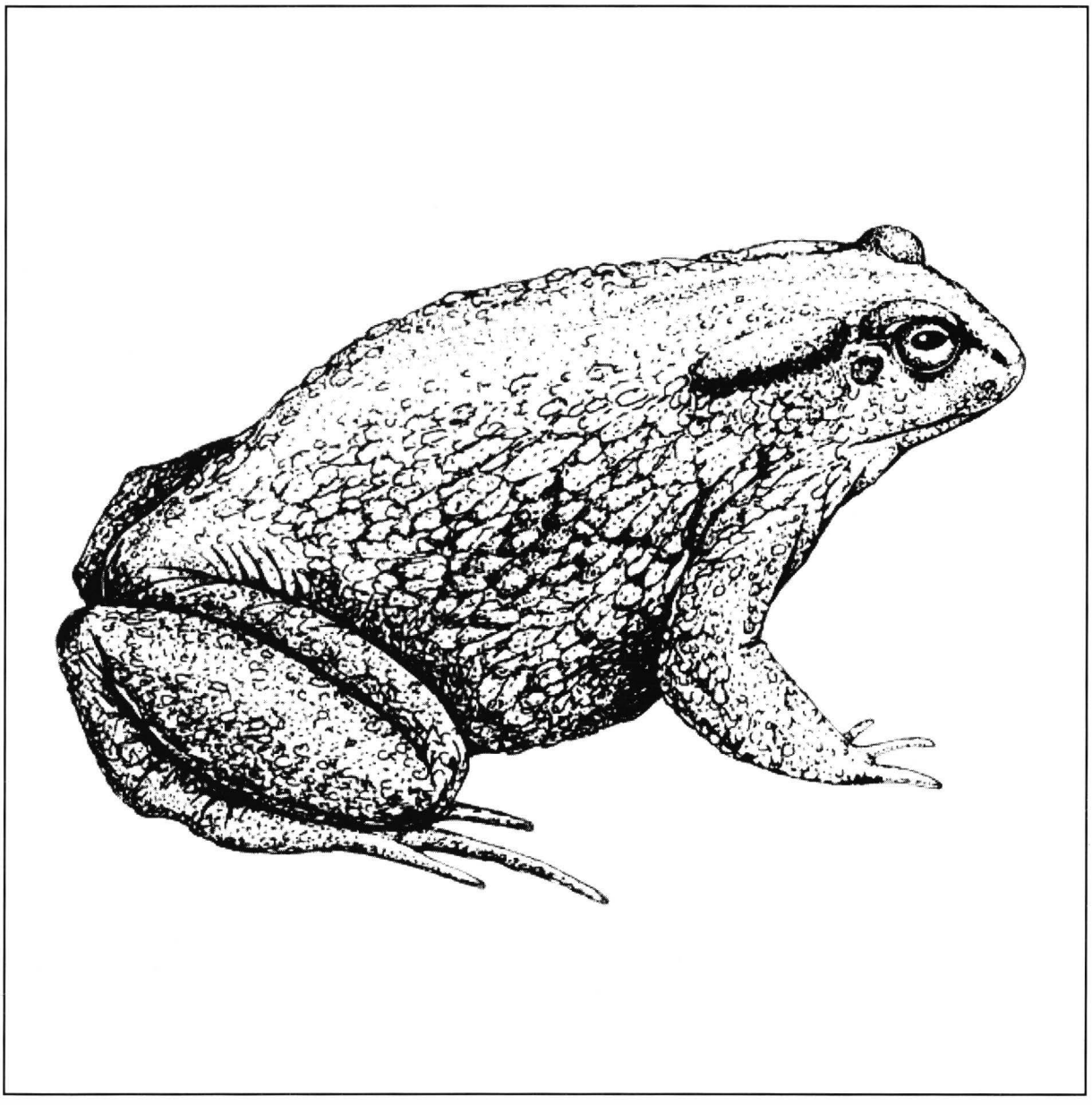
Volume 2, Number 3

July 1992
ISSN 0268-0130

THE HERPETOLOGICAL JOURNAL



Published by
THE BRITISH HERPETOLOGICAL SOCIETY

Indexed in
Current Contents

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

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

BRITISH HERPETOLOGICAL SOCIETY

FRONT COVER: Common Toad, *Bufo bufo* (D. Leeton)

REVIEW:

ASSESSING EFFECTS OF PESTICIDES ON AMPHIBIANS AND REPTILES: STATUS AND NEEDS

RUSSELL J. HALL[1] AND PAULA F. P. HENRY[2]

[1] *U.S. Fish and Wildlife Service, Mail Stop 725, ARLSQ, 1849 C Street, N. W. Washington, DC 20240, U.S.A.*

[2] *U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Maryland, 20708, U.S.A.*

(Accepted 5.9.91)

ABSTRACT

Growing concern about the decline of certain amphibian populations and for conservation of amphibians and reptiles has led to renewed awareness of problems from pesticides. Testing amphibians and reptiles as a requirement for chemical registration has been proposed but is difficult because of the phylogenetic diversity of these groups. Information from the literature and research may determine whether amphibians and reptiles are adequately protected by current tests for mammals, birds, and fish. Existing information indicates that amphibians are unpredictably more resistant to certain cholinesterase inhibitors, and more sensitive to two chemicals used in fishery applications than could have been predicted. A single study on one species of lizard suggests that reptiles may be close in sensitivity to mammals and birds. Research on effects of pesticides on amphibians and reptiles should compare responses to currently tested groups and should seek to delineate those taxa and chemicals for which cross-group prediction is not possible. New tests for amphibians and reptiles should rely to the greatest extent possible on existing data bases, and should be designed for maximum economy and minimum harm to test animals. A strategy for developing the needed information is proposed. Good field testing and surveillance of chemicals in use may compensate for failures of predictive evaluations and may ultimately lead to improved tests.

INTRODUCTION

The U.S. Fish and Wildlife Service (1990) recently released a list of endangered and threatened wildlife species that included 19 taxa of amphibians and 101 taxa of reptiles. More than a decade ago, a committee of scientists (Anon., 1977) reported to the European Committee for the Conservation of Nature and Natural Resources that at least 30% of Europe's amphibian species and 45% of its reptile species were in danger of extinction. Similarly, an earlier assessment in the United States (Bury, Dodd & Fellers, 1980) estimated that continued survival of at least 16% of native salamanders and 7% of frogs and toads was in jeopardy. More recent studies by the U.S. Fish and Wildlife Service (Corn, Stolzenburg & Bury, 1989) of the effects of acid precipitation on amphibian populations of the Rocky Mountain region noted declines of several species, but current evidence does not implicate acidification as a primary cause of these declines. Worldwide concern over declining amphibians prompted the U.S. National Research Council to sponsor a conference in February 1990 to summarize evidence and to seek explanations for the declines (Borchelt, 1990). Measures to conserve amphibians and reptiles have been slow, and threats from toxic chemicals in the environment are among the threats that have received insufficient attention. The success of governments in ameliorating the effects of toxic chemicals on other biota leads to the conclusion that it may also be possible to protect amphibians and reptiles.

Governments, particularly in the developed countries, use scientific evidence that certain chemicals are harmful to biota to restrict or even eliminate their use. The organochlorine pesticides, generally broad-spectrum, persistent pesticides, some of which accumulate in animal tissues, are a well-known example. The harm caused in the decades when information on effects was accumulating resulted in the requirement of batteries of tests to register new chemicals for sale and use. The process involved research that revealed mechanisms and extent of the harm, legislation, and regulations for enforcement of restricted applications to prevent a recurrence of past problems. The extent of continued losses of fish and wildlife determine the effectiveness of regulated applications of chemicals.

Losses of fish and birds attracted the most attention and initiated legislation and regulations for the application of chemicals. Concern was especially high for mammals because of the obvious implications for human health. Regulations specified fish, birds, and mammals as test subjects and required tests to reveal the kinds of toxicity that had been observed in natural populations of these organisms. If amphibians and reptiles were ever considered, it was presumed that tests conducted on fish, birds, and mammals would yield a range of toxicity data that, when evaluated and implemented with appropriate safety factors, would also protect these other groups.

The U.S. Environmental Protection Agency (EPA) recently (draft Revised FIFRA Guidelines Document - Subdivision E, March 1988) began consideration of pre-registration testing of chemicals for their acute lethal toxicity to amphibians and reptiles, using frogs (*Rana* spp. tadpoles) and adult lizards (*Anolis carolinensis*). In the proposal, works by Hall & Swineford (1980) and Hall & Clark (1982) were cited as examples of acceptable protocols for both classes. Several questions are raised by this proposal. Are existing safeguards adequate to protect amphibians and reptiles? Would requirement of the kinds of pre-registration testing proposed confer additional safeguards? Are more effective and more efficient tests available? And, lacking adequate information on which to answer the foregoing questions, how can the information needed be obtained?

We have reviewed the scientific evidence of the toxicity of chemicals, evaluated the adequacy of existing knowledge for protection of herpetofauna, and identified a strategy for research to provide missing information.

PROBLEMS IN EVALUATING RISK

Diversity of Amphibians and Reptiles

Taxonomic diversity. Reptiles are more diverse than any of the other land vertebrates. Crocodilians, for example, are more closely related to birds than to turtles. If relatedness is a good predictor of hazard of chemicals, effective predictors for all reptiles do not exist. Pough, Heiser & McFarland (1989), relying on the work of Smithson (1985) and others, stated that precise relationships among the major groups of vertebrates cannot be determined. Nevertheless, all indications are that the major groups of amniotes are both distinct and diverse. An *Anolis* lizard is probably not a good predictor of responses of crocodilians and turtles, for example, even though all three are in the same vertebrate class. Likewise amphibians and reptiles are likely to respond differently to chemicals.

Ecological diversity. Amphibians have complex life cycles, and more opportunities for exposure to chemicals and more potential routes of exposure than other vertebrates. Likewise, diversity in life history and survival strategies of amphibians and reptiles is extreme, as, for example, the differences in adaptations between iguanas and amphisbaenids illustrate.

Physiological diversity. As amniotes, reptiles can be expected to share many physiological and biochemical characteristics with birds and mammals, but as poikilotherms, can be expected to differ in their response to various environmental conditions. Amphibians likewise are physiologically diverse with different adaptations at different stages to accommodate morphological and ecological changes through the life cycle.

Geographic diversity. Amphibians and reptiles occupy a great variety of climatic and ecological zones, but achieve their greatest biological importance in the tropics. Most evaluations of effects of toxic chemicals on them have been done with temperate species.

Selectivity of Chemicals

Chemicals intended for use in the environment are screened on the basis of selective toxicity. An ideal pesticide is highly toxic to target pest organisms and non-toxic to other organisms. Of greatest concern to producers of pesticides is mammalian toxicity as it predicts hazard to humans. Most successful pesticides are highly toxic to invertebrate animals or to target kinds of plants and generally less toxic to vertebrates. The degree of toxicity to vertebrates varies, however, both among chemicals and among different groups of vertebrates. The following examples use toxicity values obtained from a summary of reports on the comparative toxicity of many pesticides to a variety of animal species (Kenaga, 1979) to illustrate some differences among selectively toxic chemicals. *Carbaryl* is a widely used and effective insecticide of very low toxicity to most vertebrates. *Parathion*, a broad-spectrum non-systemic insecticide, is highly toxic to birds and mammals, but of low hazard to fish. *Azinphos-methyl*, a non-systemic insecticide and acaricide, is much more toxic to birds than to fish. *Trifluralin*, a herbicide of low toxicity to birds and mammals, is among the most toxic chemicals to fish. The potential interaction of the great natural diversity among amphibians and reptiles and the intentional selectivity engineered into pesticides can result in diverse responses and in unpredicted hazards.

The Nature of Data on Chemical Hazards

Most available information on the hazard of chemicals to vertebrates is in data bases that were generated on groups of animals other than amphibians and reptiles. Probably the greatest volume is on mammals, primarily laboratory rodents. Another very large volume of information exists on fish. Less information is available on birds, but much of the knowledge from avian studies is relatively useful because of its focus on hazards to wild animals in the natural environment. For the great majority of chemicals, there is no information on hazards to amphibians or reptiles.

RESEARCH ON AMPHIBIANS AND REPTILES

Past research on hazards of environmental chemicals to amphibians and reptiles has not revealed complete answers to the questions posed, but may indicate how answers may be found.

Power, Clark, Harfenist & Peakall (1989) recently summarized research on the susceptibility of amphibians to toxic chemicals. The wide variety of studies included acute toxicity tests with 211 different pollutants, a variety of effects in the laboratory with 154 different substances or conditions, and field studies of the effects of 54 different pollutants. Test species or protocols have not been standardized. Use of 45 different species of amphibians in acute toxicity tests was reported under widely varied test conditions. The authors discussed the problems of selection of test species, life stages, and endpoints, and the common problems of test media, holding, rearing, and testing conditions, and length of the observation period. Multiple test species were suggested until more is known about interspecific differences in sensitivities. Available test guidelines were identified as inadequate.

An earlier summary of the literature on the effects of environmental contaminants on reptiles (Hall, 1980) was reasonably complete, but almost entirely concerned with organochlorines. Much of it was based on observations following field applications or on reports of chemical residues in tissues.

Until recently, it was commonly stated that research does not support the argument that any species of amphibian or reptile is more susceptible to any chemical contaminant than other kinds of vertebrates. The literature on environmental contamination is devoid of compelling evidence that adult and larval amphibians are more sensitive to chemicals than other land and aquatic vertebrates (Table 1). In fish culture, however, amphibians are often regarded as pests and the literature of fish culture and fish control reveals that at least two chemical toxicants are selectively toxic to amphibians. TFM (3-trifluoromethyl-4-nitrophenol) is a toxicant developed in the 1950s as a selective control for the sea lamprey (*Petromyzon marinus*) in the Laurentian Great Lakes region of North America. Howell (1966) and Gilderhus & Johnson (1980) observed that mudpuppies (*Necturus maculosus*) and frog larvae are routinely killed by field applications of TFM, and suggested that they are perhaps

as sensitive as the target lamprey. Bioassays by Chandler & Marking (1975) indicated that larvae of gray treefrogs (*Hyla versicolor*), leopard frogs (*Rana pipiens*), and bullfrogs (*R. catesbeiana*) are 1.2 to 8.2 times as sensitive to TFM as several fish species for which comparable data (Marking & Olson, 1975) are available. Kane, Stockdale & Johnson (1985) and Kane & Johnson (1989) found that TFM is four times more toxic to bullfrog larvae than to fathead minnows (*Pimiphales promelas*) and that young larvae are selectively killed by TFM in ponds inhabited by fish.

Helms (1967) reported on experiments with another toxicant, formalin, to control tadpoles in fish production ponds, and Carmichael (1983) found that treatment with formalin selectively removes tadpoles in raceways containing fingerling largemouth bass (*Micropterus salmoides*). In fact, the tadpoles were more sensitive to formalin than nine species of fish tested by Bills, Marking & Chandler (1977) and far more sensitive than four of five species representing major invertebrate groups (Table 2). Only ostracods (*Cypridopsis* sp.) were more sensitive than tadpoles. The other arthropod species, the backswimmer (*Notonecta* sp.) and the freshwater prawn (*Palaemonetes kadiakensis*), were highly resistant. Because formaldehyde is a major air pollutant that readily dissolves in water to produce formalin, its increasing concentrations could conceivably have significant effects on amphibians in the environment.

Some research has questioned the validity of traditional toxicological methods and endpoints in evaluations involving amphibians and reptiles. Standard toxicological methods were developed over many years for homeothermic vertebrates, and a separate set was developed for use with fish. These methods may not be useful for amphibians and reptiles. Temperature regimes for toxicity tests on poikilotherms have been questioned. The review by Power *et al.* (1989) revealed that tests on amphibians have been conducted over a wide range of temperatures. Tests with death as an endpoint in relatively inactive species have been questioned because behavioural effects were sometimes noticeable at far below lethal exposure. For example, Hall & Swineford (1981) found that larvae of *Ambystoma opacum* were debilitated at levels of toxaphene far below lethal concentrations and probably would not have survived in the wild, although many ultimately recovered in the laboratory. Preliminary results from our laboratory indicate behavioural anomalies in *A. maculatum* exposed to cholinesterase inhibitors at one order of magnitude below lethal levels. Behavioural changes affecting feeding or escape could have lethal effects in the field. Studies of teratogenic effects (Cooke, 1981) indicated that abnormalities in amphibian larvae are produced at sublethal concentrations.

A moderate amount of work has been done on amphibian larvae, principally anuran tadpoles. Anurans are remarkably resistant to some cholinesterase inhibitors, the class of pesticides currently in greatest use, apparently many times more so than other vertebrates (Hawkins & Mendel, 1946; Edery & Schatzberg-Porath, 1960; Andersen, Aaraas, Gaare & Fonnum, 1977). Tailed amphibians may share this resistance; preliminary evaluations with *Ambystoma maculatum* larvae performed in our laboratory indicate similar resistance. Resistance permits the accumulation of tremendous body burdens that may be harmful to predators (Hall & Kolbe, 1980; Hall, 1990). Resistance appears to result from an inability of the chemicals to structurally bind with and inhibit amphibian cholinesterases (Potter & O'Brien, 1964; Wang & Murphy, 1982). Resistance may be fortuitous or may be an adaptation to naturally-occurring cholinesterase inhibitors in the environment; recent studies of *Anabaena flos-aquae*, a freshwater alga, revealed that it produces a sufficiently powerful cholinesterase inhibitor to kill livestock drinking from waters that support blooms (Cook, Beasley, Lovell, Dahlem, Hooker, Mahmood & Carmichael, 1989). Some results indicate that the apparent resistance to cholinesterase inhibitors may not apply to all amphibian species and all chemicals. For example, Sanders (1970) found the organophosphate carbophenothion to be the most toxic of 16 chemicals to tadpoles of the chorus frog (*Pseudacris triseriata*). Cooke (1981) implicated the carbamate oxymyl in the production of deformities in larvae of *Rana temporaria*, the European common frog. Although some or most synthetic cholinesterase-inhibiting chemicals may not unduly harm amphibians, the responses of amphibians could not have been predicted from results of testing other vertebrates, and another class of compounds could produce equally atypical results. Responses might be skewed toward lethality (as with TFM).

Almost no experimental evaluations of the sensitivity of reptiles to environmental chemicals have been made, although field studies were common in the era of organochlorines. In a study of the sensitivity of the green anole, *Anolis carolinensis*, to four organophosphates (Hall & Clark, 1982), responses seen were close to those of mallards and rats (Table 3). The study was intended to test the widely-held notion of "cold-blooded" and "warm-blooded" patterns of response to cholinesterase inhibitors.

A series of investigations summarized by Cooke (1981) related exposure to environmental pollutants and the occurrence of deformities in tadpoles, and indicated that the production of such deformities can be a sensitive indicator of pollution by certain chemicals. Cooke (1981) reported development and testing of a protocol in which caged tadpoles in waters receiving runoff or spray drift from agricultural fields could indicate chemical treatments hazardous to amphibians. Dumont, Shultz, Buchanan & Kao (1983) developed what they called the "FETAX" test, a protocol using embryos of the clawed frog (*Xenopus laevis*) as an assay for teratogenicity of chemicals and mixtures of contaminants. The primary purpose of the proposed test was neither screening agricultural chemicals nor protection of amphibian populations, but it might be adaptable for these purposes. Despite indications that examining production of abnormalities in embryos and larvae may be an economical and powerful tool for identifying chemical hazards, it has not been extensively used for testing new chemicals.

In summary, far too little is known to conclude that safety standards for other kinds of vertebrates are adequate for the protection of amphibians and reptiles. Existing evidence indicates that amphibians are more sensitive to a selective piscicide and to a prophylactic fishery chemical than most fish commonly tested, and strikingly more resistant to some cholinesterase-inhibiting compounds (Table 1) than other classes of vertebrates. Neither response could have been predicted from tests conducted on other vertebrate classes. Susceptibility of reptiles to selective pesticides is virtually unknown. Preliminary information (Hall and Clark, 1982) suggests similarity in responses to other amniote vertebrates, but this conclusion is based on the exposure of only one lizard species to four chemically similar compounds.

	BULLFROG	MALLARD	B/M
CARBAMATES			
BAYGON	595	9.4	63
CARBARYL	>4000	>2564	-
MEXACARBATE	566	3.0	190
NABAM	420	>2560	<0.2
ORGANOPHOSPHATES			
CHLORPYRIFOS	>400	76	>5
DEMETON	562	7.2	78
DIAZINON	>2000	3.5	>570
DICROTOPHOS	2000	4.2	476
PHORATE	85	0.6	140
TEMEPHOS	>2000	79	>25
TEPP	112	3.6	31
OTHER			
DDT	>2000	>2240	-
SODIUM MONO-FLUOROACETATE	54	5.9	9
STRYCHNINE	2.2	2.0	1.1

TABLE 1. Acute oral toxicity (LD_{50} in mg/Kg) of pesticides to bullfrogs (*Rana catesbeiana*) and mallards (*Anas platyrynchos*), and the relative sensitivity of bullfrogs compared to mallards. Mallards are, for example, 63 times as sensitive to Baygon as are bullfrogs. Data from Tucker & Crabtree (1970).

ORGANISM	LC_{50} (mg/l)			
	24 h	48 h	72 h	96 h
Ostracods[2] (*Cypridopsis* sp.)	1.15			1.05
Tadpoles of Three Species of Amphibians[3]	22 - 70	21 - 59	21 - 59	
Leopard Frog Larvae[4] (*Rana berlandieri*)	~40			
Four Species of Fish[3]	>70 - 87	49 - >100	45 - >100	
Six Species of Fish[2]	141 - 389			62.1 - 173
Largemouth Bass[4] (*Micropterus salmoides*)	~150			
Snail[2] (*Heliosoma* sp.)	710			93
Bivalves[2] (*Corbicula* sp.)	800			126
Freshwater prawn[2] (*Palaemonetes kadiakensis*)	1105			465
Backswimmer[2] (*Notonecta* sp.)	4500			835

1- In all tests, commercial formalin stated to be approximately 37% formaldehyde was used. 2- data from Bills *et al.* (1977). 3- data from Helms (1967) 4- data from Carmichael (1983)

TABLE 2. Toxicity of formalin[1] to aquatic organisms

	PARATHION	METHYL PARATHION	MALATHION	AZINPHOS-METHYL
ANOLIS	8.9	82.7	2324	98
MALLARD	2.1	10	1485	136
RAT	16	26	1840	15

TABLE 3. Acute oral toxicities (LD_{50} in mg/Kg) of four organophosphorus pesticides to three species of vertebrates. Data on *Anolis* from Hall & Clark (1982). Data on mallard and rat from Kenaga (1979)

PREREGISTRATION TESTING

Routine preregistration testing would be costly in financial terms and in animal subjects. Before making a commitment, the predictive value of generated data should be known. Amphibians may be expected to be exposed to chemicals in the water or by food and appropriate tests must expose larvae and transformed individuals by different media. One limited study (Hall & Swineford, 1979) compared routes of uptake in adult toads. Conclusions were that exposure to the organochlorine methoxychlor through water could be significant even in highly terrestrial amphibians. A battery of tests is therefore required, and some or all tests might have to be used for each chemical, depending on expected distribution of chemicals after their release into the environment. More than one species would be necessary to account for interspecies variability. For reptiles, route of exposure and choice of life stages present fewer problems, but predictability from one group to another is largely unknown. Until variability among and within groups is better assessed, species of many groups must be tested.

Presently, assurance that preregistration testing leads to conclusions of reasonable confidence would require many types of tests with several species, developmental stages and protocols, for each chemical. Compounding the difficulty is the need to expose subjects in a variety of unconventional ways and the necessity to obtain test subjects from nature, with attendant concerns for quality assurance and conservation.

An alternative to an elaborate array of laboratory tests for registration could be carefully designed and monitored field tests that expose a variety of free-living amphibians and reptiles to each chemical in an environmentally realistic manner, consistent with anticipated use of the chemical and cognizant of life history events that might modify hazard. The relative values of field tests, controlled field experiments (also known as *mesocosm tests*), and controlled laboratory tests were discussed by Hoffman, Rattner & Hall (1990). There are tradeoffs that make it impossible to simultaneously maximize environmental realism, repeatability, and predictive value. Testing schemes that best resemble actual exposure in one field situation may be of least value in predicting effects when the chemical is used in a different situation. Nevertheless, there is a role for careful field testing that cannot easily be filled by any combination of experimental procedures in the laboratory. Furthermore, hybrid testing methodologies that, for example, use caged tadpoles to monitor effects of operational pesticide applications (Cooke, 1981) may optimize advantages of the different kinds of tests.

One solution to the problem of preregistration testing is to perform the research to determine first whether preregistration testing of amphibians and reptiles is necessary and, if required, how the required information can be obtained most efficiently.

A RESEARCH STRATEGY

The goal of this research is to determine what kinds of new information are necessary to protect amphibians and reptiles from the hazards of environmental chemicals.

Research should examine the relative sensitivity of major groups of amphibians and reptiles to the major groups of environmental contaminants. Of primary concern is inherent (taxonomic) variability in responses; for this reason *in vitro* investigations may be preferred. Endpoints need not be lethality, but must be repeatable. Ecological diversity contributes greatly to risk in the field, but is of less immediate concern than basic differences in sensitivity because ecological effects in modifying toxicity can often be projected across species. For example, amphibians with aquatic larvae are probably more susceptible to water-borne pesticides than those that develop on land.

Chemicals with selective toxicity should be examined first. Differences in toxic rank are important because they permit comparison of data from aquatic and terrestrial tests. Data on toxic rank can be determined from a variety of *in vitro* procedures to reduce costs. Research should determine whether abnormalities in embryos or larvae are indicators of general toxicity, or whether they are caused by wholly different mechanisms than those resulting in acute lethality. If teratogenesis is a good general indicator of hazard, it may provide the basis of a low cost routine screening.

If sensitivity of one group of amphibians or reptiles falls outside the range of observed sensitivities in the other groups commonly tested, further investigations should determine to what extent the sensitive amphibian or reptile represents its taxonomic group and to what extent responses of the group could be predicted from data routinely collected on other vertebrates.

Once there is a reasonable base of knowledge on ranges of expected responses of different groups of amphibians and reptiles to different classes of chemicals, it may not be necessary to perform complete series of tests on all chemicals. New chemicals introduced or those undergoing reevaluation can be given one or more spot tests to determine whether responses fall within expected limits. Those that fall within expected limits may not require further testing, while those that exceed expected limits may need detailed evaluation.

Data, statistics, computer models and risk assessments are only of limited value in predicting the hazards that chemicals may pose to natural populations. Chemicals that have appeared safe based on laboratory tests have been implicated in dieoffs in the field. For example, dimethoate and methamidophos were thought to pose little risk to birds, but each chemical caused mortality of sage grouse (*Centrocercus urophasianus*) feeding in treated fields (Blus, Staley, Henny, Pendleton, Craig, Craig & Halford, 1989). Additional research should seek better understanding of the ways in which ecological factors modify chemical risks. For wildlife in general, there is inadequate knowledge of the significance to populations of transient sublethal effects or of subtle changes that may go undetected in the laboratory. In predicting hazards from the kinds of information most often available, there is no substitute for knowledgeable biologists who are experienced with chemical contaminants and who are familiar with amphibians and reptiles as they live in the field. Laboratory investigations, therefore, should provide a guide, but should not obviate the need for well designed field tests and vigilance by biologists after registration. Good field testing and surveillance of chemicals in use can, moreover, improve the quality of testing in the laboratory by providing feedback on the quality of risk assessments that may lead to their improvement.

ACKNOWLEDGEMENTS

We thank P. H. Albers, A. S. Cooke, R. Eisler, M. J. Melancon, E. J. Rockwell, and an anonymous reviewer for comments on the manuscript. Many of the ideas expressed here were first presented as part of a symposium at the First World Congress of Herpetology, and preparation of those ideas as a manuscript owes much to the encouragement of M. R. K. Lambert. Much of the work reported here was supported by the U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, and was encouraged and guided in its early stages by L. F. Stickel.

REFERENCES

Andersen, R. A., Aaraas, I., Gaare, G. & Fonnum, F. (1977). Inhibition of acetylcholinesterase from different species by organophosphorus compounds, carbamates and methylsulphonylfluoride. *General Pharmacology* **8**, 331–334.

Anonymous (1977). Amphibia and reptiles endangered. *Marine Pollution Bulletin* **8**, 51.

Bills, T. D., Marking, L. L. & Chandler, J. H., Jr. (1977). Formalin: its toxicity to nontarget aquatic organisms, persistence, and counteraction. *U.S. Fish and Wildlife Service Investigations in Fish Control* **73**, 1–7.

Blus, L. J., Staley, C.S., Henny, C. J., Pendleton, G. W., Craig, T. H., Craig, E. H. & Halford, D. K. (1989). Effects of organophosphorus insecticides on sage grouse in southeastern Idaho. *Journal of Wildlife Management* **53**, 1139–1146.

Borchelt, R. (1990). Frogs, toads, and other amphibians in distress. *National Research Council News Report* **40**, 2–5.

Bury, R. B., Dodd, C. K., Jr. & Fellers, G. M. (1980). Conservation of the amphibia of the United States: a review. *U.S. Fish and Wildlife Service Resource Publication* **134**.

Carmichael, G. J. (1983). Use of formalin to separate tadpoles from largemouth bass fingerlings after harvesting. *Progressive Fish-Culturist* **45**, 105–106.

Chandler, J. H. & Marking, L. L. (1975). Toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to selected aquatic invertebrates and frog larvae. *U.S. Fish and Wildlife Service Investigations in Fish Control* **62**, 3–7.

Cook, W. O., Beasley, V. R., Lovell, R. A., Dahlem, A. M., Hooser, S. R., Mahmood, N. A. & Carmichael, W. W. (1989). Consistent inhibition of peripheral cholinesterases by neurotoxins from the freshwater cyanobacterium *Anabaena flos-aquae*: studies of ducks, swine, mice and a steer. *Environmental Toxicology and Chemistry* **8**, 915–922.

Cooke, A. S. (1981). Tadpoles as indicators of harmful levels of pollution in the field. *Environmental Pollution, Series A* **25**, 123–133.

Corn, P. S., Stolzenburg, W. & Bury, R. B. (1989). Acid precipitation studies in Colorado and Wyoming: Interim report of surveys of montane amphibians and water chemistry. *U.S. Fish and Wildlife Service Biological Report* **80** (40.26).

Dumont, J. N., Schultz, T. W., Buchanan, M. V. & Kao, G. L. (1983). Frog embryo teratogenesis assay: *Xenopus* (FETAX) — a short-term assay applicable to complex environmental mixtures. In *Short-term Bioassays in the Analysis of Complex Environmental Mixtures III* 393–405. Waters, M.D., Sandhu, S. S., Lewtas, J., Claxton, L., Chernoff, N. & Nesnow, S. (eds.) New York: Plenum Press.

Edery, H. & Schatzberg-Porath, G. (1960). Studies on the effects of organophosphorus insecticides on amphibians. *Archives of International Pharmacodynamics* **124**, 212–224.

Gilderhus, P. A. & Johnson, B. G. H. (1980). Effects of sea lamprey (*Petromyzon marinus*) control in the Great Lakes on aquatic plants, invertebrates and amphibians. *Canadian Journal of Fisheries and Aquatic Science* **37**, 1895–1905.

Hall, R. J. (1980). Effects of environmental contaminants on reptiles: a review. *U.S. Fish and Wildlife Service Special Scientific Report - Wildlife* **228**.

Hall, R. J. (1990). Accumulation, metabolism and toxicity of parathion in tadpoles. *Bulletin of Environmental Contamination and Toxicology* **44**, 629–635.

Hall, R. J. & Clark, D. R., Jr. (1982). Responses of the iguanid lizard *Anolis carolinensis* to four organophosphorus pesticides. *Environmental Pollution (Series A)* **28**, 45–52.

Hall, R. J. & Kolbe, E. (1980). Bioconcentration of organophosphorus pesticides to hazardous levels by amphibians. *Journal of Toxicology and Environmental Health* **6**, 853–860.

Hall, R. J. & Swineford, D. (1979). Uptake of methoxychlor from food and water by the American toad (*Bufo americanus*). *Bulletin of Environmental Contamination and Toxicology* **23**, 335–337.

Hall, R. J. & Swineford, D. (1980). Toxic effects of endrin and toxaphene on the southern leopard frog *Rana sphenocephala*. *Environmental Pollution (Series A)* **23**, 53–65.

Hall, R. J. & Swineford, D. M. (1981). Acute toxicities of toxaphene and endrin to larvae of seven species of amphibians. *Toxicology Letters* **8**, 331–336.

Hawkins, R. D. & Mendel, B. (1946). True cholinesterases with pronounced resistance to eserine. *Journal of Cellular and Comparative Physiology* **27**, 69–85.

Helms, D. R. (1967). Use of formalin for selective control of tadpoles in the presence of fishes. *Progressive Fish-Culturist* **29**, 43-47.

Hoffman, D. J., Rattner, B. A. & Hall, R. J. (1990). Wildlife Toxicology. *Environmental Science and Technology* **24**, 276-283.

Howell, J. H. (1966). The life cycle of the sea lamprey and a toxicological approach to its control. In *Phylogeny of Immunity* 263-270. Smith, R. T., Meischer, P. A. & Good, R. A. (eds.) Gainesville: University of Florida Press.

Kane, A. S. & Johnson, D. L. (1989). Use of TFM (3-trifluoromethyl-4-nitrophenol) to selectively control frog larvae in fish production ponds. *Progressive Fish-Culturist* **51**, 207-213.

Kane, A. S., Stockdale, T. M. & Johnson, D. L. (1985). 3-trifluoromethyl-4-nitrophenol (TFM) control of tadpoles in culture ponds. *Progressive Fish-Culturist* **47**, 231-237.

Kenaga, E. E. (1979). Acute and chronic toxicity of 75 pesticides to various animal species. *Down to Earth* **35**, 25-31.

Marking, L. L. & Olson, L. E. (1975). Toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to nontarget fish in static tests. *U.S. Fish and Wildlife Service Investigations in Fish Control* **60**, 1-27.

Potter, J. L. & O'Brien, R. D. (1964). Parathion activation by livers of aquatic and terrestrial vertebrates. *Science* **144**, 55-57.

Pough, F. H., Heiser, J. B. & McFarland, W. N. (1989). *Vertebrate Life*. New York: MacMillan.

Power, T., Clark, K. L., Harfenist, A. & Peakall, D. B. (1989). A review and evaluation of the amphibian toxicological literature. *Canadian Wildlife Service Technical Report Series* **61**.

Sanders, H. O. (1970). Pesticide toxicities to tadpoles of the western chorus frog *Pseudacris triseriata* and Fowler's toad *Bufo woodhousii fowleri*. *Copeia* **1970**, 246-251.

Smithson, T. R. (1985). The morphology and relationships of the Carboniferous amphibian *Eoherpeton watsoni* Panchen. *Zoological Journal of the Linnean Society* **85**, 317-410.

Tucker, R. K. & Crabtree, D. G. (1970). Handbook of toxicity of pesticides to wildlife. *U.S. Fish and Wildlife Service Resource Publication* **84**.

U.S. Fish and Wildlife Service (1990). Endangered & threatened wildlife and plants. *Code of Federal Regulations* 50 CFR 17.11 & 17.12.

Wang, C. & Murphy, S. D. (1982). Kinetic analysis of species difference in acetylcholinesterase sensitivity to organophosphate insecticides. *Toxicology and Applied Pharmacology* **66**, 409-419.

HERPETOLOGICAL JOURNAL, Vol. 2, pp. 72-76 (1992)

ASSIMILATION OF ENERGY, PROTEIN AND FATTY ACIDS BY THE SPECTACLED CAIMAN *CAIMAN CROCODILUS CROCODILUS* L.

JOHN DAVENPORT[1], TIMOTHY J. ANDREWS AND GILES HUDSON.

School of Ocean Sciences, University of Wales at Bangor, Marine Sciences Laboratories, Menai Bridge, Gwynedd LL 59 5EY, North Wales, U.K.

[1]*Present address: University Marine Biological Station, Millport, Isle of Cumbrae, Scotland, KA28 0EG*

ABSTRACT

At 30±1°C, caimans (*Caiman crocodilus*) ate a mean satiation ration of 8.2% body weight. Mean total gut clearance time (TGCT) was 136 h; mean gastric emptying time (GET) was 97 h. These data indicate that caimans eat considerably less food than salt-water crocodiles (*Crocodylus porosus*) of similar size, not because their meal size was less, but because they take much longer to process food. Assimilation efficiencies for protein, energy (cals) and dry mass were 91.8%, 68.2% and 62.0% on a diet of sheeps' hearts and 90.2%, 69.2% and 64.7% in caimans fed on fish. These efficiencies are all significantly lower than those found in salt-water crocodiles. Hard particulate material is retained within the stomach by a powerful pyloric sphincter, but caimans appear not to eat gastroliths deliberately. Evidence is presented to show that polyunsaturated fatty acids (PUFAs), especially 22:5ω3, are assimilated and incorporated into tissues. Fish-fed caimans showed more 20:5ω3 and 22:6ω3 in their tissue than liver-fed animals. It has previously been suggested that species of the Family Crocodylidae (believed to be of largely marine ancestry) can assimilate PUFAs while members of the Family Alligatoridae (probably of prolonged freshwater ancestry) cannot. The results of this study deny this clearcut distinction and indicate that any differences in lipid metabolism amongst crocodillians are likely to reflect ecological considerations rather than taxonomic patterns.

INTRODUCTION

It has been suggested that living true crocodiles (Family: Crocodylidae) have evolved from predominately marine ancestors, while alligators and caimans (Family: Alligatoridae) have a long history of life in freshwater ecosystems. The main evidence for this hypothesis lies in the observations of Taplin & Grigg (1981) and Taplin *et al.*, (1982), who found that true crocodiles have lingual glands which secrete salt, while members of the Alligatoridae do not. However, there is some supporting evidence from nutritional studies. Garnett (1985) suggested that the salt-water crocodile (*Crocodylus porosus*) has a nutritional requirement for the long chain polyunsaturated fatty acids (PUFAs) found in marine fish, while Ferguson (1981) and Lance (1982) both indicate that alligators (*Alligator mississippiensis*) are unable to assimilate these marine fatty acids. the main aim of the study reeported here was to determine whether another member of the Alligatoridae, the spectacled caiman, *Caiman crocodilus*, could incorporate long chain fatty acids into its tissues. In addition, it was decided to investigate appetite, gut transit, plus protein and energy assimilation in the caiman, for comparison with the data recently presented for the salt-water crocodile by Davenport *et al.*, (1990).

MATERIALS AND METHODS

COLLECTION AND MAINTENANCE

Ten spectacled caimans were obtained from biological suppliers. At the time of the study the animals weighed between 240 and 600 g. They were held in running fresh water at the experimental temperature of 30±1°C. Five of the animals (used in gut transit studies) were fed on sheep heart and ox liver (offered daily) while the other five were divided into two groups. Two were fed on pigs' liver, while the other three were fed on whole chopped herring. Feeding was continued for one month prior to experimentation.

APPETITE AND TRANSIT

Satiation ration was estimated in the caimans by depriving them of food for 4 days and then offering food from preweighed rations until they were satiated.

Gut transit of food was investigated in two ways. Five animals were each given a meal of minced ox liver containing chromic oxide (2% w/w). Thereafter they were fed normally on unlabelled ox liver. The animals were held separately and their faeces collected and inspected twice per day. The presence of green (i.e. chromic oxide labelled) faeces was recorded. This experiment allowed calculation of total gut clearance time (TGCT) and an estimate of gastric emptying time (GET). Another two animals were fed a meal labelled with 2% (w/w) barium sulphate plus numbers of barium/polystyrene spheroids (1 mm diameter) and lead glass beads (0.4 mm diameter). X-rays were obtained before and immediately after the meal; further X-rays were taken after 4, 8, 12, 19, 24, 52, 74, 96, 144, 168 and 192 h. After the radio-opaque meal they were offered unlabelled food each day. This experiment provided information about the behaviour of material within the various parts of the gut.

ASSIMILATION OF NUTRIENTS

Assimilation of energy, protein and dry mass was studied in the group which had been fed initially upon sheeps' heart and later upon whole herring. Fatty acid assimilation was investigated in the two groups fed upon pigs' liver and whole herring repectively. Each group was fed upon chromic-oxide labelled food (2% w/w) which had been finely minced and reminced so that the food and label were evenly mixed. Labelled food was offered to the animals every 48 h. Uneaten food was removed after 4 h. Animals were fed for a week (a period longer than the TGCT) on labelled food and then faecal samples were collected from each animal (by placing them in individual tanks with

broad mesh floors). Animals were inspected several times per day; only freshly voided faeces were collected. Feeding and faecal collection continued until adequate amounts of material were available for chemical analysis. The five animals being studied for fatty acid assimilation were then killed humanely; tissue samples were taken from the tail muscle and a large abdominal fat body found in all the caimans. For comparative purposes, tail muscle and adipose tissue samples from three specimens of *Crocodylus porosus* (stored frozen from the earlier study of Davenport *et al*., 1990) were also analysed for fatty acid composition.

Food and faecal samples were dried at 70°C to constant weight. Chromic oxide content was measured by the method of McGinnis & Kasting (1964). Energy content was determined by the wet oxidation method of Ivlev (1935). Protein content was estimated by measuring total nitrogen (micro-Kjeldahl technique). Lipids were extracted from dried food, faecal and tissue samples and the total lipid content determined gravimetrically as described by Folch *et al*., (1957). Once the lipid content had been determined, fatty acid composition was studied. Samples were transmethylated prior to fatty acid analysis by heating with an excess of boron trifluoride methanol reagent (15% BF_3) for 1h at 100°C. Fatty acid methyl esters (FAME) were extracted into pentane, evaporated to dryness with oxygen-free nitrogen and dissolved in hexane with a known amount of tricosanoic acid meythl ester as standard. Analysis was carried out by capillary gas chromatography, using a Carlo Erba 6180 gas chromatograph fitted with a flame ionization detector and a DB Wax 30 W capillary column, 25 m, 0.32 mm, I.D. and an on-column cold trap injector. FAME were identified using commercially available standards, graphical techniques and systematic separation factors (Ackman, 1972). Lipid classes were investigated by thin layer chromatography, using pre-cooled plates (silica-gel 60). The plates were then developed in petroleum spirit: diethyl ether (95:5 v/v) and visualised with iodine vapour. Samples were co-eluted with a methyl ester standard (178-1; Sigma Chemicals).

RESULTS

Appetite and Gut Transit

At 30±1°C, the caimans ate a mean satiation ration of sheeps' heart of 8.2% body weight (SD 4.3%). The mean TGCT (time between chromic oxide-labelled meal and appearance of last labelled faeces) was 136 h; the mean GET (time between appearance of first and last labelled faeces) was 97 h. X-radiography showed that food moved through the gut in a similar fashion to the salt-water crocodile (Davenport, *et al*., 1990). The stomach is large and drum shaped; the meal is totally confined within it until about 4 h after the meal when some barium sulphate label moves into the duodenum. Barium sulphate labelled material passes rapidly through the small intestine into the large intestine and rectum (some material reaching the rectum in as little as 12 h), but the barium spheroids and glass beads remain within the stomach, lying close to the pyloric sphincter. Clearly, as in the salt-water crocodile, a powerful sphincter sorts particulate material; only fluid and fine material passes into the small intestine. In the X-ray plates it was noticeable that the larger barium spheroids lay anterior to the glass beads which were packed next to the pyloric sphincter. After 52 h no barium sulphate was present in the small intestine, but the particulate material was again dispersed throughout the stomach (the caimans having eaten non-labelled material which now filled the stomach). Barium sulphate and a few particles filled the rectum. After 74 h no barium sulphate could be seen in any part of the gut and about 25% of the spheroids and glass beads had passed into the colon. There was no evidence that the pyloric sorting mechanism could differentiate between spheroids and beads. As each new meal was eaten a few particles left the stomach; one animal had lost all particles after 168 h, but the other still had substantial numbers in the stomach after 192 h.

Assimilation

Energy, protein and dry mass. Assimilation efficiencies were calculated as described by Maynard & Loosli (1969):

$$\% \text{ efficiency} = \frac{[Cr_2O_3]:[\text{nutrient in food}]}{[Cr_2O_3]:[\text{nutrient in faeces}]} \times 100$$

On a diet of sheeps' heart the assimilation efficiencies for protein, energy (cals) and dry mass were 91.8% (SD 3.6%), 68.2% (SD 5.6%) and 62.0% (SD 7.4%) respectively. On a diet of fish (herring) the mean assimilation efficiency for protein was 90.2% (SD 2.0%), for energy (cals) 69.2% (SD 4.1%) and for dry mass 64.7% (SD 4.6%). There was clearly no significant difference in rate of assimilation of these nutrients from the contrasting diets.

Fatty acids. Fatty acid data for diets, faeces and tissue samples are presented in Tables 1 and 2. As expected there were marked differences between the fatty acid compositions of the diets. Pigs' liver contained a far higher proportion of the C18 fatty acids than the herring diet with approximately ten times the proportion of linoleic acid (18:2ω6) and seven times the percentage of 18:0. The fish diet was rich in long chain fatty acids, particularly 22:6ω3. Rather surprisingly, the pigs liver contained some 22:6ω3 (perhaps because the pigs had been fed on fish meal at some stage), but at much lower concentrations than the herring.

In considering the faecal data, it must be remembered that they represent proportions of the unassimilated lipid, plus any fatty acids excreted by the caiman or synthesized by gut bacteria. Although both diets contained hardly any of the short chain saturated fatty acid 12:0, substantial amounts of faecal lipid were made up of this fatty acid which was probably of bacterial origin. Of particular interest are the low levels of 22:6ω3 in the faeces, indicating assimilation of this PUFA, particularly in the fish-fed animals.

There are some noticeable differences between the fatty acid composition of tissues of caimans fed on contrasting diets, providing further indication that dietary incorporation of fatty acids has a role to play in the species. Fish-fed caimans showed more 20:5ω3 and 22:6ω3 in their tissues than liver-fed animals, indicating that these fatty acids are incorporated from the diet. However, in the case of 22:6ω3, the tissue levels are still well below dietary concentrations (especially in the fish-fed animals), suggesting that this fatty acid has a low saturation point.

In Table 3, the tissue fatty acid composition of fish-fed caimans and salt-water crocodiles are compared. The tissue compositions are remarkably similar, and there are no signifi-

	Fatty acid content (mean % total lipid)			
Fatty acid	Diet	Faeces	Tail muscle	Fat body
12:0	T	52.5	8.6	0.4
14:0	0.1	T	0.6	1.2
15:0	T	T	T	0.2
16:0	13.5	5.4	7.0	9.0
16:1ω7	0.7	0.2	4.4	0.7
17:0	0.7	0.1	0.7	0.6
18:0	21.7	10.2	9.2	13.4
18:1ω9	11.4	3.9	11.5	21.2
18:1ω7	2.2	1.4	4.2	4.6
18:2ω6	10.3	2.7	4.2	7.1
19:0	0.4	-	-	T
18:3ω3	0.6	0.2	0.4	0.6
18:4ω3	T	0.1	0.1	0.3
20:0	0.1	T	T	T
20:1ω9	0.2	T	1.4	1.7
20:4ω6	11.6	2.9	4.2	3.0
20:5ω3	1.0	0.3	0.6	0.5
22:1ω11	0.1	1.3	0.3	0.1
22:5ω3	2.5	1.2	1.0	0.9
22:6ω3	3.6	1.4	1.6	2.1

T = trace quantities; - indicates none detected

TABLE 1: Fatty acid data for caimans fed on pigs' liver

	Fatty acid content (mean % total lipid)			
Fatty acid	Diet	Faeces	Tail muscle	Fat body
12:0	0.7	29.6	3.2	2.5
14:0	2.1	0.1	0.2	1.7
15:0	0.3	T	T	0.2
16:0	16.7	5.8	5.0	5.7
16:1ω7	3.8	0.4	0.3	1.2
17:0	0.4	T	0.2	0.4
18:0	3.5	0.9	6.9	14.2
18:1ω9	4.6	0.7	7.2	23.9
18:1ω7	2.5	0.4	1.0	5.3
18:2ω6	0.8	0.2	2.2	5.9
19:0	-	-	-	0.1
18:3ω3	0.5	T	0.2	0.6
18:4ω3	1.4	0.3	0.2	0.2
20:0	0.1	1.1	0.8	0.4
20:1ω9	0.1	2.4	0.1	0.5
20:4ω6	2.6	2.4	2.0	5.4
20:5ω3	8.5	1.4	7.5	0.9
22:1ω11	0.7	0.6	0.2	1.3
22:5ω3	0.3	0.3	0.5	1.1
22:6ω3	19.8	1.6	3.7	3.6

T= trace quantities; - indicates none detected

TABLE 2: Fatty acid data for caimans fed on whole herring

	Fatty acid content (mean % total lipid)			
	A) *Caiman crocodilus*		B) *Crocodylus porosus*	
Fatty acid	Tail muscle	Fat body	Tail muscle	Fat body
12:0	3.2	2.5	8.5	1.3
14:0	0.2	1.7	1.4	T
15:0	T	0.2	T	T
16:0	5.0	5.7	3.9	1.5
16:1ω7	0.3	1.2	0.4	1.5
17:0	0.2	0.4	T	0.1
18:0	6.9	14.2	2.1	2.1
18:1ω9	7.2	23.9	4.5	1.3
18:1ω7	1.0	5.3	1.3	1.5
18:2ω6	2.2	5.9	3.7	0.3
19:0	-	0.1	-	1.0
18:3ω3	0.2	0.6	0.4	T
18:4ω3	0.2	0.2	0.3	T
20:0	0.8	0.4	2.1	0.2
20:1ω9	0.1	0.5	4.5	T
20:4ω6	2.0	5.4	4.8	6.7
20:5ω3	7.5	0.9	0.7	T
22:1ω11	0.2	1.3	3.0	0.3
22:5ω3	0.5	1.1	O.5	1.4
22:6ω3	3.7	3.6	4.8	2.3

T = trace quantities; - indicates none detected

TABLE 3. Comparison of fatty acid composition of tissues of caimans and salt-water crocodiles fed on fish diets.

cant differences between the levels of 22:5 and 22:6 fatty acids, suggesting that there is no systematic difference in PUFA requirement or uptake between the species. Only in the C18 fatty acids are there differences, with the caimans demonstrating greater incorporation.

DISCUSSION

The appetite of caimans (ca. 8% body wt. satiation ration) is similar to that of salt-water crocodiles (10% body weight satiation ration), but the mean TGCT of 136h is appreciably longer than that of *Crocodylus porosus* (97 h) and the GET is more than twice as long (97 h vs. 40 h). Taken together these observations indicate that young caimans eat considerably less food than young salt-water crocodiles of similar size. Assimilation rates for protein, energy and dry mass were all significantly lower in caimans (91.8, 68.2, 62.0% respectively) than in juvenile salt-water crocodiles (97.4, 85.2, 77.5% respectively), so a picture emerges of caimans eating less, taking longer to digest food and being less efficient in doing so. Generally speaking it would seem that salt-water crocodiles are faster moving, more active animals which must have a high metabolic rate and initial growth rate since they reach a much larger final size than the caimans; selection pressure in favour of fast and efficient food processing has presumably been greater in *Crocodylus porosus*. Perhaps the use of gastroliths by salt-water crocodiles is also related to the relative efficiency of digestion: Davenport *et al.*, (1990) found that salt-water crocodiles would deliberately eat small stones, and suggested that this would help in the breakup of insect and crustacean prey organisms. However, the caimans studied here showed little tendency towards this habit and would not eat stones, though small amounts of wood and paint flakes from their holding tanks were found in the stomachs of caimans post mortem (much less than in *Crocodylus porosus* held in the same tanks). This finding is consistent with the observations of Skoczylas (1976) and Schaler & Crawshaw (1982) who found no stones or other hard material in the stomachs of wild *Caiman crocodilus*.

The fatty acid data show that caimans, like salt-water crocodiles, are capable of assimilating PUFAs from the diet and incorporating them into the tissues. Garnett (1985) stated that *Crocodylus porosus* needed PUFAs in its diet because it could not desaturate and elongate short chain saturated fatty acids, a situation similar to that of marine fish (Ackman *et al.*, 1968). It seems likely that spectacled caimans also have a requirement for 'marine' PUFAs, presumably satisfied by the crustacean component in their diet. The findings of Ferguson (1981) and Lance (1982) that alligators are not capable of absorbing marine PUFAs clearly do not apply to all members of the family Alligatoridae, and in any case merit further study since some young alligators eat considerable quantities of the marine/estuarine blue crab *Callinectes sapidus* (Chabreck, 1971) which must be rich in PUFAs. In both caimans and salt-water crocodiles, relatively small quantities of PUFAs are incorporated into tissues by comparison with the concentration in the flesh of marine fish, even though substantial quantities of PUFAs are assimilated across the gut wall. The excess PUFAs, assimilated but not incorporated into structural materials, are presumably used as an energy source, or as the precursors of more complex molecules.

ACKNOWLEDGEMENTS

The authors are grateful to the Nuffield Foundation for funding from its Small Grants Scheme.

REFERENCES

Ackman, R. G.. (1972). The analysis of fatty acids and related materials by gas-liqiud chromatography. In *Progress in the chemistry of fats and other lipids,* 165-285. Holan, R. T. (ed). Oxford: Pergamon Press.

Ackman, R. G., Tocker, C. S. & McCachlan, J. (1968). Marine phytoplankton fatty acids. *Journal of the Fisheries Research Board of Canada* **25**, 1603-1620.

Chabreck, R. H. (1971). The food and feeding habits of alligators from fresh and saline environments in Louisiana. *Proceedings of the Annual Conference of the Southeastern Game and Fish Commission* **20**, 102-110.

Davenport, J., Grove, D. J., Cannon, J., Ellis, T. R. & Stables, R. (1990). Food capture, appetite, digestion rate and efficiency in hatchling and juvenile *Crocodylus porosus*. *J. Zool.* **220**, 569-592.

Ferguson, M. W. J. (1981). The value of the American alligator (*Alligator mississippiensis*) as a model for research in craniofacial development. *Journal of Craniofacial, Genetic and Developmental Biology* **1,** 123-144.

Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226,** 497-509.

Garnett, S. (1985). Fatty acid nutrition of the estuarine crocodile *Crocodylus porosus. Comp. Biochem. Physiol*. **81B,** 1033-1035.

Ivlev, V. S. (1935). Eine mikromethode zur bestimmung des kaloreinghalts von nahrstoffen. *Biochem. Z.* **275**, 49-55.

Lance, V. (1982). Trace elements, Vitamin E, and Glutathione peroxidase in the American alligator, *Alligator mississippienesis*. *Proceedings of the Annual Alligator production Conference* **2**, 17-30.

McGinnis, A. L. & Kasting, R. (1964). Comparison of gravimetric and chromic oxide methods for measuring percentage utilization and consumption of food by phytophagus insects. *J. Insect Physiol*. **10,** 989-995.

Maynard, L. A. and Loosli, K. J. (1969). *Animal nutrition*. 6th Edition. New York: McGraw Hill Book Co.

Schaler G. B. & Crawshaw, P. G. (1982). Fishing behaviour of the Paraguayan caiman (*Caiman crocodilus*). *Copeia* **1982**, 66-72.

Skoczylas, R. (1978). Physiology of the digestive tract. In *Biology of the Reptilia: Physiology B*, pages 589-717. Gans, C. (ed). London and New York: Academic Press.

Taplin, L. E. and Grigg, G. C. (1981). Salt glands in the tongue of the estuarine crocodile *Crocodylus porosus*. *Science* **212**, 1045-1047.

Taplin, L. E., Grigg, G. C., Harlow, P., Ellis, T. M. & Dunson, W. A. (1982). Lingual salt glands in *Crocodylus acutus* and *C. johnstoni* and their absence from *Alligator mississippiensis* and *Caiman crocodilus*. *J. Comp. Physiol*. **B 149**, 43-47.

HERPETOLOGICAL JOURNAL, Vol. 2, pp. 77-81 (1992)

GLOBAL CORRELATES OF SPECIES RICHNESS IN TURTLES

JOHN B. IVERSON

Dept. of Biology, Earlham College, Richmond, Indiana 47374, USA

(Accepted 14.11.90)

ABSTRACT

The relationships between maximum total and maximum freshwater turtle species richness versus twelve environmental factors hypothesized to be correlated with richness were examined for 42 river drainage basins on five continents. The only highly significant correlate was annual rainfall. Latitude, temperatures, and basin area and discharge were not significantly correlated with species richness. These results are interpreted in light of current theoretical determinants of species diversity.

INTRODUCTION

Although some authors have provided gross descriptions of global species richness patterns in turtles (e.g. Darlington, 1948, 1957; Mittermeier, 1972; Pritchard, 1979), quantitative analysis of richness patterns is available only on local geographic scales (e.g. Huheey, 1965, for Illinois; Rogers, 1976; Owen & Dixon, 1989, for Texas; Schall & Pianka, 1978, for Iberia). Quantitative work on a broader scale has been impeded by the lack of published information on the precise distributions of many of the world's turtles. The publication of preliminary distribution maps for all turtle species (Iverson, 1986) now makes a quantitative study of the correlates of global turtle species richness possible.

This study was undertaken to test several specific predictions about expected correlates with species richness that were based on biogeographic theory and/or patterns observed in other vertebrate taxa. I hypothesized that species richness should be greatest in the tropics (e.g. see Pianka, 1966; Kiester, 1971; MacArthur, 1972; Schall & Pianka, 1977; Stevens, 1989); in drainage basins with large areas (e.g. see Swift *et al.*, 1986; McAllister *et al.*, 1986; Angermeier & Schlosser, 1989) or with large discharge rates (e.g. Livingstone *et al.*, 1982); in areas with warmer winter and/or summer temperatures (Schall & Pianka, 1977, 1978); or with a low annual range of temperature variation (Schall & Pianka, 1977, 1978); and in areas with high rainfall (Rogers, 1976; Schall & Pianka, 1978; Owen & Dixon, 1989) or low seasonal variation in amount of rain. These predictions are here tested with maximum species richness data for 42 drainage basins representing all continents inhabited by turtles.

METHODS

Turtle species density maps were prepared by outlining the range of each turtle species as plotted in Iverson (1986), and manually overlaying those range maps to produce species richness isopleth (= species density) maps by turtle family and continent (Iverson, 1992). Approximately 60 river basins representing the major drainages on every continent were selected for analysis, and the site of maximum total (i.e. freshwater and terrestrial) turtle species richness in each basin pinpointed. The following environmental variables were compiled from records from the city nearest to that site as reviewed in Ruffner (1978) or Ruffner & Bair (1987): mean daily maximum and minimum temperatures in winter and summer (January or July, depending on hemisphere), difference in mean daily temperature in summer (January or July) compared to that in winter (a measure of seasonal variation in temperature), mean annual rainfall, mean monthly rainfall in the wettest month, mean monthly rainfall in the driest month, and the difference in mean rainfall in the wettest month compared to that in the driest month.

Drainage area and discharge data for each basin were then compiled, primarily from Showers (1973), but checked and supplemented when necessary with data from Tamayo and West (1964), Snead (1980), Berra (1981), Livingstone *et al.* (1982), and Swift *et al.* (1986). In addition to maximum total species richness for each basin, aquatic species richness was also determined for the same location based on habitat preferences in Ernst & Barbour (1989). Species richness in each of the (primarily aquatic) families Chelidae (Austro-American sidenecked turtles), Pelomedusidae (Afro-American sidenecked turtles), Kinosternidae (American mud and musk turtles), Trionychidae (Softshell turtles), Bataguridae (Batagurine turtles) and Emydidae (Pond turtles) were also determined for the same location. A nearly complete data set was thus compiled for a total of 42 basins (Appendix 1).

Although the data set is possibly flawed by (1) assuming the accuracy of distributions plotted by Iverson (1986); (2) using only the location of maximum richness in each basin; and (3) being forced to use climatic data from the nearest large city for which records are available (and thus not precisely at the location of maximum diversity), it is believed to be representative enough to be of value in this preliminary investigation of global correlates of species density in turtles. Correlation analysis between environmental variables and species richness values was performed with Statview™ Software on a Macintosh™ computer. Because 12 correlations were generated for each taxonomic group, the significance level was adjusted to $P < 0.004$ (0.05/12) to account very conservatively for the experiment-wise error rate (Sokal & Rohlf, 1981).

RESULTS

Turtle species richness is greatest in the lower Ganges-Brahmaputra river basin in India and Bangladesh (ca. 23° N latitude; seventeen aquatic and two terrestrial species) and in the lower Mobile River basin in Alabama in the United States (ca. 31°N latitude; sixteen aquatic and two terrestrial; see also Iverson, 1992). The results of the correlation analysis of total species richness, total aquatic species richness, and within family species richness versus the twelve environmental characters (Table 1) reveal that the only consistent predictor of species richness was annual rainfall (Fig. 1).

Annual rainfall was significantly correlated with species richness for all taxa combined, for all aquatic taxa combined, for trionychids, and for all non-trionychid taxa combined (Table 1). Only trionychid richness was positively correlated with seasonal variance in rainfall.

Surprisingly, neither latitude nor basin area nor discharge rate were significantly correlated with species richness in any group. Of the several measures of temperature, none showed a significant correlation with species richness for any taxonomic group, although the relationships between winter temperature and total and trionychid species richness approaches significance. Precipitation amount during the wettest month is positively correlated with overall species richness, but among families, only with trionychid richness. Precipitation during the driest month is positively correlated with richness for emydid turtles and all aquatic non-trionychid turtles combined. When the effects of latitude were removed by partial correlation analysis, only total annual rainfall ($r = 0.55$; $P< 0.0001$) and driest month rainfall ($r = 0.51$; $P= 0.0005$) were significantly correlated with total species richness.

DISCUSSION

Few of my original predictions were supported by the data compiled here. Perhaps the most surprising is the weak correlation between richness and latitude; however, this may be related to the lack of a continental land mass between about 10° N and 10°S latitude in the Oriental zoogeographic region. In addition, within-family patterns are complicated because some families are high-latitude families that have only recently dispersed into the tropics (e.g. the Kinosternidae and Emydidae, which both evolved primarily in isolation in North America; Darlington, 1948, 1957). Thus richness in these families is not inversely correlated with latitude (or positively correlated with winter temperatures); indeed, the signs of the correlation coefficients (though not statistically significant) are the opposite of those predicted.

Given that freshwater fish species richness has been found to be correlated with both lake (Barbour & Brown, 1974) and basin area (McAllister *et al.*, 1986; Swift *et al.*, 1986), as well as basin discharge (Livingstone *et al.*, 1982), it was surprising that neither basin area nor basin discharge were positively correlated with turtle species richness at any taxonomic level. This may be because the richness value used in this analysis represents only the maximum local value for a particular basin and not the total for the entire basin; however, given the distributions figured by Iverson (1986), it is doubtful that even total turtle species diversity for each basin would be correlated with basin area.

Schall & Pianka (1977, 1978) have shown that turtle species density is positively correlated with mean July temperature in Iberia and mean annual temperature in the United States and Australia. However, my global analysis does not reinforce the effect of temperatures (summer, winter or annual) on density, except that winter temperatures and annual seasonality in temperature approach being significantly correlated with trionychid species richness. This is particularly interesting since the family Trionychidae is atypical among turtles in lacking temperature-dependent sex determination (Ewert & Nelson, 1991) and apparently lacking the ability of hatchlings to overwinter in the nest in temperate regions (Gibbons & Nelson, 1978). Whether these characteristics are related must await further study. Comparisons of correlation coefficients of the various measures of temperature with species richness for the various samples (even though not statistically significant at $P< 0.004$) do suggest that summer temperatures have less affect on turtle species richness than winter temperatures.

Rogers (1976, in Texas), Schall & Pianka (1978, in the United States and Australia) and Owen & Dixon (1989, in Texas) all demonstrated a positive correlation between turtle species richness and annual precipitation, although Schall & Pianka (1977) found the opposite trend in Iberia. However, the latter is obviously an artifact of the peninsular nature of Iberia as well as the multitude of mountain barriers that prevent dispersal away from the Mediterranean coastal plain. Schall & Pianka (1978) also noted a negative correlation between richness and variability in precipitation across years. The strongest correlations revealed in this study related turtle species richness to annual rainfall. However, although I had predicted that seasonal (i.e. within year) variation would be inversely correlated with richness (the climatic stability hypothesis; see Pianka, 1988), the actual pattern may be exactly the opposite (at least for some taxa).

The positive effects on richness of both amount of and seasonal variation in rainfall (though the latter may only affect the trionychids) provide some support for the spatial heterogeneity hypothesis (Pianka, 1988). More rain and more seasonality in rain presumably would result in more variety in habitats through the course of a year - from rivers, streams, lakes, and ponds to marshes and bogs, to meadows and ephemeral pools. In addition, more rain and more seasonality in rain within a basin (e.g., the Irawaddy, Mekong, and Ganges basins) also imply a greater likelihood of habitat disturbance through flooding, and thus the disturbance hypothesis (e.g. see Petraitis *et al.*, 1989) may also be supported by these data. These basins indeed do have very high turtle species densities, with the world's highest being in the lower Ganges-Brahmaputra basin. However, further study will be necessary to test adequately the applicability of either the spatial heterogeneity or disturbance hypotheses.

Unfortunately, the data so far available on other factors do not permit a testing of several of the mechanisms hypothesized to explain diversity (reviewed by Pianka, 1988), e.g., the evolutionary time hypothesis, the ecological time hypothesis, the productivity hypothesis (see Currie, 1990), the competition hypothesis, the predation hypothesis (see Arnold, 1972), the climatic stability and stability in primary production hypotheses (see Pianka, 1966, 1988), andthe climatic predictability hypothesis (though the latter might be tested with annual flood stage data). They also give no hint as to why the maximum diversity of turtles today (13 genera in the lower Ganges) was so far exceeded by diversity in Montana (ca. almost 48°N latitude) during the Upper Cretaceous (at least 18 genera and subgenera in the Hell Creek formation; Hutchison & Archibald, 1984), especially when at least 72-83% of Cretaceous taxa survived the Cretaceous-Tertiary boundary event (Hutchison & Archibald, 1984).

In summary, although a latitudinal gradient in species richness has been demonstrated for numerous plant and animal taxa (review in Stevens, 1989), no such pattern is evident in turtles. Likewise, although basin area and discharge are often highly correlated with species density in other aquatic taxa (e.g. Livingstone *et al.*, 1982; Swift *et al.*, 1986; McAllister *et al.*, 1986; and Angermeier &

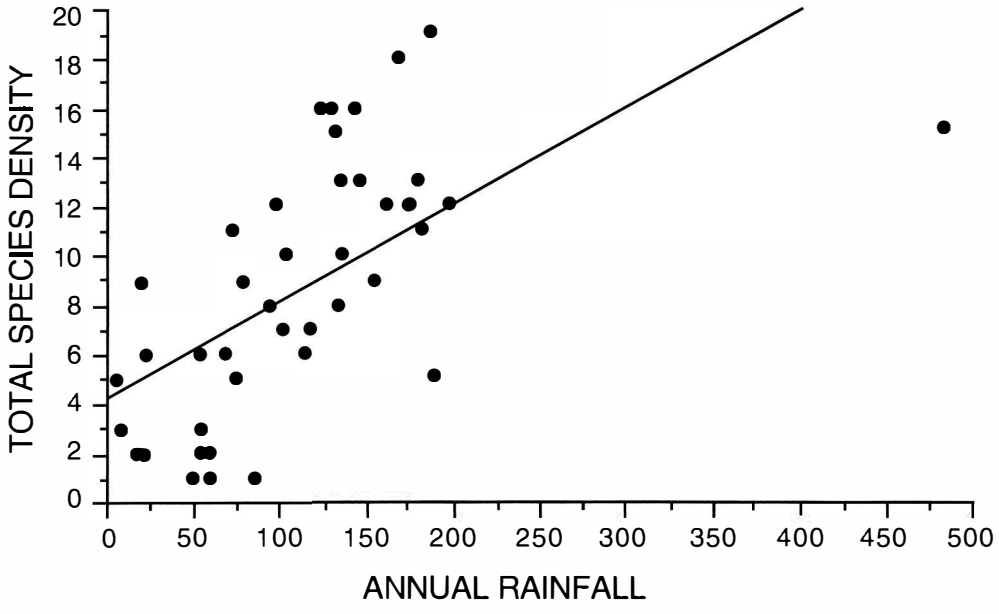


Fig. 1. Relationship between annual rainfall (mm) and maximum species richness for 42 river basins. Least squares regression equation is $y = 0.039x + 4.303$ ($N = 42$; $r = 0.62$; $P < 0.0001$).

Factor	Overall (N=42) r	Overall (N=42) P	Aquatic (N = 42) r	Aquatic (N = 42) P	Chelid (N = 7) r	Chelid (N = 7) P	Pelomedusid (N = 10) r	Pelomedusid (N = 10) P	Kinosternid (N = 14) r	Kinosternid (N = 14) P	Trionychid (N = 28) r	Trionychid (N = 28) P	Batagund (N = 15) r	Batagund (N = 15) P	Emydid (N = 16) r	Emydid (N = 16) P	All aquatic but Trionychid (N = 42) r	All aquatic but Trionychid (N = 42) P
	Level of species Richness																	
Latitude	-0.37	0.016	-0.29	0.06	-0.18	0.70	-0.71	0.023	+0.34	0.23	-0.44	0.020	-0.14	0.62	+0.03	0.92	-0.23	0.15
Basin area	+0.08	0.62	+0.09	0.58	+ 0.51	0.25	+0.59	0.08	-0.42	0.14	+0.05	0.82	-0.17	0.55	-0.01	0.96	+0.11	0.50
Discharge	+0.22	0.18	+0.26	0.12	+0.51	0.25	+0.27	0.48	-0.41	0.14	+0.26	0.21	-0.08	0.80	-0.15	0.57	+0.30	0.06
Winter max	+0.42	0.006	+0.34	0.029	+0.20	0.66	+0.50	0.15	-0.09	0.75	+0.52	0.005	+0.24	0.39	-0.08	0.77	+0.27	0.08
Winter min	+0.38	0.013	+0.31	0.049	+0.08	0.86	+0.27	0.45	-0.19	0.52	+0.50	0.007	+0.20	0.48	-0.26	0.33	+0.26	0.10
Sum max	+0.09	0.57	+0.16	0.31	+0.65	0.12	+0.29	0.42	+0.15	0.61	-0.22	0.27	-0.23	0.41	+0.25	0.35	+0.17	0.29
Sum min	+0.30	0.054	+0.33	0.031	-0.01	0.98	+0.07	0.84	+0.16	0.59	+0.26	0.19	+0.23	0.40	+0.14	0.61	+0.27	0.09
Seasonal difference	-0.35	0.023	-0.26	0.10	-0.14	0.77	-0.25	0.49	+0.23	0.42	-0.50	0.007	-0.23	0.41	+0.37	0.16	+0.22	0.17
Total rain	+0.62	0.001	+0.60	0.001	+0.10	0.83	+0.74	0.015	+0.12	0.68	+0.53	0.004	+0.59	0.022	+0.61	0.012	+0.52	0.0004
Wettest month	+0.45	0.003	+0.42	0.006	-0.30	0.52	+0.39	0.27	+0.05	0.87	+0.56	0.002	+0.47	0.08	+0.33	0.22	+0.30	0.06
Driest Month	+0.36	0.02	+0.41	0.007	+0.09	0.85	+0.49	0.15	+0.19	0.51	-0.23	0.24	-0.10	0.72	+0.68	0.004	+0.53	0.0003
Seasonal range	+0.41	0.007	+0.37	0.016	-0.33	0.46	+0.33	0.36	+0.01	0.97	+0.57	0.002	+0.48	0.07	+0.14	0.61	+0.24	0.13

TABLE 1. Correlations among measures of turtle species richness and environmental factors (see text for explanations). N = number of basins in sample. Statistical significance including experiment-wise error rate is $P < 0.05/12$ or $P < 0.0042$ (see text).

Schlosser, 1989), these relationships are also lacking among turtles. The only consistent correlate of turtle species richness identified in this study was total annual rainfall. Unfortunately, the precise mechanism through which rainfall might affect turtle diversity is not yet clear. However, even that variable explains only about 38% of the variation in turtle species richness in the 42 basins sampled, suggesting that other factors (e.g., dispersal history or past environmental conditions) may be more important in determining turtle species richness than those discussed here.

ACKNOWLEDGEMENTS

This research was supported by Earlham College, the Joseph Moore Museum, the Howard Hughes Medical Institute, the PEW Memorial Trust, the Ford Foundation, and my family. Miranda Stern was instrumental in developing the distributional data base on which the range maps used in this analysis were based. The critical comments of A.R. Kiester on an early draft are greatly appreciated.

REFERENCES

Angermeier, P. L. & Schlosser, I. J. (1989). Species-area relationships for stream fishes. *Ecology* **70**, 1450-1462.

Arnold, S. J. (1972). Species densities of predators and their prey. *Amer. Natur.* **106**, 220-236.

Barbour, C. D. & Brown, S. H. (1974). Fish species diversity in Lakes. *Amer. Natur.* **108**, 473-489.

Berra, T. M. (1981). *An atlas of distribution of the freshwater fish families of the world.* Lincoln: Univ. Nebraska Press.

Currie, D. J. (1991). Energy and large-scale patterns of animal and plant species richness. *Amer. Natur.* submitted.

Darlington, P. J. (1948). *Zoogeography: The geographical distribution of animals.* New York: Wiley.

Darlington, P. J. (1957). The geographical distribution of cold-blooded vertebrates. *Quart. Rev. Biol.* **23**, 105-123.

Ernst, C. H. & Barbour, R. W. (1989). *Turtles of the world.* Washington DC: Smithsonian Institution Press.

Ewert, M. A. & Nelson, C. E. (1991). Sex determination in turtles: Diverse patterns and some possible adaptive values. *Copeia* **1991**, 50-69.

Gibbons, J. W. & Nelson, D. H. (1978). The evolutionary significance of delayed emergence from the nest by hatchling turtles. *Evolution* **32**, 297-303.

Huheey, J. E. (1965). A mathematical method of analyzing biogeographical data. I. Herpetofauna of Illinois. *Amer. Midl. Natur.* **73**, 490-500.

Hutchison, J. H. & Archibald, J. D. (1984). Turtle diversity across the K/T boundary, N.E. Montana. *Ann. Meeting Geol. Soc. Amer., Reno, Nevada.* (Abstract).

Iverson, J. B. (1986). *Checklist with distribution maps of the turtles of the world.* Richmond, Indiana: Iverson Publ.

Iverson, J. B. (1992). Species richness maps of the turtles of the world. *Smithsonian Herpetol. Inform. Service* **88**, 1-18.

Kiester, A. R. (1971). Species density of North American amphibians and reptiles. *Syst. Zool.* **20**, 127-137.

Livingstone, D. A., Rowland, M. & Bailey, P. E. (1982). On the size of African riverine fish faunas. *Amer. Zool.* **22**, 361-369.

MacArthur, R. H. (1972). *Geographical ecology: Patterns in the distributions of species.* New York: Harper and Row.

McAllister, D. E., Platania, S.P., Schueler, F.W., Baldwin, M. E. & Lee, D. S. (1986). Ichthyofaunal Patterns on a Geographic Grid. In: *The zoogeography of North American freshwater fishes*, 17-51. Hocutt, C.H. & Wiley E. O. (eds.). New York: Wiley and Sons.

Mittermeier, R. A. (1972). Zoogeography of fossil and living turtles. *Australian Natur. Hist.* 265-269.

Owen, J.G. & Dixon., J. R. (1989). An ecogeographic analysis of the herpetofauna of Texas. *Southwest. Natur.* **34**, 165-180.

Petraitis, P. S, Latham, R. E. & Niesenbaum, R. A. (1989). The maintenance of species diversity by disturbance. *Quart. Rev. Biol.* **64**, 393-394.

Pianka, E. R. (1966). Latitudinal gradients in species diversity: A review of concepts. *Amer. Natur.* **100**, 33-46.

Pianka, E. R. (1988). *Evolutionary ecology* , 4th edn. New York: Harper and Row.

Pritchard, P. C. H. (1979). *Encyclopedia of turtles.* Neptune, New Jersey: T.F.H. Publ.

Rogers, J. S. (1976). Species density and taxonomic diversity of Texas amphibians and reptiles. *Syst. Zool.* **25**, 26-40.

Ruffner, J. A. (1978). *Climates of the United States.* Detroit, Michigan: Gale Research Co.

Ruffner, J. A. & Bair, F. E. (1987). *The weather almanac.* Detroit, Michigan: Gale Research Co.

Schall, J. J. & Pianka, E. R. (1977). Species densities of reptiles and amphibians on the Iberian Peninsula. *Doñana Acta Vertebrata* **4**, 27-34.

Schall, J. J. & Pianka, E. R. (1978). Geographical trends in numbers of species. *Science* **201**, 679-201.

Showers, V. (1973). *The world in figures.* New York: Wiley and Sons.

Snead, R. E. (1980). *World atlas of geomorphic features.* Huntington, New York: Robert E. Krieger Publ.

Sokal, R. R. & Rohlf, F. J. (1981). *Biometry.* San Francisco: Freeman and Co.

Stevens, G. C. (1989). The latitudinal gradient in geographical range: How so many species coexist in the tropics. *Amer. Natur.* **133**, 240-256.

Swift, C. C., Gilbert, C. R., Bortone, S. A., Burgess, G. H. & Yerger, R. W. (1986). Zoogeography of the freshwater fishes of the southeastern United States: Savannah River to Lake Pontchartrain. In *The zoogeography of North American freshwater fishes*, 213-255. Hocutt, C.H. & Wiley, E. O. (eds.). New York: Wiley and Sons.

Tamayo, J. L. & West, R. C. (1964). The hydrography of Middle America. In: *Handbook of Middle American Indians. Vol. 1. Natural environment and early cultures*, 84-121. West, R.C. & Wauchope, R. Austin: Univ. Texas Press.

APPENDIX 1.

Species density and environmental data for 42 river basins. Temperatures are in Celcius; rainfall in cm.

Basin	Contin-nent[a]	Climate data source	Lati-tude	Basin area (x 1000 km²)	Basin discharge (x 100 m³/sec)	Mean daily temperature[b]				Range annual monthly mean	Mean ann-ual rain	Mean monthly rainfall			Total turtle species	Aquatic turtle species
						Winter max[b]	Winter min[b]	Summer max[b]	Summer min[b]			Wettest month	Driest month	Range		
Amazon	NA	Manaus	3	6150	1750	31.7	23.9	33.9	23.9	1.1	181.1	26.2	3.8	22.4	13	12
Amur	AS	Khabarovsk	48	2050	98	-18.9	-25.0	23.9	17.2	42.5	48.8	10.4	0.5	9.9	1	1
Apalachicola	NA	Apalachicola	30	49	6	16.7	8.9	31.1	23.9	14.7	142.7	21.6	6.1	15.5	16	15
Chao Phyra	AS	Bangkok	14	150	—	31.7	19.4	32.2	24.4	2.8	146.8	35.6	0.3	35.3	13	11
Colorado	NA	Yuma	33	637	1	19.4	4.4	41.7	26.1	21.9	7.6	1.3	0	1.3	3	2
Congo	AF	Leopoldville	4	3822	390	27.2	17.8	30.6	21.1	3.3	135.4	19.6	0.3	19.3	10	8
Danube	EU	Bucharest	44	773	64	0.6	-6.7	30.0	16.1	23.3	57.9	9.7	2.8	6.9	2	1
Fly	AU	Port Moresby	9	55	90	28.3	22.8	31.7	24.4	0.8	101.1	19.3	1.8	17.5	7	7
Ganges	AS	Dacca	24	1621	385	25.0	13.3	31.7	26.1	9.7	187.7	33.8	0.5	33.3	19	17
Indus	AS	Karachi	25	1178	36	25.0	12.8	32.8	27.2	11.1	19.8	8.1	0.3	7.9	9	7
Irawaddy	AS	Moulmein	17	409	127	31.7	18.3	28.3	23.3	0.8	483.1	120.7	0.5	120.1	15	12
Kristna	AS	Hyderabad	17	259	—	29.4	15.0	30.6	22.8	4.4	75.2	16.5	0.8	15.7	5	4
Magdalena	SA	Cartagena	10	260	80	28.9	22.8	31.1	25.6	2.5	93.5	27.4	0	27.4	8	7
Mekong	AS	Saigon	11	811	120	31.7	21.1	31.1	23.9	1.1	198.4	33.5	0.3	33.3	12	10
Mississippi	NA	Little Rock	35	3222	173	10.0	-1.1	33.9	21.7	23.4	125.0	13.7	7.2	6.5	16	15
Mobile	NA	Mobile	31	111	11	16.1	5.0	32.8	22.8	17.2	170.2	22.6	6.6	16.0	18	16
Murray-Darling	AU	Adelaide	35	1072	4.7	15.0	7.2	30.0	16.1	11.7	53.6	7.6	1.8	5.8	3	3
Narmada	AS	Ahmadabad	23	98	12.3	29.4	14.4	33.9	26.1	8.1	74.4	31.0	0	31.0	5	4
Niger	AF	Enugu	6	2092	57	32.2	22.2	28.3	21.7	2.2	181.6	32.5	1.3	31.2	11	8
Nile	AF	Lira	2	2802	26	32.8	16.1	27.2	16.1	2.8	154.2	25.4	1.8	23.6	9	6
Orange	AF	Port Nolloth	29	677	3	16.7	7.2	19.4	11.7	3.6	5.8	0.8	0.3	0.5	5	1
Orinoco	SA	Bolivar	8	880	180	32.2	22.2	32.2	23.9	0.8	97.3	18.0	1.8	16.3	12	10
Parana	SA	Buenos Aires	27	3100	229	21.7	11.7	33.9	21.7	11.1	117.9	14.2	3.8	10.4	7	6
Po	EU	Venice	45	75	15	6.1	0.6	27.8	19.4	20.3	84.8	9.4	5.1	4.3	1	1
Potomac	NA	Washington	39	36	3.1	6.7	-1.1	30.6	20.6	22.8	103.6	12.4	6.4	6.1	10	9
Red	AS	Hanoi	21	120	—	20.0	14.4	33.3	26.1	12.5	176.3	38.6	2.0	36.6	12	10
Rhone	EU	Marseille	43	99	15	11.7	3.3	25.6	14.4	12.5	58.9	9.4	1.5	7.9	1	1
Rio Grande	NA	Brownsville	26	471	1	21.7	11.1	33.9	24.4	12.8	68.3	12.7	2.5	10.2	6	4
Sao Francisco	SA	Benin	13	611	27	26.1	20.6	30.0	23.3	3.3	190.0	28.4	6.6	21.8	5	3
Savannah	NA	Savannah	32	26	3.2	16.1	3.9	32.8	21.7	17.2	130.0	20.1	4.8	20.3	16	14
Senegal	AF	Dakar	15	440	—	26.1	17.8	31.1	24.4	5.8	54.1	25.4	0	25.4	6	4
Si	AS	Canton	23	448	116	18.3	9.4	32.8	25.0	15.0	161.5	26.9	2.3	24.6	12	12
St. Johns	NA	Jacksonville	30	21	0.9	19.4	7.2	33.3	22.8	14.7	136.1	19.6	4.3	15.2	13	11
St. Lawrence	NA	Detroit	42	1316	130	0	-7.2	28.3	17.2	24.7	78.7	8.6	4.6	4.1	9	8
Suwannee	NA	Gainesville	29	23[c]	4	20.6	8.3	32.8	21.7	12.6	133.4	19.6	4.6	15.0	15	13
Tigris	AS	Abadan	30	1105	28.6	17.8	6.7	44.4	27.2	23.6	19.3	4.6	0	4.6	2	2
Volga	AS	Astrakhan	46	1380	77	-5.0	-10.0	29.4	20.6	32.5	16.3	1.8	1.0	0.8	2	1
Volta	AF	Accra	5	388	11.7	30.6	22.8	27.2	22.8	1.7	72.4	17.8	1.5	16.3	11	8
Yangtze	AS	Shanghai	31	1827	322	8.3	0	32.8	23.9	24.2	114.3	17.8	3.8	14.0	6	6
Yaqui	NA	Guaymas	27	70	0.9	23.3	13.9	35.6	27.8	13.1	23.9	6.9	0	6.9	6	4
Yellow	AS	Tientsin	37	771	15	0.6	-8.9	32.2	22.8	31.7	53.3	19.3	0.3	19.1	2	2
Zambezi	AF	Zomba	16	1331	160	22.2	11.7	26.7	18.3	5.6	134.4	30.7	0.5	30.2	8	6

[a] AF = Africa; AS = Asia; AU = Australia; EU = Europe; NA = North America; SA = South America

[b] Winter = January in northern hemisphere, July in southern hemisphere; vice versa in summer

[c] Estimated from map

HERPETOLOGICAL JOURNAL, Vol. 2, pp. 82–85 (1992)

EFFECTS OF INGESTED RADIO TRANSMITTERS ON *BUFO BUFO* AND *RANA TEMPORARIA*.

R. S. OLDHAM AND M. J. S. SWAN.

Department of Applied Biology and Biotechnology, Leicester Polytechnic, Scraptoft, Leicester LE7 9SU, UK.

(Accepted 26.11.90)

ABSTRACT

Fourteen adult common frogs (*Rana temporaria*) and 40 common toads (*Bufo bufo*) were forced fed with 2.5 g radio transmitters. The transmitters lodged in the stomach and were regurgitated after 2 to 13 days in the frog and 2 to 38 days in the toad. They did not significantly affect either feeding rates or mass fluctuations of the toads. Reception range was up to 100 m and battery life 33 to 47 days. Twenty three toads tracked on a daily basis during the summer showed displacement of between 0 and 108 m, animals commonly staying in one place for several days.

INTRODUCTION

Radio transmitters of 3 g or less are available commercially (see Kenward (1987) for list of suppliers) and have been used for tracking and physiological investigation of small animals by many workers (e.g. snakes: Henderson, Nickerson & Ketcham, 1976; turtles: Schubauer & Gibbons, 1978; toads: van Gelder & Krammer, 1985; Sinsch, 1988).

External attachment of transmitters is commonly used in tracking vertebrates; collars have been used for mammals (e.g. Ormiston, 1985), harnesses and tail clips for birds (e.g. Bray & Corner, 1972) and adhesive tapes for snakes (Ikeda, Iwai, Wada & Hayashi, 1979). In anurans external attachment presents problems since they have no apparent neck, a thin epidermis which is sporadically sloughed and a subcutaneous cavity which gives the animal considerable freedom of movement within its skin. A latex harness developed for *Bufo bufo* (Nuland & Claus, 1981; van Gelder, Aarts & Staal, 1986) was unsuccessful in dense vegetation or in water. If not fitted properly the harness caused irritation. Similarly Gittins (*pers. comm.*) used a flexible plastic harness with limited success.

The problems of hindrance and skin irritation can be solved by surgical implantation of the transmitter (e.g. voles: Smith, 1980; snakes: Reinert & Cundall, 1982; Weatherhead & Anderka, 1984; Tiebout & Cary, 1987; *Bufo bufo*: Olders *et al.*, 1985; *B. calamita*: Sinsch, 1988). This procedure, however, may cause trauma (Fitch & Shirer, 1971), reduced activity (Weatherhead & Anderka, *op. cit.*), infection (Olders *et al.*, 1985) and contravention of vivisection laws (Morris, 1980). Sinsch (1988), however, reported no abnormal behaviour after surgical implantation and complete healing by the conclusion of tracking experiments in *Bufo calamita*. Force-feeding provides a method of internal placement without recourse to surgery. Tagging devices have been swallowed and retained by snakes (e.g. Fitch & Shirer, 1971; Kroll, Clark & Albert, 1972; Ai, Olivier, Ambid & Saint-Girons, 1975; Henderson *et al.*, 1976; Kephart, 1980; Reinert and Kodrich, 1980; Priede, 1980) and toads (Pearson & Bradford, 1976). Pearson & Bradford (1976) do not comment upon the effects of the transmitters on the toads.

In our studies of the habitat requirements of *Bufo bufo* and *Rana temporaria* radio-tracking was used in conjunction with pitfall trapping. The detailed results of these investigations will be reported elsewhere; the purpose of the present paper is to describe the radio-tracking procedure.

Experiments with external harnesses proved unsatisfactory and an ingestion technique was investigated. Transmitter retention times, food consumption and body mass changes of internally labelled anurans, measured in the laboratory, are documented. Preliminary field trials are also described.

MATERIALS AND METHODS

EQUIPMENT

The equipment comprised transmitters ("small" 2.0 g and "large" 2.5 g; Fig.1) manufactured by Biotrack, Wareham, Dorset, UK; a portable receiver and a Yagi antenna from Mariner Radar, Lowestoft, Suffolk, UK (Fig. 2), operating within the frequency range 173.20 to 173.35 MHz. The transmitters are supplied with disconnected battery leads and were stored under refrigeration.

The equipment was tested on open playing fields where an audible signal was received from 200 m using a hand-held transmitter, of either size, 1m above ground level. To overcome the problem of exact transmitter location in dense vegetation a loop aerial permitting use within about 0.1 m^2 of the signal source was used (Fig. 2).

The transmitters had a working life of approximately 47 days (large transmitters) and 33 days (small transmitters) at the ranges cited above. Beyond this time the signal was weaker and detection harder.

INTERNAL PLACEMENT

Dummy transmitters were eased down the throats of frogs and toads, whose mouths had been prised open using the corner of an index card. The swallowing reflex, indicated by the sinking of the eyeballs, drew the package down the oesophagus. Both species were restrained by wrapping tissue around their bodies to secure the front legs. The process was not difficult with either species, but easier with frogs. Occasionally the transmitter was rejected immediately; in this case it was reinserted.

After ingesting the transmitters, 40 toads and 14 frogs were placed singly in vivaria (0.07 to 0.10 m^2 floor area) and kept at a range of temperatures between 7 and 20 °C between August and

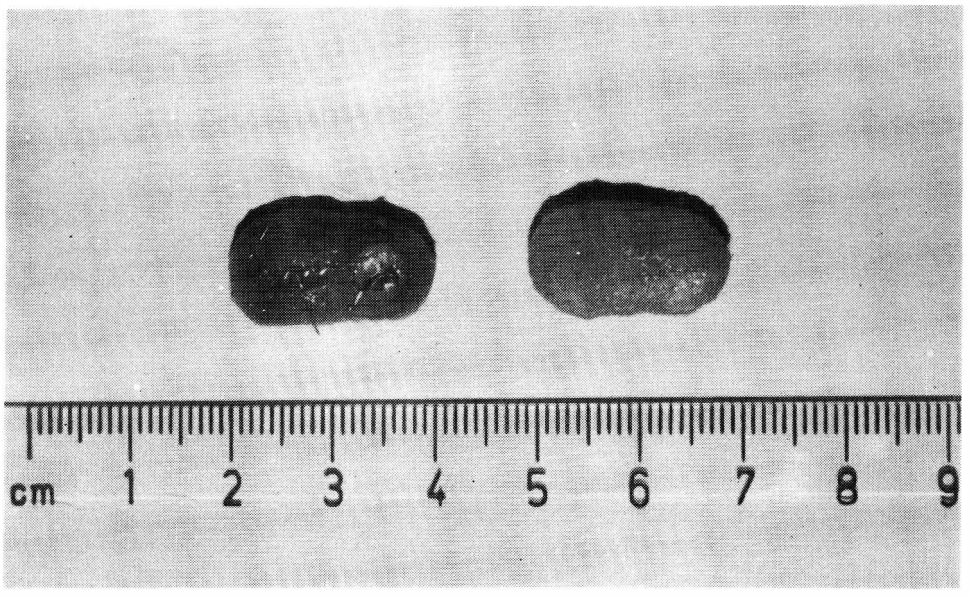


Fig. 1. Transmitter potted in silicone aquarium sealant, with battery terminals unconnected (left); dummy transmitter (right).

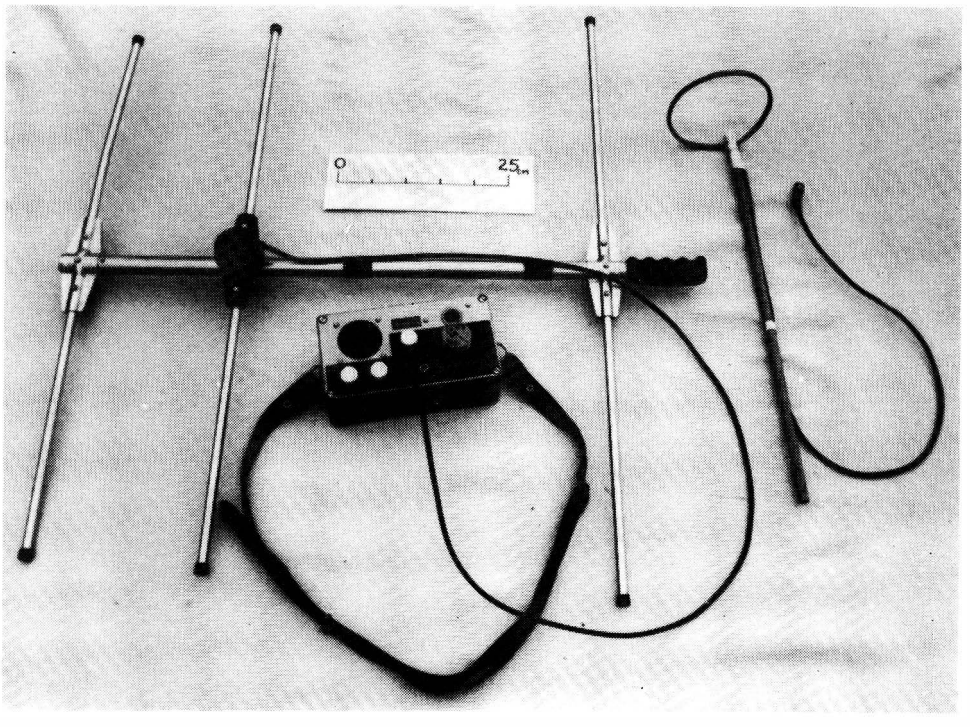


Fig.2. Portable receiving equipment. Yagi antenna and receiver and loop aerial.

December. The animals were examined daily and if the transmitters were rejected they were reinserted. If reinsertions are included the toads were used in a total of 92 retention trials and the frogs in 21 trials. A further 25 toads and 15 frogs were used as controls. All were weighed at intervals throughout the experiments.

A known number of house cricket nymphs (*Acheta domestica*) was provided daily as food in each vivarium and any left were counted at the next feeding time. It was thus possible to estimate the number consumed each day.

RESULTS

Effects of Transmitters

After periods ranging between 2 and 38 days all the dummy transmitters were deposited on the vivarium floor. To assess whether they were regurgitated or defaecated an additional nine toads and two frogs (masses between 25 g and 55 g) were dissected 3 to 16 days after ingestion of transmitters. All 11 had retained them within their stomachs, the pylorus being too narrow to permit passage.

Toads had median retention times of 13 days (range 2 to 38 days) and frogs six days (range 2 to 13 days, Table 1). Thirty-seven (69%) of the animals regurgitated the transmitters within two weeks; the remainder retained them for over three weeks. Reinsertion of transmitters into toads (an additional 52 trials) produced a similar array of retention times. Neither frogs nor toads showed significant differences in retention times between animals kept at 13, 16 and 19 °C ($P>0.05$). However, an additional four frogs and two toads kept at 7 °C became torpid, neither eating crickets nor regurgitating transmitters.

With transmitters in their stomachs neither frogs nor toads ate significantly fewer cricket nymphs than the control animals ($P>0.05$, Table 2). The slight apparent difference between the two groups of toads in Table 2 was caused by reduced feeding in some animals during the few days following initial implantation of the transmitter. On the other hand, on several occasions toads were observed to feed within minutes of implantation.

Mass changes during the experiments ranged from 11% decrease to 41% increase in the toads (Table 3) and from 0% to 12% increase in the frogs. There was no consistent relationship between animal size and percentage mass change ($P>0.05$) and toad mass loss did not correlate with length of dummy retention time ($P>0.05$).

FIELD TRIALS

Between 1982 and 1987 field trials were conducted with 23 toads in Leicestershire. All took place during the toads' active season, in spring and summer, but not in the migratory phase. All the animals were released within 25 m of their site of capture and were followed, usually at daily intervals, for up to 22 days. They were detected from a range of up to 150 m. Distances between recorded positions ranged between 0 m and 108 m. During tracking, animals were commonly detected in scrub, on one occasion beneath 10 cm of water, once buried at a depth of 5 cm in soil and once in a retreat amongst rocks. In nine of the 23 instances both the toads and the transmitters were lost. In a further five cases transmitters were regurgitated by the toads and retrieved separately. The remainder were recaptured complete with transmitters, which they subsequently regurgitated.

Most of the tagged animals inhabited woodland, scrub or gardens. In five instances, however, toads were located in habitats on the verge of woodland/scrub and arable fields. These were observed to forage in the fields during the night and hide in the scrub, up to 20 m distant, during daylight. The nocturnal phase of this behaviour was confirmed, for large numbers of toads, by independent torchlight searches.

	Sex	*n*	Mass range (g)	Days retained Range	Median
B. bufo	M	23	18 - 53	2 - 38	15
	F	17	17 - 88	2 - 38	13
R. temporaria	M&F	14	11 - 47	2 -13	6

TABLE 1. Retention times of dummy transmitters in laboratory toads and frogs.

	Mean no. crickets eaten per day						
	Experimental group			Control group			Mann-Whitney U test
	n	Mean	S.D.	*n*	Mean	S.D.	*P*
B. bufo	40	1.4	0.41	25	1.6	0.57	>0.05
R. temporaria	14	2.5	0.71	15	2.4	0.67	>0.05

TABLE 2. Effect of transmitters on toad and frog food consumption during experiments lasting between 21 and 44 days.

		n	% Mass change range	median	
B. bufo	with transmitter	40	-11 to+41	+4	$P>0.05$
	control	25	-2 to+18	+9	
R. temporaria	with transmitter	14	0 to +12	+5	$P>0.05$
	control	15	+2 to +4	+3	

TABLE 3. Effects of transmitters on mass changes in toads and frogs in experiments lasting between 7 and 45 days.

DISCUSSION

The mean transmitter retention times in the laboratory, of 16 and 6 days for toads and frogs respectively, seemed to allow sufficient time for tracking both species. This was confirmed for toads by field trials in which the animals were detected in a range of habitats.

Laboratory evidence indicated that the transmitters had no effect upon the feeding and associated mass changes of toads. There are no reports in the literature of similar trials for amphibia, but Reinhert & Kodrich (1982) believed that the appetite of the grass snake *Sisturus* was unaffected by an ingested transmitter. However, the possibility of reduced field activity consequent upon the presence of the transmitter, as reported for *Agkistrodon* and *Coluber* by Fitch & Shirer (1971) cannot be excluded.

A serious disadvantage of the method was the high frequency of loss of expensive transmitters, despite thorough searches. This was particularly frustrating since it was impossible to know whether the loss was animal specific (predation, deep burial, extensive wandering etc.) or equipment specific (e.g. failed battery). This problem can be overcome by continuous monitoring but, aside from the practical difficulties of nocturnal work, this incurs the problems of animal disturbance and habitat damage.

The field trials suggest that toads are relatively sedentary during their summer terrestrial phase. The trials also show that toads will forage at night in arable fields and retreat to scrub refugia during the daylight. Van Gelder *et al.* (1986) describe a similar activity pattern during the migration of toads in the Netherlands. The fact that they do not avoid bare ploughed land is of significance in terms of conservation since such cultivation may not be inimical to toads. If they retreat from the field during the day they are unlikely to suffer physical damage by machinery. At the same time it seems important that areas of rough grass and scrub be retained as refugia in intensively cultivated land.

ACKNOWLEDGEMENTS

The work was conducted whilst MJSS was in receipt of a NERC studentship. We are also grateful for the enthusiastic field assistance provided by H. Gratrex, C. Parker, A. Perrins, J. Sedgeley, C. Smith and D. Hilton-Brown; for preparation of the loop ariel by B. Rigg and for help with the manuscript by J. Arntzen, D. Bullock and A. Smithson.

REFERENCES

Ai B., Olivier, H., Ambid, L. & Saint-Girons H. (1975). Enregistrement continu par biotelemetrie de la temperature interne de Reptiles et de Mammiferes hibernants de petite taille. *C. R. Acad. Sc. Paris.* **280**, 1015-1018.

Bray O. E. & Corner G. W. (1972). A tail clip for attaching transmitters to birds. *J. Wild. Manag.* **36**, 640-642.

Fitch H. S. & Shirer H. W. (1971). A radiotelemetric study of spatial relationships in some common snakes. *Copeia* **1971**, 118-128.

Gelder J. J. van, Aarts H. M. J. & Staal H. W. M. (1986). Routes and speed of migrating toads (*Bufo bufo* L.): a telemetric study. *Herpet. J.* **1**, 111-114.

Gray H. R. & Haynes J. M. (1979). Spawning migration of adult chinook salmon (*Oncorhynchus shawyshu*) carrying external and internal radio transmitters). *J. Fish. Res. Board Can.* **36**, 1060-1064.

Henderson R. W; Nickerson M. A. & Ketcham S. (1976). Short term movements of the snakes *Chironius carinatus*, *Helicops angulatus* and *Bothrops atrox* in Amazonian Peru. *Herpetologica* **32**, 304-310.

Ikeda K; Iwai N; Wada Y; & Hayashi Y. (1979). Technological development of the radio tracking system for studies of Habu movement. *Snake* **11**, 122-123.

Kenward R. E. (1987). *Wildlife radio tagging.* London: Academic Press.

Kephart D. G. (1980). Inexpensive telemetry techniques for reptiles. *J. Herpet.* **14**, 285-290.

Kroll C. K; Clarke D. R. & Albert J. W. (1972). Radiotelemetry for studying thermoregulation in free-ranging snakes. *Ecology* **54**, 454-456.

Morris P. (1980). An elementary guide to practical aspects of radio tracking mammals. In *A handbook on biotelemetry and radio tracking*, 161-168. Amlaner, C. J. & MacDonald, D. W. (Eds.). Oxford: Pergamon Press.

Nuland G. J. van & Claus P. F. H. (1981). The development of a radio tracking system for anuran species. *Amphibia-Reptilia* **2**, 107-116.

Olders J. H. J; van Gelder J. J. & Krammer J. (1985). A thermo-sensitive transmitter for radio tracking small animals. *Neth. J. Zool.* **35**, 479-485.

Ormiston B. G. (1985). Effects of a subminiature radio collar on activity of free-living white footed mice. *Can. J. Zool.* **63**, 733-735.

Pearson O. P. & Bradford D. F. (1976). Thermoregulation of lizards and toads at high altitudes in Peru. *Copeia* **1976**, 155-170.

Power J. H. & McCleave J. D. (1980). Riverine movements in hatchery reared Atlantic salmon (*Salar salar*) upon return as adults. *Env. Biol. Fish* **5**, 3-13.

Priede I. G. (1980) An analysis of objects in telemetry studies of fish in the natural environment. In *A handbook on biotelemetry and radio tracking*, 105-118. Amlaner, C. J. & MacDonald, D. W. (Eds.). Oxford: Pergamon Press.

Reinert H. K. & Cundall D. (1982). An improved surgical implantation method for radio-tracking snakes. *Copeia*, **1982**, 702-705.

Reinert H. K. & Kodrich W. R. (1982). Movements and habitat utilization by the Massasauga, *Sisturus catenatus catenatus. J. Herpet.* **16**, 162-171.

Schubauer J. P. & Gibbons J. W. (1978). Home range and movement patterns of *Chrysemys scripta* (Reptilia: Emydidae) in a thermally altered lake in South Carolina. *Assoc. Southeast Biol. Bull. (USA)* **25**, 73.

Sinsch U. (1988). Temporal spacing of breeding activity in the Natterjack toad, *Bufo calamita. Oecologia* **76**, 399-407.

Smith H. R. (1980). Growth, reproduction and survival in *Peromyscus leucopus* carrying intraperitoneally implanted transmitters. In *A handbook on biotelemetry and radio tracking*, 367-374. Amlaner, C. J. & MacDonald, D. W. (Eds.). Oxford: Pergamon Press.

Tiebout H. M. & Cary J. R. (1987). Dynamic spatial ecology of the water snake, *Nerodia sipedon. Copiea* **1987**, 1-18.

Weatherhead P. J. & Anderka F. W. (1984). An improved radio transmitter and implantation technique for snakes. *J. Herpet.* **18**, 264-269.

HERPETOLOGICAL JOURNAL, Vol. 2, pp. 86-89 (1992)

SEASONAL AND DIEL CYCLES OF ACTIVITY IN THE RUIN LIZARD, *PODARCIS SICULA*

AUGUSTO FOÀ[1], GIANLUCA TOSINI[2] AND ROGER AVERY[3]

[1]*Dipartimento di Scienze del Comportamento Animale e dell'Uomo, Università di Pisa, via A Volta 6, 56126 Pisa, Italy*

[2]*Museo Zoologico "La Specola", Sezione del Museo di Storia Naturale, Università di Firenze, via Romana 17, 50125 Firenze, Italy*

[3]*Department of Zoology, The University, Bristol BS8 1UG, UK*

(Accepted 2.1.91)

ABSTRACT

Adult ruin lizards, *Podarcis sicula*, at a study site near Pisa, Italy, were seen during all months of 1988, but in significantly reduced numbers in January, February, November and December. The length of the diel period during which individuals were observed varied from 2-3 h in mid-winter to 14 h in July and August. High activity, defined as any one-hour period of the day during which the total number of lizards observed over three days of observation in any month was greater than the 95% confidence limits for the overall mean, was bimodal from April to October inclusive. Bimodality in the activity pattern was particularly pronounced in the hottest months, as low activity, when numbers observed were lower than the C.L. for the overall mean, occurred during the central hours of the day (1200-1400 h) in July and August. Low activity was also recorded at the beginning and end of the active period in all months from February to November. The diel cycles of juveniles (lizards less than 6 months old) appeared to be less structured than those of adults.

INTRODUCTION

Investigations of the inter-relationships between climate, habitat and activity pattern are of crucial importance in understanding the ecology and behaviour of many ectotherms; this is especially true of diurnal lizards (Porter, Mitchell, Beckman & DeWitt, 1973; Avery, 1976; Porter & Tracy, 1983). The activity component requires that the study animals should be relatively easy to observe, unless there is some indirect means for determining their movements. In many Mediterranean regions the presence of man over many millenia has resulted in a substantial reduction in vegetation cover. This has created environments which are apparently particularly favourable for lizards, and the animals often occur at high densities. The combination of open environments and large numbers makes the lizards fairly easy to observe, and they are thus very suitable for studies of activity patterns and other behaviour. Observations of activity patterns in the field not only help to disclose important aspects of the ecology, ecophysiology and spatio-temporal organisation of behaviour, but can also greatly contribute to our understanding of the adaptive significance of circadian and other rhythms in natural environments (e.g. Janik, 1987; Underwood, 1990).

This paper describes the seasonal and diel cycles of activity in a population of ruin lizards, *Podarcis sicula*, inhabiting the coastal plains at Pisa, Italy. It is planned that this is to be one of a series of papers, which will include both field and laboratory studies, on the physiology and ecology of rhythmic behaviour in the species.

MATERIALS AND METHODS

The work was carried out during the period January-December 1988 at the field station of the Dipartimento di Scienze del Comportamento Animale e dell'Uomo of Pisa University, located 1.5 km from the Ligurian sea in an area of reclaimed marshland with well-drained, sandy soils. The 150 m^2 study site is in a flat meadow and incorporates the foundations of a dismantled poultry pen. The surrounding vegetation is mostly mown grass, but isolated plants of mullein, *Verbascum sinuatum* L., and a few trees and shrubs, provide shade and refuge. The activities of many of the individual lizards in the study population centred on the foundations, in which perforated bricks and other debris provided abundant shelter.

The term activity is used in this paper in a broad sense (see Discussion). We measured as an index of lizard activity the total number of lizards which could be observed in a specified unit of time. We defined as "active" all those lizards whose presence could be visually detected within the study area. This included (i) moving lizards (ii) immobile lizards which were basking (iii) other immobile lizards which could be detected, e.g. hidden beneath vegetation. The data were obtained as follows: one observer walked through the study area along standard paths, counted the number of lizards, and attributed each sighting to one of the categories adult male, adult female, subadult, juvenile. This was repeated every hour, from sunrise to sunset, for three days every month for a total of twelve months (N=468). The units in which the data were compiled were total number of lizards observed within a specified hour over three days. The soil temperature was measured at the point at which each lizard was first observed. Times were always recorded as European Standard Time; this is one hour ahead of Greenwich Mean Time (GMT).

RESULTS

Adult *P. sicula* were observed in all months of the year, but activity in January and December was sporadic and only very few individuals were seen (Table 1). Numbers were also significantly reduced during February and November (based on the null hypothesis of equal numbers in all months from February to November, χ^2=149, P<0.001). Adult males and adult females showed similar monthly cycles of activity (G-test with STP analysis, P<0.05) and the sexes have been combined for further analysis.

Month	Total number of adults	Total number of juveniles	Maximum soil temperature (°C)
January	2	-	-
February	40	-	25.6
March	127	-	28.3
April	213	-	33.4
May	187	-	39.1
June	186	-	36.6
July	139	30	42.8
August	148	100	40.9
September	160	72	40.0
October	174	33	32.5
November	42	4	22.9
December	3	0	-

TABLE 1. Monthly values for total number of lizards observed and maximum recorded soil temperatures.

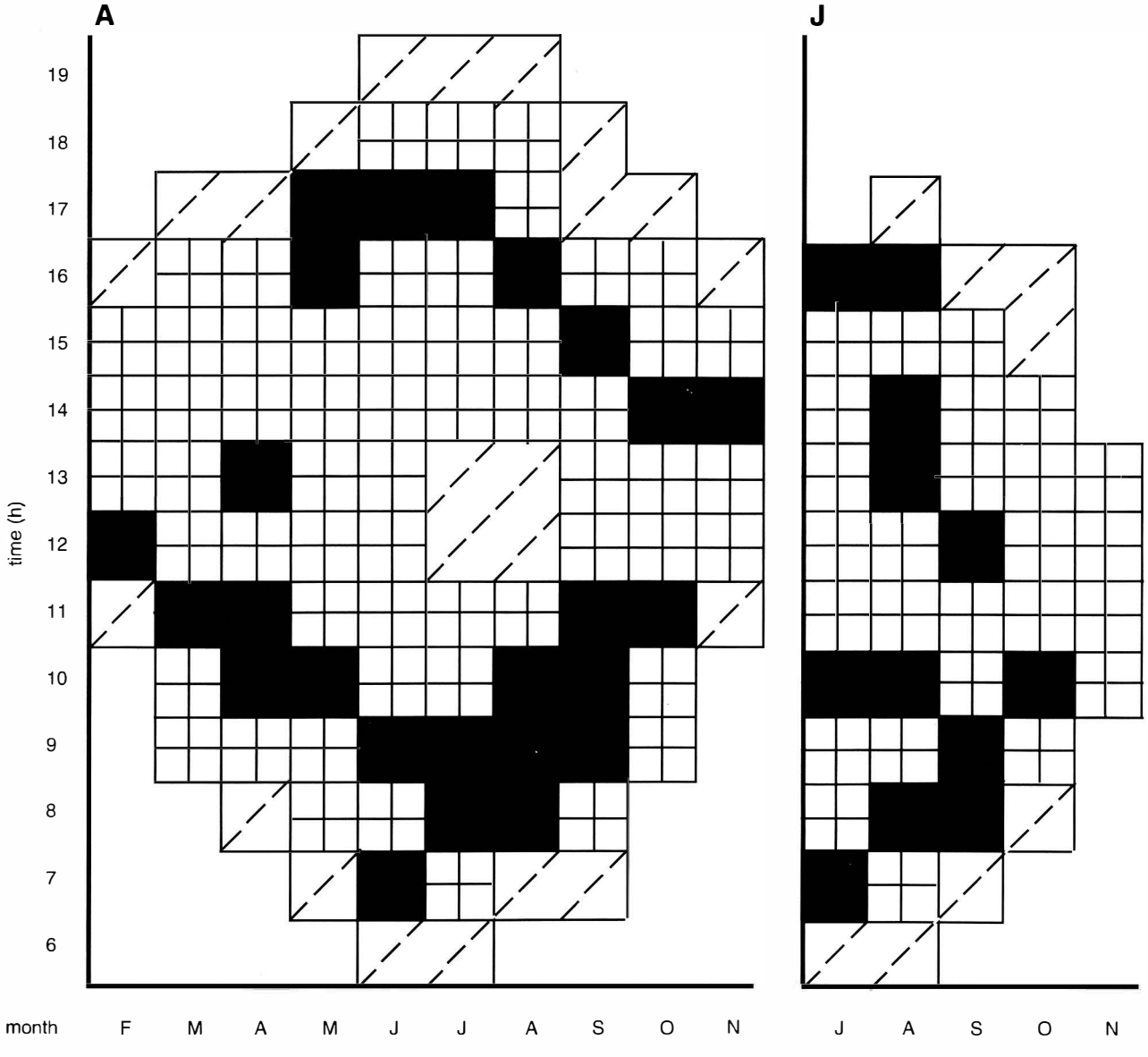


Fig. 1. Seasonal (monthly) and diel activity of *P. sicula*: adults (A) on the left, juveniles (J) on the right. January and December are not shown because of very small sample sizes. Black squares represent high activity, diagonal hatching low activity and cross-hatching intermediate levels of activity (see text for definitions).

The length of the period during which adults were observed varied seasonally, from 2-3 h in January and December to 14 h in June and July (Fig. 1). Activity during the latter months occupied most of the period from sunrise to sunset; during January and December less than one half of this period was utilised, with intermediate proportions in intervening months (Fig. 1). There was considerable variation in the number of lizards actually observed from hour to hour, much probably due to sampling error. In order to remove this error to demonstrate any underlying trends, the mean and 95% confidence limits for the overall hourly totals within each month were calculated. Any individual total which lay outside the 95% confidence limits of the mean was designated "high activity" if above the mean, "low activity" if below it. Fig. 1 demonstrates a clear seasonal pattern in the distribution of both high and low activity.

High activity occurred during the middle period of the day in February, March and November. From April to October there were two peaks of high activity, separated by progressively increasing periods of time until June and decreasing periods of time from August to October. Low activity was concentrated at the beginning and end of the active period throughout the year (except in January and December, when sample sizes were too small to detect any hourly differences). There was also a period of low activity from 1200-1400 h in July and August; these are the months when maximum recorded soil temperatures reached their highest levels (Table 1). The presence of such low midday activity shows that the bimodal pattern was more pronounced in the hottest months of the year.

Juvenile lizards first appeared in July. They gave the impression of a less structured diel cycle, with comparatively high levels of activity maintained for long periods. This is partially borne out by the data (Fig. 1). Juveniles did not show a period of significantly reduced activity at midday in July and August.

DISCUSSION

The data presented in this paper show that adult *P. sicula* at Pisa conform to the patterns of diel and seasonal activity which have been recorded in a wide range of generalised lacertid lizards from southern Europe, including other populations of the species from elsewhere within its geographical range (for *P. sicula*: Avery, 1978; Ouboter, 1981; Henle, 1988; Van Damme, Bauwens, Castilla & Verheyen, 1990; for other lacertid species, examples include Gruber & Schutze-Westrum, 1971; Busack, 1976; Pough & Busack, 1978; Perez-Mellado & Salvador, 1981; Perez-Mellado, 1983; Bowker, 1986; Pollo Mateos & Perez-Mellado, 1989). This pattern is characterised by sporadic activity during the cool winter months, long periods of activity with peaks during the mid-morning and late afternoon during the hottest months of the summer.

Juvenile *P. sicula*, however, do not appear to conform so clearly to this pattern. Subadults (i.e. sexually immature lizards between six and eighteen months of age) probably have diel activity patterns similar to those of juveniles, but the data were too limited for detailed analysis. There was some evidence that subadult numbers decreased during March-June due to competition for territorial space with adults (*P. sicula* is a territorial species, see Verbeek, 1971 and review of literature in Henle & Klaver, 1986).

An important limitation of this, as of almost all of the studies of activity cycles of lacertid lizards cited above, is that "activity" is only partially defined. An "active" lizard is one which can be observed (see Materials and Methods). It is not in any kind of retreat or burrow, but may actually be engaged in a variety of behaviours. These include hunting for food, sexual and competitive interactions, sentinel predation, basking and periods immobile in shade during the hotter parts of the day. Only the first two involve a high portion of movement; in the last three, the "active" lizard may actually be immobile, sometimes for quite long periods. Different behaviours are associated with different probabilities that a lizard will be observed. Basking lizards are exposed to direct solar radiation and so the probability that they will be observed is high. Shade-seeking lizards, on the other hand, undoubtedly have a lower probability of being observed. This difference in observability is the likely explanation for the asymmetry in "high activity" seen in most months of the year and illustrated in Fig. 1. It is associated with basking, which occupies longer periods during the early morning, when both solar radiation and air/substrate temperatures are low, shorter periods during the late afternoon when solar radiation is low but air/substrate temperatures comparatively high, and very short periods (which can include zero) during the middle of the day when both solar radiation and air/substrate temperatures are high. This problem of differences in observability is probably unavoidable in any study based on scan samples (Altmann, 1974). For a more detailed interpretation it is necessary at the least to know the behaviour of an individual lizard during the period immediately prior to recording. This can be achieved by continuous observation of focal animals, but is inevitably a great deal more time-consuming.

The seasonal differences in activity patterns recorded here have been interpreted as responses to environmental conditions. There is some experimental evidence, however, that they may have an endogenous component. When, for example, *P. sicula* captured during the hottest months (July-August) were tested under constant temperature (29°C) and darkness in the laboratory, 71% of the individuals (10 out of 14) continued to display a bimodality in spontaneous locomotor activity; in a cooler period of the year, i.e. when *P. sicula* were captured between end of September and beginning of October, only 13% of the lizards (2 out of 15) tested under the same laboratory conditions was still bimodal (Foà, in preparation). This makes the species a particularly suitable model animal to study and test hypotheses relating to the internal or external coincidence of photoperiod and other environmental variables (e.g. Pittendrigh & Daan, 1976) in the induction, maintenance and experimental manipulation of rhythmic behaviour and seasonal activity patterns.

REFERENCES

Altmann, J. (1974). Observational study of behaviour: sampling methods. *Behaviour* **49**, 227-267.

Avery, R. A. (1976). Thermoregulation, metabolism and social behaviour of Lacertidae. In *Morphology and Biology of Reptiles*, 245-259. Bellairs, A. d'A. and Cox C. B. (eds). London: Linnean Society Symposium series no. 3.. Academic Press.

Avery, R. A. (1978). Activity patterns, thermoregulation and food consumption in two sympatric lizard species (*Podarcis muralis* and *P. sicula*) from central Italy. *J. Anim.Ecol.* **47**, 143-158.

Bowker, R. G. (1986). Patterns of thermoregulation in *Podarcis hispanica* (Lacertilia: Lacertidae). In *Studies in Herpetology*, 621-626. Rocek, Z. (ed.). Proc. Europ. Herpet. Meeting, Prague, 1985. Prague: Charles University.

Busack, S. D. (1976). Activity cycles and body temperatures of *Acanthodactylus erythrurus*. *Copeia* **1976**, 826-830.

Gruber, U. F. and Schultze-Westrum, T. (1971). Zur Taxonomie und Okologie der Cycladen-Eidechse (*Lacerta erhardii*) von den Nordlichen Sporaden. *Bonn zool. Beitr.* **22**, 33-48.

Henle, K. (1988). Dynamics and ecology of three Yugoslavian populations of the Italian wall lizard (*Podarcis sicula campestris* de Betta) (Reptilia, Lacertidae). *Zool. Anz.* **220**, 33-48.

Henle, K. & Klaver, C. J. J. (1986). *Podarcis sicula* (Rafinesque-Schmaltz, 1810) - Ruineidechse. In *Handbuch der Reptilien und Amphibien Europas*. Band 2/II, Echsen (Sauria) III (Lacertidae III: *Podarcis*), 254-342. Bohme, W. (ed.) Wiesbaden: AULA Verlag.

Janik, D. S. (1987). *Circadian organization in the Desert Iguana*. PhD dissertation, University of Oregon.

Ouboter, P. E. (1981). The ecology of the island lizard *Podarcis sicula salfii*: :correlation of microdistribution with vegetation coverage, thermal environment and food-size. *Amphibia-Reptilia* **2**, 243-257.

Perez-Mellado V. (1983). Activity and thermoregulation in two species of Lacertidae: *Podarcis hispanica* (Steindachner, 1970) and *P. bocagei* (Seoane, 1884). *Ciencia Biol.Syst.Port.* **5**, 5-12.

Perez-Mellado, V. and Salvador, A. (1981). Actividad y termoregulacion estival de *Podarcis pityusensis* Bosca, 1883 (Sauria: Lacertidae) en Ibiza y Formentera. *Amphibia-Reptilia* **2**, 181-186.

Pittendrigh, C. S. & Daan, S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents. V. Pacemaker structure: a clock for all season. *J. comp. Physiol.* **106**, 333-355.

Pollo Mateos, C. & Perez-Mellado, V. (1989). Activity and thermoregulation in three Mediterranean species of Lacertidae. *Herpet. J.* **1**, 343-350.

Porter, W. P., Mitchell, J. W., Beckman, W. A. & DeWitt, C. B. (1973). Behavioral implications of mechanistic ecology: thermal and behavioral modeling of desert ectotherms and their microenvironment. *Oecologia (Berl.)* **13**, 1-54.

Porter, W. P. and Tracy, C. R. (1983). Biophysical analyses of energetics, time-space utilization and distributional limits. In *Lizard Ecology: Studies of a Model Organism*, 55-83. Huey, R. B., Pianka, E. R. and Schoener, T. W. (eds.) Cambridge, Mass.: Harvard UP.

Pough, F. H. & Busack, S. D. (1978). Metabolism and activity of the spanish fringe-toed lizard (Lacertidae: *Acanthodactylus erythrurus*). *J. Thermal Biol.* **3**, 203-205.

Underwood, H. (1990). The pineal and melatonin: regulators of circadian function in lower vertebrates. *Experientia* **46**, 120-128.

Van Damme, R., Bauwens, D., Castilla, A. M. and Verheyen, R. F. (1990). Comparative thermal ecology of the sympatric lizards *Podarcis tiliguerta* and *Podarcis sicula*. *Acta Oecologica* **11**, 503–512.

Verbeek, B. (1972). Ethologische Untersuchungen an einigen europaischen Eidechsen. *Bonn zool. Beitr.* **23**, 122-151.

HERPETOLOGICAL JOURNAL, Vol. 2, pp. 90-93 (1992)

EGG PRODUCTION IN THE SMOOTH NEWT *(TRITURUS VULGARIS)*

J. M. R. BAKER

Brain and Behaviour Research Group, Department of Biology, The Open University, Walton Hall, Milton Keynes, MK7 6AA, U.K.

(Accepted 21.1.91)

ABSTRACT

The relationship between body size and fecundity in female smooth newts *(Triturus vulgaris)* was examined by counting the numbers of eggs oviposited, to resolve several controversies. First, whether body size is positively correlated with fecundity. Second, whether ovarian oocyte counts represent the numbers of ova oviposited by females in any particular year. In addition, the hypothesis that body size is positively related to the rate of ovipositon was tested. Female body size was positively correlated with clutch size and rate of oviposition. Numbers of ova oviposited by individuals were of a similar order of magnitude to the estimates of clutch size obtained from ovarian oocyte counts in previous studies. A median oviposition rate of 7.2 ova per day was recorded during the oviposition period.

INTRODUCTION

Among amphibians, clutch volume or mass has been shown to be positively related to female body size, which has lead to the conclusion that female body size constrains clutch size (Crump & Kaplan, 1979; Kaplan & Salthe, 1979). Within species it has become axiomatic that large female body size is naturally selected due to the associated fecundity advantage (e.g. Howard, 1988; Woolbright, 1989).

Smooth newts produce many single eggs over a long period of time and wrap each egg within vegetation (Smith, 1973), so that precise counts of numbers of eggs produced by individual females are difficult to obtain. Hence investigations of fecundity the smooth newt have relied on ovarian oocyte counts, making the assumption that the yolked ova in the ovaries immediately prior to mating represent the reproductive potential of an individual for that year (Bell, 1977). Bell (1977) found that larger females contained more ovarian oocytes than small females. Verrell (1986) and Verrell & Francillon (1986) have found that female body size is positively correlated with number of ovarian oocytes. However, Hagström (1980) was not able to find any relationship between body size and clutch size, in Norwegian smooth newts.

The assumption that ovarian oocyte number represents actual clutch size may not be justifiable. Hagström (1980) reported that female smooth newts that he captured in ponds, after he assumed that oviposition had ceased, still contained yolked oocytes. It is possible that not all females complete oviposition synchronously, so that these newts may have still been ovipositing. However, Harrison (1985) found that females moving away from a breeding pond in July also contained yolked oocytes.

To resolve the above controversies, actual clutch size records are needed. In addition to the problems of prolonged oviposition periods and cryptic deposition of ova, work in this area has also keen hampered by the fact that, once removed from their natural breeding ponds, female smooth newts tend to lose reproductive condition and cease oviposition (pers. obs.). This may explain why attempts to investigate ovipositional parameters within this species and other congenerics, in an aquarium situation, have produced results that are at odds with the ovarian clutch size counts. Ovarian oocyte counts predict that *Triturus* species should oviposit several hundreds of eggs (100-400, *T. vulgaris,* (Bell, 1977); approximately 200-300, *T. vulgaris* and *T. cristatus* (Hagström, 1980); 100-500, *T. vulgaris* (Verrell, 1986); 130-470, *T. vulgaris* (from graph in Verrell & Francillon, 1986); 240, *T. vulgaris* (Harrison, 1985)).

However, only very low numbers of ova have actually been deposited in aquaria Wimpenny (1951) recorded mean values of 2.75 and 0.76 ova for *T. cristatus and T. vulgaris,* and Verrell (1986) recorded counts of 25-80 for *T. vulgaris.* There is clearly a discrepancy between ovarian clutch sizes and oviposited clutch counts. Verrell (1986) explains his low clutch sizes as being the number of ova produced on a single insemination only.

Another anomaly is that the rates of oviposition recorded in aquaria are lower than might be expected. oviposition periods of natural populations last between approximately 75 and 125 days (90 days for *T cristatus, T. helveticus* and *T. vulgaris* (Smith, 1973);125 days for *T. vulgaris* (Bell & Lawton, 1975); 75-90 days for *T. marmoratus* (Diaz-Paniagua, 1989)) and so the rate of oviposition should fall within the range of 0.8 (100 ova in 125 days) and 6.7 (500 ova in 75 days) ova per day. However, Arntzen & Hedlund (1990) found rates of 0.33 for *T. cristatus* and 0.74 for *T: marmoratus.*

The present study records actual clutch sizes of individual female smooth newts, selected to represent the full body size range of a natural population. They were maintained under semi-natural conditions to avoid loss of reproductive condition during the course of the study. The study was performed to test the hypothesis that clutch size is positively related to maternal body size and to establish whether oviposited clutch counts are similar to ovarian counts from previous studies.

This study also allowed an examination of rates of oviposition and hence the testing of a second hypothesis, that rate of ovipositon is positively related to female body size. In an observational study, Diaz-Paniagua (1989) found that large female *Triturus marmoratus pygmaeus* were more efficient at oviposition than small females, in terms of ova produced per oviposition attempt, and they also invested less time in failed attempts than smaller females.

Costs associated with ovipositional behaviour of Triturus species have not been investigated, but observation of smooth newts in a natural pond in the Milton Keynes area suggests that such costs may exist. I Towards the end of one reproductive period (May 1990), females were seen ovipositing during daylight

hours, so that females in this particular pond were easily visible in shallow water, at the pond's edge searching for oviposition sites and were also seen ovipositing just below, or at, the water's surface. This latter operation sometimes involved individuals rolling onto their sides and backs, clearly exposing the non-cryptically coloured ventral surfaces. Such behaviour may expose females to a greater risk of predation than does their otherwise secretive nature. Diaz-Paniagua (1989) sees the potential advantages of efficient oviposition in large female *Triturus marmoratus pygmaeus* as being energetic savings and as leaving more time in which to search for new oviposition sites. However, if there are increased chances of predation associated with ovipositional behaviour, then efficient oviposition may also be advantageous in minimising these dangers. Diaz-Paniagua's work predicts that the more efficient ovipositors will be large females.

METHODS

To ensure that females were captured before the start of oviposition, newts were collected at a drift fence, as they migrated towards a pond in Milton Keynes (7.3.89 - 27.3.89). Twelve females, selected by eye to cover a wide range of body sizes, were individually maintained outside in plastic aquaria, measuring (39 x 20 x 25 cm). These aquaria were placed in a larger tub of water (see Fig. 1) to ensure that the newts experienced a temperature regime similar to that of a small pond. Each tank was furnished with one clay pot refuge and two pieces of 'weed'. This 'weed' consisted of strips of polythene (20 x 2 cm) which were cut along one edge to create a fringe. This design allowed females to carry out their normal oviposition behaviour of wrapping ova in some flexible material, which also prevented the newts from eating the ova, and at the same time facilitated ease of collection of ova. Weights were placed on one end of each strip to anchor the 'weed' to the substrate, so that the polythene strips spanned the whole depth of the aquarium. The newts were fed on an *ad libitum* basis on zooplankton that was cultured in the large tub and *Tubifex* obtained from a commercial supplier.

Males, captured in funnel traps at the same breeding pond, were introduced every week, for two or three days, to ensure that all oocytes were fertilised. After the two or three days these males were returned to the pond.

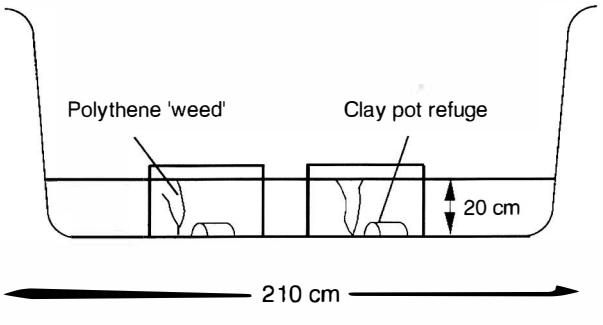


Fig. 1. Diagram of aquaria containing individual females, within a larger tub of water.

Tanks were inspected for ova daily. As soon as any ova were seen in any tank, that individual female was weighed (to 0.1 g). After this, ova were collected every 1-3 days, removing them from the substrate by gently peeling away the polythene. Females were judged to have oviposited a complete clutch on the basis of changes in external characters and behaviour. On completion of oviposition the tail fin decreases in size, the skin becomes granular rather than smooth, the cloaca becomes reduced in size and dome-shaped rather than flat-topped, dome-shaped, and the newts often float at the water surface. These characteristics are also associated with the resumption of terrestrial life.

Once a female had completed oviposition she was anaesthetised in MS-222 (Sandoz) and snout-vent length (SVL) and total length (TL) were measured to the nearest 0.5 mm. Newts were anaesthetised only after oviposition, to ensure that any possible adverse effects of anaesthesia could not act on oviposition.

RESULTS

FEMALE BODY SIZE AND CLUTCH SIZE

Ten of the twelve females produced ova. Descriptive data of the body sizes and clutch sizes of these newts is given in Table 1. In the following statistical analyses, data from only the ten ovipositing newts were used. Pearson product-moment correlation analyses were used to detect whether there was a relationship between both female mass and the number of ova produced and between SVL and the number of ova produced. One-tailed tests were adopted because of the uni-directional nature of the hypotheses being tested.

There were significantly positive correlations between both measures of female body size and the numbers of ova produced. For female body mass and clutch size r=0.61, P<0.05, 8 d.f. For SVL and clutch size, r=0.60, P<0.05, 8 d.f..

RATE OF OVIPOSITION

To test the hypothesis that larger females are able oviposit faster than small females, a measure of oviposition rate was needed. This was calculated by dividing the clutch size by the number of days between the first and last ova produced (oviposition period), for each individual female. Descriptive data on oviposition period and oviposition rate are given in Table 2. A Pearson product-moment correlation analysis showed that female body mass was significantly positively correlated with oviposition rate (r=0.89, P<0.01, 8 d.f.). Larger females were able to oviposit at a faster rate than small females. Data on the maximum number of ova produced per night, by each female, are also given in Table 2, as 'peak rate oviposition', to show the maximum rate at which the females in the present study were able to oviposit.

DISCUSSION

The present study confirms the results of Bell (1977), Verrell (1986) and Verrell & Francillon (1986) that there is a positive association between female body size and clutch size in smooth newts. Larger females oviposited more ova than did small females. It is also notable that the two newts that did not oviposit were both particularly small, weighing 1.1 and 1.7 g. It is possible that these two females were immature, being below the

	Mean	Median	S .D.	Range
SVL (mm)	45.9	45.3	3.816	40.0 - 52.0
TL (mm)	85.5	85 .2	8.82	70.0 - 99.5
Mass (g)	2.7	2.4	0.822	1.6 - 4.2
No. ova oviposited	300	252	189.4	88 - 637

TABLE I. Mean, median, standard deviation and range of body sizes and numbers of ova produced by ten *T. vulgaris*.

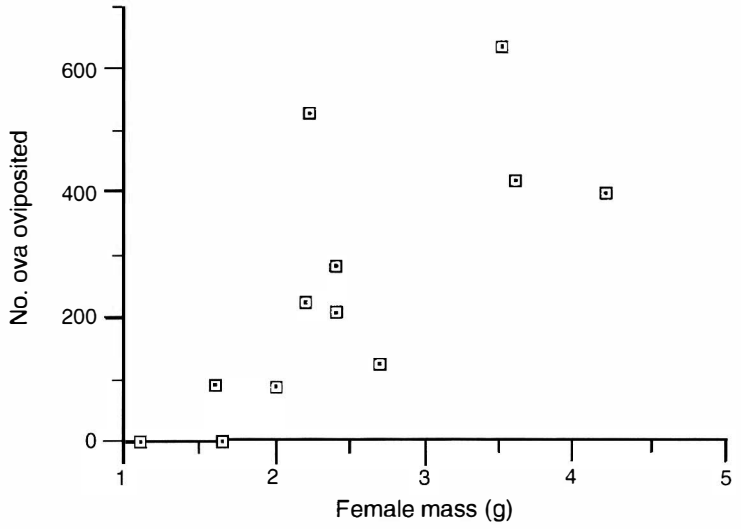


Fig. 2. Relationship between female body size (mass) and the number of ova oviposited. Data from the two non-ovipositing females were not included in the statistical analysis.

minimum body size for sexual maturity. It has previously been thought that juvenile smooth newts do not migrate to ponds (Verrell, 1985). It is possible that juvenile smooth newts have not been recognised as inhabiting the aquatic environment because only the largest juveniles adopt this strategy and the size range of these newts tends to be similar to the small end of the adult body size range.

The clutch size data of the present study also suggests that the assumption that ovarian oocyte counts represent actual clutch sizes is valid. The numbers of ova oviposited by newts in the present study (88-637 ova) are of a similar order of magnitude to estimates of clutch size generated from previous ovarian oocyte counts, from populations in Oxfordshire or Buckinghamshire, made at the beginning of breeding seasons (approximately 100-400 recorded by Bell (1977), 100-500 recorded by Verrell (1986), 130-470 from graph in Verrell & Francillon (1986)). Thus I would conclude that female *T. vulgaris* from local populations are quite able to oviposit all ova yolked at the beginning of a season, during that season.

Harrison (1985) working on *T. vulgaris* at Llysdinam, mid-Wales, recorded mean clutch sizes of 239 (yolked ova carried by immigrating females) or 190 (obtained by subtracting the number of yolked ova in emigrating females from the number in immigrating females) which seem much lower than the mean in the present study (300). However the Llysdinam newts are smaller than those found in the Milton Keynes area, with mean total length (TL) of 79 mm for immigrants and 76 mm for emigrants. Hence the difference in clutch sizes between local newts and those from mid-Wales may be a reflection of differences in body size. Hagström's (1980) low ovarian counts of approximately 200 may reflect the shorter foraging and/or breeding seasons of *T. vulgaris* in Sweden.

Harris (1987) notes that female *Notophthalmus viridescens dorsalis* actually oviposit rather more ova than ovarian oocyte counts would predict. Harris interprets this observation as evidence that females may be able to yolk up ova during the oviposition period, depending on food availability. If female *T. vulgaris* can adopt a similar strategy of vitellogenesis then this would explain the presence of yolked ova in the ovaries immediately after the breeding period, as found by Hagström (1980), Harrison (1985) and Verrell et al. (1986). This does not seem an unreasonable possibility, when it is considered that adult smooth newts are reported to perform most feeding and annual growth, as well as courtship, during the aquatic phase (Verrell, 1987).

The mean and median rates of oviposition (8.7 and 7.2 ova per day) are higher than values predicted using data from ovarian oocyte counts and records of the lengths of population oviposition periods (0.8 to 6.7 ova per day). The relatively high daily rate of oviposition recorded in the present study is probably an accurate measurement of natural oviposition rates of females in the field, and is higher than estimated by dividing

	Mean	Median	S.D.	Range
Oviposition period (days)	36.9	40.0	19.69	11 - 74
Mean oviposition rate (ova per 24 hours)	8.7	7.2	4.64	3.9 - 17.3
Peak rate oviposition (ova per 24 hours)	32.3	32.0	13.07	16 - 54

TABLE 2. Mean, median, standard deviation and range of ovposition periods, mean rates of oviposition and peak rates of oviposition for ten female *T. vulgaris*.

clutch sizes by oviposition periods of whole populations, because oviposition is not exactly synchronous. Mean and median oviposition periods for individual newts are 36.9 and 40 days respectively (see Table 2), whereas the length of time that elapsed between the first and last ova produced by the sample as a whole was 84 days. Thus, an individual female actually oviposits over only a fraction of the population oviposition period.

The finding that oviposition rate is positively correlated with female body size is consistent with the results of Diaz-Paniagua (1989) that large female newts can oviposit at a higher rate than small females. Diaz-Paniagua found that this difference occurred because small female *T. marmoratus pygmaeus* were less efficient at oviposition behaviour. It is not known whether this is also true of *T. vulgaris*, or whether small females are physiologically and/or energetically less able to sustain a high rate of oviposition.

ACKNOWLEDGEMENTS

This study was conducted whilst in receipt of a NERC studentship. Thanks are due to Leigh Gillett, Richard Griffiths, Lottie Hosie and Caroline Pond for helpful comments on this work, and to Tim Halliday for supervision.

REFERENCES

Arntzen, J. W. & Hedlund, L. (1990). Fecundity in the newts *Triturus cristatus, T. marmoratus* and their natural hybrids in relation to species coexistence. *Holarc. Ecol.* **13,** 325-332 .

Bell, G. (1977). The life of the smooth newt (*Triturus vulgaris*) after metamorphosis. *Ecol. Monogr.* **47**, 279-299.

Bell, G. & Lawton, J. H. (1975). The ecology of the eggs and larvae of the smooth newt. (*Triturus vulgaris* (Linn.)) *J. Anim. Ecol.* **44**, 393-424.

Crump, M. L. & Kaplan, R. H. (1979). Clutch energy partitioning of tropical tree frogs (*Hylidae*). *Copeia* **1979**, 626-635.

Diaz-Paniagua, C. (1989). Oviposition behaviour of *Triturus marmoratus pygmaeus*. *J. Herpet.* **23**, 159-163.

Hagström, T. (1980). Egg production of newts (*Triturus vulgaris* and *Triturus cristatus*) in Southwestern Sweden. ASRA Journal **1**, 1-8.

Harris, R. N. (1987). An experimental study of population regulation in the salamander, *Notophthalmus viridescens dorsalis* (Urodela: Salamandridae). *Oecologia* **71**, 280-285 .

Harrison, J. D. (1985). *Aspects of the ecology of amphibian larvae.* University of Wales Ph.D. Thesis.

Howard, R. D. (1988). Sexual selection on male body size and mating behaviour in American toads, *Bufo americanus. Anim. Behav.* **36**, 1796-1808.

Kaplan, R. H. & Salthe, S. N. (1979). The allometry of reproduction: an empirical view in salamanders. *Amer. Nat.* **113**, 671-689.

Smith, M. (1973). *The British Amphibians and Reptiles.* (*5th* Ed.). London: Collins.

Verrell, P. A. (1985). Return to water by juvenile amphibians at a pond in southern England. *Amphibia-Reptilia* **6**, 93-96.

Verrell, P. A. (1986). Male discrimination of larger, more fecund females in the smooth newt, *Triturus vulgaris. J. Herpet.* **20**, 416-422.

Verrell, P. A. (1987). Growth in the smooth newt (*Triturus vulgaris*) during the aquatic phase of the annual cycle. *Herpet. J.* **1**, 137-140.

Verrell, P. A. & Francillon, H. (1986). Bodysize, age and reproduction in the smooth newt, *Triturus vulgaris. J. Zool.* **210**, 89-100.

Verrell P. A., Halliday, T. R. & Griffiths, M. L. (1986). The annual reproductive cycle of the smooth newt (*Triturus vulgaris*) in England. *J. Zool. Lond* **(A) 210**, 101-119.

Wimpenny, R. S. (1951). The effect of vegetation on the breeding of newts, *Molge cristata* and *Molge vulgaris. J. Anim. Ecol.* **20,** 98-100.

Woolbright, L. L. (1989). Sexual dimorphism in *Eleutherodactylus coqui*: selection pressures and growth rates. *Herpetologica* **45**, 68-74.

HERPETOLOGICAL JOURNAL, Vol. 2, pp. 94-98 (1992)

HERPETOFAUNA OF PLEISTOCENE (IPSWICHIAN) DEPOSITS AT SELSEY, WEST SUSSEX:THE EARLIEST BRITISH RECORD OF *BUFO CALAMITA*

J. ALAN HOLMAN

Michigan State University Museum, East Lansing, Michigan 48824-1045, U.S.A.

(Accepted 31.1.91)

ABSTRACT

Pleistocene deposits at Selsey, West Sussex, thought to represent Substage II of the Ipswichian Interglacial Age, have yielded five herpetological taxa: *Bufo bufo*, *Bufo calamita*, *Rana* sp., *Emys orbicularis*, and *Natrix natrix*. This is the earliest British record of *Bufo calamita* and extends its temporal range in Britain back about 105,000 years. The presence of *Emys orbicularis* indicates that mean July temperatures in Britain were probably at least two degrees Celsius warmer than today. The anurans and the pond tortoise indicate the presence of a lotic wetland situation The anurans (outside of the breeding season) and the grass snake could have lived in a grassy area near the wetland.

INTRODUCTION

The Pleistocene deposits at Selsey, West Sussex, (Fig. 1) are noteworthy because flint flakes from the site provide almost the only evidence of humans from the early part of the Ipswichian stage (Stuart, 1988), the last interglacial episode of the Pleistocene. Fossil mammals have been reported from the Selsey Site (West *et al.*, 1960), but herpetological remains (other than those of the European pond tortoise, *Emys orbicularis*, (Stuart, 1979) have never been recorded. Recent collections made by personnel of the Institute of Archaeology, London, have yielded remains of three taxa of anurans and a snake. Although this is a small herpetofauna, it is significant, because it has provided the earliest fossil record of the natterjack toad, *Bufo calamita*, from Britain. This record extends the knowledge of the natterjack in Britain back about 105,000 years. These herpetological remains form the subject of the present paper, along with comments about the ancient distribution of *Bufo calamita* in Britain and Europe.

THE SELSEY, WEST SUSSEX, SITE

At Selsey in West Sussex along the English Channel, Pleistocene sediments occur in a channel cut into the Eocene Bracklesham Beds. These deposits are located on the foreshore between the Lifeboat House and the Holiday Camp on Selsey Bill (West *et al.*, 1960). These landmarks are still depicted on ordnance Survey Landranger Series. Sheet 197, (1990). The area is at about SZ 859924 on this map.

Stuart (1982) has discussed the Selsey beds based on pollen spectra outlined in West *et al.* (1960). The earliest horizon covers the period of time from the late Wolstonian cold stage to Ipswichian subzone 1a (an early part of the Ipswichian interglacial stage). During this interval, birch woodland and pine replaced the late cold stage, herb-dominated vegetation. Horse remains (Equus ferus Boddaert) were taken from sediments within this interval, but the fossils were not assigned to any specific vegetational zone.

The later horizons, zones Ib to early IIb have yielded typical mid-Ipswichian taxa, plus the evidence of *Homo sapiens*. Vertebrates recorded from these zones by Stuart (1982) include pond tortoise (*Emys orbicularis* Linnaeus), humans (*Homo sapiens* Linnaeus represented by flint flakes), beaver (*Castor fiber* L.), extinct straight-tusked elephant (*Palaeoloxodon antiquus* Falconer & Cautley), and extinct (non-woolly) rhinoceros (*Dicerorhinus hemitoechus* (Falconer)).

The new amphibian and reptile fossils come from two collections, both equated with Ipswichian subzone II, and thus indicating mid-Ipswichian times. The first collection contains two species of *Bufo* and came from a peaty, organically-rich exposure which was referred to as the "Peat Bed" locality by the collectors. The second collection contains a *Rana* and a *Natrix* and came from an exposure of dark gray organic clayey silt. The principle collectors of this material were R. Fowler and S. Parfitt of the Institute of Archaeology, London.

SYSTEMATIC PALAEONTOLOGY

Abbreviations used in the numbering system used for the newly reported Selsey herpetological bones are as follows: IAL (Institute of Archaeology, London), SA (Selsey Amphibians), SR (Selsey Reptiles) - followed by individual specimen numbers.

Class Amphibia
Order Anura
Family Bufonidae

Two modern workers, Böhme (1977) and Sanchiz (1977) have studied the osteology of the European Bufonidae as it applies to the interpretation of fossil members of this family. Terminology for structures on individual bones follows these publications. The Family Bufonidae comprises a large anuran assemblage, but only one genus, *Bufo*, occurs in the Pleistocene and modern fauna of Britain and Europe.

Genus *Bufo* Laurenti

Three species of *Bufo*, *B. bufo*, *B. calamita*, and *B. viridis* occur in the Pleistocene and modern fauna of Britain and Europe. Two of these, *B. bufo* and *B. calamita* have been identified from the Selsey Site. Fossil *Bufo* mainly occur in the form of individual cranial and postcranial bones. I have

SITE	AGE	MINIMUM NUMBER OF INDIVIDUALS
Whitemoor Channel, Bosley, E. Cheshire. Holman and Stuart (1991)	Early Flandrian, ca 10,000 – 8,800 B.P.	2
Ightham Fissures, Sevenoaks, Kent. Holman (1985)	?Early Flandrian	12
Cow Cave, Chudleigh, Devon. Holman (1988)	?Early Flandrian	16
Selsey, West Sussex Holman (this paper)	Ipswichian II ca 115,000 B.P.	3

TABLE 1: Distribution of fossil *Bufo calamita* in Britain.

consistently found that the ilium is the the most reliable of all of the individual anuran bones upon which to make taxonomic identifications (vide Holman, 1985 for a discussion of this). Moreover, the ilium appears to be a very reliable bone for distinguishing the three modern and Pleistocene species of British and European *Bufo* (Sanchiz, 1977; Holman, 1989). Böhme (1977) and Sanchiz (1977) have found characters in other anuran postcranial elements that allow them to distinguish between different taxa of living and fossil British and European forms. Sanchiz (1977) has done an especially comprehensive study on the genus *Bufo* in the Tertiary of Europe in which he outlines differences in *Bufo* postcranial elements other than the ilium.

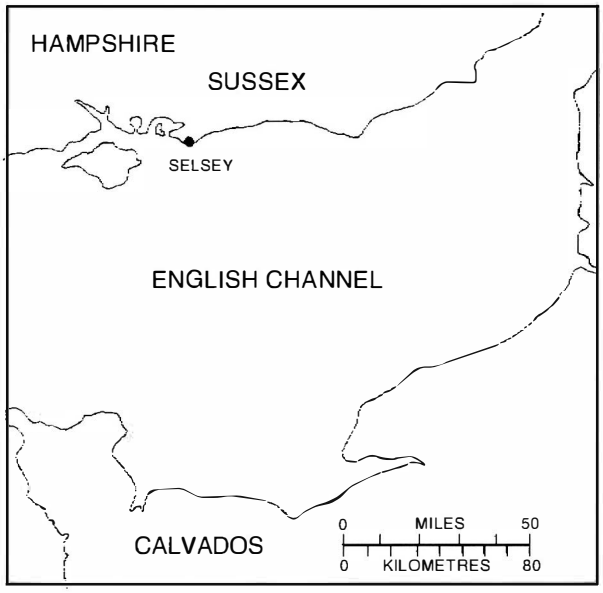


Fig. 1. General Location of the Selsey, West Sussex, site.

Bufo bufo (Linnaeus)

Material. Two left and three right humeri IALSA-1-5; three left and one right ilia IALSA-6-9 (Fig. 3b); three left and two right femora IALSA-10-14; three left and two right tibiofibulae IALSA-15-19; four calcania IALSA-20-23; one urostyle IALSA-24.

Remarks. This section will first deal with osteological criteria used herein to separate *B. bufo* Selsey site fossils from those of *B. calamita.* These criteria follow Sanchiz (1977) and Holman (1989) and have been confirmed as much as possible by comparison of the fossils with modern skeletons of both species. The terminology used for structures on individual bones also follows Sanchiz (1977) and Holman (1989).

Humerus. The humerus of *B. bufo* appears to lack a definable paraventral crest (Sanchiz, 1977, p. 81) whereas *B. calamita* has a rudimentary paraventral crest. In case this character is a reflection of sexual dimorphism, it should be pointed out that all of the Selsey fossils identified as *B. bufo* on the basis of this character appear to be males based on the enlarged distal flange, and that the one humerus identified as *B. calamita* appears to be a female based on the lack of a distal flange.

Ilia. Three characters are used to separate *B. bufo* from *B. calamita* (vide Fig. 3 and Holman, 1989, Fig. 1). The first character is that the ilial prominence of *B. bufo* is low and rounded; sometimes low and roughened; or sometimes even in the shape of a low, irregular, sharpened crest. The ilial prominence of *B. calamita* on the other hand is higher and is triangular in shape. The second character involves the lack of a ridge on the anteroventral part of the ilial shaft in *B. bufo* which is present as the so-called "*calamita* ridge" in *B. calamita* (Holman, 1989). It should be pointed out that a groove is present above the ridge in *B. calamita* (Fig. 3a and Holman, 1989, fig. 1) and that this groove might be as specifically diagnostic as the ridge itself. Finally, the third character is that the pars descendens is much less

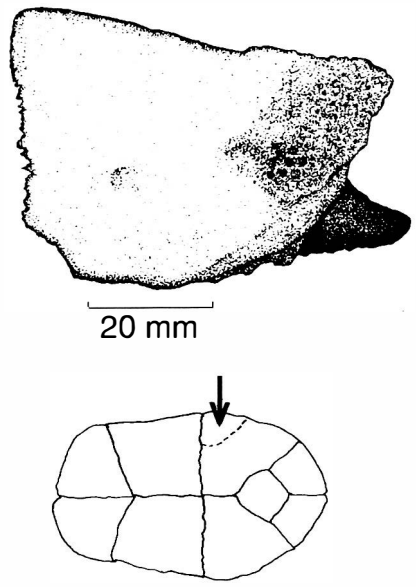


Fig. 2. Portion of a large right hyoplastron of *Emys orbicularis* Natural History Museum, London, Number BMR 9287, redrawn from Stuart (1979). The position of the fossil in life is indicated by an arrow on an outline drawing of a modern *E. orbicularis* plastron in ventral view.

extensive in *B. bufo* than in *B. calamita*, both anterior and ventral to the acetabular fossa. Quite probably, both of these characters relate to the different gaits of the two animals. *B. bufo* usually progresses by making a series of short hops, whereas *B. calamita* appears to be making "mouselike dashes".

Femora. In *B. bufo* the crista femoris is low and tends to be flattened proximally. In *B. calamita* and *B. viridis* this crest appears as a sharpened ridge. Another character pointed out by Sanchiz (1977) involves the the fact that the crista femoris of *B. bufo* is undivided rather than triangular in shape.

Tibiofibulae. The internal border of the tibiofibula in *B. bufo* has a sharp ridge on its internal border; this ridge is more roughened in *B. calamita* and *B. viridis* (Sanchiz, 1977, p. 81).

Calcanea. The calcanea of *B. bufo* are rounded rather than roughened and more flattened as in *B. calamita*. One might caution here that this might be a sexually variable character.

Urostyle. Sanchiz (1977) gives ratios based on measurements of the urostyle that he has found will separate the three species of British and European *Bufo*. These ratios are derived from dividing the height of the cotyles x 100 divided by the width of the cotyles. This ratio is given as 50.0 for *B. bufo*, 43.6 for *B.calamita*, and 41.3 for *B. viridis* (Sanchiz, 1977, table p. 78). The ratio reflects the fact that the cotyles of *B. bufo* are less wide and more high in *B. bufo* than in the other two species.

In the Selsey *B. bufo* urostyle the width of the cotyles is 3.0 mm, the height of the cotyle is 1.7, giving a Sanchiz ratio of 56.7. This appears to fall in line with *B. bufo* rather than the other two species, even though it exceeds the value of *B. bufo*.

Bufo calamita Laurenti

Material. Right humerus IALSA-25; two left and two right ilia IALSA-26-29 (Fig. 3a); two left and three right femora IALSA-30-34; two left tibiofibulae IALSA-35-36; one calcaneum IALSA-37.

Remarks. Characters for distinguishing the individual bones of *B. calamita* from *B. bufo* were given in the above section on *B. bufo*.

Bufo sp. indet.

Material. Presacral vertebra from region IV to VIII of Sanchiz (1977, p. 77) IALSA-38; two right radio-ulnae IALSA-39-40; three phalanges IALSA-41-43.

Remarks. These are elements that I am unable to identify to the specific level. They probably represent either *B. bufo* or *B. calamita*.

Family Ranidae
Genus *Rana*

The identification of individual skeletal elements of the genus *Rana* have been discussed by Böhme (1977)

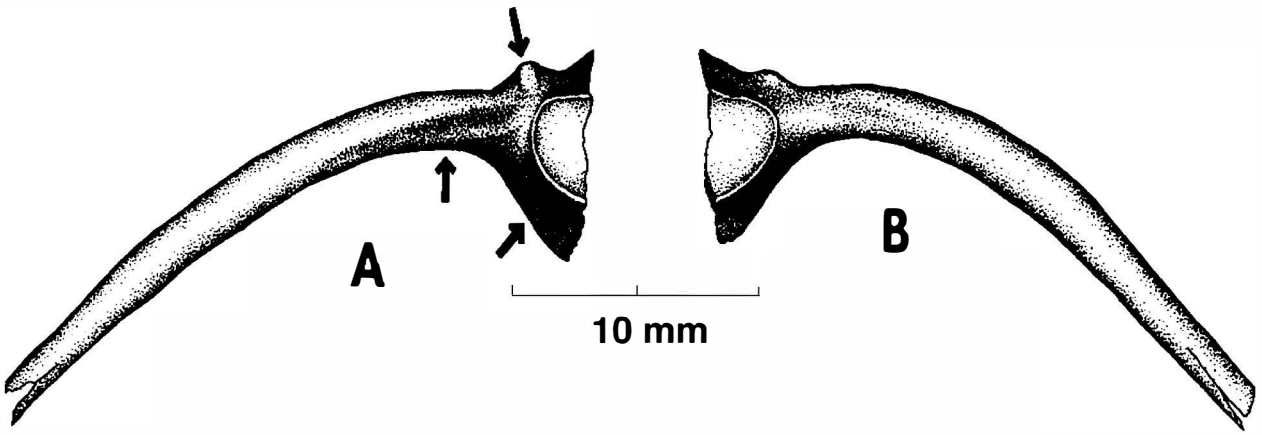


Fig. 3. A, Left ilium of *Bufo calamita* IALSA-29 from the Selsey, West Sussex, Site. B, Right ilium of *Bufo bufo* IALSA-O9 from the Selsey, West Sussex, Site.

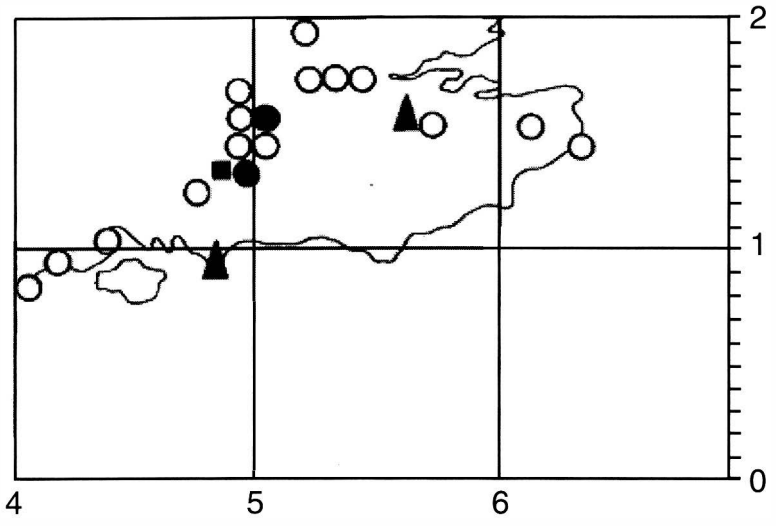


Fig. 4. Historic and fossil records of *Bufo calamita* in southeastern England redrawn and modified from Holman & Stuart (1991). Triangles indicate fossil records. Open circles indicate historic records up to and including 1959. Closed circles indicate records from 1960 through 1969. Squares indicate records from 1970 to 1983.

Rana sp. indet.

Material. Femur (with proximal and distal ends missing) IALSA-44.

Remarks. The femur of *Rana* is much larger and more curved than in species of European *Hyla* and longer and more gracile than in the three British and European species of *Bufo*. I am unable to identify this element to the specific level.

Class Reptilia
Order Testudinata
Family Emydidae
Emys orbicularis Linnaeus

Material. Part of a large right hyoplastron (Fig. 2); Natural History Museum, London, No. BMR 9287.

Remarks. This element was reported by Stuart (1979). It was found in 1961 in detritus mud which indicates zones Ib IIb of the Ipswichian.

Order Serpentes
Family Colubridae
Genus *Natrix* (Linnaeus)

Szyndlar (1984) has discussed the identification of British and European species of *Natrix* on the basis of individual vertebrae.

Natrix natrix (Linnaeus)

Material. Fragmentary anterior trunk vertebra IALSR 45.

Remarks. The vertebra has a more extensive and robust hypapophysis than in British and European *Vipera* and has the base of the parapophyseal process more massive than in *Natrix maura* and *tessellata* (Szyndlar, 1984, p. 26).

DISCUSSION AND SUMMARY

The Selsey, Sussex, Ipswichian strata have produced a small herpetological assemblage consisting of *Bufo bufo*, *Bufo calamita*, *Rana* sp, *Emys orbicularis*, and *Natrix natrix*. The most significant records are those of *B. calamita* and *Emys orbicularis*.

Bufo calamita, the natterjack toad, is one of the three most endangered herpetological species in Britain today. Since its first mention by Pennant in 1776 (Smith, 1973) the natterjack has been reported widely but locally in England and southwestern Scotland. Its habitat is now mainly coastal dune sites, but it was formerly also more common on inland heaths. It also occurs in southwestern Ireland. In the past few decades the natterjack has disappeared from many localities where it was formerly present (Holman & Stuart, 1991, fig. 5).

The present comprehensive range of *Bufo calamita* is from Iberia across to north-central Europe where it extends northward to about 55 degrees in Britain and to about 58 degrees in south Sweden and Estonia (Arnold & Burton, 1978). The natterjack lives in a wider range of habitats in the southwestern part of Europe where it is also more abundant than it is in northern areas. In northern and northeastern regions it lives in habitats with sandy soils that produce warmer microclimates (Beebee, 1983). According to Beebee, all of the habitats outside of the Iberian Peninsula are similar in that they have well-drained soil and low vegetation that facilitates insolation of the ground. Thus, *Bufo calamita* is considered primarily a species of southwestern Europe that has been able to extend its range northeastwards by exploiting locally warm habitats.

Sanchiz (1977) has discussed the fossil record of the Bufonidae in the Tertiary of Europe. He concludes that the family came to Europe as an Asiatic immigrant and arrived at about the boundary between the Oligocene and the Miocene. Of the

three modern European species, *B. viridis* is known in Europe since the middle Miocene and *Bufo bufo* and *Bufo calamita* since the upper Miocene. The fact that these three modern species were established by upper Miocene times is of considerable interest, but it is not surprising, considering the ancient origin of many species of anura in Europe (Sanchiz, in preparation for Handbuch Paläoherpetologie and pers. comm.).

There are no Tertiary records of any *Bufo* species in Britain, but this does not preclude their presence in the area during this geological period. In fact, since Britain was a peninsular appendage of Europe during most of the Cenozoic, it would seem possible that the two modern species of Britain could have extended westward into Britain by very late Miocene times.

Nevertheless, the Selsey, West Sussex. record of *Bufo calamita* represents the earliest fossil record of the natterjack in Britain, at about 115,000 years ago (Stuart, 1982, 1988).

Based on other vertebrate fossil remains in British Ipswichian Substage II faunas (Stuart, 1982) which include lion, hyaena, extinct (non-woolly) rhino, hippo, and European pond tortoise, one might expect a warmer climate than occurs in Britain today. Thus it might be suggested that *Bufo calamita* might have been able to exploit a wider variety of habitats in Britain during Ipswichian II times than they do at present. It is noted here that there are no historical records for *Bufo calamita* in the immediate vicinity of the fossil site (Fig. 4).

All of the other fossil records of *B. calamita* in Britain are from sites that are thought to represent the early Flandrian (Holocene) interglacial stage (Table 1). The author eagerly awaits more evidence of the fossil occurrence of this unique British amphibian.

The European pond tortoise, *Emys orbicularis*, is of considerable interest because its presence in fossil interglacial sites in Britain indicates that July temperatures were at least two degrees warmer than at present. *Emys orbicularis* is particularly characteristic of Ipswichian deposits in Britain (Stuart, 1979, 1982; Hallock, Holman & Warren, 1990; Holman & Clayden, 1990), and has been sparingly found at other interglacial sites including the Cromerian, Hoxnian, and early Flandrian (Stuart, 1979, 1982; Holman, Stuart & Clayden, 1990).

It is difficult to say much about the palaeoecological conditions indicated by the Selsey herpetofauna because only five taxa are known. Nevertheless, the presence of lotic wetland conditions are indicated by the three anuran species that would have needed such situations for breeding purposes (Frazer, 1983). Moreover, the European pond tortoise prefers still or slow-moving water with abundant aquatic vegetation (Arnold and Burton, 1978). The grass snake *Natrix natrix* and the anurans (outside of the breeding season) could have lived in nearby grassy areas.

ACKNOWLEDGEMENTS

I wish to thank Mark Roberts and Simon Parfitt of the Institute of Archaeology, London, for allowing me to study the Selsey Pleistocene herpetological fossils. The United States Science Foundation (NSF BSR-851-5665) supported my work in Britain in 1987 and the National Geographic Society (NGS 4150-89) supported my work in Britain in 1990. Irene Rinchetti made the drawings.

REFERENCES

Arnold, E. N. & Burton, J. A. (1978). *A Field Guide to the Reptiles and Amphibians of Britain and Europe*. London: Collins.

Beebee, T. J. C. (1983). *The Natterjack Toad*. Oxford University Press.

Böhme, G. (1977). Zur Bestimmung quartarer Anuran Europas an Hand von Skellettelementen. *Wissenschaftliche Zeitschrift der Humboldt-Universität zu Berlin, mathematik, Naturwissenshaft* **26**, 26-32

Frazer, D. (1983). *Reptiles and Amphibians in Britain*. London: Collins.

Hallock, L. A., Holman, J. A. & Warren, M. R. (1990).Herpetofauna of the Ipswichian Interglacial Bed (Late Pleistocene) of the Itteringham Gravel Pit, Norfolk, England. *Journal of Herpetology* **24**, 33-39.

Holman, J. A. (1985). Herpetofauna of the late Pleistocene Fissures near Ightham, Kent. *Herpetological Journal* **1**, 26-32.

Holman, J. A. (1988). Herpetofauna of the late Devensian/early Flandrian Cow Cave Site, Chudleigh, Devon. *Herpetological Journal* **1**, 214-218.

Holman, J. A. (1989). Identification of *Bufo calamita* and *Bufo bufo* on the basis of skeletal elements. *British Herpetological Society Bulletin* **29**, 54-55.

Holman, J. A. & Clayden, J. D. (1990). A late Pleistocene interglacial herpetofauna near Shropham, Norfolk. *British Herpetological Society Bulletin* **31**, 31-35.

Holman, J. A., Stuart, A. J. & Clayden, J. D. (1990). A middle Pleistocene herpetofauna from Cudmore Grove, Essex, England, and paleogeographic and paleoclimatic implications. *Journal of Vertebrate Paleontology* **10**, 86-94.

Holman, J. A. & Stuart, A. J. (1991). Amphibians of the Whitemoor Channel early Flandrian Site near Bosley, East Cheshire; with remarks on the fossil distribution of *Bufo calamita* in Britain. *Herpetological Journal* **1**, 568-573.

Sanchiz, F. B. (1977). La Familia Bufonidae (Amphibia, Anura) en el Terciario Europea. *Trabajos sobre Neogeno-Cuatanario* 8, 75-111.

Stuart, A. J. (1979). Pleistocene occurrences of the European pond tortoise in Britain. *Boreas* **8**, 359-371.

Stuart, A. J. (1982). *Pleistocene Vertebrates in the British Isles*. London & New York: Longman.

Stuart, A. J. (1988). *Life in the Ice Age*. Aylesbury: Shire Publications Ltd.

Szyndlar, Z. (1984). Fossil snakes from Poland. *Acta Zoologica Cracoviensa* **28**, 1-156.

Smith, M. A. (1973). *British Amphibians and Reptiles (5th Edition)*. London: Collins.

West, R. G., Sparks, B. W. & Sutcliffe, A. T. (1960). Intercoastal interglacial deposits of the English Channel. *Philosophical Transactions of the Royal Society of London B* **243**, 95-133.

SHORT NOTES:

HERPETOLOGICAL JOURNAL, Vol. 2, pp. 99-101 (1992)

MORPHOLOGICAL VARIATION IN RUSSELL'S VIPER IN BURMA AND THAILAND

WOLFGANG WÜSTER, SATOKO OTSUKA*, ROGER S. THORPE AND ANITA MALHOTRA

Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen, AB9 2TN, Scotland, U.K.

**Present address: 4-9-7 Asahigaoka, Yotsukaido City, Chiba 284, Japan.*

(Accepted 1.11.90)

Russell's viper (*Vipera russelli*) is one of the most widespread venomous snakes in southern Asia. It is an important cause of snakebite mortality and morbidity in many areas, including Thailand and especially Burma (Looareesuwan, Viravan & Warrell, 1988; Warrell, 1989). The symptoms of Burmese and Thai Russell's viper bite differ considerably: bites in Burma result, among other symptoms, in pituitary infarction, generalized capillary permeability and primary shock (Myint-Lwin *et al.* 1985), whereas bites in Thailand result in intra- vascular haemolysis (Warrell, 1986, 1989).

In some venomous snake species complexes, venom differences have been found to be related to taxonomic differentiation of the populations concerned; in these cases, venom differences were accompanied by morphological differences, for instance in *Echis* (Warrell & Arnett, 1976) and in the Asiatic cobra complex (Wüster 1990; Wüster & Thorpe, 1989, 1991). However, in the case of Russell's viper, previous workers have found relatively little morphological differentiation between Burmese and Thai Russell's vipers (Anon. 1987; Warrell, 1989), and both populations are generally included in the subspecies *V. r. siamensis* Smith, 1917 (e.g. Harding & Welch, 1980; Warrell, 1989).

The aim of this paper is to investigate the pattern of geographic variation in Russell's viper in Burma and Thailand, and to show that advanced taxonomic techniques can lead to a clearer assessment of the pattern of morphological variation than univariate statistics.

Preserved *V. russelli* specimens were borrowed from a number of museums in Europe and the United States. Twenty-three scalation and colour pattern characters were recorded from all specimens. In order to record the position of characters along the body of the snakes, the ventral scales were numbered from the head to the vent. The position or length of a character along the body is recorded as the ventral scale at the level of which it is situated. This is then converted to %ventral scale (%VS) position in order to compensate for variation in the number of ventral scales. The width or height of colour pattern characters on the dorsum of the specimens, is recorded as the number of dorsal scale rows on which they encroach. This is then expressed as a percentage of the number of dorsal scale rows at the level of the character (%DS width).

Principal components analyses (PCAs) were run separately for each sex, using all specimens with good locality records, and 21 characters showing geographic variation across the entire *V. russelli* complex (Table 1). In addition, a two-way analysis of variance (ANOVA) was run on the specimens of both sexes and populations, in order to determine which characters show statistically significant differences between the two populations.

The ordination of the individual specimens along the first two principal components is shown in Fig. 1. In both sexes, the Burmese and Thai specimens are clearly separate, to the extent that there is no overlap between the males, and very little between the females of the two populations.

The results of the two-way ANOVA are shown in Table 2. Out of 21 characteristics investigated, 12 show significant differences between the Thai and Burmese populations.

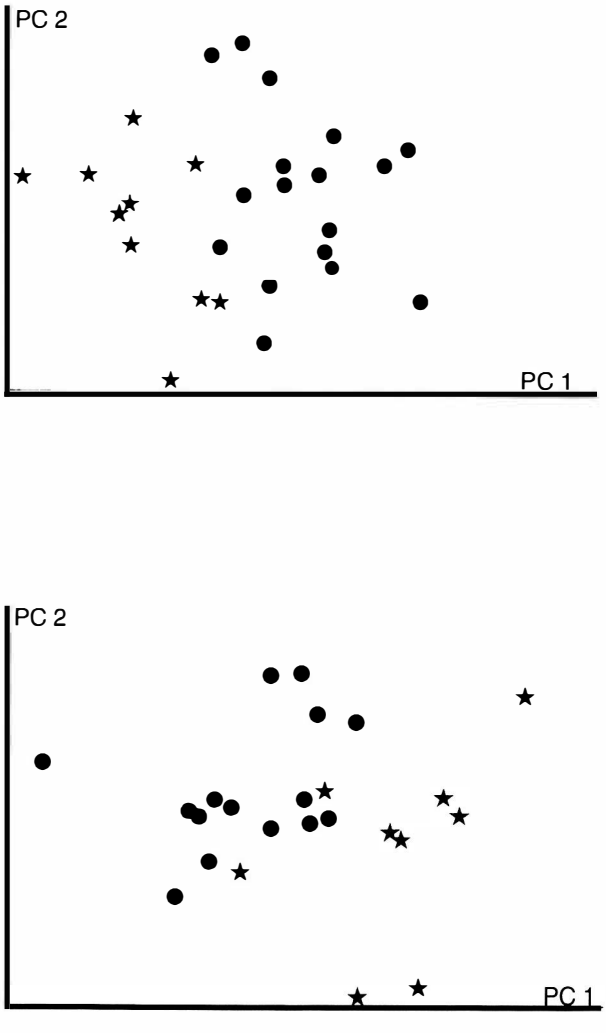


Fig. 1. PCA results: ordination of the Burmese (circles) and Thai (stars) Russell's viper males (top) and females (bottom) along the first two principal components.

This study shows that the Thai and Burmese populations of *V. russelli* differ considerably in their morphology. Although no single character can discriminate with absolute certainty between the two populations, there are significant differences in more than half the characters examined, including "standard" taxonomic characters such as the number of subcaudals. The differentiation in the overall phenotype is such that the PCAs reveal no overlap between male specimens, and very little between the female specimens of the two populations. The use of multivariate techniques allows a better assessment of the degree of morphological differentiation between populations than do univariate methods, as it analyses the pattern of variation in all characters used simultaneously (see Thorpe, 1987, for a review).

The reports suggesting only minor morphological differences between Thai and Burmese Russell's vipers have been held up in some cases as an example of extreme venom differentiation unaccompanied by morphological differentiation. We have shown here that the morphological differences are more important than was hitherto realised, although they are much more subtle than the very startling differences in venom effects. Nevertheless, it is important to emphasise the fact that there are significant differences, since it shows that detailed studies of the population systematics of venomous snakes can be used as a predictor of venom variation.

Acknowledgements. We wish to thank the curators and staff of the following natural history museums for enabling us to examine the specimens in their care (acronyms according to Leviton, 1980): AMNH, BM, CAS, FMNH, FSM, MCZ, MHNG, NHMW, NHRM, RMNH, ZFMK, ZMH, ZMUC. We particularly wish to acknowledge the help and cooperation of the staff of the British Museum (Natural History) during visits by the authors. This study was partly funded by NERC (WW) and the Leverhulme Trust (RST). We also wish to thank an anonymous reviewer for his comments on the manuscript.

1.	Number of ventral scales.
2.	Number of subcaudal scales.
3.	Number of dorsal scale rows at 25% VS length.
4.	Number of dorsal scale rows at 50% VS length.
5.	Number of dorsal scale rows at 75% VS length.
6.	Number of dorsal scale rows at 100% VS length.
7.	Number of supralabials.
8.	Number of infralabials.
9.	Number of scales contacting eye.
10.	Number of scales between supraoculars.
11.	Number of scales between chin shields and first ventral scale.
12.	Number of scales contacting rostral.
13.	Number of lateral spots between head and vent.
14.	Number of dark spots on the five ventrals situated at 50% VS length.
15.	% Infralabials with dark spot.
16.	%DS width of mid-dorsal spot row at 50% VS length.
17.	%DS width of lateral spot row at 50% VS length.
18.	%VS length of lateral spot at 50% VS length.
19.	%DS height of upper edge of lateral spot row at 50% VS length.
20.	Width of dorsolateral head spot (in scales).
21.	Number of scales between dorso-lateral head spots.

TABLE 1. List of *Vipera russelli* characters used in principal components analysis.

	Burma		Thailand		Significance
	M (n = 17)	F (n = 15)	M (n = 10)	F (n = 9)	
1.	159.2 ± 3.33	160.7 ± 3.24	158.8 ± 4.71	161.4 ± 4.64	n/s
2.	46.9 ± 2.10	41.2 ± 2.86	51.7 ± 5.54	48.0 ± 3.89	P<0.0001
3.	26.6 ± 0.70	26.5 ± 0.83	28.0 ± 0.82	27.7 ± 1.00	P<0.0001
4.	28.9 ± 0.78	28.9 ± 0.52	30.3 ± 1.25	29.9 ± 0.81	P<0.0001
5.	22.9 ± 1.27	23.7 ± 1.18	24.1 ± 0.88	24.4 ± 1.01	P<0.005
6.	20.8 ± 0.66	21.1 ± 0.88	21.4 ± 0.70	21.6 ± 0.86	P<0.05
7.	10.4 ± 0.45	10.7 ± 0.42	10.4 ± 0.39	10.6 ± 0.55	n/s
8.	13.1 ± 0.51	13.5 ± 0.46	12.8 ± 0.48	13.3 ± 0.71	n/s
9.	12.8 ± 1.04	12.8 ± 0.65	13.4 ± 0.70	13.6 ± 0.74	P<0.01
10.	8.1 ± 1.11	8.8 ± 0.41	8.1 ± 0.74	8.6 ± 0.88	n/s
11.	6.3 ± 0.77	6.2 ± 0.86	5.4 ± 0.52	6.1 ± 0.60	P<0.05
12.	6.2 ± 0.44	6.5 ± 0.52	5.8 ± 0.42	6.2 ± 0.67	P<0.05
13.	26.3 ± 1.73	25.3 ± 2.80	23.2 ± 2.03	25.0 ± 1.48	P<0.01
14.	13.6 ± 3.28	12.2 ± 1.74	13.7 ± 1.77	12.4 ± 1.74	n/s
15.	96.4 ± 4.32	94.9 ± 5.85	95.7 ± 9.09	100.0 ± 0.00	n/s
16.	34.1 ± 4.95	36.4 ± 3.31	28.4 ± 2.31	30.3 ± 1.88	P<0.0001
17.	21.0 ± 2.90	22.1 ± 1.84	23.2 ± 2.80	21.9 ± 2.00	n/s
18.	2.5 ± 0.43	2.4 ± 0.47	2.5 ± 0.49	2.5 ± 0.34	n/s
19.	26.3 ± 2.04	26.9 ± 1.46	28.0 ± 1.38	27.2 ± 1.26	P<0.05
20.	7.0 ± 10.00	7.6 ± 0.62	6.8 ± 0.53	6.8 ± 0.79	P<0.05
21.	3.1 ± 0.49	3.1 ± 0.52	3.0 ± 0.67	2.6 ± 0.88	n/s

TABLE 2. Geographic differences in individual characters. Data are means ± SD, and significance of geographic differences of all characters. Numbers of characters as in Table 1. n/s, not significant (P>0.05).

REFERENCES

Anonymous (1987). The flying death of Rajputana - systematics of dangerously venomous snakes. In *Report of the British Museum (Natural History)*, 1984-1985. London: British Museum (Natural History).

Harding, K. A., & Welch, K. R. G. (1980). *Venomous snakes of the World. A checklist.* Oxford: Pergamon Press.

Leviton, A. E. (1980). Museum acronyms -second edition. *Herpetological Review* **11**, 93-102.

Looareesuwan, S., Viravan, C. & Warrell, D. A. (1988). Factors contributing to fatal snake bite in the tropics: analysis of 46 cases in Thailand. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 930-934.

Myint-Lwin, Phillips, R. E., Tun-Pe, Warrell, D. A., Tin-Nu-Swe & Maung-Maung-Lay. (1985). Bites by Russell's viper (*Vipera russelli siamensis*) in Burma: Haemostatic, vascular, and renal disturbances and response to treatment. *The Lancet* **2**, 1259-1264.

Thorpe, R. S. (1987). Geographic variation: a synthesis of cause, data, pattern and congruence in relation to subspecies, multivariate analysis and phylogenesis. *Bolletino di Zoologia* **54**, 3-11.

Warrell, D. A. (1986). Tropical snake bite: clinical studies in southeast Asia. In *Natural toxins - Animal, plant and microbial.* 25-45. Harris, J.B. (Ed.) Oxford: Clarendon Press.

Warrell, D. A. (1989). Snake venoms in science and clinical medicine 1. Russell's viper: biology, venom and treatment of bites. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83**, 732-740.

Warrell, D. A. & Arnett, C. (1976). The importance of bites by the saw-scaled or carpet viper (*Echis carinatus*): epidemiological studies in Nigeria and a review of the world literature. *Acta Tropica* **33**, 307-341.

Wüster, W. (1990). *Population evolution of the Asiatic cobra (Naja naja) species complex.* Unpublished PhD thesis, University of Aberdeen.

Wüster, W. & Thorpe, R. S. (1989). Population affinities of the Asiatic cobra (*Naja naja*) species complex in Southeast Asia: reliability and random resampling. *Biological Journal of the Linnean Society* **36**, 391-409.

Wüster, W. & Thorpe, R. S. (1991). Asiatic cobras: systematics and snakebite. *Experientia.* **47**, 205-209.

HERPETOLOGICAL JOURNAL, Vol. 2, pp. 101-103 (1992)

THE SAND LIZARD, *LACERTA AGILIS*, IN ITALY: PRELIMINARY DATA ON DISTRIBUTION AND HABITAT CHARACTERISTICS

MASSIMO CAPULA AND LUCA LUISELLI

Dipartimento di Biologia Animale e dell'Uomo, Università "La Sapienza" di Roma, Via Borelli 50, 00161 Roma, Italy

(Accepted 30.1.91)

Lacerta agilis Linnaeus is a lacertid lizard whose wide range extends from NE Iberia and W France to central Asia through most of Europe. This species is rare or absent from the European regions characterized by a Mediterranean climate, such as most of the Iberian Peninsula, the Italian Peninsula and S Balkans (Arnold & Burton, 1978; Jablokov, Baranow & Rozanow, 1980; Bischoff, 1984, 1988). The occurrence of the sand lizard in northern Italy has never been reported in the existing distribution accounts on the species (Bischoff, 1984, 1988).

In this note preliminary data on the known distribution and habitat characteristics of the species in northern Italy are reported.

Distribution. Lacerta agilis has been observed and collected only in one locality of NW Italy (Piedmont) and in two localites of NE Italy (Friuli). Fig. 1 shows the approximate locations of the sites at which specimens of *L. agilis* were encountered (for conservation reasons the precise localities of the *L. agilis* populations are not reported). In the locality 1 (numbers refer to Fig. 1), which is sited 50 km NW of Cuneo (Cottian Alps, NW Italy), an adult male was collected; this specimen is now preserved in the Collection of the "Craveri" Natural History Museum of Bra (Cuneo, Piedmont) (Lapini, Morisi, Bagnoli & Luiselli, 1989). In the locality 2, which is sited 6 km east of Tarvisio (Carnic Alps, NE Italy) the species was first observed in July 1987 and then collected in August 1989. Two specimens (two adult females) from this locality are now preserved in the Collection of the Museo Friulano di Storia Naturale of Udine (Friuli). In the locality 3, which lies 14 km south of Tarvisio (Julian Alps, NE Italy), were observed two specimens (two females) during herpetological investigations carried out in the periods July 1987 and August 1989, but none of these specimens was collected and preserved, due to the apparent rarity of the species.

Both the specimens from NE Italy, and those from NW Italy can be probably ascribed to the nominal form, i.e. *Lacerta agilis agilis.* In fact, according to Bischoff (1988) and Rahmel (1988) the subspecific status of *Lacerta agilis argus* - which could occur at least in NE Italy (see Bischoff, 1984) - cannot be supported, since there are no definitive diagnostic characters (morphometric and/or meristic) between this subspecies and the nominal form.

Habitat characteristics. Locality 1 is a broad alpine valley; the grass vegetation of the pastures in which the specimen of *Lacerta agilis* was collected belongs to the *Nardetum strictae*

association. The SW slope of the valley is covered by larch woods, while the NE slope is poorly wooded. This area lies very close to the border between Italy and France, and is geographically connected to the Ubayette Valley (SW France); the latter valley is not far from the locality of Barcelonnette, where the occurrence of *Lacerta agilis* was previously detected (Naulleau, 1978; Castanet, 1989). Owing to the scarcity of the observations carried out on the species in this area, at present we cannot express any hypothesis on the status and density of the local sand lizard population.

Locality 2 is close to the border between Italy and Austria, and is geographically connected to the Gail Valley (SW Austria), which is also inhabited by *Lacerta agilis* (Bischoff, 1984; Cabela & Tiedemann, 1985). The locality 3 lies very close to the border between Italy and Yugoslavia, not far from some Slovenian sites where the sand lizard is known to occurr (e.g., Zelenci, near Podkoren, and Triglav Massif) (Brelih & Dzukic, 1974; Gregori, 1980).

The habitat occupied by *Lacerta agilis* in NE Italy is very similar to that described for the species in central Europe (Podloucky, 1988), i.e. forest margins, field and road edges combined with hedges and/or scrub, and ruderal areas with open shrub vegetation, often with a southern exposure. In localities 2 and 3 *L. agilis* seems to be rather secretive and coexists with *Lacerta vivipara*; the other reptiles observed in the area are: *Anguis fragilis*, *Natrix natrix*, *Coronella austriaca* and *Vipera berus* (Darsa, 1972; Stergulc, 1987).

Lacerta agilis is a new species to the Italian herpetological fauna. The localities discovered in this country establish that the sand lizard is present also on the southern slope of the Alpine Massif.

Although our data show that the distribution of this lacertid lizard in northern Italy is still poorly known, the small number of *Lacerta agilis* specimens encountered up to now could indicate that the species is presumably rare, and that the density of the local populations is relatively low. This working hypothesis needs further investigations.

Since *L. agilis* occurs in some regions of SW Austria (e.g., Tirol, Kärnten: Cabela & Tiedemann, 1985), NW Yugoslavia (e.g., Slovenia: Pavletic, 1964; Brelih & Dzukic, 1974), and southern Switzerland (e. g., Valais and Engadina: Schneppat & Schmocthen, 1987), which are all sited close to the borders of northern Italy, it can be inferred that this species is present in a number of Italian alpine localities maybe more numerous than the three at present known.

Acknowledgements. The authors are gratefully indebted to Claudio Anibaldi, Claudio Bagnoli and Luca Lapini for their valuable help in the field.

REFERENCES

Arnold, E. N. & Burton, J. A. (1978). *A Field Guide to the Reptiles and Amphibians of Britain and Europe*. Glasgow: Collins.

Bischoff, W. (1984). *Lacerta agilis* Linnaeus 1758 - Zauneidechse. In *Handbuch der Reptilien und Amphibien Europas, Band 2/I Echsen (Sauria) II, (Lacertidae II: Lacerta)*, 23-68. Böhme, W. (ed.). Wiesbaden: AULA Verlag.

Bischoff, W. (1988). Zur Verbreitung und Systematik der Zauneidechse, *Lacerta agilis* Linnaeus, 1758. *Mertensiella* **1**, 11-30.

Brelih, S. & Dzukic, G. (1974). Catalogus faunae Jugoslaviae. IV/2. Reptilia. *Con. Acad. Sci. Rep. Slov., Ljubljana* **4**(2), 1-33.

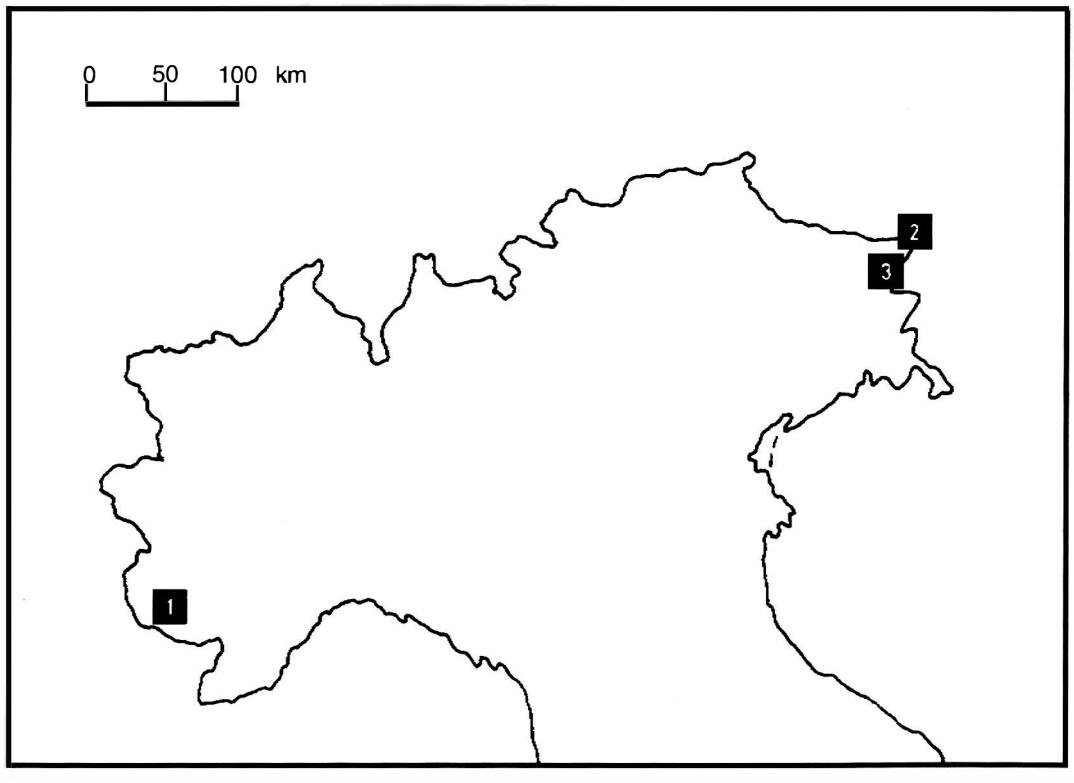


FIG. 1. Locality records of *Lacerta agilis* in northern Italy.

Cabela, A. & Tiedemann, F. (1985). Atlas der Amphibien und Reptilien Österreichs (Stand 1984). *Neue Denkschr. Naturhis. Mus. Wien* **4**, 1-80.

Castanet, J. (1989). *Lacerta agilis.* In *Atlas de repartition des Amphibiens et Reptiles de France*, 126-127. Castanet, J. & Guyetant, R. (eds.). Paris: S. H. F.

Darsa, M. (1972). Anfibi e Rettili di Fusine. *Hyla, Notiz. U.E.I.*, **2** (1), 3-13.

Gregori, J. (1980). Zelenci, izvir Save Dolinke. *Kulturni in naravni spomeniki Slovenije, Zbirka vodnikov, Ljubljana* **97**, 1-31.

Jablokow, A. W., Baranow, S. S. & Rozanow, A. S. (1980). Population structure, geographic variation and microphylogenesis of the Sand Lizard (*Lacerta agilis*). In *Evolutionary Biology* **12**, 91-127. Hecht, M. K., Steere, W. C. & Wallace, B. (eds.). New York, London: Plenum Press.

Lapini, L., Morisi, A., Bagnoli, C. & Luiselli, L. (1989). *Lacerta agilis* Linnè, 1758, specie nuova per la fauna italiana (Reptilia, Squamata, Lacertidae). *Gortania, Atti Mus. Friul. Storia Nat.* **10** (1988), 205-212.

Naulleau, G. (1978). Lézard des souches ou Lézard agile. In *Atlas preliminaire des Reptiles et Amphibiens de France*, 86-87. Castanet, J. (ed.). Montpellier: S. H. F.

Pavletic, J. (1964). Vodozemci i gmizavci. *Hrvatski Narodni Zooloski Muzej, Zagreb* 1-37.

Podloucky, R. (1988). Zur Situation der Zauneidechse Lacerta agilis Linnaeus, 1758 in Niedersachsen - Verbreitung, Gefährdung und Schutz. *Mertensiella* **1**, 146-166.

Rahmel, U. (1988). Untersuchungen zum Unterartcharakter von *Lacerta agilis agilis* Linnaeus, 1758 und *Lacerta agilis argus* (Laurenti, 1768). *Mertensiella* **1**, 31-40.

Schneppat, U. & Schmocker, H. (1983). Die Verbreitung der Reptilien im Kanton Graubünden. *Jber. Natf. Ges. Graubünden* **100**, 47-133.

Stergulc, F. (1987). Anfibi e Rettili della Foresta di Tarvisio. Observazioni preliminari sul popolamento erpetologico di alcuni habitat forestali e montani. In *Vertebrati della Foresta di Tarvisio. I - Saggio Faunistico,* 19-61. Tarvisio: Ministero Agricoltura e Foreste, Corpo Forestale della Stato.

BOOK REVIEWS:

Ecophysiology of Desert Arthropods and Reptiles. J. L. Cloudsley-Thompson. Springer-Verlag, Berlin etc., 203 pp. (1991). £60.00, cloth.

Reptiles have figured prominently in scientific studies of deserts during the past sixty years. There are many reasons for this. Reptiles in deserts are often relatively abundant - certainly when compared with most other vertebrates - and can often be comparatively easy to observe. The effects of harsh climatic conditions on the lives of the animals can be conceptually straightforward: it is easier to visualise the effects of searing heat on a lizard - it may die! - than the results of the subtle interplay of competition, predation, parasitism and environment in shaping the physiology and behaviour of a lizard in a tropical forest or a Mediterranean scrubland.

Reflecting this emphasis of studies, there have been a number of books on desert reptiles during the past decade. They all bear the imprint of the interests and viewpoints of their individual authors. The present book (which also deals with desert arthropods, the two groups being given about equal treatment), is no exception. It could perhaps be summed up as scientific natural history, covering a variety of topics such as *Thermal Regulation and Control* (chapter 4), *Water Balance and Nitrogenous Excretion* (chapter 5) and *Seasonal Activity and Phenology* (chapter 6) in a readable and interesting way. The natural history aspect is emphasized by about seventy monochrome photographs, mostly I would guess taken by the author himself. They increase one's pleasure in reading the book, but I am not convinced that they are strictly necessary.

I must make it clear that I am not using the term "natural history" in the perjorative sense in which it is now sometimes employed. The book does not lack intellectual rigour, and as with many of Cloudsley-Thompson's more serious writings, the reference list is extremely comprehensive. Any book which has "Trophic Level Patterns of Process-Functioning" as a subheading (page 22) is certainly not lightweight!

R. A. Avery
University of Bristol

Neotropical Wildlife Use and Conservation. John G. Robinson & Kent H. Redford (Eds.) (1991) 520 pp. University of Chicago Press, Chicago and London. £49.50 cloth: £22.50 paper.

Several books have been published recently on the sustainable utilisation of wildlife but none has been confined to the neotropics, which have tended to be neglected in discussions of wildlife exploitation. Of the forty-seven authors of the chapters in this book, eighteen live and work in the countries concerned so that one is often reading a first-hand account of the issues being discussed. The theme of the book is that the conservation of wildlife depends on its being used and that wildlife that has no value to local people is unlikely to survive. This is not always appreciated by conservationists in the developed world who may be unaware of the essential role that wild animals have played in the lives of people in tropical societies. The authors include social scientists as well as biologists so that the needs of local people are not overlooked in discussions of wildlife preservation. Many of the chapters show that these are not irreconcilable objectives although certain aspects of the wildlife trade, particularly that concerning live birds, leave much to be desired.

The chapters of the book are grouped into seven "Parts" comprising *Framing the issues, Subsistence hunting, Market hunting and collecting, Wildlife farming and ranching, Sport hunting, Commercial uses* and *The future.* Reptiles are mentioned in several of the chapters but six are concerned solely with reptiles - two on sea turtles, two on caiman and two on lizards. Some of the discussions about the sustainability of the yields come to different conclusions. The exploitation of nesting olive ridley turtles in some beaches of Costa Rica appears to remove only a small proportion of eggs but in Honduras, the loss to egg-collectors can reach 100%. The hunting of Paraguayan caiman for hides was found to have an adverse effect on reproductive success due to disturbance of nesting females. The more successful exploitation of the spectacled caiman in Venezuela may be due to the preferential taking of large males. Proposals to restock areas by releasing young from eggs hatched in captivity are not supported on the

grounds that the caiman are already at carrying capacity. This seems unlikely to continue to be the case once the population is appreciably reduced by cropping.

The book can be highly recommended for its broad coverage and balanced viewpoint. It can have only a beneficial effect on the conservation of neotropical wildlife.

S. K. Eltringham
University of Cambridge

The Habitat of Sand Lizards Lacerta agilis at Merseyside. A. S. Cooke (1991). Research and Survey in Nature Conservation No. 41. 70pp. Nature Conservancy Council, Peterborough, U.K. £6.00, paper.

This publication by A. S. Cooke is one of the Nature Conservancy Council's series entitled *Research and Survey in Nature Conservation*. This particular volume lives up to that title's name in that it is indeed about research and survey. Although 70 pages long, there are only 21 pages of text; the remaining pages being made up of preliminaries, references, a list of other titles in the series and 38 pages of tables and graphs.

The status of the sand lizard in Britain (with a fragmented distribution in the south of England) surely needs no introduction and the isolated population of sand lizards on the coastal dunes of Merseyside (on the northwest coast of England) is well known. In this report, Cooke describes an ecological study of the Merseyside sand lizards which he undertook in the 1980's. He used the well-tried and successful method of collecting data on a range of variables within a certain defined area around each sand lizard that was detected. Observations were made in eight study sites and in three different habitat types. This method generates a lot of information on population habitats and that information has been extensively and well analysed by Cooke. Indeed, sufficient data has been collected for habitat restoration and management. The data could also be used as a base-line for a monitoring programme and I would venture to suggest that a monitoring programme is a must.

The report is an account of much needed, well executed research and the overall presentation of the report cannot be criticised. An analysis of the habitat was the objective but that objective was kept fairly narrow with regard to the structure of the habitat. It would have been useful to extend the research into levels and abundance of prey and into conditions for egg-laying and incubation.

There is a common problem with this series (*Research and Survey in Nature Conservation*) and that is the NCC (as it was) never seemed to be sure if they were aimed at the specialist audience or the lay-audience. Consequently the style suffers and that is certainly the case with Cooke's report. In some parts the report is good objective science but in other parts he seems to be writing for a more general audience. However, my impression is that this volume will be welcomed by anyone concerned with the conservation of reptiles in Britain and that it will also be of interest to those researching on lizard ecology.

However and despite the good science, will this report be used as a basis for the future conservation and management of the Merseyside sand lizards?

Ian Spellerberg
University of Southampton

OBITUARY:

PROFESSOR J. W. PATTERSON

It is sad to report the death of Professor Jerry Patterson from a inoperable brain tumour on 24 July 1991, after a short illness. Jeremy William Patterson was born in Croydon, Surrey on 31 December 1944. He was interested in reptiles from an early age, and joined the British Herpetological Society in 1963. Jerry made many contributions to the herpetology of Britain, Europe and Southern Africa, after a training in general and applied zoology.

Jerry attended St Catherine's College, Cambridge from 1964 to 1967, gaining a B.A. honours degree in Natural Sciences (Zoology). He then took an M.Sc. (with distinction) in Medical Parasitology at the London School of Hygiene and Tropical Medicine (LSHTM), part of the University of London. After his M.Sc. was completed in 1969, Jerry stayed at the LSHTM to work for a Ph.D. This was awarded in 1972, for the thesis "The influence of juvenile hormone mimics on the metamorphosis and fertility of mosquitoes, and other bloodsucking insects". The work, designed to control the insect vectors of tropical diseases, continued during a postdoctoral fellowship at LSHTM from 1971 to 1974. The major results are to be found in a series of seven papers from 1973-1979, mostly in the prestigious *Journal of Insect Physiology*.

Jerry's opportunity to do herpetological research came when he went to the University of Nottingham in 1974. He worked as a postdoctoral fellow with Peter Davies until the end of 1977. Jerry's interests at that time centred on the thermal biology of lizards, and their adaptations to cool climates. Much of the fieldwork was done at Calpe in Spain as described in Jerry's first herpetological paper, published in the *British Journal of Herpetology* in 1977. Most of this work was published in the late 1970s, although some continued to appear up to the time of his death.

Perhaps the major finding of this period was that the pattern of acclimation to temperature in lizards differs from the classic pattern described for aquatic ectotherms. Lizards regulate their daytime body temperatures to high levels, at all seasons when they are active. However, they have no control over body temperature at night, and so this fluctuates substantially through the year. Lizards compensate for these fluctuations by a seasonal change of metabolism at low, but not a high, temperatures.

Jerry's contribution to British herpetology was made during this period, when he worked on *Lacerta vivipara* and *Anguis fragilis*. These lizards were selected as cool temperature examples to pair with the warm temperate *Podarcis hispanica* and *Chalcides bedriagai* from Spain. Jerry was also interested in the British species in their own right. He investigated how the common lizard copes with the long winter, and various aspects of the ecology of the slow worm, an animal in which he had a longstanding interest.

In December 1977 Jerry left for Southern Africa, where he worked for the remainder of his career. His first appointment was as a lecturer in the University of Zambia, which he held until 1980. Jerry then moved to Chancellor College, University of Malawi, where he stayed until 1988, being successively a lecturer, senior lecturer and reader. From 1989 until his death he was an Associate Professor at the University of Zimbabwe.

Most of Jerry's research in Africa was herpetological. In Malawi, he began a study of the adaptations of tropical lizards, work which he was actively publishing at the time of his death. The first results were an extension of his work in Europe. He found that montane skinks which experienced wide annual variation in climate showed acclimation to temperature, but individuals of the same species from lower altitudes did not. He proposed that the low altitude skinks had never been exposed to the cold, and so to selection for the ability to acclimate.

The later African papers focused more on ecological energetics. They marked the progression of his thought from purely physiological questions of growth in insects, through the ecophysiology of adaptation to cold, to purely ecological questions of reproductive allocation. The period from 1989 was particularly productive, and promised to surpass his publication activity of the late 1970s. Jerry's early death is a substantial loss to herpetology in respect of the publication of this earlier work, as well as of his current research.

Jerry was a mine of information on the herpetofauna of Southern Africa, and had many active projects at the time of his death. These ranged from the seasonality of amphibian reproduction, the comparative biochemistry of jumping muscles in anurans, to lizard reproduction and thermal biology. Jerry was always concerned with the care of animals used in his research. It is characteristic that during his final illness, before its seriousness was diagnosed, his major concern was for a group of *Pachydactylus tigrinus*, to be used in a comparison of diurnal and nocturnal geckoes.

Jerry was invited to present a paper at the First World Congress of Herpetology in Canterbury in 1989.

Subsequently he gave papers at meetings of the American Physiological Society and the IUCN African Amphibian Working Group; a measure of the breadth of his knowledge. Jerry also published on biological research and education in Africa. He was a respected figure in Southern African biology, for example being the external examiner in Zoology for the National University of Lesotho. He is survived by a wife and two sons.

Adrian Hailey
University of Zimbabwe

Editor's note: Copies of Professor Patterson's herpetological bibliography are available on request from the editor.

ANNOUNCEMENT:

Applications published in the Bulletin of Zoological Nomenclature

The following Applications were published on 19 December 1991 in Vol. 48, Part 4 of the *Bulletin of Zoological Nomenclature*. Comment or advice on these applications are invited for publication in the *Bulletin* and should be sent to the Executive Secretary, I.C.Z.N., c/o The Natural History Museum, Cromwell Road, London SW7 5BD, U.K.

Case 2552
***Anniella pulchra* Gray, 1852 (Reptilia, Squamata): proposed designation of a neotype**

Robert W. Murphy
Department of Ichthyology and Herpetology, Royal Ontario Museum, I00 Queens Park, Toronto, Ontario, Canada M5S 2C6

Hobart M. Smith
Department of EPO Biology, University of Colorado, Boulder, Colorado 80309. U.S.A.

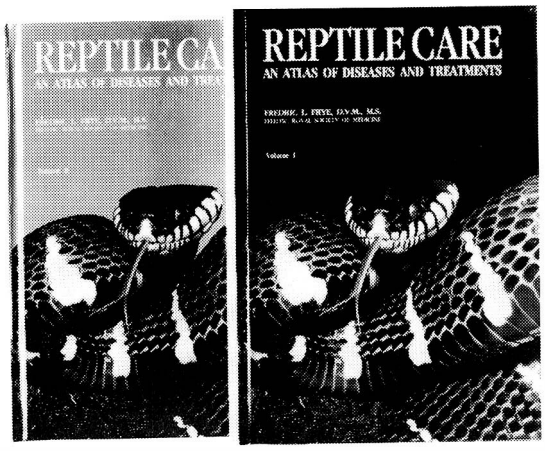
The purpose of this application is to conserve the specific name of *Anniella pulchra* Gray, 1852 in accordance with its accustomed understanding and usage by the designation of a neotype. *A. pulchra* is the type species of *Anniella* Gray, 1852, a genus of fossorial, legless lizards from California and Baja California (Norte), Mexico.

EDITOR'S NOTE:

The Editor is grateful to the following for refereeing manuscripts in 1991:

E. N. Arnold, B. Banks, T. Beebee, W. Branch, G. Brown, J. Cloudsley-Thompson, A. Cooke, J. Cooper, C. Cummins, R. van Damme, J. Davenport, M. Ferguson, T. Graham, A. Hailey, T. Halliday, W. Hodl, U. Joger, A. Malhotra, C. McCarthy, R. Meek, E. Moll, G. Packard, B. Pierce, C. Reading, P. Smith, I. Spellerberg, M. Swan, G. Underwood, B. Viertel, R. Wassersug, K. Wells, P. Weygoldt, P. Zwart.

REPTILE CARE

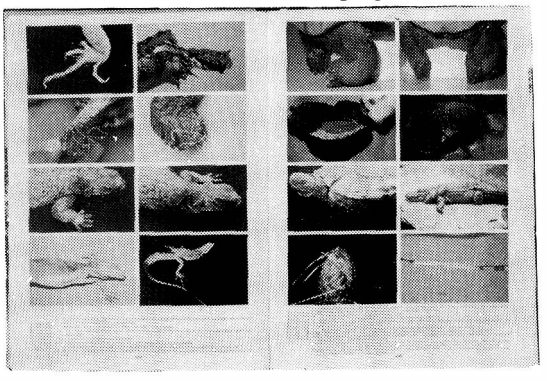


An Atlas of Diseases and Treatments

"A masterpiece that sets high standards for veterinary textbooks" Dr I F Keymer.

Interest in the keeping and husbandry of reptiles has been growing very rapidly over recent years. One of the results of this is that veterinary surgeons, herpetologists and others interested in reptiles are confronted more and more often with medical situations involving these diverse and complex creatures. Dr Fredric Frye is recognised throughout the world as one of the most experienced specialists in the veterinary treatment of exotic species. His new book brings together the very latest understanding about the diagnosis and treatment of disease and injury in reptiles. Reptile Care is a big book (10¼" x 14"), it is published in two volumes and contains 650 pages. In addition to the comprehensive text the book is made particularly valuable by over 1,850 beautifully reproduced full colour technical photographs. It is a book that is very necessary and that fills an important gap in available knowledge. It will be invaluable to all who are involved in the care and husbandry of captive reptiles.

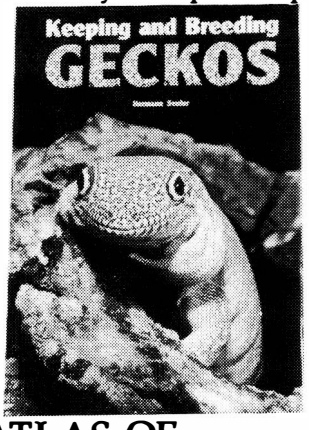
Price £125.00 Code 632



Keeping and Breeding GECKOS

Until now information about Geckos, and particularly, good advice has been hard to come by. This new book brings together the very latest information in a concise and understandable form and will be invaluable to both the interested amateur as well as the dedicated herpetologist.

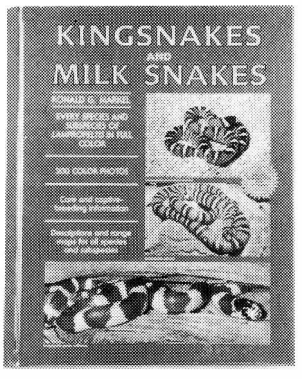
Price £22.95 Code 625

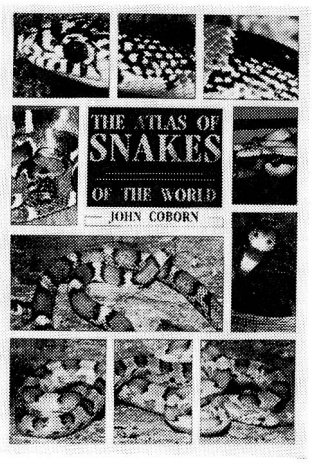


KINGSNAKES AND MILK SNAKES

"If Peter Stafford's book on Pythons and Boas marks a major step forward in herpetological books Markel's book is a quantum leap...for the kingsnake enthusiast this is the book for which you have been waiting."
David Blatchford.

Price £20.95 Code 136





THE ATLAS OF SNAKES of the World.

The Atlas discusses all of the families, subfamilies and genera of snakes and illustrates them with over 1400 full colour photographs.

Price £89.95 Code 620

Title	Code	Price
Pythons and Boas (*Stafford*)	139	£15.49
Atlas of Reptiles and Amphibians for the Terrarium	133	£89.95
Encyclopedia of Reptiles and Amphibians	140	£24.95
Diseases of Reptiles (*Dr E Elkan*)	635	£17.95
Breeding Terrarium Animals (*Elke Zimmermann*)	143	£24.95
Encyclopedia of Turtles (*Pritchard*)	144	£42.95

Post and Packing Free.
Offer Closes 30.9.92.
To order, please complete coupon (or write enclosing relevant details) and send to PSL MAILSALES, FREEPOST, Bideford, Devon, EX39 2YZ.

Please send me the following book/s. I enclose a cheque/P.O. made payable to MAILSALES

Code	Title	Price
	Total	

Name_____
Address_____
_____
_____
_____ Post Code_____

Or Debit my Access/Visa No:

Expiry Date ____

Registered in England No 2212066

Signature_____ HJ6/92

THE HERPETOLOGICAL JOURNAL

INSTRUCTIONS TO AUTHORS

(revised January 1992)

1. The *Herpetological Journal* publishes a range of features concerned with reptile and amphibian biology. These include: full papers (no length limit); reviews and mini-reviews (generally solicited by a member of the editorial board); short notes; controversies, under 'Forum' (details available from the Editor); and book reviews. Faunistic lists, letters and results of general surveys are not published unless they shed light on herpetological problems of wider significance.

2. *Three* copies of all submissions, and illustrations, should be sent to the Editor. All papers will be subject to peer review by at least two referees

3. Authors should consult a recent issue of the Journal regarding style. Papers should be concise with the minimum number of tables and illustrations. They should be written in English and spelling should be that of the *Oxford English Dictionary*. Papers should be typed or produced on a good-quality printer (at least near-letter quality, avoid worn ribbons), and double-spaced with wide margins all round. Typesetting is greatly assisted if accepted manuscripts can be supplied on microcomputer diskettes. Authors are therefore strongly encouraged to produce manuscripts using a wordprocessor (preferably on a PC-compatible microcomputer).

4. For all papers the title page should contain only the following: title of paper; name(s) of the author(s); address of the Institution where the work was done; a running title of 5 words or less. The text of the paper should begin on page 2 and be produced in the following order: Abstract, Text, Acknowledgements, References, Appendices. Full papers and reviews should have the main text divided into sections. Short notes (generally less than six manuscript pages and accompanied by a single data set) should be produced as continuous text. The first subhead will be centred in capitals, the second shouldered in lower case, and the third run on in italics. Footnotes are not permitted.

5. The usual rules of zoological nomenclature apply.

6. Tables are numbered in arabic numerals, e.g. Table l; they should be typed double spaced on separate sheets with a title/ short explanatory paragraph underneath.

7. Line drawings and photographs are numbered in sequence in arabic numerals, e.g. Fig. 1. Colour photographs can only be included at cost to the author. If an illustration has more than one part each should be identified as (a), (b), etc. The orientation and name of the first author should be indicated on the back. They should be supplied camera-ready for uniform reduction of one-half on A4 size paper. Line drawings should be drawn and fully labelled in Indian ink, dry-print lettering or laser printed. A metric scale must be inserted in micrographs etc. Legends for illustrations should be typed on a separate sheet.

8. References in the text should be given as in the following examples: "Smith (1964) stated ..."; "...as observed by Smith & Jones (1963)." "...as previously observed (Smith, 1963; Jones, 1964; Smith & Jones, 1965)". For three or more authors, the complete reference should be given at the first mention, e.g. (Smith, Jones & Brown, 1972), and *et al.* used thereafter (Smith *et al.*, 1972). For the list of references the full title or standard abbreviations of the journal should be given. The following examples will serve to illustrate the style and presentation used by the Journal.

   Bellairs, A. d'A. (1957). *Reptiles.* London: Hutchinson.

   Boycott, B. B. & Robins, M. W. (1961). The care of young red-eared terrapins (*Pseudemys scripta elegans*) in the laboratory. *British Journal of Herpetology* **2**, 206–210.

   Dunson, W. A. (1969a). Reptilian salt glands. In *Exocrine glands*, 83–101. Botelho, S. Y., Brooks, F. P. and Shelley, W. B. (Eds). Philadelphia: University of Pennsylvania Press.

   Dunson, W. A. (1969b). Electrolyte excretion by the salt gland of the Galapagos marine iguana. *American J. Physiol.* **216**, 995–1002.

9. Final acceptance of a paper will depend upon the production by the author of a typescript and illustrations ready for the press. However, every assistance will be given to amateur herpetologists to prepare papers for publication.

10. Proofs should be returned to the Editor by return of post. Alterations should be kept to the correction of errors; more extensive alterations will be charged to the author.

11. Twenty-five offprints and one complimentary copy of the Journal are provided free of charge. Further copies (minimum of twenty-five) may be purchased provided that they are ordered at the time the proofs are returned.

12. All submissions are liable to assessment by the editorial board for ethical considerations, and publication may be refused on the recommendation of this committee. Contributors may therefore need to justify killing or the use of other animal procedures, if these have been involved in the execution of the work.

THE HERPETOLOGICAL JOURNAL

Volume 2, Number 3 1992

CONTENTS

Herpetological Journal vol. 2, no. 2 was published on 30 April 1992