement would have been responses to local factors; perhaps determined by thermoregulatory needs or movement between places offering shelter. However in just under half the cases there was the tendency to disperse, with over half the recorded movement during a period resulting in movement away from the original point of capture. Over 12% (5) of observations were of snakes whose net movement was 75% or more of their total distance moved. The daily rate of 'dispersion' too indicates a slow, but gradual movement away from any particular location.

These data indicate that *C. austriaca* do not, as a rule, occupy fixed dens for prolonged periods (compared with, for example, *Coluber viridiflavus* (Ciofi, Chelazzi & Della Santina, 1992)). However they move through the habitat at differing rates; some staying resident in a general area for some while whilst others will pass through an area quite quickly. Gravid female snakes seemed to be more frequently encountered in restricted areas, often in association with a favourable aspect for basking and some even showed repeated use of certain features for over night refuges. However the data reported here indicate that this is not always the case (and some female snakes were quite mobile).

These observations are further corroborated by looking at the incidence of recaptures of smooth snakes at the two study sites during the course of the three year study period (Gent, 1988). In total 111 different animals were captured by hand at the two sites (52 at Site 1 and 59 at Site 2). Of these 81 (73.0%) were only seen in one year, 25 in two years (22.5%) and only 5 (4.5%) were seen in all three years of the study. Further, excluding radio-tracking data, 53 were only caught on one occasion (47.7%) and 91 (82.0%) were caught five times or less. Only thirteen animals (11.7%) were caught ten or more times.

This generally restricted movement behaviour shown by *C. austriaca* is pertinent to those involved with habitat management. The low movement of the species means that animals are likely to remain within a limited home range during the short term. Sites should therefore be managed to ensure that all features needed by the species are available within relatively discrete patches; thus feeding and thermoregulatory requirements and the provision of shelter and protection should all be available within a limited area. Nonetheless, the actual ranges used by the species during the longer term are likely to be relatively large. Thus a further consideration arising from the nature of movement of the species is that the likely impact of habitat fragmentation on the species will be greater than it would be for a species that is able to travel much greater distances and move much more rapidly.

The behaviour also has notable implications for survey and site assessment. Observations of smooth snakes in a particular part of a site at any one time are likely to considerably under represent the total population that uses that area.

ACKNOWLEDGEMENTS

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SHORT NOTES

THE EFFECT OF SODIUM NITRATE ON THE GROWTH AND SURVIVAL OF TOAD TADPOLES (*BUFO BUFO*) IN THE LABORATORY

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Amphibians have been proposed as potential biological indicators of pollutants, because of their biphasic lifestyles and permeable skins (Blaustein & Wake, 1990), and yet there is relatively little information available regarding their susceptibility to chemical hazards (Hall & Henry, 1992). Nitrate in water can contribute towards eutrophication (Mason, 1991), and laboratory tests have shown that it can also be toxic to invertebrates (Camargo & Ward, 1992) and fish (Westin, 1974). It is not known whether nitrate has an effect on amphibians, but it has been suggested that anions may adversely affect larval growth (Ireland,1991). The present experiment investigated the effect of a nitrate salt on the growth and survival of tadpoles of the common toad (*Bufo bufo*).

Sections were taken from six spawn strings deposited in a pond in Northamptonshire, England. These sections were allowed to hatch in a plastic tub, filled with tap water, and placed outdoors. After hatching, larvae were captured on a random basis, and total length (TL) was measured. Thirty-nine larvae, each measuring 10 mm TL, were then selected from the random sample. At this time all larvae had attained Gosner stage 25. The selected larvae were assigned randomly to one of three groups, each group being reared in different water conditions. The first group was grown in distilled water only, as a control. The remaining experimental groups were grown in two different concentrations of sodium nitrate solution. These two treatments were made up by dissolving sodium nitrate in distilled water to make solutions of 40 and 100 ppm of nitrate ion. The 40 ppm level was chosen because this is similar to the maximum recorded levels in ponds on agricultural land in Northamptonshire (unpubl. data) at times of the year when amphibian larvae can be found in the water. The higher level of 100 ppm was chosen in order to magnify any effects that may have been too subtle to detect at lower levels. The pH of the distilled water and sodium nitrate solutions was measured, to ensure that variation in levels of sodium nitrate was not confounded by a pH effect on larval growth. The pH fluctuated between 5.57 and 7.47. Distilled water had the lowest pH values, whilst adding sodium nitrate increased pH. The maximum difference between the pH of distilled water and the high nitrate condition on any single recording period was 1.0.

The tadpoles were reared individually. Each tadpole was grown, in a clear, food-quality, plastic beaker, containing 500 ml of either distilled water or nitrate solution. The beakers were placed on plastic trays in a blocked arrangement to ensure that any positional effects were evenly spread over all three treatments. Water temperature fluctuated between 19°C and 24°C. The solutions or water were replaced after seven and thirteen days. The plastic beakers were also changed at these intervals to prevent algal growth. The total length (TL) of each tadpole was measured on these days. Tadpoles were fed a food pellet (0.02 g JMC Aquatics cat fish pellet food) on days 0, 7 and 13. There was always some food left in the beakers at the time of replacing the beakers, and so growth was assumed not to be limited by food. The tadpoles were exposed to a natural photoperiod via the laboratory windows.

The growth of tadpoles in the three groups is shown in Fig. 1a. An analysis of variance of total length at seven days was carried out using Genstat (Genstat 5 Committee, 1987). Block effects are significant ($F_{12,15}=2.98$, $P<0.05$), and when these are removed, there is a significant treatment effect, $F_{2,15}=79.71$, $P<0.001$. Tukey tests, as described in Zar (1984), show that tadpoles in the control group are significantly bigger than those in the 40 ppm nitrate group ($q=9.177$, $P<0.05$) and that tadpoles in the 40 ppm nitrate group are significantly bigger than those in the 100 ppm nitrate group ($q=6.557$, $P<0.05$). Survival of tadpoles in the three groups is shown in Fig. 1b. To test whether a greater proportion of tadpoles in the nitrate solutions had died compared to those in distilled water, a chi-square analysis of survivors and mortalities on day 13 (the day on which the last larva in the 100 ppm nitrate solution died) was performed. Data from the two nitrate concentrations were pooled. Mortalities were significantly more frequent in the nitrate solutions than in distilled water, $\chi^2=23.4$, $P<0.001$ (with Yates' correction).

Sodium nitrate solution appears to have clear, adverse effects on the growth and survival of *Bufo bufo* tadpoles grown in the laboratory. Sodium nitrate caused a decrease in the rate of growth and caused higher mortality than was found in tadpoles grown in distilled water. The magnitude of the effect of sodium nitrate on growth may have been exaggerated by the growth of a *Saprolegnia* fungus on uneaten food pellets. The tadpoles in the nitrate solutions tended to feed less than those in distilled water, allowing the fungus to grow on food that
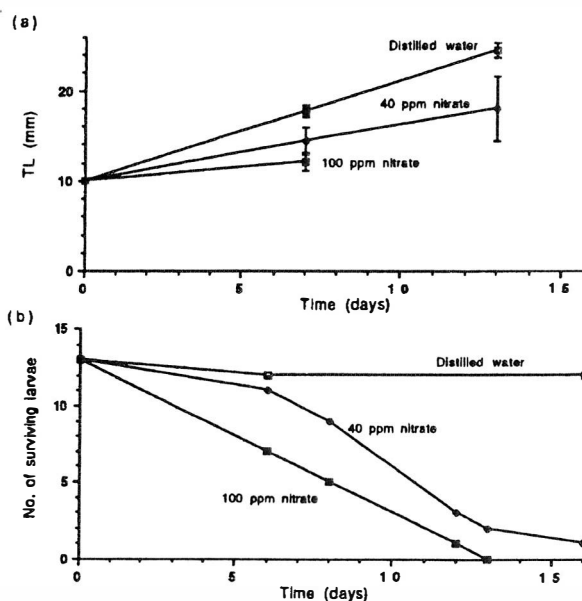

Fig. 1. (a) Growth of larvae of *Bufo bufo* in distilled water and two sodium nitrate solutions. Error bars represent one standard deviation. (b) Survival of larvae of *Bufo bufo* in distilled water and two sodium nitrate solutions.

was undisturbed by tadpole feeding. Hence it is possible that the low rate of feeding by tadpoles in the nitrate solutions allowed the *Saprolegnia* to grow, and this in turn may have further reduced the feeding activity of the tadpoles. The fungus did not appear to affect the tadpoles directly.

From this study it is not possible to conclude which ions are responsible for the observed effects on the growth and survival of toad tadpoles, since both nitrate and sodium ions co-vary between the treatments. Ireland (1991) has found that sodium ions may reduce growth rates in the larvae of *A. maculatum* and so it is quite possible that sodium ions were also responsible for the effects observed in the present experiment. We are currently seeking to separate the effects that sodium and nitrate ions may have on amphibian larvae (Baker & Waights, in prep.)

The present results should be treated with some caution. Firstly, dissolving sodium nitrate in distilled water causes a shift in pH from slightly acidic to slightly alkaline. pH can affect the growth of anuran larvae; it is generally observed that low pH reduces growth (Freda, 1986; Böhmer & Rahman, 1990). However, the lower pH of the distilled water in the present experiment was in fact associated with enhanced growth. Therefore, it is unlikely that the pH shift in the present experiment is sufficient to explain the mortalities and reduced growth that were recorded. It should also be noted that toads are frequently found breeding in ponds with an alkaline pH (pH 7-9 unpubl. data). The second note of caution is that effects detectable in a laboratory situation do not always translate to the field. Toxicity to aquatic organisms varies with temperature and water quality (Mason, 1991). The present experiment was carried out in a laboratory, rearing tadpoles at a relatively constant, high temperature. These conditions differ from those of a pond, which may either increase or decrease the magnitude of the effect of nitrate on toad larvae. Finally, it should be noted that the nitrate concentrations used in this experiment are nominal. Actual nitrate levels may change over time, due to the presence of the food pellets and due to the activities of the toad tadpoles.

It is desirable to find out whether nitrates in natural breeding sites can cause adverse effects on toad tadpoles, either directly through mortality or through reduced growth rate. There is a body of work that has shown that rapid growth or attainment of large body size during the larval phase reduces the risk of predation (Cooke, 1974; Heyer, McDiarmid, & Weigman, 1975; Caldwell, Thorpe & Jervey, 1980; Travis, Keen & Julianna, 1985) and is critical to the survival and reproductive success of individuals during later stages of the life cycle (Berven & Gill, 1983; Smith, 1987; Semlitsch, Scott and Pechmann, 1988). Hence the response of reduced growth observed in the laboratory may have important consequences for larvae in the natural situation.

In nature, any adverse effect of nitrates may be attenuated due to the timing of the amphibian breeding and seasonal fluctuations in nitrate levels. Amphibian populations most likely to be exposed to high levels of solutions of nitrate salts are those breeding in ponds on agricultural land. Nitrate levels may become high due to fertilizer run-off from surrounding land. However, the amphibian larval stage may avoid the peak levels which will occur during the winter months. During the winter there is little plant growth to assimilate the nitrate fertilizer, and water run-off is at its highest due to the lack of plant transpiration and respiration (Mason, 1991). Hence it remains to be seen whether amphibian larval populations on agricultural land can be adversely affected by the application of nitrate fertilizers. However, the clear effects of sodium nitrate demonstrated in the present study suggest that the subject of amphibian sensitivity to nitrates is worthy of future investigation. We are carrying out further work in this area, using more realistic and carefully-monitored growth conditions.

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TEST OF AN ELECTRONIC INDIVIDUAL TAG FOR NEWTS

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No satisfactory technique for the individual marking of urodeles has been discovered, and this methodological problem limits ethological and behavioural studies. Joly & Miaud (1989) report that rings and jaw tags may cause necrosis and may entangle the newt in aquatic vegetation; the regenerative capability of newts limits the use of toe-clipping (toes are regenerated within months), and of freeze- or heat-branding (scars last only a few weeks) to short-term studies. Hagström (1973) identified individual newts by comparing their belly patterns with a photographic archive of the patterns of the local newt population; however it is impractical to distinguish more than 100 individuals, and some species lack recognizable belly patterns. Autotransplantation of skin patches from the belly to the back (Andreone, 1986) can be used only for large species, is laborious, and cannot be accomplished in the field.

Joly & Miaud (1989) described a skin staining technique, and stated that spots tattooed on the belly remain readable for 2-3 years. We tested skin staining with unsatisfactory results. Seven alpine newts *Triturus alpestris*, and three Italian crested newts *Triturus carnifex* were anaesthetized, and spots were drawn on their bellies with hypodermic injections of alcian blue using an odontological anaesthetic injector. All the newts, kept in aquaria, were unharmed and survived, but the spots disappeared completely within 2-5 months in three of the newts, and after eight months the spots drawn on all the other seven newts had considerably faded and were hardly recognizable.

Camper & Dixon (1988) proposed the use of microchips for marking amphibians and reptiles. We have tested a permanent electronic tag that is being widely used for the individual tagging of animals in veterinary and farming practice on a sample of newts, kept in aquaria. The tag is a passive "transponder" chip encapsulated in glass, (external size 1.8 x 12 mm, weight 100 mg). The tag's unique, 8 digit alphanumeric code is read by a hand-held scanner (1.1 kg with batteries) which emits a low frequency magnetic field that activates the passive transponder, which has no power source of its own and hence has an unlimited lifespan. The tag codes may be read directly on the scanner, or may be sent to a computer port. The scanner can read the tag from a distance of 20 cm, through water and through organic tissues.

In order to test the tolerance of these tags by urodeles, on 16 December 1991 we inserted tags intraperitoneally in 11 metamorphosed Italian warty newts (*Triturus carnifex*), seven metamorphosed alpine newts (*T. alpestris*), and six branchiate alpine newts (non-metamorphosed individuals still retaining external gills, and presumably born either in the same or in a previous year). The tags were implanted using a special syringe, and each operation lasted a few seconds; the newts were under anaesthetic (chloretone in water). These newts were kept together in the same aquaria with control, non-tagged metamorphosed Italian warty newts ($n=9$), metamorphosed alpine newts ($n=7$), and branchiate alpine newts ($n=4$) until 15 April 1992. The newts had been divided into three size classes, and within each class individuals were assigned at random to the tagging or the control group. The newts were fed twice a week with earthworms and fly larvae. From 15 April to 28 May 1992 all the newts were kept in a controlled outside pond. During all these periods the newts were checked and weighed weekly.

Only one of the marked newts, the smallest branchiate alpine newt weighing only 1.3 g, died within one day due to tagging. All the other newts survived, and their wounds from the tag insertion had healed completely in two weeks, without infection. Later in the study period, 1 marked and 2 control newts died of unknown causes.

The intraperitoneal tags apparently did not impair movement by the tagged newts. In order to check the effect of the
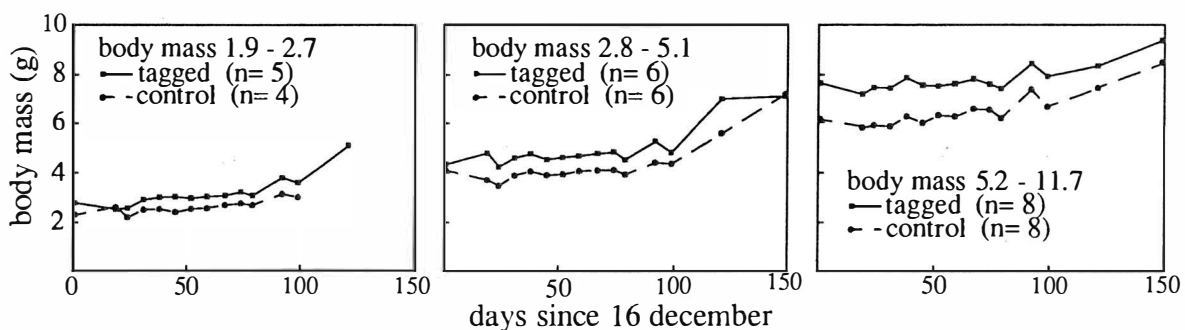

FIG. 1. Average body mass of marked and control newts, divided into three size classes. The class with body mass < 2.7 g included metamorphosed male or female alpine newts and branchiate alpine newts; class 2.8-5.1 g metamorphosed male or female alpine newts and Italian warty newts; class > 5.1 g metamorphosed male or female Italian warty newts.

tags, we compared the body mass changes of the marked and the control newts during their residency in the aquaria and in the outside pond (Fig. 1). The tagged and the control groups in the three size classes did not differ significantly in their initial, or final body mass (Mann-Whitney tests: $P > 0.05$). Body mass changes were tested by the Wilcoxon sign test on the body mass increments respective to the first day, in each of the three size classes of Fig. 1, and again no significant difference was found between marked and control newts (all tests: $P > 0.05$). In May all the newts had acquired their full reproductive characters and they were engaged in courtship.

We conclude that these electronic tags may be used for individual marking of newts above 2 g in body mass, with the advantages of a permanent marking and of an easy insertion in the field. Recently, Sinsch (1992a, 1992b) marked toads using electronic tags: microchips similar to those used by us were suitable for toads of snout-vent lenght exceeding 30 mm, and coded wires for newly metamorphosed toadlets.

The tags and the scanners we used are marketed by Euro I.D. (Grossbullesheimer Str. 56, 5350 Euskirchen 16, Germany). Similar tags (slightly smaller, 2.1 x 10 mm and 63 mg) and scanners (less suitable for our purposes) are marketed by FishEagle Co. (Lechlade, Gloucestershire, GL7 3QQ, England). The prices are approximately 10 US$ per tag and 2,000 US$ per scanner.

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FIRST RECORD OF THE POLYODONT SNAKE *SIBYNOPHIS GEMINATUS GEMINATUS* (BOIE, 1826) FROM THE PHILIPPINES, WITH A DISCUSSION OF *SIBYNOPHIS BIVITTATUS* (BOULENGER, 1894)

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Up to now one species of *Sibynophis* was known from the Philippines. It was described in 1894 by Boulenger as *Polyodontophis bivittatus*, from Palawan/Philippines. This rare species is endemic to the Province of Palawan, occuring on Palawan, Culion and Busuanga Islands (Taylor, 1922; Leviton, 1964; Alcala, 1986).

S. geminatus (Boie, 1826) is widely distributed in parts of Indonesia, Malaysia, Singapore and Sabah (Boulenger, 1893, 1896; Leviton, 1964). One subspecies, *S. g. insularis*, was described from Poeloe-Weh/North Sumatra (Mertens, 1927). Only the type is known (SM 22094).

A freshly killed *Sibynophis* was obtained on June 7th 1990 in Languyan/TawiTawi/Sulu-Archipelago/Philippines. It was killed by a local villager in the early afternoon while it was crossing a small trail in a rainforest (lowland forest of the molave type; see Dickerson, 1928), near a rivulet.

Description. Female; total length 530 mm; tail length 210 mm; weight 21 g; maxillary teeth 37; 9 upper labials right, 8 left; upper labials 3, 4, 5 bordering orbit; 2 anterior temporals right, 1 left; 151 ventrals; 116 subcaudals. In other scalation characteristics it agrees with *S. geminatus* and *S. bivittatus*.

Colouration. Reddish-brown above, getting darker towards the tail, colour extending to the outer edges of the ventrals; dorsolaterally a row of dark spots on both sides, which are anteriorly framed with white, and become confluent on the tail, forming dark longitudinal stripes; ventral surface yellowish, a dark spot on each side of each ventral scale; head brown with irregular black markings; upper labials white, bordered above by a black stripe which passes through the eye to the neck; dark nuchal bar, containing two lighter spots.

The colours, especially the reddish tinge of the dorsal side and the yellowish tinge of the ventral side, are fading in alcohol. For the species determination, the described *Sibynophis* was compared with *S. bivittatus* and *S. g. geminatus*.

In Table 1 are listed those characteristics, which are used for the differentiation between *S. bivittatus* and *S. g. geminatus*, and *S. g. geminatus/S. g. insularis* respectively (using the data from Boulenger, 1893, 1894, 1896; Taylor, 1922; Mertens, 1927; and Leviton, 1964). The low minimum number of subcaudals given for *S. g. geminatus* should be used with reservation since the tail of *Sibynophis* breaks off easily (Mertens, 1927) and museum specimens often have incomplete tails.

The type of *S. g. insularis* is clearly distinguishable from *S. g. geminatus*, by its lower scale and teeth counts. However, the variability within this subspecies is still unknown.

The variations in scalation between *S. g. geminatus* and *S. bivittatus* are insignificant as the ventral and subcaudal counts of *S. bivittatus* lie within the range of *S. g. geminatus*, and the ranges of the head scalation found in each overlap. Even in the very small collection in the Senckenberg Museum, deviations from the supposedly species-specific upper labial numbers occur in both species (see Table 2). Obviously such deviations are not as rare as Boulenger, Taylor, and Leviton state. Only SM 17106 has the species-specific head scalation, while SM 17105 and SM 17107 both show the characteristics of the other species on one side.

Regarding colouration, the differences between the species are also weak. All *S. bivittatus* are distinctively striped, never spotted, while from *S. g. geminatus* striped, spotted, and combined patterns are known. Boulenger (1893, 1896) recognized five colour forms: one with light stripes (from Java), three with a different combination of stripes and spots (from Singapore, Java, Sumatra, Borneo, the Malay Archi-

	S. bivittatus	*S. g. geminatus*	*S. g. insularis* (1 specimen)
Maxillary teeth	39 - 43	35 - 48	33
Upper labials	8 (rarely 9)	9 (rarely 8)	7/8
Labials bordering orbit	3,4,5 (rarely 4,5 or 4,5,6)	4,5,6 (rarely 3,4,5)	3,4/3,4,5
Ventrals	145 - 155	144 - 183	140
Subcaudals	110 - 112	89 - 145	tail incomplete
Light interocular bar	present	absent	absent
Light nuchal bar	absent	mostly present	absent
Light dorsolateral stripes	distinct	mostly present	slight
Dorsolateral spots	absent	often present	present

TABLE 1. Comparison between *Sibynophis bivittatus*, *Sibynophis geminatus geminatus* and *Sibynophis geminatus insularis*. Data from Boulenger (1893, 1896), Taylor (1922), Mertens (1927) and Leviton (1964).

	S. bivittatus (SM 17107, Culion)	S. g. geminatus (SM 17105, Java)	S. g. geminatus (SM 17106, Java)
Upper labials	8/9	9/8	9/9
Labials bordering orbit	4,5/4,5,6	4,5,6/3,4,5	4,5,6/4,5,6
Ventrals	147	163	167
Light interocular bar	present	absent	absent
Light nuchal bar	absent	absent	absent
Light dorsolateral stripes	present	present	present
Dark dorsolateral spots	absent	absent	absent

TABLE 2. Characteristics of three *Sibynophis* specimens from the Senckenberg Museum.

pelago), and one without stripes (from Sarawak and Sabah/ north Borneo). A light interoccipital bar is only known from *S. bivittatus*, while most *S. g. geminatus* have a light nuchal bar instead. The unstriped colour form has a dark nuchal collar.

Based on a comparison of scalation characteristics, the specimen from TawiTawi cannot be clearly assigned to one or other of the nominal species. However, its colouration indicates it belongs to the unstriped form of *S. g. geminatus* from northern Borneo, with a dark nuchal collar. This is not unexpected, the distance between eastern Sabah and TawiTawi is less than 100 km. A relationship between the TawiTawi form and the Palawan species is unlikely, as they are separated by the middle Visayan islands and Mindanao where *Sibynophis* is unknown. Also, the TawiTawi specimen shows none of the "*bivittatus*" colour features. It consequently seems that both Philippine *Sibynophis* populations reached their present ranges independently from Borneo. Since *Sibynophis* shows differentiation on Palawan, but not on TawiTawi, the colonization of Palawan may have occured earlier, or/and this island may have been continously separated from Borneo for longer.

It would be interesting to know whether or not striped and spotted specimens occur within single populations of *S. geminatus*, so that the taxonomic value of this feature could be assessed. However, at the moment the sample sizes from the different regions are too small, and localities, especially for the older material, are not specific enough.

The criteria used to distinguish *S. bivittatus* from *S. g. geminatus* are very weak. They have a high interspecific variability in many features and overlap in many of these. If further collections and investigations of *Sibynophis* from Borneo and Palawan do not result in the discovery of more reliable criteria for separating them, *S. bivittatus* should be regarded as a subspecies of *S. geminatus*.

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