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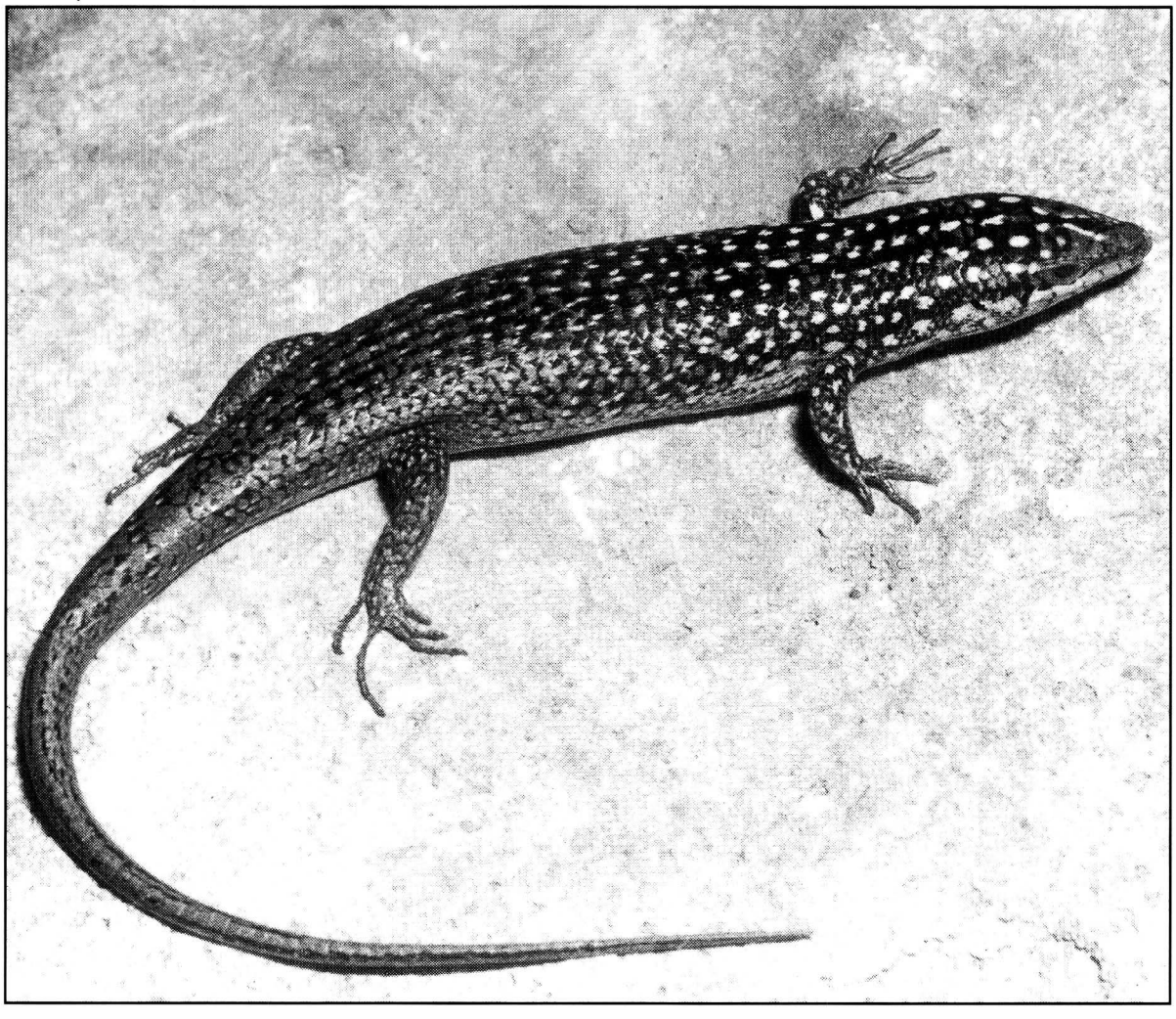

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FRONT COVER: *Mabuya vezo* (see Ramanamanjato *et al.*, pp. 65-71)

HERPETOLOGICAL JOURNAL, Vol. 9, pp. 37-42 (1999)

GROWTH AND ENERGETICS OF EMBRYOS OF THE GECKO, *PHYLLODACTYLUS MARMORATUS*, A SPECIES WITH HARD-SHELLED EGGS

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We measured water contents, growth of embryos and metabolic rates in hard-shelled eggs of the Australian gecko, *Phyllodactylus marmoratus*, throughout incubation to make comparisons between (1) the proportional water content at oviposition of eggs of *P. marmoratus* and flexible-shelled eggs of lizards; and (2) the dry-mass specific energy consumption during development in *P. marmoratus* and lizards with flexible-shelled eggs. Egg contents (i.e. excluding eggshell) contained nearly 80% water, higher than reported for any other squamate reptile. Eggs were laid at embryonic stages 26/27-29, which is slightly earlier than for most other lizards. Incubation lasted 79-84 days at 25 °C and net water loss averaged just under 3 mg. Metabolism reflected the size of embryos, with little growth and low rates of oxygen consumption during the first third of incubation. Thereafter, growth and oxygen consumption increased, with oxygen consumption slowing after day 70. This pattern is similar to that of other species of lizard. Water content of embryos fell from above 90% early in incubation to around 70% at hatching. Thus, the embryonic metabolic scaling factor was different when based on embryonic wet and dry mass. The dry-mass specific energetic cost of development in *P. marmoratus* was lower than other lizards, but this result was not related to having a hard-shelled egg. The respiratory exchange ratio suggests that embryonic metabolism is based on mixed protein and lipid, a pattern similar to that in flexible-shelled eggs of lizards, but different from birds.

Key words: *Phyllodactylus*, gecko, embryonic development.

INTRODUCTION

Geckos of the subfamily Gekkoninae are unusual among lizards because they lay eggs with hard calcareous shells (Packard, Tracy & Roth, 1977). Hard-shelled eggs of geckos have low conductances to gases (Dunson & Bramham, 1981; Dunson, 1982), which enables them to incubate in dry environments, such as under the bark of trees or in crevices among rocks, without desiccating. The unusual eggshell and "nest" environment suggest that other aspects of their development may be unusual, also.

The flexible-shelled eggs of most lizards absorb water during development (Packard, Packard & Boardman, 1982; Packard, Packard, Miller, Jones & Gutzke, 1985) and it is possible that many species must absorb water for proper development (Vleck, 1991; Ji, 1992). In contrast, water uptake by hard-shelled eggs of reptiles is minimal, if it occurs at all (Packard *et al.*, 1977). One would expect, therefore, that the water content of hard-shelled eggs might be higher than that of flexible-shelled eggs at the time of oviposition because the hard-shelled eggs are unable to supplement their water content during incubation.

The marbled gecko, *Phyllodactylus marmoratus*, is an Australian species of gekkonine lizard that lays hard-shelled eggs. It occurs in the "wetter parts of southern Australia" (Cogger, 1992). The reproductive biology of *P. marmoratus* is well known (King, 1977; Doughty & Thompson, 1998), with mating occurring in autumn and females storing sperm over winter. Ovulation and fertilization using stored sperm occur in spring. This reproductive strategy makes *P. marmoratus* an ideal subject for studies of eggs, because females can be collected in spring and maintained in the laboratory in the absence of males until oviposition occurs.

We quantified the water content of fresh eggs, measured changes in embryonic water contents, and described embryonic growth in hard-shelled eggs of *P. marmoratus* to compare available data for species with flexible-shelled eggs. In addition, we measured embryonic metabolism (rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) throughout development to compare metabolic ontogeny in *P. marmoratus* with species of lizards with flexible-shelled eggs. These measurements allowed us to estimate total energy expenditure during development and to identify the energy substrate that fuels embryonic development. In particular, we compared the mass-specific energy consumed during development of embryonic *P. marmoratus* and lizards with flexible-shelled eggs.

MATERIALS AND METHODS

Gravid female geckos were collected in November, 1993, from beneath the bark of River Red Gum trees, *Eucalyptus camaldulensis*, along the banks of the River Murray in South Australia, between Murray Bridge and Blanchetown. Lizards were transported to the Univer-

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sity of Adelaide on the day of capture and housed individually in containers of 130 mm x 220 mm x 75 mm high in a room at 20-25°C, as described by Doughty & Thompson (1998). A window in the room ensured a natural light cycle and each container was heated from beneath at one end with electric heating cable to 32°C for 12 hours of the light phase.

Containers were inspected every morning for animal maintenance and to check for eggs, which were immediately removed from the cage without rotation. A unique number was written on each egg with a 3B graphite pencil and the egg was weighed on a mg balance. Eggs were placed in a box containing moistened Terra-lite grade 3 vermiculite with a water potential of -150 kPa, as determined using thermocouple psychrometry with a Wescor HR-33T microvoltmeter and C52 sample chamber. Eggs were placed on the surface of the vermiculite with 13-20 eggs per box. Each incubation box had a closely fitting lid and was incubated at 25±0.2°C. Individual eggs and the incubation box were weighed weekly and any mass loss from the box, assumed to be due to water loss (Packard, Packard, Miller & Boardman, 1988), was compensated for by the addition of distilled water. Incubation boxes were moved within the incubator daily to control for any undetected temperature variation within the incubator.

Six eggs were weighed and frozen whole on the day of laying (day zero) and subsequently lyophilized to give water content of whole eggs. Ten other eggs were dissected on the day of oviposition and the embryos were separated from the contents and the shells. Fresh whole wet egg mass and separate dry masses of eggshell and contents were determined for these eggs. Twenty-nine eggs, covering most embryonic stages, were dissected for embryonic staging between day 3 and day 75, after having their rate of metabolism measured (see below). A further nine eggs were dissected during incubation without metabolic rate being measured. Embryos were staged according to the scheme of Dufaure and Hubert (Porter, 1972), weighed to 0.01 mg and stored frozen until lyophilized in a Dynavac Freeze Drier Model FD-5. Water content was then calculated by subtraction. Wet and dry mass determination of yolk-free embryos and hatchlings exclusive of extra-embryonic fluid allowed calculation of water content of embryonic tissues.

In addition to the eggs that were dissected throughout incubation, we measured the rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) approximately twice per week in 14 eggs throughout incubation, using closed system respirometry as described by Thompson & Russell (1998). Eggs were sealed into glass jars of known volume at 25°C. Gas samples from the jars were analysed using an Ametek S-3A/II oxygen analyser and Ametek CD-3A carbon dioxide analyser. Output from the analysers was recorded on an IBM compatible PC computer using Datacan (Sable System Software). Barometric pressure was measured with a Compensiert

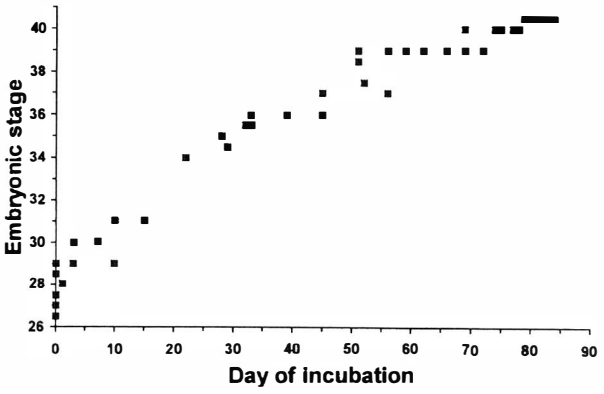

FIG. 1. Embryonic stage measured against time of incubation for *Phyllodactylus marmoratus* at 25 °C. Stages are based on tables of Dufaure and Hubert (Porter, 1972). Intermediate stages are arbitrarily assigned a 0.5 score (i.e. stage 38/39 is scored as 38.5) and hatchlings are scored as stage 40.5.

barometer and all values of $\dot{V}O_2$ and $\dot{V}CO_2$ converted to STPD. Volume of gas in the respirometry chambers was adjusted for the volume of the egg by assuming an egg density of 1 (Douglas, 1990). Respiratory exchange ratio (RE) was calculated by dividing $\dot{V}CO_2$ by $\dot{V}O_2$. Total energetic cost of development was estimated by plotting $\dot{V}O_2$ for individual eggs against incubation period, joining the points and calculating the area of the enclosed polygon. A mean value was then calculated from these individual estimates.

On the day of hatching, embryos were killed by cervical dislocation. Wet mass was measured to 0.01 mg, the hatchlings were dissected and internal yolk removed and weighed separately. All samples were stored frozen until lyophilized.

Means are presented ±1 SE and comparisons between means made using *t*-test. Linear regressions were done using the method of least squares and statistical significance was assumed if $P < 0.05$.

RESULTS

Phyllodactylus marmoratus layed one (26%) or two (74%) eggs. Incubation period at 25°C was 79-84 d (mean = 81.4±0.3 d, mode = 81 d, $n = 18$). Eggs were laid at embryonic stage 26/27-29 with most (seven of ten) being at stage 27 or 27/28. Most differentiation oc-

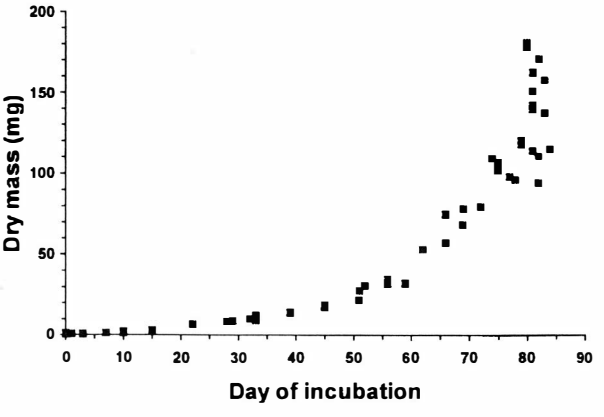

FIG. 2. Dry mass of embryos and yolk-free hatchlings of *Phyllodactylus marmoratus* during incubation.

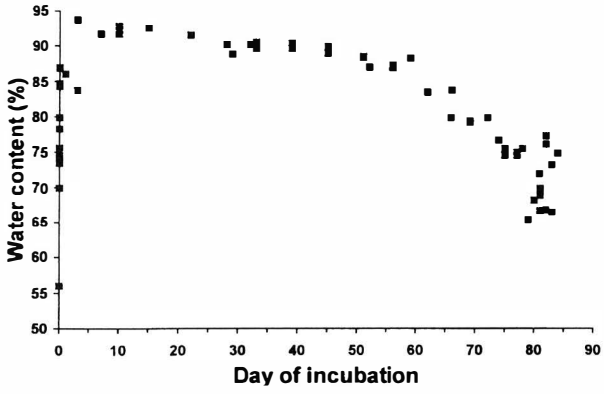

FIG. 3. Water content of embryos of *Phyllodactylus marmoratus* throughout incubation.

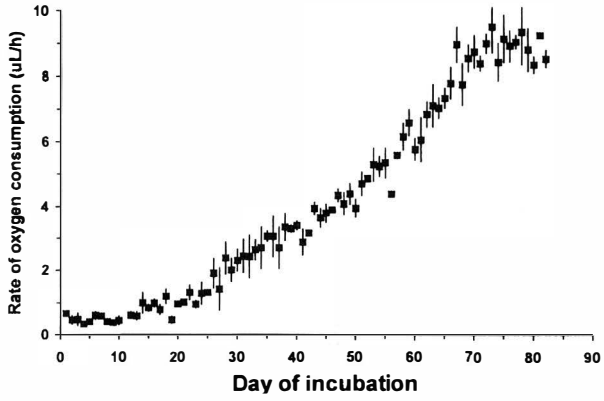

FIG. 4. Mean metabolic rates of eggs of *Phyllodactylus marmoratus* oviposited in the laboratory throughout incubation. Bars represent 1 SE.

curred during the first 50 days and most embryos had reached the penultimate embryonic stage (Stage 39) by day 50 (Fig. 1). Embryonic growth was very slow until about day 50, after which growth accelerated rapidly and continued to increase until hatching (Fig. 2). Water content varied among day 0 embryos (Fig. 3). Water content of fresh whole eggs was 65.7±1.6% (n=6) and of the egg contents (i.e. egg minus the eggshell) was 77.9±0.9% (n=10). Initial mass of eggs killed on day 0 was not significantly different from initial mass of eggs allowed to hatch (t = 0.43, P>0.05). Water content of embryos was above 90% (Fig. 3) from day 3 to about day 50, after which it fell to 70.6±1.0% (n = 14; range = 65.4 - 77.4%) at hatching. The decrease in water content (Fig. 3) mirrors the increase in embryo size (Fig. 2). Egg mass remained almost constant during incubation. Mean initial egg mass was 628 ±8 mg (Table 1) for all eggs available and mean change in egg mass was a fall of 2.7±0.5 mg (range = -6 - +1 mg, n = 18) for all eggs that hatched. Yolk-free wet mass of hatchlings was 473±17 mg and yolk-free dry mass was 137±7 mg (Table 1).

Metabolic rates (Fig. 4) reflected embryonic growth (Fig. 2). Rates rose slowly from about 0.5 μLh^{-1} to about 1.0 μLh^{-1} for the first 25 days (or 30% of incubation). Thereafter, metabolic rate increased rapidly to reach a plateau of 8-10 $\mu L.h^{-1}$ between day 70 and hatching (Fig. 4). Integration of metabolic curves gave an estimate of 78.1±1.5 ml of O_2, equivalent to 1.51 ±0.03 kJ of energy (Table 1). Thus, the dry mass-specific energy consumption during development was 11.3 ±0.5 kJg^{-1} (Table 1).

TABLE 1. Total oxygen consumption, its energy equivalent and energy consumed per g (mass-specific cost) of dry mass by eggs of *Phyllodactylus marmoratus*. Means are given ±1SE.

	Mean±SE	N	Range
Wet egg mass (mg)	628±8	74	499-774
Hatchling wet mass (mg)	473±17	14	356-571
Hatchling dry mass (mg)	137±7	14	95-181
Total VO_2 (ml)	78.7±1.53	14	67.35-87.87
Energy equivalent (J)	1508±29	14	1292-1684
Mass-specific cost (kJg^{-1})	11.3±0.5	14	8.8-15.2

A linear regression for RE plotted against day of incubation had a slope not significantly different from zero (F = 0.29, 1 df, P = 0.592) and an intercept of 0.77. For interpretation of our value of R, we have assumed that all metabolism is aerobic, that the only metabolic substrates are lipid and protein, and that the nitrogenous waste is stored as urea (Thompson & Russell, 1998). Based on the energetic equivalent of oxygen for different respiratory substrates (Schmidt-Nielsen, 1990), we estimate from an RE of 0.77 that embryonic energy metabolism in *P. marmoratus* is derived 40% from lipid and 60% from protein and has an energy equivalent of 19.163 kJL^{-1} of O_2.

DISCUSSION

EMBRYONIC STAGE, GROWTH AND DIFFERENTIATION

Embryos of *P. marmoratus* are at a slightly earlier stage of development (about stage 27) than is typical for eggs of lizards (Shine, 1983). The reason for the difference between *P. marmoratus* and other species is not known, but may be common to geckos with hard-shelled eggs. Although the embryonic stage at oviposition was not actually reported in hard-shelled eggs of *Gekko japonicus*, it is very likely that, like *P. marmoratus*, they are at a relatively early stage of development because rates of oxygen consumption are too low to be detected when the eggs are first laid (Ji, 1992).

Differentiation of embryos is rapid after oviposition. Embryos are at the stage (29-31) normally associated with oviposition in lizards by day 5-10, although there is clearly some variation presumably associated with variation in stage at oviposition (Fig. 1). Differentiation continues rapidly until stage 36 at about 35 days. Thereafter, differentiation proceeds more slowly, with embryos spending longer at each stage, a pattern that is common among lizards (Thompson & Stewart, 1997).

In contrast to differentiation, growth of embryos is slow for the first half or more of incubation, after which

it accelerates (Fig. 2). This pattern in *P. marmoratus* is almost identical to that for *I. iguana* (Ricklefs & Cullen, 1973), a comparison that is particularly relevant given the similarities of incubation periods in *I. iguana* and *P. marmoratus*. The general pattern for these species is similar to that of altricial birds (Vleck & Hoyt, 1991), which is surprising given that lizards are essentially precocial. Embryonic growth of a dragon lizard, *Pogona barbata* (as *Amphibolurus barbatus*) (Packard *et al.*, 1985) shows broad similarity to growth of *I. iguana* and *P. marmoratus*, although there may be a slowing of growth towards the end of incubation to give a logistic growth curve, rather than the exponential curve of *I. iguana* (Vleck & Hoyt, 1991). Growth in the skink *Eumeces fasciatus* slows even more than in *P. barbata* late in development (Thompson & Stewart, 1997) to give a pattern similar to that of precocial birds. Calculation of individual growth rates for *P. marmoratus,* using the methods described by Hoyt (1987), shows that there is a decline in growth rate after a peak of 4.2 mg dry mass/day on day 71, although this decline is obscured by variation in sizes of embryos in Fig. 2.

The time of most rapid growth (Fig. 2) coincides with the time of slowest differentiation in *P. marmoratus* and *E. fasciatus* (Thompson & Stewart, 1997; Fig. 1) and with the most rapid loss of percentage water content of embryos in *P. marmoratus* and *I. iguana* (Ricklefs & Cullen, 1973; Fig. 3). Presumably, this is the time of ossification of bone and deposition of fat from reserves in the yolk, both tissues with low water contents.

Water Content and Mass Changes During Development

Eggs of *P. marmoratus* change in mass very little during development, with a mean net loss of water of only 2.7±0.5 mg. Flexible-shelled eggs incubated at a water potential similar to that used for *P. marmoratus* would gain water for the first half or more of incubation and then lose some prior to hatching, resulting in a net uptake of water during incubation (Packard *et al.*, 1985). No gain of mass was measured at any time during development. The small loss of mass in *P. marmoratus* presumably reflects the low shell conductance of hard-shelled eggs of geckos (Dunson & Bramham, 1981; Dunson, 1982) and is probably typical of hard-shelled eggs of other gekkonine lizards. The metabolism of both lipid and protein results in the production of water (Withers, 1992). Since there was a small net loss of water during development, all the metabolic water generated during development must also have been lost.

Although the water contents of eggs of many species of birds have been reported as a percentage of mass of egg contents, excluding the shell, it is not clear that data for most reptiles do not include the shell (Vleck, 1991). Knowledge of the mass and water content of eggs independent of the shell is important in reptiles because some species, such as *P. marmoratus,* with a calcareous shell have a greater relative shell mass than others with flexible-shelled eggs that lack extensive deposits of calcium. There are, however, some data for water content of egg contents for flexible-shelled eggs for comparison with eggs of *P. marmoratus*. The mean water content of fresh eggs (excluding the shell) for four species of squamates (the agamid lizard, *Pogona barbata*, the snake *Coluber constrictor* (Packard *et al.*, 1985) and the skinks *Eumeces fasciatus* (Thompson & Stewart, 1997), *Menetia greyii* (Thompson & Russell, 1998)) is 64.5±3.9% (range: 59.0 - 75.9%). Only one of those is above 70% (*M. greyii*) and all contain a smaller proportion of water than eggs of *P. marmoratus*. Thus, eggs of *P. marmoratus* contain relatively more water than other species so far studied, but comparative data are few. Eggs of another species with hard-shelled eggs, *Gekko japonicus*, are reported to be 74% water, but the report does not make clear whether the measurement includes the eggshell (Ji, 1992). If the value represents shell-free egg contents, then the water content of *G. japonicus* and *P. marmoratus* are very similar; if the value includes the shell, then eggs of *G. japonicus* would contain proportionally more water than eggs of *P. marmoratus*, strengthening the suggestion that hard-shelled eggs have high relative water contents at the time of oviposition. The water content of flexible-shelled eggs of squamates is much lower than that of altricial (mean = 84.3% water) and precocial (mean = 74.7% water) birds (Vleck, 1991), whereas the water content of eggs of *P. marmoratus* is similar to, and well within the range of values for precocial birds (Vleck & Hoyt, 1991). Since water uptake does not occur in eggs of *P. marmoratus* during development, and since neonates of *P. marmoratus* are precocial in an avian sense, this similarity is not unexpected.

The variation in estimates of water content of embryos on day zero reflects the very small mass (Fig. 2) and fragility of early embryos. Thus, small errors in measurements from inadvertent inclusion of small amounts of yolk may result in proportionally large errors in the estimate of water content. Nevertheless, all estimates on day zero are lower than those from days 3 - 50, so it is likely that early embryos rapidly take up water. Embryos of *Iguana iguana* also take up water during the first few days of incubation (Ricklefs & Cullen, 1973). Thereafter, although embryonic *I. iguana* generally contain relatively more water than *P. marmoratus*, they show a similar decline in relative water content (Ricklefs & Cullen, 1973).

Metabolic Rates

Because of the large change in percent-age water content of embryos during development, we plotted regressions of rates of oxygen consumption against wet

and dry mass for all embryos and hatchlings that were killed immediately after measurements (n = 35). The resulting regressions are:

$$\ln \dot{V}O_2 = 0.18 + 0.69 \ln \text{wet mass}$$
($r^2 = 0.89$, $F = 273.39$, 1 df, $P < 0.001$)

$$\ln \dot{V}O_2 = 2.30 + 0.45 \ln \text{dry mass}$$
($r^2 = 0.88$, $F = 235.05$, 1 df, $P < 0.001$)

where $\dot{V}O_2$ is in $\mu L h^{-1}$ and dry mass is in mg. A result of the changes in water content of embryos during development is that the metabolic scaling factor for $\dot{V}O_2$ is different for wet embryos (0.69) and dry embryos (0.45). Both scaling factors are lower than the 0.76 for dry mass of the scincid lizard *Eumeces fasciatus* (Thompson & Stewart, 1997). The relevance of the different scaling factors in *P. marmoratus* and *E. fasciatus* is not known.

The shape of the curve that describes the increase in $\dot{V}O_2$ during incubation is similar to that for other species of lizards (e.g. Thompson & Stewart, 1997) with little rise for the first part of incubation (about 14 days in *P. marmoratus*), followed by a rapid rise until late in development when the rate of increase slows to reach a plateau. There appears to be no decline in $\dot{V}O_2$ late in incubation (Fig. 4) as there is in some species of reptiles (Thompson, 1989). $\dot{V}O_2$ levels off at about the same time (day 71, Fig. 4) that growth rate declines, a pattern typical of precocial birds (Hoyt, 1987) and some other lizards (Thompson & Stewart, 1997).

The dry mass-specific energy consumption during development in *P. marmoratus* is lower (11.3±0.5 kJg^{-1}) than shown in any other species of lizard (range: 12.4 $kJ.g^{-1}$ for *Eumeces fasciatus* to 19.6 kJg^{-1} for *Morethia adelaidensis*) (Ji, 1992; Thompson & Russell, 1999; Thompson & Stewart, 1997; Thompson, Speake, Russell & McCartney, 1998*a*; Vleck & Hoyt, 1991) but is within the range (9.7-21.9 kJg^{-1}) for birds (Booth & Thompson, 1991). This result, however, is probably not associated with the hard eggshell, because the dry mass-specific cost of development in another gecko with hard-shelled eggs, *Gekko japonicus*, is 15.2 kJg^{-1} (Ji, 1992), close to the mean of 15.6 1.1 kJg^{-1} reported for lizards (Thompson & Russell, 1999).

Respiratory Exchange Ratio and Metabolic Substrate

An important result from the metabolic measurements is that RE is above 0.71, confirming that mixed protein and lipid is used as a metabolic substrate during incubation. Similar values of RE have been reported in other lizards (Thompson & Russell; 1999; Thompson & Stewart, 1997). Considering the relatively lower energy density of protein compared to lipid (Schmidt-Nielsen, 1990) and the small importance of protein as a metabolic substrate for embryonic birds and turtles (Romanoff, 1967; Rahn & Ar, 1974; Thompson, Speake, Russell, McCartney, 1998*b*), it is puzzling why lizards rely so heavily on metabolism of protein to fuel embryonic development. Greater reliance on lipids as a metabolic fuel would enable a smaller egg of equivalent energy density to be produced. It appears, however, that female *P. marmoratus* are able to accommodate a range of egg sizes (Doughty & Thompson, 1998). The striking similarity of the proportion of lipids and protein used as metabolic substrates during development in *P. marmoratus* and species with flexible-shelled eggs (Vleck & Hoyt, 1991; Thompson & Stewart, 1997; Thompson & Russell, 1999) suggests that the utilization of a mixed metabolic substrate during development may be general in lizards.

CONCLUSION

This study has shown that the hard-shelled eggs of *P. marmoratus* share aspects of embryonic growth, metabolism and metabolic substrates with other lizards. The main differences thought to be associated with having a hard eggshell are the initial water content and loss of water during incubation. The utilization of protein as a major metabolic substrate during development is similar to other species. Further comparative study of the relationships of metabolism of protein, metabolic water production and net water exchanges with the environment in both hard-shelled and flexible-shelled eggs during incubation is required to understand the basis of the difference in the relative use of lipids and proteins as energy substrates in embryos of lizards, compared to turtles (Thompson *et al.*, 1998*b*) and birds (Rahn & Ar, 1974).

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REPRODUCTIVE TRAITS OF TWO SYMPATRIC VIVIPAROUS SKINKS (*MABUYA MACRORHYNCHA* AND *MABUYA AGILIS*) IN A BRAZILIAN RESTINGA HABITAT

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The reproductive cycles, fat body cycles and some life-history traits of the sympatric viviparous skinks *Mabuya macrorhyncha* and *M. agilis* were compared in a seasonal "restinga" habitat of south-eastern Brazil. Both male and female reproductive and fat body cycles are very similar between species, with gestation lasting 9-12 months and parturition occurring during the early wet season. Clutch size of *M. macrorhyncha* was smaller than that of *M. agilis*. Females mature at a larger size in *M. macrorhyncha* than in *M. agilis*, but males of both species appear to mature at similar sizes. In both species, females are larger than males, but the latter have proportionately larger heads. Reproductive traits of *M. agilis* are typical of Neotropical *Mabuya*, but those of *M. macrorhyncha* have some peculiarities, one of which (small clutch size) is believed to result from constraints imposed by its morphological adaptation (i.e. relatively flattened body plan) to bromelicolous habits.

Key words: Reproduction, life history, *Mabuya*, Brazilian skink

INTRODUCTION

The genus *Mabuya* is one of the most speciose and widely distributed genera of the Scincidae, with about 90 species found over most of tropical Africa, Asia, and America (Shine, 1985; Nussbaum & Raxworthy, 1994). Not surprisingly, this genus is also extremely diverse ecologically, with species utilizing a wide variety of habitats and microhabitats (e.g. Huey & Pianka, 1977; Castanzo & Bauer, 1993; Nussbaum & Raxworthy, 1994, 1995; Ávila-Pires, 1995; Vrcibradic & Rocha, 1996). Concomitant with such geographical and ecological radiation, lizards within this genus have developed a considerable diversity of reproductive characteristics in different reproductive modes that includes both oviparity and viviparity (Fitch, 1970, 1985; Vitt & Blackburn, 1983; Shine 1985; Blackburn & Vitt, 1992). Although both viviparous and oviparous *Mabuya* species occur in the Old World, only viviparous forms are found in the New World (Fitch, 1985; Shine, 1985; Vitt & Blackburn, 1983, 1991; Blackburn & Vitt, 1992). Actually, New World *Mabuya* seem to constitute a monophyletic group derived from an African lineage (Greer, 1970; Shine, 1985; Bauer, 1993) and, apparently, all of its species possess a peculiar type of viviparity characterized by an extreme degree of placental nutrient transfer that is unique among squamates (Shine, 1985; Blackburn & Vitt, 1992). The great diversity of reproductive features among *Mabuya* species makes the study of reproduction in lizards of this genus very informative, as many questions about ecological and evolutionary reproductive responses may be addressed. Special interest should be paid to the analysis of reproductive patterns of sympatric *Mabuya*, from which present differences may highlight those historical forces that may select for certain reproductive characteristics. Indeed, differences in reproductive characteristics among sympatric and allopatric populations of congeners have been reported for this genus in Africa (Huey and Pianka, 1977; Pianka, 1986).

At many localities along the coastal sand plains of south-eastern Brazil, which are characterized by sand-dune habitats (restinga), two species of *Mabuya* usually occur sympatrically: *Mabuya macrorhyncha* and *M. agilis* (Araújo, 1991, 1994; Rocha & Vrcibradic, 1996; Vrcibradic & Rocha, 1996). Some information on reproductive traits exists for the former species (Vanzolini & Rebouças-Spieker, 1976; Rebouças-Spieker & Vanzolini, 1978; Zanotti, Sant'Anna & Latuf, 1997) and virtually none for the latter. Although living sympatrically in these habitats and being similar in body size, these species differ markedly in morphology and microhabitat use, with *M. macrorhyncha* showing some tendency to scansoriality and living mainly on and among ground bromeliads. On the other hand, *M. agilis* is a ground-dweller which basks and forages on leaf litter (Rocha & Vrcibradic, 1996; Vrcibradic & Rocha, 1996). The comparatively more flattened body and head, and longer fingers in *M. macrorhyncha* have been suggested to be adaptative traits related to its habit of living among bromeliads (Vrcibradic & Rocha, 1996). Flattening of the body plan to suit ecological specializations (such as life in rock crevices) may impose some reproductive constraints to females of some lizard species (Broadley, 1974; Vitt, 1981).

We analysed the reproductive ecology and sexual dimorphism of *Mabuya macrorhyncha* and *M. agilis*, specifically addressing the following questions: (1) Do the two sympatric species differ in their reproductive and fat body cycles at Barra de Maricá? (2) Does litter size differ between species and is it correlated with fe-

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male body size within species? (3) At what size do males and females attain sexual maturity in each species? (4) Within species, is monthly variation in fat body mass more conspicuous in females than in males, considering the low energetic demands of sperm production (see Blem, 1976) compared to gestation? (5) Are the species sexually dimorphic?

MATERIALS AND METHODS

STUDY AREA AND CLIMATE

Field work was carried out at the Barra de Maricá restinga (22° 57' S, 43° 50' W), 38 km east of Rio de Janeiro city in the Rio de Janeiro State, SE Brazil. Restingas are Quaternary sand-dune habitats covered with herbaceous and shrubby vegetation, common along the Brazilian coast (Suguio & Tessler, 1984; Eiten, 1992). The area has marked tropical seasonality (Fig. 1), with a wet season between October and March and a dry season between April and September (Franco *et al.*, 1984; Rocha, 1992). The mean annual temperature varies between 22 and 24°C and the mean annual rainfall ranges from 1000 to 1350 mm (Nimer, 1979).

COLLECTING METHODS AND ANALYSIS

Lizards were collected monthly from May 1989 to April 1992 with an air rifle. Shots were always directed to the head and neck of the lizards, in order to kill them immediately. Each lizard was then immediately transferred to a plastic sac containing cotton soaked in ether. This was done in order to quickly anaesthetize lizards that may not have been killed instantaneously and to ensure a painless death. Shooting was by far the most efficient method we found to collect *Mabuya* in a restinga area characterized by large patches of vegetation. Catching them by hand or with elastic bands when they are basking is very difficult (*M. macrorhyncha* usually basks on the spiny-edged leaves of ground bromeliads) and noosing them is practically impossible (the lizards are very skittish and usually retreat to the interior of thickets of vegetation or bromeliad patches when they sense any disturbance). The characteristics of the study area also make the use of pitfall and drift fence traps not feasible, since the patches of vegetation are too dense compared to another restinga areas where we have successfully used such technique to catch skinks (see Vrcibradic & Rocha, 1995 for more details).

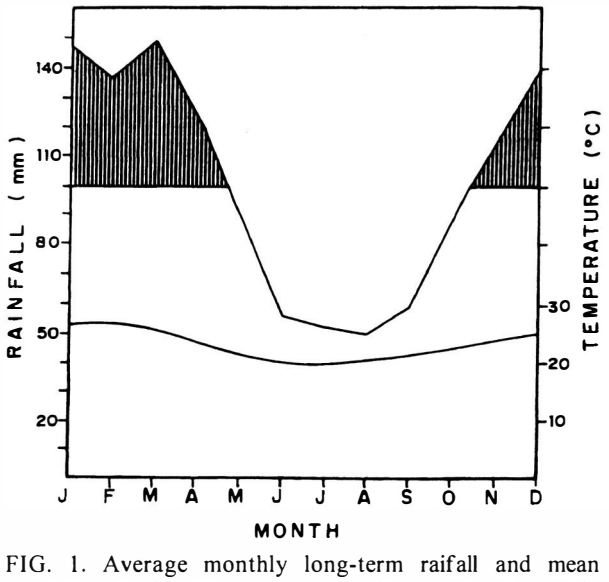

FIG. 1. Average monthly long-term raifall and mean temperature of Barra de Maricá, Rio de Janeiro, Brazil. Extracted from Rocha (1992).

To ensure sufficient data were collected for statistical analyses, the number of skinks collected per month usually ranged from one to ten (September 1991 was an exception). We believe the impact caused on the skink population was negligible, since we sampled only a small portion of the available habitat during the study. Also, on subsequent visits to the area, after the monthly collections had ceased, we did not note any visible decrease in the frequency of skinks sighted per day (both species are fairly abundant in the area).

Each collected lizard was weighed (to the nearest 0.01 g) with a Pesola balance, prior to fixation in 10 % formalin. The snout-vent length (SVL), head length (HL), head width (HW), mouth length (ML) and head height (HH) of each lizard was measured using vernier calipers (to the nearest 0.1 mm). Specimens were then dissected for sex determination and excision of reproductive organs (including embryos) and fat bodies (the few lizards whose organs were damaged by the shot were not considered).

We counted and measured ovarian follicles, oviductal ova and embryos of each female, for both species. The reproductive state of each female was assessed according to the following categories (modified from Patterson, 1990). *Stage 1*: no yolking follicles; no ova established in oviducts; *Stage 2*: yolking follicles; no ova established in oviducts; *Stage 3*: ova or embryo sacs (less than 4 mm in diameter) in oviducts; *Stage 4*: embryo sacs more than 4 mm, chorioallantois established, embryos undeveloped; *Stage 5*: embryos occupying $\geq$ 50 % of embryo sac; eyes and limb buds (or limbs) evident (Stages 30 - 36 of Dufaure & Hubert, 1961); *Stage 6*: well formed (near-term or term) foetuses (Stages 37 - 40 of Dufaure & Hubert, 1961)

Females were considered reproductively active if they contained ova or embryos in the oviducts (stages 3 to 6). Mean brood size was estimated for each species using data from all females containing oviductal ova or embryos. To evaluate the extent to which female body size affects brood size, we performed a linear regression of brood size on female SVL.

For each male, we recorded the longest and shortest axes of each testis and estimated testis volume using the formula for an ellipsoid (Mayhew, 1963). To assess reproductive condition of males, paraffin sections were taken from the middle of the left testis (including the epidydimes) and stained with haematoxylin and eosin. Males were considered reproductively active if spermatozoa were present either in testes or in the epididymes.

All linear measurements of gonads and embryos were taken with digital calipers (to the nearest 0.1 mm). The combined mass of both fat bodies (for each sex, in both species) was recorded using a Mettler eletronic balance (to the nearest 0.001 g).

To assess the monthly variation in male testis volume (expressed as the averaged volume of the two testes in each male) and in fat body mass for both sexes, we calculated the residuals of the regressions of testis volume on SVL (both log-transformed) and of fat body mass on SVL (*idem*), respectively, and took the mean value (plus 1 SD) of the residuals for each month (only sexually mature lizards were included for this purpose). Residuals of fat body mass of adults were correlated with residuals of mean testis volume (in males) and with mean embryo sac diameter (in females) using regression analysis, to evaluate the degree of usage of fat reserves throughout the reproductive cycle. For *M. macrorhyncha* (the monthly sample sizes of *M. agilis* are too small), the effect of three environmental variables (total monthly rainfall, mean monthly temperature and photoperiod) on mean monthly testis volume (expressed as mean value of residuals; see above) was analysed by regression analyses. Due to the lack of published data on the temporal gap between environmental changes and changes in lizard testicular activity, we assumed a time-lag of two months because such physiological responses to environmental variation are unlikely to be immediate. Data on average monthly rainfall and mean monthly temperatures for a 38-year period (1931-1968) were obtained from the Departamento Nacional de Meteorologia station of Niterói, located ca. 19 km west of the study area.

We tested for intersexual differences in lizard SVL within each species using one-way analysis of variance (ANOVA). HL, ML, HW and HH were compared between sexes in each species through analysis of covariance (ANCOVA), using SVL as the covariate.

To increase our sample sizes for analyses involving clutch sizes and morphometric variables (including SVL), for both species, we included data from lizards collected sporadically in Barra de Maricá both before (in 1986) and after (in 1995 and 1996) the study period.

Descriptive statistics are expressed throughout the text as mean ± standard deviation. Nomenclature of other *Mabuya* species mentioned in this paper follow Ávila-Pires (1995).

RESULTS

FEMALE REPRODUCTIVE CYCLE

Brood size of *M. macrorhyncha* averaged 2.66 ± 0.63 (range 2-4; $n = 38$; Fig. 2a) and was significantly correlated with female SVL ($r^2 = 0.258$; $F_{1,35} = 12.16$; $P = 0.001$).

The smallest female *M. macrorhyncha* containing oviductal ova measured 59.9 mm in SVL. Another female of the same size (collected on 25 November 1996) had undeveloped oviducts and contained no vitellogenic follicles in its ovaries, which suggests that

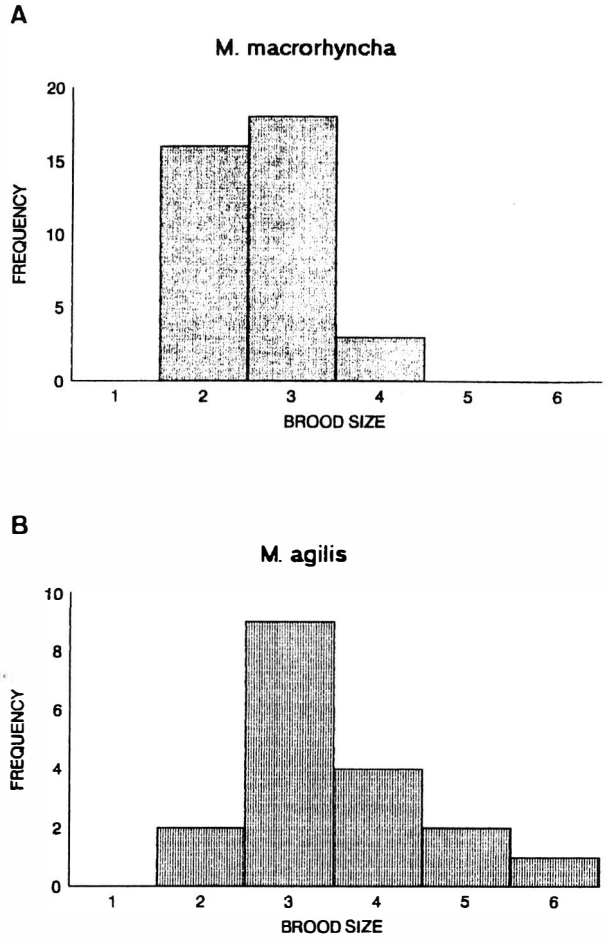

FIG. 2 . Frequency of brood sizes (expressed as number of broods for each brood size) among *M. macrorhyncha* (a) and *M. agilis* (b) at Barra de Maricá, Rio de Janeiro, Brazil.

it was not sexually mature. Three females contained embryos/ova in different development stages simultaneously (respectively, in stages 3 and 4, 3 and 5, and 3, 4 and 5). Two females (SVLs = 68.0 and 72.8 mm) containing well-formed foetuses were collected in late October 1991 (Fig. 3a); another female collected on 5 December 1996 contained three well-formed foetuses. The smallest individual in our sample, a male (36.3 mm SVL; umbilical scar present), was collected on 13 December 1991. Yolking ovarian follicles of *M. macrorhyncha* ranged in diameter from 0.9 mm to 2.2 mm; the smallest oviductal ova were 2.3 mm in diameter. Three well-formed foetuses from a female collected in October 1991 ranged from 24.9 to 25.7 mm in SVL. Ovulation apparently occurs from December to March and implanted ova undergo little increase in size until about June, when embryos begin their rapid development phase until they are ready to be born, about October-November (Fig. 4a).

Brood size of *M. agilis* averaged 3.50 ± 1.04 (range 2-6; $n = 18$; Fig. 2b) and was not significantly correlated with female SVL ($r^2 = 0.131$; $F_{1,16} = 2.41$; $P = 0.14$).

The smallest female *M. agilis* with oviductal ova had a SVL of 49.2 mm. Females with well-formed embryos were collected in mid-September 1989, early and late

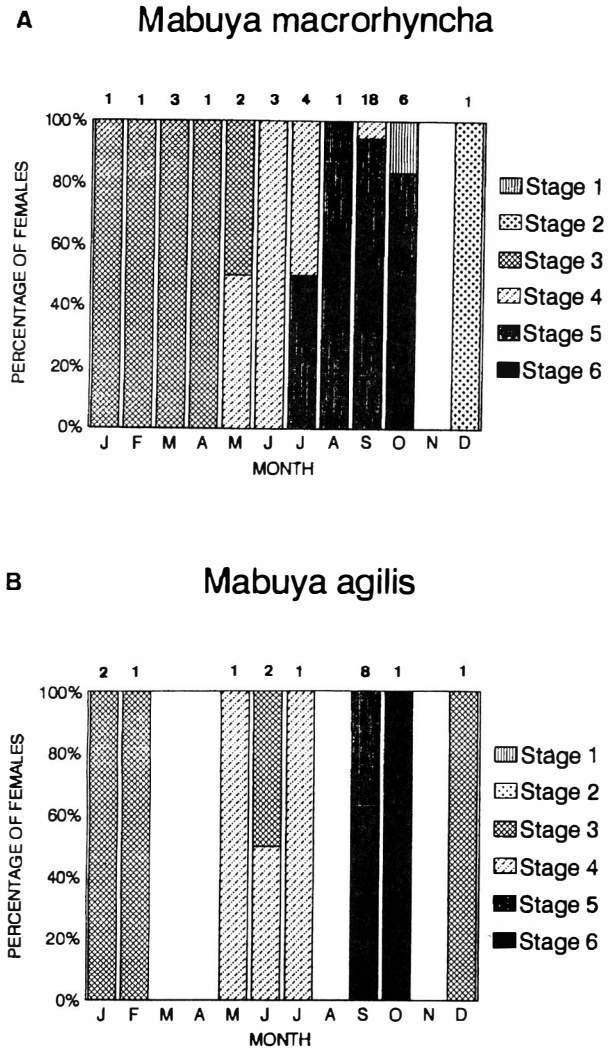

FIG. 3. Percentages of sexually mature female *M. macrorhyncha* (a) and *M. agilis* (b) in each reproductive stage (see text) for each month during the study period (May 1989-April 1992) at Barra de Maricá, Rio de Janeiro, Brazil. Monthly sample sizes are expressed by numbers above bars and represent pooled data from different years.

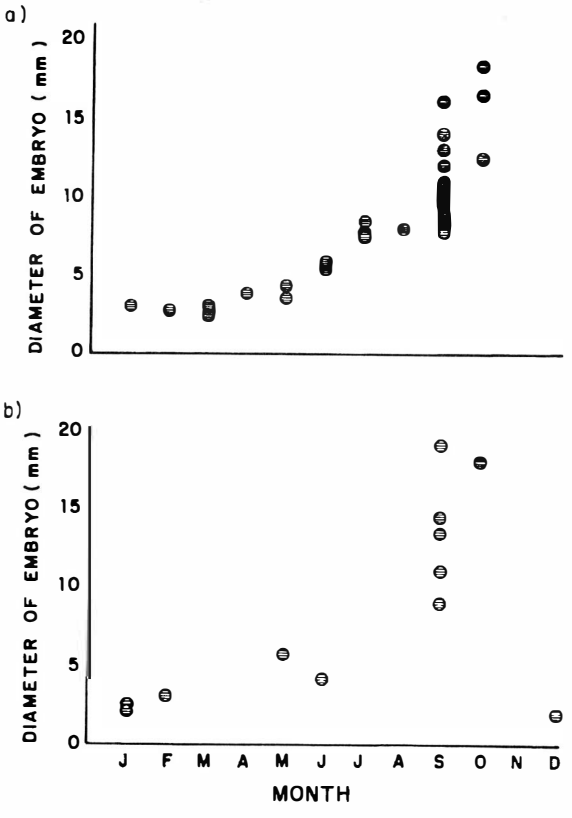

FIG. 4. Monthly distributions of embryo size (expressed as maximum diameter of the largest oviductal ova or embryo sac for each female) for *M. macrorhyncha* (a) and *M. agilis* (b) at Barra de Maricá, Rio de Janeiro, Brazil.

September 1991, and late October 1991 (Table 2), and were all larger than 70 mm in SVL. The three females in reproductive stage 5, from September 1991 (see Fig. 3b) ranged from 63 to 68 mm in SVL. The smallest individual in the sample, a female (SVL = 47.6 mm) was collected on 13 May 1991 and very young individuals (SVL $\leq$ 45 mm) were seen in the field on 30 December 1991. One female containing enlarged follicles (about to be ovulated) and no implanted ova was collected on 28 November 1995. Yolking follicles ranged from 1.1 to 2.2 mm in diameter and the smallest oviductal ova measured 1.5 mm. Four well-formed fetuses from a female collected in October 1991 ranged in SVL from 24.7 to 26.7 mm. Ovulation in *M. agilis* appears to begin in November and ova apparently stay relatively small until May-June, when rapid embryonic growth begins (Figs. 3b and 4b). Embryos are well developed by September-October and parturition apparently occurs mainly during October-November. Brood size of *M. agilis* was significantly greater than that of *M. macrorhyncha* (ANOVA: $F_{1,54} = 14.15$; $P < 0.001$). Most (ca. 90 %) of the females containing oviductal ova or embryos, in both species, also had vitellogenic follicles in their ovaries.

TABLE 1. Values of morphological characters of *Mabuya macrorhyncha* at Barra de Maricá, Rio de Janeiro, Brazil. *F*-ratios test for differences between the sexes using ANCOVA, with SVL as the covariate. *** $P<0.001$.

	Head Width		Mouth Length		Head Height		Head Length	
	males	females	males	females	males	females	males	females
n	45	49	52	55	42	42	51	54
Mean	8.31	8.46	10.15	10.07	5.42	5.44	14.03	13.93
SD	0.93	0.74	0.92	0.80	0.79	0.61	1.28	1.03
Range	5.2-9.4	6.7-9.5	6.8-11.5	6.9-11.2	3.2-6.9	3.4-6.4	9.3-15.8	9.8-15.6
F-ratio	28.71***		63.36***		17.77***		78.87***	

TABLE 2. Values of morphological characters of *Mabuya agilis* at Barra de Maricá, Rio de Janeiro, Brazil. *F*-ratios test for differences between the sexes using ANCOVA, with SVL as the covariate. ***$P<0.001$.

	Head Width		Mouth Length		Head Height		Head Length	
	males	females	males	females	males	females	males	females
n	20	18	21	19	20	18	21	18
Mean	8.35	7.91	9.45	9.12	6.45	6.00	13.75	12.93
SD	0.48	0.68	0.53	0.66	0.39	0.62	0.72	0.94
Range	7.4-9.5	6.4-8.6	8.6-10.8	7.2-9.8	5.8-7.0	4.4-6.8	12.4-15.6	10.6-13.9
F-ratio	35.72***		25.48***		37.72***		66.98***	

MALE REPRODUCTIVE CYCLES

The smallest male *M. macrorhyncha* containing spermatozoa in the lumina of seminiferous tubules and/or epididymes (from 28 October 1991) had a SVL of 55.9 mm. However, both this individual, and a slightly larger one (58.4 mm SVL; collected 29 September 1991) contained very few mature spermatozoa.The monthly distribution of residuals of the testis-volume - SVL regressions (Fig. 5a) showed that testes are at their largest from November through January, decrease in size from February onwards, remaining small until August or September, when they begin to enlarge again.

The smallest male *M. agilis* (collected on August 1986) measured 55.5 mm in SVL and had its testes and epididymes filled with spermatozoa, indicating that it was sexually mature. The monthly testicular cycle in Fig. 5b suggests that testes are in a regressed state during mid-dry season months (May-July), start the size increase in August and remain enlarged from September through March, shrinking thereafter.

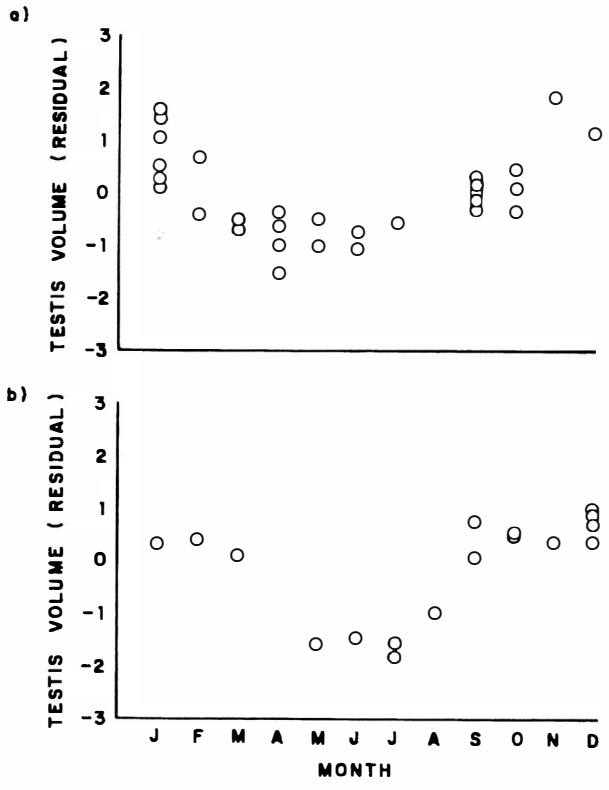

FIG. 5. Monthly distributions of testis volume (expressed as the residuals of the log testis volume-log SVL regression) for *M. macrorhyncha* (a) and *M. agilis* (b) at Barra de Maricá, Rio de Janeiro, Brazil.

The relationships between mean monthly testis volume of *M. macrorhyncha* and the three environmental variables tested were positive in all cases. The regressions of testis volume on temperature ($r = 0.93$, $P < 0.001$) and on rainfall ($r = 0.94$, $P < 0.001$) were both significant, whereas the regression of testis volume on fotoperiod ($r = 0.01$, $P = 0.76$) was not.

FAT BODY CYCLES

Monthly variation in fat body mass was somewhat similar between sexes in both species (Fig. 6). A pattern is more evidently seen in female *M. macrorhyncha*, whose fat reserves appear to reach a peak during the middle of the dry season and decrease after August-September (Fig. 6a).

In male *M. macrorhyncha*, the variation in fat body mass was not significantly correlated to the variation in testis volume ($r = 0.03$, $F_{1,29} = 0.02$, $P = 0.88$). In females, fat body mass was negatively and significantly related to embryo sac diameter ($r = -0.42$, $F_{1,27} = 5.67$, $P < 0.05$).

In *M. agilis*, the variation in fat body mass was not significantly correlated with either testis volume (in males; $r = -0.38$, $F_{1,10} = 1.67$, $P = 0.226$) or mean embryo sac diameter (in females; $r = -0.4$, $F_{1,10} = 1.90$, $P = 0.198$), though the relationship was negative in both cases.

SIZE AND SEXUAL DIMORPHISM

Mean adult SVL of *M. macrorhyncha* (66.8 ± 4.52 mm; $n = 98$) was not statistically different from that of *M. agilis* (66.8 ± 7.28 mm; $n = 41$) (ANOVA: $F_{1,137} = 0.0$, $P = 0.997$). The monthly distribution of sizes for each species is shown in Fig. 7.

Male and female *M. macrorhyncha* ranged in SVL from 36.3 to 72.2 ($n = 54$) and from 41.7 to 77.0 mm ($n = 59$), respectively. Adult males (i.e. ≥ 55.9 mm) averaged 65.1 ± 3.77 mm ($n = 43$) in SVL, whereas adult females (i.e. ≥ 59.9 mm) averaged 68.7 ± 4.04 mm ($n = 50$). For lizards with SVLs of 55.9 mm (i.e. the size of the smallest adult male) or larger, sexes differed significantly in mean SVL (ANOVA: $F_{1,94} = 10.92$, $P =$

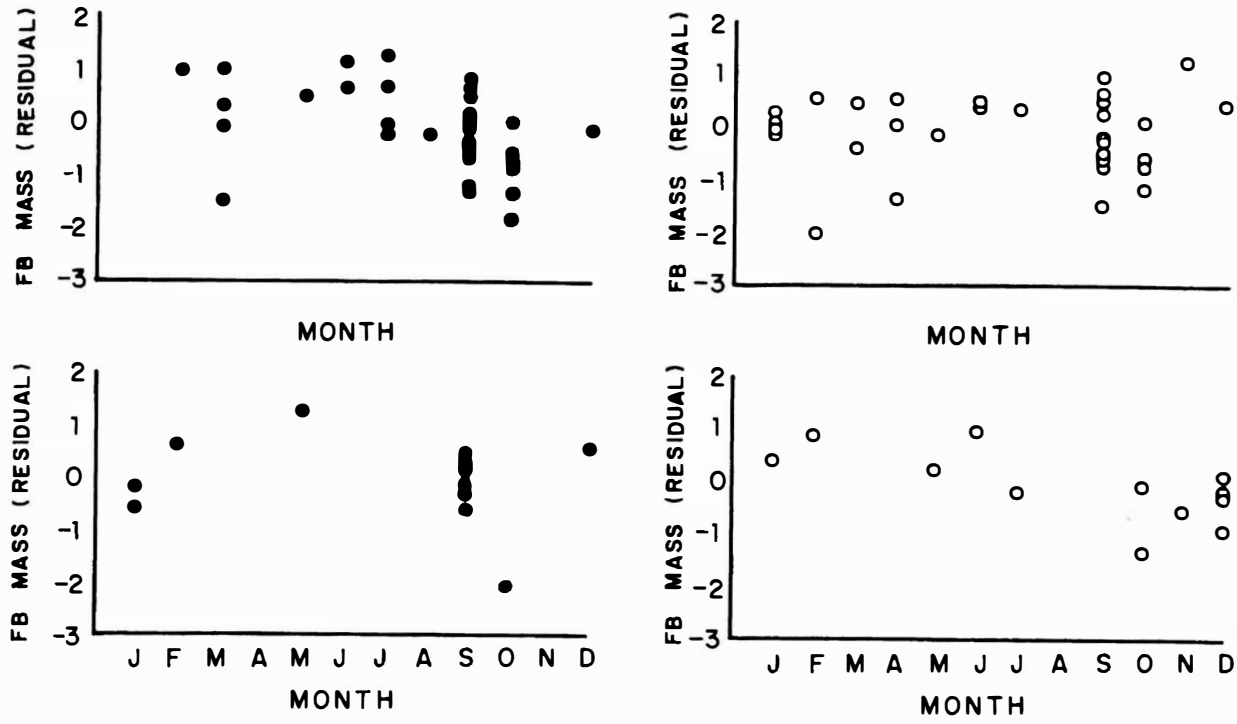

FIG. 6. Monthly distributions of fat body mass (expressed as the residuals of the log fat body mass-log SVL regression) for *M. macrorhyncha* (females - upper left; males - upper right) and *M. agilis* (females - lower left; males - lower right) at Barra de Maricá, Rio de Janeiro, Brazil.

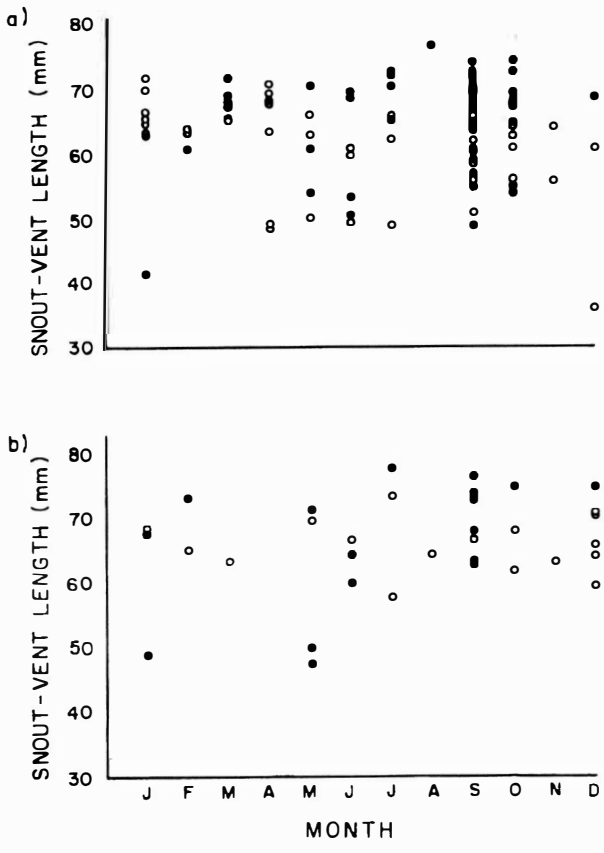

FIG. 7. Monthly distribution of SVL (in mm) of male (open circles) and female (closed circles) *M. macrorhyncha* (a) and *M. agilis* (b) at Barra de Maricá, Rio de Janeiro, Brazil.

0.001), with females reaching larger sizes than males. Sexes differed in the relative values of HL, ML, HW and HH, with higher values for males (Table 1).

Male *M. agilis* examined in this study averaged 66.1 ± 4.74 mm (range 55.5 - 73.8 mm; $n = 21$) in SVL and were all adult-sized. Females ranged from 47.6 to 77.9 mm in SVL ($n = 19$), with only two individuals smaller than 60 mm. Excluding these juvenile-sized individuals, average SVL of female *M. agilis* was 70.7 ± 5.29 mm ($n = 17$) and was significantly larger than that of males (ANOVA: $F_{1,36} = 7.87$, $P < 0.005$). Sexes differed in the relative values of all head dimensions tested, with males attaining higher values (Table 2).

DISCUSSION

Brood sizes of both species at Barra de Maricá (especially *M. macrorhyncha*) were relatively small compared to other Neotropical *Mabuya* species (see Table 3) and to various Old World congeners, both viviparous and oviparous (e.g. Fitch, 1970; Barbault, 1976; Huey & Pianka, 1977; Simbotwe, 1980; Patterson, 1990; Flemming, 1994; Huang, 1994). Brood size of *M. agilis* was comparable to that reported by Somma & Brooks (1976) for *M. mabouia* in the Caribbean island of Dominica, but their data are from only seven gravid females. *Mabuya macrorhyncha* had an even smaller and less variable brood size (usually two or three, rarely four) than its sympatric congener, though it equalled that of a closely related (and yet undescribed) species from north-east Brazil (Stevaux,

1993) (see Table 3). Similarly low values have been observed for allopatric populations of *M. macrorhyncha* in a number of areas (both on the continent and on islands) along the coast of São Paulo state, south-east Brazil, by Vanzolini & Rebouças-Spieker (1976) (Table 3). It appears that, in general, *M. macrorhyncha* gives birth to fewer offspring than its other New World congeners (except for the aforementioned undescribed species), a fact that may have ecological implications. This species is strongly associated with ground bromeliads, which they use as basking and foraging sites and as refugia from predators (Rebouças-Spieker, 1974; Rocha & Vrcibradic, 1996; Vrcibradic & Rocha, 1996), and has a relatively flattened body plan (compared to the ground-dwelling *M. agilis*), which presumably facilitates its movement amidst bromeliad leaves (Vrcibradic & Rocha, 1996). A reduction in the number of offspring and, consequently, in the burden of the brood carried by females (whose body height obviously increases during pregnancy), may be advantageous in a lizard with such characteristics. Indeed, in the caatinga of north-east Brazil, the rock-crevice specialist tropidurid lizard *Tropidurus* (=*Platynotus*) *semitaeniatus* (which has a flattened morphology to suit its microhabitat requirements) has a reduced clutch size (usually two) compared to the sympatric round-bodied tropidurid *Tropidurus hispidus* (referred to as *T. torquatus*), an extreme habitat-generalist, whose clutch ranges from 3 to 14 eggs (Vitt, 1981).

It is interesting to note that three female *M. macrorhyncha* contained embryos/ova in more than one developmental stage (including simultaneous occurrence of stage 3 ova and stage 5 embryos), which may suggest that asynchronous embryo development within a female may occasionally occur in that species (it is also possible, however, that the oviductal ova in those particular females had, for some reason, failed to develop further and remained small; in any case, those ova looked normal, with no signs of degeneration). Asynchronous development among embryos within females is previously unreported for South American *Mabuya*, and may be another peculiarity of *M. macrorhyncha*. It is also probable, however, that egg reabsorption may have taken place, as it has been reported for the closely related *Mabuya* sp. of north-east Brazil (Stevaux, 1993).

We found brood size to be significantly related to female SVL in *M. macrorhyncha* at Barra de Maricá, which did not occur among the populations of this species studied by Vanzolini & Rebouças-Spieker (1976) at the São Paulo coast. We cannot say, however, if this represents actual differences between southern and northern populations of *M. macrorhyncha*, or if other factors such as sample size may be taken into account. In the case of *M. agilis*, on the other hand, we believe that the absence of a relationship may be a result of the small sample size (the presence of juvenile-sized females with implanted ova is unlikely to have affected

TABLE 3. Reproductive characteristics of some Neotropical *Mabuya* species. The letter (I) designates insular populations. *, pooled data from two or more localities; *a*, mean, range in parentheses; *b*, time at which parturition occurs; *c*, calculated from Table 4 of the referenced paper; *d*, as *M. mabouia*; *e*, as *M. bistriata*.

Species	*n*	Brood Size[a]	Reproductive season[b]	Locality	Source
M. agilis	18	3.5 (2-6)	Oct-Nov	Maricá, SE Brazil	Present study
M. bistriata	5	- (4-8)*	?	Amazonian Brazil	Ávila-Pires (1995)
M. caissara	17	5.0 (2-8)[c]	Nov-Dec	Ubatuba, SE Brazil	Vanzolini &Rebouças-Spieker (1976)
M. caissara	14	5.6 (3-9)[c]	Nov-Dec	Caraguatatuba, SE Brazil	*Ibid.*
M. caissara	10	4.0 (2-6)[c]	Nov-Dec	São Sebastião, SE Brazil	*Ibid.*
M. caissara	12	4.8 (3-6)[c]	Nov-Dec	Bertioga, SE Brazil	*Ibid.*
M. frenata	12	4.0 (1-8)	?	Araguaia, Cent. Brazil	Vitt (1991)
M. frenata	113	4.9 (2-8)	Aug-Nov	Valinhos, SE Brazil	Vrcibradic & Rocha (1998)
M. heathi	131	5.0 (2-9)	Sept-Nov	Exu, NE Brazil	Vitt & Blackburn (1983)
M. mabouia (I)	7	3.3 (3-5)	?	Dominica, West Indies	Somma & Brooks (1976)
M. macrorhyncha	11	2.4 (2-5)[c]	Dec-Feb	Enseada, SE Brazil	Vanzolini &Rebouças-Spieker (1976)
M. macrorhyncha	12	3.2 (1-6)[c]	Dec-Feb	Peruíbe, SE Brazil	*Ibid.*
M. macrorhyncha (I)	35	3.3 (2-4)[c]	?	Buzios, SE Brazil	*Ibid.*
M. macrorhyncha (I)	51	2.2 (1-4)[c]	?	Qu. Grande, SE Brazil	*Ibid.*
M. macrorhyncha	38	2.7 (2-4)	Nov-Dec	Maricá, SE Brazil	Present study
M. nigropunctata[d]	-	- (3-7)	Aug-Nov	Iquitos, Peru	Dixon & Soini (1975)
M. nigropunctata[d]	10	5.2 (4-6)	?Mar-Aug	Santa Cecilia, Ecuador	Duellman (1978)
M. nigropunctata[e]	94	4.7 (2-9)*	Aug-Sept	Amazonian Brazil	Vitt & Blackburn (1991)
M. unimarginata	7	5.2 (2-7)	?	Costa Rica (Pacific slope)	Fitch (1985)
M. sp.	76	2.6 (1-4)	Jan-Feb	Cabaceiras, NE Brazil	Stevaux (1993)

the correlation, since only one female smaller than 60 mm was present in our sample of "gravids", and it had only three ova). Brood size is significantly affected by female body size in other Brazilian *Mabuya* species (Vanzolini & Rebouças-Spieker,1976; Vitt, 1991; Vitt & Blackburn, 1983, 1991; Stevaux, 1993; Vrcibradic & Rocha, 1998), and it is quite surprising that, in our study, *M. agilis* did not show such a relationship, whereas *M. macrorhyncha*, with their smaller and less variable broods, did.

Like other Brazilian *Mabuya* species whose reproduction has been reasonably well-studied (Vitt & Blackburn, 1983, 1991; Vrcibradic & Rocha, 1998), *M. agilis* attains reproductive maturity at small body sizes (i.e. about 49 mm SVL), presumably when only a few months old (see Blackburn & Vitt, 1992). *Mabuya macrorhyncha*, on the other hand, apparently does not reproduce in its first year, as suggested by our data and by Vanzolini & Rebouças-Spieker (1976). The latter authors also mentioned that the smallest reproductive females in their samples were about 60 mm in SVL, which agrees with our data. Similar patterns have been reported by Stevaux (1993) for the closely related *Mabuya* sp. This relatively delayed reproduction of the *M. macrorhyncha* lineage relative to other congeners (including the sympatric *M. agilis*) is difficult to interpret in the light of our data and deserves further study. Nevertheless, the gestation periods of both *M. macrorhyncha* and *M. agilis* are apparently identical, spanning between nine and twelve months. The pattern of embryonic growth is also apparently similar between the two species, with little increase in ovum diameter during the first five or six months, followed by rapid embryonic growth thereafter, as in other Brazilian *Mabuya* species (Blackburn & Vitt, 1992; Stevaux, 1993; Vrcibradic & Rocha, 1998). The reproductive cycle of *M. macrorhyncha* appears to lag about one month behind that of *M. agilis*: of the 17 gravid females of the former species collected in September, all were in stage 5, whereas five of the eight gravid *M. agilis* from the same month were in stage 6 (i.e. bore well-formed embryos). Thus, *M. macrorhyncha* breeds somewhat later than *M. agilis*, with parturition probably beginning in late October or early November and apparently extending into December, when that of *M. agilis* may have already ceased. Unfortunately, we have very few adult females of both species from the period November-February, so that it is not possible to determine when parturition actually ceases in each of them. It is also interesting to note that the three smallest gravid *M. agilis* from September were in stage 5, while the five largest were in stage 6, suggesting that first-year females of this species may breed somewhat later than older females, as reported by Blackburn, Vitt & Beuchat (1984) for *M. heathi*.

Although late-stage embryos were found in six *M. agilis* and two *M. macrorhyncha*, none of these appeared large enough to be full-term. Among São Paulo populations, neonate *M. macrorhyncha* are apparently born at a SVL of 32-34 mm (Vanzolini & Rebouças-Spieker, 1976; Zanotti *et al.*, 1997). Three term embryos taken from a female *M. agilis* from the restinga of Grumari, located about 160 km west of Barra de Maricá, averaged 30.6 ± 0.38 mm in SVL (Vrcibradic, unpubl. data), which suggests that the young of this species are born at a SVL of at least 31 mm. The lack of neonate-sized individuals of both species in our sample further obscures our understanding of when parturition actually occurs.

The breeding periods of *M. agilis* and *M. macrorhyncha* are short and well defined, like those of other Neotropical *Mabuya* (see table 3), which would supposedly place them into the category of "non-continuous" breeders, according to the classification of Sherbrooke (1975). Indeed, based on that work, Rocha (1994) referred to *M. heathi* and *M. nigropunctata* (= *M. bistriata*) as having non-continuous reproduction. Although testis cycles in males of the Barra de Maricá species are clearly seasonal and non-continuous, application of such terms to female cycles may not be appropriate: the simultaneous presence of vitellogenic follicles and implanted ova or embryos in the species studied by us indicate that reproduction may actually be continuous, with ovulation occurring shortly or immediately after parturition (almost all sexually mature females of both species were reproductively active). The production of tiny, yolk-poor follicles by female neotropical *Mabuya* is energetically unexpensive, and may occur simultaneously with gestation, which is very long and accounts for those lizards having annual reproduction.

Males of the two *Mabuya* species, unlike females, apparently attain sexual maturity at similar SVLs (55-56 mm), although the minimum reproductive size of male *M. agilis* may be overestimated, since it represented the smallest male in the whole sample. Testis cycles overlap considerably between the two species, with maximum gonadal activity during the wet season, coincident with the period of parturition and ovulation in females. It appears that, for some reason, testes of *M. agilis* suffer a greater reduction in size during the dry season compared to *M. macrorhyncha*. Reproductive cycles (both of males and females) of the two species overlap almost completely, which suggests that they may be regulated by the same factors, such as environmental cues and/or food availability (see Rocha, 1992 and Stevaux, 1993). Environmental variables such as rainfall, temperature and photoperiod seem to strongly influence the testis cycle of *M. macrorhyncha*, whose response to the variation of the first two apparently takes about two months (it is quite puzzling that the response to photoperiod was different). Males of another Brazilian species, *M. frenata*, also respond significantly to the above variables with a time-lag of two months, but the relationship is negative in this inland form (Vrcibradic & Rocha, 1998). Similarly, testis size of male *M. heathi* from north-east Brazil increases as the dry season progresses and decreases when conditions

get wetter (Vitt & Blackburn, 1983), showing a trend opposite to that of *M. macrorhyncha* (and of its close relative, *Mabuya* sp.; see Stevaux, 1993). Maybe the male cycles of neotropical *Mabuya* are more strongly tied to the female cycles than to direct external influences (male peak spermiogenesis always coincides with female late parturition-early ovulation periods), but a better understanding of the effects of environmental cues on male cycles of tropical lizards is needed before any conclusions can be drawn.

Monthly variation in fat body mass does not seem to be too important for male reproductive activity in either species, although a comparison of Figs. 5b and 6 (lower right) suggest an inverse relationship between testis and fat body cycles in *M. agilis* that could, perhaps, be clearer if the sample sizes were larger. A comparison of Figs. 4b and 6 (lower left) is even more suggestive and, again, we believe that the small sample size of *M. agilis* was responsible for the lack of a significant correlation between fat body mass and embryo size. This was not the case for *M. macrorhyncha*, for which the correlation was significant and showed that fat reserves undergo a decrease in mass during the rapid growth phase of the embryos, as in other Brazilian *Mabuya* (see Blackburn & Vitt, 1992; Stevaux, 1993; Vrcibradic & Rocha, 1998). The considerable increase in size of the conceptus of *M. macrorhyncha*, which is similar to that of other New World congeners (Vitt & Blackburn, 1983, 1991; Blackburn & Vitt, 1992; Stevaux, 1993; Vrcibradic & Rocha, 1998), indicates that reproduction is more expensive energetically for females than for males, which may explain the differential importance of fat reserves among sexes (e.g. Gaffney & Fitzpatrick, 1973; Jameson, 1974; Ortega, 1986; Ramirez-Pinilla, 1991; Huang, 1997).

The pattern of sexual dimorphism in size and shape observed for the two species at Barra de Maricá, with females reaching larger body sizes, but having relatively smaller head dimensions than males, agrees with that of other South American *Mabuya* species (Rebouças-Spieker, 1974; Vitt & Blackburn, 1983, 1991; Stevaux, 1993; Vrcibradic & Rocha, 1998). Sexual dimorphism in *M. macrorhyncha* is also clear from Tables 6 to 11 of Rebouças-Spieker (1974), concerning populations from the São Paulo coast. Large female body size among neotropical *Mabuya* may be the product of evolutionary pressures acting to increase the number and volume of offspring carried by the females (e.g. Fitch, 1981; Vitt & Blackburn, 1983, 1991). Such a pressure would not be expected to be strong in *M. macrorhyncha*, whose broods are usually of only two or three (see Vitt (1981) for data on the small-brooded Tropidurid *Tropidurus semitaeniatus*),but even so, females of this species have SVLs larger than those of males and positively correlated to brood size. It is possible that small brood size in *M. macrorhyncha* is a derived character, acquired after its ancestors adapted to a bromelicolous mode of life (see above), a view opposed to that of Rebouças-Spieker (1974), who proposed that this species is an ancestral form, based on biogeographical analyses. Naturally, biochemical and genetic comparisons with other New World species are needed before any conclusions can be drawn. Among male neotropical *Mabuya*, attainment of relatively large heads has been suggested to be related to aggressive male-male interactions (Vitt & Blackburn, 1983; 1991). This seems a plausible explanation, though we have never witnessed such behavior in either species at Barra de Maricá (see also Stevaux, 1993 for a discussion of this topic).

We conclude that *M. macrorhyncha* and *M. agilis* have reproductive characteristics and patterns of sexual dimorphism that are typical of New World *Mabuya* in general, e.g. ovulation of minute and yolk-poor ova, gestation lasting about one year (with most of the mass increase of the embryos occurring within the four months prior to parturition), large female body size and relatively large head dimensions in males. *Mabuya macrorhyncha* has, however, some peculiar characteristics that differ from *M. agilis* and its other Neotropical congeners (except a very close relative in north-east Brazil): it does not breed in its first year (delaying first reproduction until attaining a SVL of ca. 60 mm), produces relatively small broods, and females may present asynchronous breeding (or engage in egg reabsorption). These characteristics should not be attributed to possible effects of the sympatry with *M. agilis*, since they are present in allopatric populations in São Paulo State (Vanzolini & Rebouças-Spieker, 1976), nor to the female size-brood size relationship, since the two species do not differ in adult body size. Such unique features of the *M. macrorhyncha* lineage should be better studied in order to investigate if they represent primitive traits or secondary adaptations, possibly related to the species' bromelicolous habits.

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AMPHIBIAN COLONIZATION OF NEW PONDS IN AN AGRICULTURAL LANDSCAPE

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Newly constructed ponds on farm land were surveyed for amphibians and compared with long-standing farm ponds. The frequencies of amphibian occupation of the two pond types were similar (65 and 71% respectively), but the species composition differed. *Bufo bufo* was found more frequently in new ponds than in old ponds, whereas *Triturus cristatus* and *T. vulgaris* were found less frequently in new ponds. The differences in the amphibian species assemblage between the two types of pond reflected the ponds' functions and the amphibians' dispersal abilities. New ponds were larger and tended to support fish and waterfowl more frequently than did old ponds. *Triturus cristatus* was not found in any fish ponds. Principal component and discriminant analyses of variables related to ponds and the surrounding terrestrial habitat indicated that, for *T. cristatus* and *T. vulgaris*, the location of new ponds relative to existing ponds was a significant factor in pond colonization. *Triturus cristatus* and *T. vulgaris* did not colonize ponds at distances greater than 400 m from existing ponds. *Rana temporaria* and *Bufo bufo* were not so constrained by dispersal abilities and were able to colonize new ponds at distances up to 950 m from existing ponds. *Rana temporaria* was more likely to be found in new ponds containing submerged vegetation; however, multivariate analyses could not discriminate between ponds that were, and were not, colonized by *Bufo bufo*. The results of this study are discussed with regard to the construction and management of ponds for the conservation of these amphibians.

Key words: amphibian assemblages, farm ponds, colonization, habitat characteristics

INTRODUCTION

Amphibians in Britain have exploited a variety of man-made ponds (Warwick, 1949; Banks & Laverick, 1986; Jeffries, 1991; Beebee, 1997). However, in regions where widespread species (common frogs, *Rana temporaria*, common toads, *Bufo bufo*, great crested newts, *Triturus cristatus*, palmate newts, *T. helveticus*, and smooth newts, *T. vulgaris*) have declined, habitat loss, particularly the loss of breeding ponds, seems to be a major causal factor (Cooke & Scorgie, 1983; Hilton-Brown & Oldham, 1991). In general there has been a decline in the number of ponds in Britain over the course of the twentieth century (Barr *et al.*, 1994; Oldham & Swan, 1997). However, the rate of loss has been lessened by the creation of new ponds. Seven per cent of ponds located by systematic surveys included in the National Amphibian Survey were newly created (Swan & Oldham, 1993), and since the 1960s increases in pond numbers have been recorded in some areas (Oldham & Swan, 1997). Thus, new ponds may represent a significant proportion of all ponds, and amphibian success at such sites is worthy of attention.

While the creation of new ponds in gardens has provided breeding sites for *Rana temporaria*, *Bufo bufo* and *Triturus vulgaris* (Cooke, 1975; Beebee, 1979; Beebee, 1985; Banks & Laverick, 1986; Hilton-Brown & Oldham, 1991), the suburban areas most likely to provide this sort of habitat represent only a small proportion (5.5%) of land in Great Britain (Stott *et al.*, 1993). Furthermore, in the long-term, amphibian populations in such areas may suffer reduced genetic diversity due to inhibition of movement between populations in built-up areas (Hitchings & Beebee, 1998). The fate of amphibians in agricultural landscapes is significant both because of the large area of land involved (48.7%) and also due to the potential for this land-use type to support genetically diverse amphibian populations in the long-term.

The importance of ponds in rural areas, in combination with pond losses tempered by a continuing trend of pond creation, makes amphibian colonization of new ponds on farm land an issue of interest. The purpose of the present study was to compare ponds that have been recently constructed in agricultural areas with older ponds, as amphibian breeding sites. The study compared amphibian presence/absence between samples of old and new ponds, and sought to ascertain the characteristics of new ponds that made them suitable amphibian sites.

Of particular interest was the habitat surrounding ponds. Although water quality can affect the distribution of amphibian species (e.g. Cooke & Frazer, 1976; Denton, 1991), within an area of relatively homogeneous geology the distribution of pond-breeding amphibians is less dependent on the finer-scale variation in water quality. Some pond breeding amphibians appear to be fairly insensitive to water quality, being found widely distributed throughout their ranges (e.g. *Rana temporaria* and *Bufo bufo* [Swan and Oldham,

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1993]). Amphibian presence in agricultural areas seems to be largely independent of water quality (Hecnar & McCloskey, 1996), but rather is determined by geology and the nature of adjacent terrestrial habitat (Beebee, 1985; Pavignano, Giacoma & Castellano, 1990; Laan & Verboom, 1990; Swan & Oldham, 1993; Marnell, 1998; Stumpel & van der Voet, 1998), pond vegetation (Ildos & Ancona, 1994; Stumpel & van der Voet, 1998) and age (Laan & Verboom, 1990, Stumpel & van der Voet, 1998). The present study investigated the effects of land use around newly created ponds, the presence of fish, water fowl and vegetation, and the effect of pond age on the presence of amphibians in new ponds.

A particular focus of the present study was the location of new ponds relative to existing ponds. Metapopulation ecology theory (Levins, 1969; Hanksi & Gilpin, 1991) offers a useful framework to understand, manage and conserve discontinuously distributed wildlife populations, including pond-breeding amphibian populations (McCullough, 1996). The status of amphibians within a region is dependent on the outcome of the dynamic processes of the extinction of local populations and the colonization of unoccupied ponds (Savage, 1961; Gill, 1978; Hecnar & McCloskey, 1997; Sjögren-Gulve, 1994, Edenhamn, 1996). As might be expected, amphibians living in such metapopulations are able to colonize suitable, newly created ponds (Gill, 1978; Edenhamn, 1996). A key issue for the conservation management of amphibian populations is the distance between ponds which allows colonization and the long-term persistence of local populations. The present study investigated the distance between ponds and local pond density as potential influences on the colonization of new ponds.

Of the four amphibian species found in the study area (common frogs, common toads, great crested and smooth newts), great crested newts are of particular interest since they are the most scarce and rapidly declining of the widespread British amphibians (Cooke & Scorgie, 1983; Hilton-Brown & Oldham, 1991), and the least successful in the colonization of new pond habitats (Cooke & Scorgie, 1983; Beebee, 1997).

MATERIALS AND METHODS

New ponds constructed through Countryside Commission grants were located with the help of Bedfordshire County Planning Department, Northamptonshire Planning and Transportation Department and Buckinghamshire Farming and Wildlife Advisory Group. Further new ponds were located by interviewing landowners. Ponds that resulted from restoration of existing sites were not included in this study. Pond age was established through local authority records and by interview with landowners. Seventy-eight new ponds, dispersed over 3000 km^2 of west Bedfordshire, north Buckinghamshire and Northamptonshire were surveyed. Landowners and managers were interviewed to establish the nature of amphibian and fish introductions. The presence of fish was further established while surveying the ponds for amphibians. The amphibian survey was carried out in three stages, using established techniques (British Herpetological Society, 1990; Griffiths *et al.*, 1996). The first stage consisted of circuiting the accessible shoreline, visually searching for frogs and toads, the spawn of these species and also newt eggs. The second stage repeated the visual search, after dark, using a torch. For the third stage, weed beds were swept with a pond net for newts and amphibian larvae. Funnel trapping was not used due to the logistical problems in visiting sites twice, to set and collect traps.

Pond use by waterfowl (ducks and geese) was noted. Submerged vegetation was also recorded as either present or absent, since some new ponds were devoid of aquatic weed beds.

The nearest neighbouring pond to each new pond was located from maps and by interview with the landowner. In cases where access to these ponds was possible, they were also surveyed for amphibians, providing a control sample of ponds. These ponds will be referred to as 'old' ponds. The terrestrial habitat around new ponds was analysed from 1:25 000 Pathfinder series maps. A 1 cm (=250 m) grid was superimposed over each pond location. The following four variables were recorded within a 1 km radius of the pond: built up areas (the number of grid squares containing buildings the size of an individual farm house or larger), woodland (the number of grid squares containing areas of woodland), riparian habitat (the number of times rivers, streams or canals crossed grid lines), and proximate pond density (the density of water bodies within a 1 km radius of a pond). Pond density within a 2 km radius and the distance between a new pond and the nearest neighbouring pond were also measured.

Six of the eight variables were log-transformed to normalize skewed data and all variables were relativized to ensure that variables with different means did not contribute disproportionately to the overall variance. A principal component analysis was carried out using the terrestrial habitat variables (built-up areas, woodland, riparian habitat, proximate pond density, pond density and distance to nearest neighbouring pond) to see if these habitat variables could be reduced to two vectors. Discriminant analyses were used to determine differences between ponds colonized by amphibians, and those where amphibians were not detected. For each amphibian species, presence/absence was used as the independent variable. The dependent variables used were built-up areas, woodland, riparian habitat, proximate pond density, pond density, distance to nearest neighbouring pond, pond size, pond age, presence of fish, presence of waterfowl and presence of submerged vegetation. The latter three variables were categorical (presence/absence).

All statistical tests used the probability value $\alpha=0.05$ to determine significance. For χ^2 tests of association, Yates' correction was used when expected values were less than five.

TABLE 1. The frequency of occurrence of all fish species, trout, wildfowl (ducks and geese) and amphibians in old and new ponds. χ^2 values are given for comparisons of presence and absence data between old and new ponds. * indicates $P < 0.001$.

	Old ponds (n=49)	New ponds (n=78)	χ^2
Amphibians	71%	65%	3.70
Fish	20%	54%	13.92*
Trout	0%	21%	11.50*
Waterfowl	14%	46%	13.65*

RESULTS

Forty-nine old and 78 new ponds (median age = five years, range = 1 to 20 years) were surveyed. In most cases (at least 77% of new ponds) construction was funded by Countryside Commission grants. Forty-one per cent were constructed primarily for fish or waterfowl, the remainder for other purposes such as wildlife habitat creation or aesthetic value.

The new ponds surveyed were significantly larger than the old ponds (mean sizes = 1704 and 409 m^2, respectively; t=3.15, df=125, P<0.01; ranges = 13-14 160 and 30-1060 m^2, respectively) and a greater proportion supported fish, (54%), including trout (*Salmo*) species (21%), and waterfowl (46%) (Table 1). Amphibians were found in similar proportions of old (71%) and new (65%) ponds. The distribution of the number of species found per pond was also similar between the two types of pond (χ^2=3.70, df=4, P>0.05) (Fig. 1). However, interviews with landowners and managers revealed that amphibians had been introduced to some ponds. The movement of frogspawn was the most common form of amphibian introduction (3 old and 16 new ponds) and potentially created a source of bias in the survey data. To test whether frogspawn introductions were associated with the presence of frogs, the proportion of new ponds where frogs were detected that were also sites of

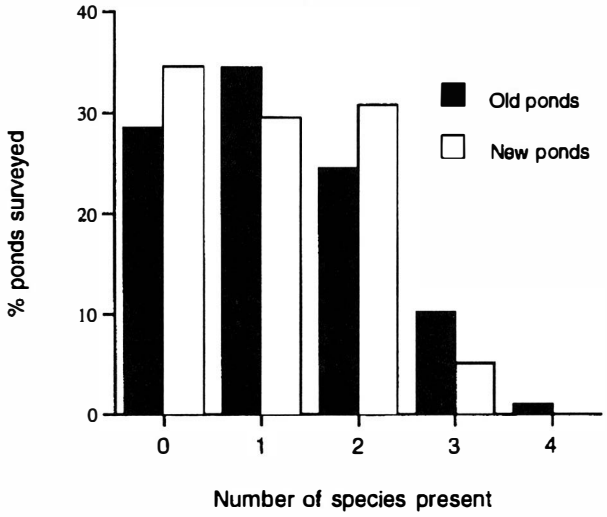

FIG. 1. The number of amphibian species occupying old and newly constructed farm ponds.

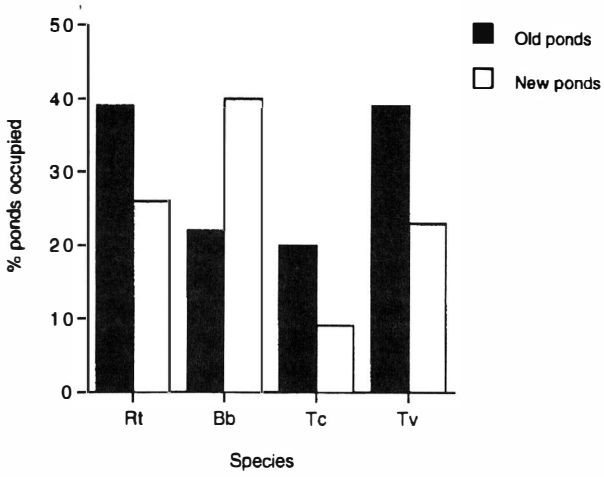

FIG. 2. The percentage of old and newly constructed ponds occupied by *Rana temporaria* (Rt), *Bufo bufo* (Bb), *Triturus cristatus* (Tc) and *Triturus vulgaris* (Tv).

introduction (9/16) was compared with the proportion of ponds where frogs had colonized naturally (16/62). The presence of frogs at new ponds was significantly associated with introductions of frogspawn (χ^2=5.412, df=1, P<0.05).

To remove any effects of frog introductions on the occupancy of new ponds, the frog occupancy data were analysed after removing all sites where frogs had been introduced. There was no significant difference in the proportions of frog presence/absence between old (39% presence) and new (26% presence) ponds (χ^2=2.173, df=1, P>0.05). An analysis of the presence/absence of toads, great crested and smooth newts revealed that the distribution of these species differed between old and new ponds (χ^2=7.625, df=2, P<0.05). Toads were found more frequently in new ponds (40%) than in old ponds (22%) whereas both *Triturus cristatus* and *T. vulgaris* occurred at lower frequencies in new (9 and 23% respectively) than in old ponds (20 and 39% respectively) (Fig. 2).

To examine the relationship between amphibian occupancy and fish and waterfowl presence, sites of frogspawn introduction were included. Similar numbers of both fish ponds and ponds utilized by waterfowl were occupied by at least one amphibian species (Table 2; χ^2=0.487, df=1, P>0.05 and χ^2=0.232, df=1, P>0.05, respectively). However, the distributions of amphibian presence/absence, by species, differed between fish, and fish-free, ponds (χ^2=11.39, df=3, P<0.01). Frogs and toads tended to be found more frequently in fish ponds, while smooth newts were found less frequently and great crested newts were never found to co-exist with fish. A similar pattern was found in ponds used by waterfowl, but this was not statistically significant (χ^2=6.64, df=3, P>0.05).

Principal component analyses were carried out for six habitat variables (built-up areas, woodland, riparian, proximate pond density, pond density and distance to nearest neighbouring pond), for the 78 new ponds. These variables reduced to two vectors repre-

TABLE 2. Amphibian occupancy of new ponds relative to the presence of fish and waterfowl. n = number of ponds, Any spp. = at least one amphibian species present, *Rt* = *Rana temporaria*, *Bb* = *Bufo bufo*, *Tc* = *Triturus cristatus*, *Tv* = *Triturus vulgaris*. Figures in brackets represent percentages.

Amphibian and fish presence	*n*	Any spp.	*Rt*	*Bb*	*Tc*	*Tv*
Fish absent	36	25 (69)	9 (25)	11 (31)	7 (19)	10 (28)
Fish present	42	27 (64)	16 (38)	20 (48)	0 (0)	8 (19)
Amphibian and waterfowl presence	*n*	Any spp.	*Rt*	*Bb*	*Tc*	*Tv*
Fish absent	42	27 (64)	11 (26)	12 (29)	6 (14)	11 (26)
Fish present	36	25 (69)	14 (39)	19 (53)	1 (3)	7 (19)

senting 68% of the variance in habitat variables. The eigenvector loadings indicate that the first axis primarily represents distance to the nearest neighbouring pond and the second axis primarily represents the amount of adjacent woodland (Table 3). Amphibian species presence/absence was then plotted against the first two axes of the principal component analyses (Fig. 3). Frog presence/absence was plotted only for the 62 ponds where introductions had not occurred. Pond occupancy by frogs and toads is spread evenly across the two principal components. However, in the case of the newts, both species appear to occur on the lower half of the first axis, indicating that newts tended to occupy new ponds in locations where the distance to the nearest neighbouring pond was small (Fig. 4).

TABLE 3. Results of principle component analysis of six variables quantifying terrestrial habitat surrounding new ponds. The variance explained by six new axes and the loadings of each original habitat variable on the first two axes are given. Wood = woodland, Rip = riparian habitat, B = built-up areas, PPD = pond density within a 1-km radius, PD = pond density within a 2-km radius, NN = distance to nearest neighbouring pond.

Axis	Eigenvalue	% of var.	cum. % of var.	Broken-stick
1	0.007	49.62	49.62	0.006
2	0.003	18.55	68.17	0.003
3	0.002	13.15	81.32	0.002
4	0.002	11.07	92.38	0.001
5	0.001	4.70	97.08	0.001
6	0.000	2.92	100.00	0.000

	Factor score coefficients for terrestrial habitat	
	Axis 1	Axis 2
Wood	-0.1637	0.7013
Rip	0.0705	0.3093
B	-0.0413	0.1021
PPD	-0.1608	-0.4869
PD	-0.0970	-0.4057
NN	0.9650	-0.0212

Discriminant analyses detected significant differences between occupied and unoccupied ponds for frogs (at all sites and sites where frogs had not been introduced) and great crested newts, but not for toads. The discriminant function for smooth newts was on the borderline of statistical significance (P=0.052). Values of Wilks' λ are given in Table 4. For frogs, univariate tests (Table 4) indicate that the presence of submerged vegetation in new ponds is associated with frog presence, for all sites and for the reduced data set excluding sites of introduction. Correlation between the original variables and those of the canonical discriminant function (Table 5) also indicate the importance of submerged vegetation. Pond age is a significantly different factor using all of the pond data for frogs, but this effect disappears when the sites of introduction are removed (Table 4).

For great crested newts, both measures of pond density and also the presence of fish are significantly different between occupied and unoccupied ponds (Ta-

TABLE 4. Values of Wilks' lamda (λ) for discriminant functions separating occupied from unoccupied ponds for *Rana temporaria*, *Bufo bufo*, *Triturus cristatus* and *T. vulgaris*. F values for univariate tests are given for significantly different variables for *Rana temporaria* (all ponds and ponds excluding sites of introductions) and *Triturus cristatus*. Age = pond age, fish = fish present, PPD = pond density within a 1-km radius, PD = pond density within a 2-km radius, SV = submerged vegetation. NS, P>0.05; *P<0.05; **P<0.01; ***P<0.001.

	Wilks' λ	df	Statistics
R. temporaria	0.669	11	χ^2=28.3**
SV	0.897	1,76	F=8.74**
Age	0.926	1,76	F=6.06*
(no introductions)	0.658	11	χ^2=22.8*
B. bufo	0.781	11	χ^2=17.4 NS
T. cristatus	0.690	11	F=26.21**
PD	0.872	1,76	F=11.19***
Fish	0.885	1,76	F=9.88**
PPD	0.948	1,76	F=4.15*
T. vulgaris	0.758	11	χ^2=19.5 NS

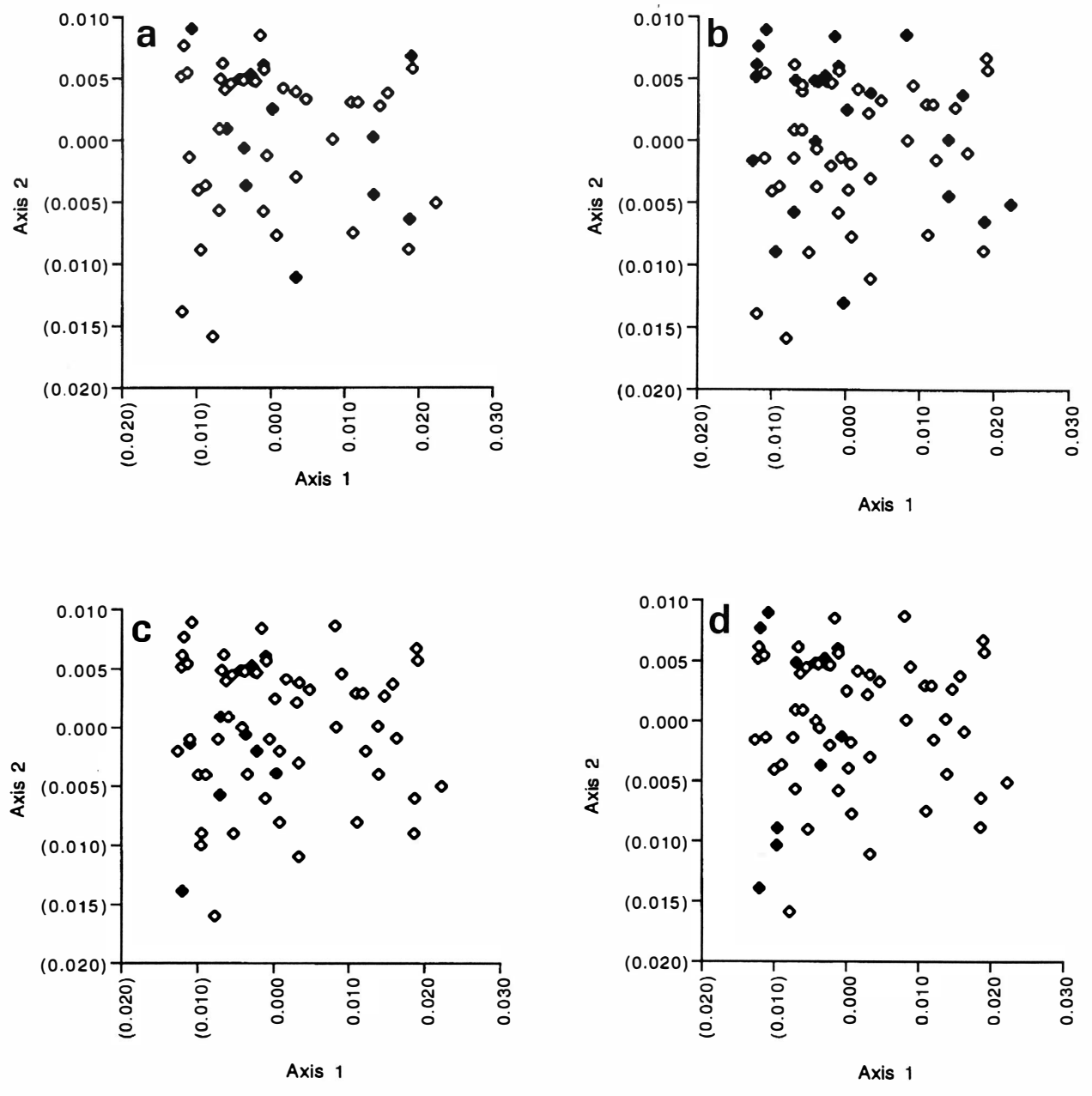

FIG. 3. The distribution of amphibian presence overlaid onto principal component analysis plots of vectors 1 and 2. (a) *Rana temporaria*, (b) *Bufo bufo*, (c) *Triturus cristatus*, and (d) *Triturus vulgaris*. Filled diamonds: present; open diamonds: absent.

TABLE 5. Pooled within-groups correlations between discriminating variables and standardized canonical discriminant function variables for the four largest correlation values, presented in decreasing order of size. *Rt* = *Rana temporaria* (all pond data), *Rt* (no intros.) = *Rana temporaria* (excluding sites of introduction), *Bb* = *Bufo bufo*, *Tc* = *Triturus cristatus*, *Tv* = *Triturus vulgaris*.

Rt		*Rt* (no intros.)		*Bb*		*Tc*		*Tv*	
SV	0.483	SV	0.466	Wood	0.532	PD	0.572	NN	0.840
Age	0.402	Fowl	0.344	PD	-0.509	Fish	-0.537	Size	0.436
B	0.261	Rip	0.280	Fowl	0.481	PPD	0.348	PPD	-0.233
Rip	0.224	Age	0.252	Fish	0.334	Fowl	-0.305	Rip	-0.216

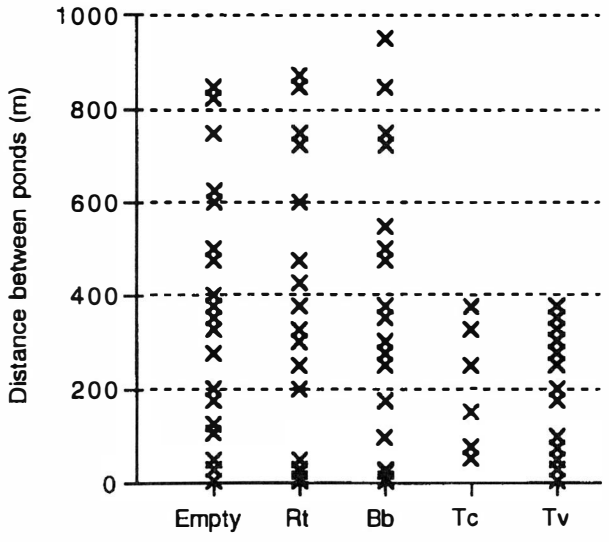

FIG. 4. The distance between old and newly constructed ponds that were either devoid of amphibians (Empty) or occupied by *Rana temporaria* (Rt), *Bufo bufo* (Bb), *Triturus cristatus* (Tc) or *Triturus vulgaris* (Tv).

ble 4). Great crested newts were most likely to colonize fish-free ponds in areas of high pond density. For smooth newts, although the discriminant function is marginally not significant, the distance to the nearest neighbouring pond is the variable most strongly correlated with the discriminant function (Table 5). New ponds colonized by smooth newts tended to be closer to the nearest neighbouring pond than those that were not colonized.

DISCUSSION

The old ponds surveyed seem typical of ponds found on agricultural land in England, in terms of amphibian occupancy and size. Cooke (1975) found frogs and toads in 33-36% and 22-35%, respectively, of ponds in agricultural areas; and the National Amphibian Survey (Swan & Oldham, 1993) found frogs, toads, great crested and smooth newts in 47, 33, 18 and 27%, respectively, of field ponds. The median size of field ponds (length x width) in the National Amphibian Survey (300 m^2) was similar to the area of old ponds in the present study (250 m^2). However, it cannot be assumed that the present study is representative of all farmed areas: regional variations exist in the pattern of amphibian occupancy of ponds in agricultural areas (e.g. Beebee, 1981).

The new ponds surveyed in this study were clearly different in nature from older ponds on farmland. This difference in part reflects the function of the ponds. It was not possible to determine the original purposes of the long-established ponds, but many ponds in the British countryside were constructed as water sources for livestock (Oldham & Swan, 1997). The new ponds surveyed were constructed for aesthetic reasons, to enhance wildlife habitat and for recreational and business purposes consistent with contemporary rural pursuits (rearing fish, angling and wildfowling). Hence the new ponds more frequently supported populations of fish (54%) and were heavily used by waterfowl (46%). Many of them were also much larger than old ponds. Although it is impossible to disentangle the confounding effects of age and size between the two groups of old and new ponds, discriminant analyses indicated that within the sample of new ponds neither of these factors was related to amphibian presence.

Although overall amphibian occupancy of old and new ponds was similar, the species composition between the pond types differed. Frogs and smooth newts were the most commonly found amphibians in old ponds (both species found in 39% of ponds), whereas in new ponds toads were the most commonly found species (40%). Occupancy of new ponds tended to be lower for frogs (26%) and significantly so for great crested and smooth newts (9 and 23%, respectively). The differing successes of the four amphibians in new ponds on farm land reflects their dispersal abilities and also the functions of the new ponds. Frogs and toads were able to colonize ponds with nearest neighbouring ponds up to 950 m away. Since the pond densities in this study area were such that the nearest neighbouring ponds were always found within a distance of 950 m, new ponds always fell within anuran colonization range. This relatively effective dispersal ability of common frogs and toads is consistent with data collected by Sinsch (1991) and Beebee (1997), except that Beebee found that frogs were more frequently found in new ponds than were toads. The reverse was true in the present study, demonstrating a greater dispersal ability for toads than found at other sites in north-western Europe (Reading *et al.*, 1991).

Newts colonized new ponds only at sites where the nearest neighbouring pond was within 400 m. This does not imply that 400 m is the maximum migratory distance from the pond of origin, but it does suggest that 400 m is an upper limit to the effective colonization distance between ponds in this particular agricultural landscape over a relatively short time-scale. In other areas newts have been found at greater distances from their ponds of origin (up to 800 m in *Triturus vulgaris* [Simms, 1969]). Amphibian colonization abilities are not absolute. Variation in the migratory limits and colonization success between study areas may be due to differences in the nature of terrestrial habitat between ponds (Reh & Seitz, 1990, Sjögren-Gulve & Ray, 1996) and also the nature of the ponds themselves. The new ponds in the present study were diverse in function and size while other studies have focused on ponds excavated more specifically for wildlife and landscape conservation (Beebee, 1997; Stumpel & van der Voet, 1998).

Many new ponds in the present study were either created specifically for, or supported, fish and/or waterfowl. Fish and waterfowl ponds were favourable for anurans but not so for newts. Fish ponds were never used by great crested newts. The positive association between fish and toads, and the converse for great crested newts has been noted in previous pond surveys (Beebee, 1979; Beebee, 1981; Dolmen, 1982; Beebee,

1985) and the differing abilities of all four amphibians to coexist with predatory fish are well-substantiated. Toad larvae are distasteful to fish (Glandt, 1984) and shoaling may also reduce the frequency of attacks (Watt *et al.*, 1997). The cryptic coloration and avoidance behaviour of frog larvae (Manteifel, 1995) may serve to keep them in microhabitats inaccessible to fish. During the present study frog larvae in trout lakes were found only in dense weed beds. Differences in the behaviour of the newt larvae explain their relative coexistences with fish; smooth newt larvae are benthic, whereas great crested newt larvae are nektonic (Dolmen, 1983), making the latter more vulnerable to fish predation.

The lack of detectable effects of terrestrial habitat, with the exception of neighbouring ponds, on amphibian colonization of new ponds is in contrast to the findings of Beebee (1985), Laan & Verboom (1990), Pavignano *et al.* (1990), and Swan & Oldham (1993). It is possible that terrestrial habitat effects were not detected because the quantification technique used in the present study was not sufficiently sensitive. Alternatively, the mixed farm land surrounding the new ponds may have provided sufficient habitat diversity such that land surrounding all new ponds was equally likely to support amphibian populations. Swan & Oldham (1993) discovered a similar trend, in that although terrestrial habitat did affect amphibian presence in ponds, within a land-use type containing a diverse habitat, habitat features were less predictive of amphibian presence.

The present study showed that amphibians are readily able to colonize new ponds on mixed farmland. However, the issue of whether amphibian presence is a measure of pond quality (Oldham & Swan, 1997) needs consideration. In Britain, areas that are species rich for a particular taxon are not necessarily so for other taxa, and species of high conservation interest do not necessarily occupy areas that are biologically diverse (Prendergast *et al.*, 1993). This may also apply at the finer scale of ponds. For example, in ponds, plant species richness does not correlate with coleopteran diversity (Wilkinson & Slater, 1995). In the present study, although new ponds were frequently colonized by toads, this does not necessarily reflect pond quality. Fourteen (18%) of the new ponds contained no submerged vegetation and presumably were of limited wildlife value. However, toads were breeding in six of these unvegetated new ponds. Future amphibian survey work will be of wider conservation interest if the relationships between amphibian presence and other measures of biological diversity or pond quality are investigated.

The data from the present study are representative of amphibian abundance and colonization abilities in an area of mixed farm land supporting a diverse range of new ponds. They suggest that, within similar landscapes, new ponds on farm land can provide suitable habitat for amphibian populations, particularly anurans. However, to benefit newts, some specifications are recommended. Ponds intended to benefit newt populations should not be stocked with fish and it may also be beneficial to avoid heavy waterfowl use of such ponds. In situations where the latter two interests are the objective of pond creation schemes, wildlife agents should advocate the construction of secondary ponds, set aside to benefit native species. New newt ponds should also be sited within 400 m of existing newt ponds. This seems to differ from Swan & Oldham's (1993) recommendation of one suitable pond per km^2. However, the closer pond proximity represents a distance over which newts have rapidly colonized new ponds; the pattern of pond occupancy reported by Swan & Oldham may have taken longer to develop.

A pond construction programme, based around a strategy of creating areas with relatively small interpond distances is also likely to benefit other species; areas of higher pond density are associated with greater plant diversity (Möller & Rördam, 1985). A proactive conservation strategy for great crested newts, based on maintaining and creating areas of high pond densities, could use this legally protected amphibian as an umbrella species, under which other pond organisms could benefit. Such a strategy is also consistent with current ideas concerning the creation of new ponds for wildlife (Williams *et al.*, 1997).

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A NEW SPECIES OF *MABUYA* FITZINGER (REPTILIA: SQUAMATA: SCINCIDAE) FROM THE ONILAHY RIVER OF SOUTH-WEST MADAGASCAR

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Mabuya vezo is described as a new white-spotted species of the *aureopunctata*-group of Madagascan mabuyas, identified by its small size and the presence of regularly arranged rows of white spots on the dorsal and dorsolateral surfaces of the neck, body, and tail. It is known from a single locality, Lavenombato, near the mouth of the Onilahy River in south-western Madagascar. *M. vezo* is a rock-dwelling species, similar in size and habitat to *M. vato,* and in general coloration to the much larger *M. aureopunctata. M. vezo* is broadly sympatric with only one member of its species-group, *M. aureopunctata*, but two species of the *elegans*-group, *M. elegans* and *M. gravenhorstii*, occur in the same area. The type locality of *M. vezo* is "fady" (taboo), which provides some degree of protection for this species, which is known from only seven specimens.

Key words: Scincidae, *Mabuya*, new species, systematics, Madagascar

INTRODUCTION

Madagascan mabuyas were most recently reviewed by Brygoo (1983) and Nussbaum & Raxworthy (1994, 1995, 1998). Currently there are nine recognized species placed in two species groups, which may not be monophyletic. The *elegans*-group, characterized by having a trapezoidal subocular scale, contains *Mabuya elegans*, *M. gravenhorstii*, and *M. madagascariensis.* Species of the *aureopunctata*-group have a rectangular subocular scale and include *M. aureopunctata*, *M. betsileana*, *M. boettgeri*, *M. dumasi*, *M. lavarambo*, and *M. vato. M. betsileana* is a problematic form known only from the holotype, and is suspected of being a mislabeled African specimen similar to, or conspecific with, *M. perrotetii* of western Africa (Brygoo, 1983).

Within the *aureopunctata*-group, *Mabuya boettgeri* and *M. lavarambo* are distinctive in both their appearance and distributions. They are the only members of the group with longitudinal body stripes. *M. boettgeri* has a unique pattern of head scales, with three supraoculars rather than the usual four; and three superciliaries rather than five (rarely six). *M. lavarambo* has an exceptionally long tail, which is more than twice the snout-vent length (less than twice the snout-vent length in other Madagascan mabuyas), and a much smaller window in the lower eyelid compared to the other Madagascan mabuyas (Nussbaum & Raxworthy, 1998). *M. boettgeri* has a north-easterly distribution in high elevation grassland and heathland, and *M. lavarambo* is restricted to the north-western satellite island, Nosy Be; whereas the white-spotted species of the group occur mostly in south-western dry forests and savannahs.

The three white-spotted species of the *aureopunctata*-group (*M. aureopunctata*, *M. dumasi*, *M. vato*) will probably prove to be monophyletic. Our herpetofaunal surveys in Madagascar are revealing complex patterns of geographic variation within the group which are complicated by apparent hybridization, both within the group and possibly with species of the *elegans*-group (Nussbaum & Raxworthy, 1995). We recently identified a new white-spotted form from near the mouth of the Onilahy River in south-western Madagascar with characteristics that cannot be attributed to geographic variation or hybridization. This new species is described below and compared in detail to other white-spotted species of the *aureopunctata*-group.

METHODS AND MATERIALS

Specimens were euthanized by injecting concentrated chlorobutanol, fixed in 10% buffered formalin, soaked in water to remove the formalin, and stored in a final solution of 70% ethanol. All measurements were taken from preserved specimens. A ruler was used to measure snout-vent length (SVL), tail length, and limb length to the nearest 1.0 mm. All other measurements were made with electronic digital calipers and recorded to the nearest 0.1 mm. Material examined is in the Museum of Zoology, University of Michigan (UMMZ), the Laboratoire de Population Terrestre, Département de Biologie Animale, Université d'Antananarivo (UADBA), and the Muséum National d'Histoire Naturelle, Paris (MNHN).

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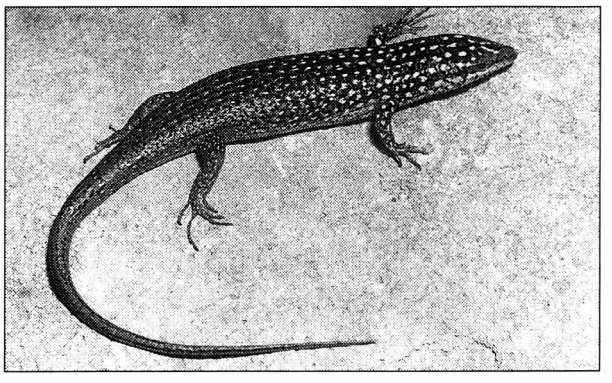

FIG. 1. Holotype (UMMZ 217100) of *Mabuya vezo* in life.

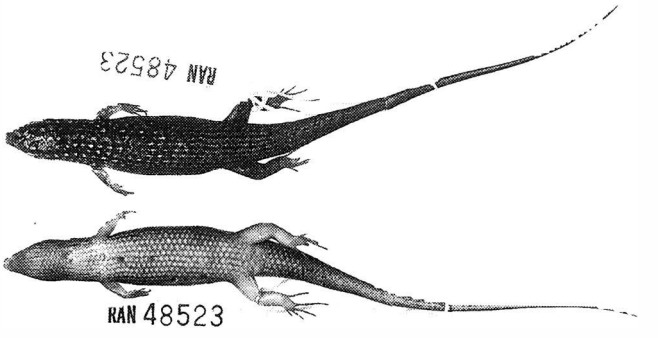

FIG. 2. Dorsal (upper) and ventral (lower) views of holotype (UMMZ 217100) of *Mabuya vezo* after 30 months in preservative.

RESULTS

MABUYA VEZO SP. NOV. (FIGS. 1 AND 2).

Holotype. UMMZ 217100 (RAN 48523), mature male, collected 9 March 1995, 0.5 km WSW of Lavenombato Village, 23° 33.5'S, 43° 48.3'E, 10 m elevation, Toliara Fivondronana, Toliara Province, Madagascar, by Jean Baptiste Ramanamanjato.

Paratypes (6). UADBA 1 (RAN 48519), UMMZ 217101-2 (RAN 48522, 48524), collected 9 March 1995 by Jean Baptiste Ramanamanjato, Achille Phillipe Raselimanana, and Angelin and Angeluc Razafimanantsoa; UMMZ 217103-5 (RAN 50271-3), collected 13 October 1995 by Jean Baptiste Ramanamanjato and Achille Phillipe Raselimanana.

Definition. A small *Mabuya* with a large, undivided, transparent disk on lower eyelid; scales of soles not spinose, subdigital scales acarinate; subocular rectangular. Ground colour of dorsal and dorsolateral surfaces of head, neck, and anterior body dark brown to nearly black, changing to light brown on posterior half of body and tail. Dorsal and dorsolateral surfaces of head, neck, body, and tail with longitudinal rows of white spots (Fig. 1); 7 rows around anterior half of neck, 11 on posterior half extending onto body and tail; more than 11 rows at midbody; a row of 7-8 large, isolated, white spots on each side beginning on supralabials and extending posteriorly after the 7th or 8th as smaller spots on each lateral body scale; lower lateral rows of white spots faint; vertebral row of white spots begins on nuchal scales. Differs from *Mabuya elegans*, *M. gravenhorstii*, and *M. madagascariensis* in having rows of white spots rather than stripes and in having a rectangular subocular scale, which is trapezoidal in the latter three; from *M. boettgeri* and *M. lavarambo* in having rows of white spots and lacking longitudinal dark and light stripes on neck and body; further from *M. boettgeri* in having 4 supraoculars (rather than 3) and 4-5 superciliaries (rather than 3); further from *M. lavarambo* in having a tail less than twice the snout-vent length (more than twice as long in *M. lavarambo*) and a larger window in the lower eyelid; from *M. betsileana* in having white spots as opposed to nearly uniform dorsal coloration and in having fewer ventral scales between mentals and cloaca, 49-53 compared to 73; from *M. dumasi* by having white spots on the dorsal surfaces of head, neck, and body, which are confined to the side of the neck in the latter species; from *M. aureopunctata* and *M. vato* in having a distinctive pattern of white spots on the dorsal and dorsolateral surfaces of the posterior half of body and tail, areas that generally lack white spots in the two latter species; further from *M. vato* in having mostly fewer scale rows around midbody (31-34 versus 34-38), mostly fewer ventral longitudinal scale rows (49-53 versus 53-58), and a light brown posterior-dorsal coloration rather than reddish bronze; and further from *M. aureopunctata* in smaller size (54 mm maximum SVL, compared to 82 mm).

Description of holotype. Specimen (Fig. 2) in good condition, tail partially regenerated, small abdominal slit on left side; hemipenes not extruded, testes white, not enlarged, apparently sexually inactive at time of capture.

Measurements and counts in Tables 1 and 2. Body length 3.1 times head length; head 1.6 times longer than wide, 1.4 times wider than deep; forelimb length 0.3 times SVL, hindlimb 0.4 times SVL.

Supranasals separated above rostral, contacting first loreal; nasal pierced by naris behind vertical suture between rostral and first supralabial; small postnasal above first supralabial, not touching second supralabial; two loreals behind nasal; first loreal above second and third supralabials, wider than tall, inferior side slightly longer than superior, anterior side higher than posterior; second loreal above third supralabial on right side, above third and fourth on left; frontonasal narrowly contacts rostral anteriorly, contacts prefrontals posteriorly and first loreal on each side; two prefrontals widely in contact with each other; one presubocular above fourth supralabial on right side, above fifth on left; two preoculars, inferior larger than superior, in front of presubocular, behind second loreal, above fourth supralabial; frontal triangular, adjacent to second and third supraocular, contacting first supraocular on left side only; four supraoculars; five

TABLE 1. Measurements (mm) of holotypes of white-spotted species of the *aureopunctata*-group. *Regenerated tail.

	Mabuya			
	vezo	*vato*	*dumasi*	*aureopunctata*
	UMMZ			MNHN
	217100	196208	203663	1456
Sex	male	male	male	unknown
Maturity	mature	mature	mature	unknown
SVL	54	52	55	39
Tail length	82*	79	87	39*
Tail width	7.5	8.0	7.8	5.2
Tail depth	6.6	5.8	7.2	3.7
Head length	14.1	14.5	14.7	10.5
Head width	8.4	8.5	8.7	6.0
Head depth	6.4	5.2	5.6	3.7
Snout length	9.3	9.9	9.8	7.5
Internarial distance	2.1	2.1	2.5	1.6
Interocular distance	5.7	4.5	5.0	4.0
Orbital length	2.7	4.5	3.5	2.9
Eye-naris distance	3.1	3.5	3.2	2.8
Eye-ear distance	3.7	4.1	4.3	3.0
Frontal length	3.4	3.5	3.4	2.6
Interparietal length	2.1	2.5	2.3	2.3
Axilla-groin length	25	23	26	18
Forelimb length	14	16	17	11
Hindlimb length	22	23	24	16
4th finger length	3.9	4.8	5.6	3.7
4th toe length	7.0	7.5	9.5	5.5

TABLE 2. Meristic data of holotypes of white-spotted species of the *aureopunctata*-group. [1] (l-r) = left-right; [2] number of ventral scales counted longitudinally from postmentals to cloaca; [3] supralabial(s) contacting first loreal; [4] Supralabial(s) contacting second loreal; [5] subdigital scales on fingers I-V of manus, left and right; [6] subdigital scales on toes I-V of pes, left and right.

	Mabuya			
	vezo	*vato*	*dumasi*	*aureopunctata*
Frontoparietals	2	2	2	2
Supraoculars (l-r)[1]	4-4	4-4	4-4	4-4
Superciliaries (l-r)	5-5	5-5	5-5	5-5
Supralabials (l-r)	5-4	4-4	4-4	4-4
Infralabials	6-6	6-6	6-6	5-5
Scale rows around midbody	33	36	32	34
Ventral scale rows[2]	51	53	52	58
First loreal/supralabial[3]	2,3	2	2	2,3
Second loreal/supralabial[4]	3,4	2,3	2,3	3,4
Keels on middorsal scales	5	5(6)	5	3
Sdm I (l-r)[5]	7-7	6-6	8-8	6-6
Sdm II (l-r)	9-11	10-10	12-12	11-11
Sdm III (l-r)	14-12	13-14	14-15	15-16
Sdm IV (l-r)	15-15	14-14	17-16	16-14
Sdm V (l-r)	10-10	9-9	10-10	10-10
Sdp I (l-r)[6]	7-7	6-7	8-8	7-6
Sdp II (l-r)	13-13	9-9	13-13	11-11
Sdp III (l-r)	15-15	16-16	17-17	16-18
Sdp IV (l-r)	?-18	19-18	21-23	20-20
Sdp V (l-r)	14-14	13-13	13-14	14-13
Scales on upper eyelid (l-r)	13-11	13-12	11-11	?
Scales on lower eyelid (l-r)	16-15	15-12	16-16	?

superciliaries, the first not contacting prefrontal; two frontoparietals meeting medially, contacting third and fourth supraoculars; interparietal triangular; two parietals in contact behind interparietal; one pair of nuchals, keeled, in contact behind and slightly left of parietal junction.

Mental and postmental wider than long; postmental adjacent to first and anterior two-thirds of second infralabials; two pairs of chin shields, anterior pair in contact with postmental and posterior one-third of second and anterior three-quarters of third infralabials, posterior pair adjacent to posterior quarter of third and anterior two-thirds of fourth infralabials.

Dorsal and lateral scales on neck and body keeled, dorsal scales on original part of tail keeled; middorsal body scales with 5-6 keels; lateral scales of neck and sacral region with 5 keels; dorsal scales of forelimbs with 4 keels, postaxial scales with 2-3 keels; dorsal scales of manus, pes, and digits acarinate; dorsal and preaxial scales of hindlimbs with 2-3 keels; ventral scales of head, neck, body, and tail smooth; all scales, except head plates and scales of soles and digits cycloid and imbricate.

Coloration after 30 months in alcohol: dorsal and dorsolateral ground color of anterior two-thirds of head yellowish brown, posterior one-third dark grey; neck and anterior half of body dark grey; posterior half of body and tail light brown; head, neck, body, and tail with white spots, most arranged in rows. Forelimbs dorsally with prominent, isolated white spots; hindlimbs with many faint white spots. Ventral surfaces whitish with small, black spots. Palms and soles brownish.

Details of white spotting as follows. On head: single greyish-white, faint spot on posterior left side of frontonasal; one white spot on posterior extremity of each prefrontal extending posteriorly onto each side of frontal, ending before posterior extremity of frontal; one oval white spot on each frontoparietal; one large, oval spot on each parietal and one large spot between parietals extending posteriorly onto nuchals; one spot on middle of each nuchal scale; supra- and infralabials light colored. White spots on anterior half of neck mostly in 7 rows, 11 rows on posterior half of neck and shoulders, as follows: one lateral row with 6 large, isolated spots anteriorly on right and 7 on left, beginning on supralabials, passing across ear openings and just above forelimb insertions, and extending as smaller spots confined to single body scales posteriorly to groin; one dorsolateral row on each side above lateral row beginning behind eyes, passing across temporals and extending dorsolaterally along body and tail, each spot occupying half of 2-4 adjacent scales; three middorsal rows beginning at level of parietals and extending posteriorly along body and tail. In addition to these 7 rows, a row is inserted between the lateral and

TABLE 3. Morphometric (mm) variation in *Mabuya vezo* paratypes. * Broken tail, parts lost; ** regenerated tails, not broken; *** damaged scale.

	UADBA	UMMZ				
	1	217101	217102	217103	217104	217105
Sex	male	male	male	female	female	male
Maturity	mature	mature	mature	immature	mature	mature
SVL	51	50	46	36	53	46
Tail length	57*	77**	11*	60	75**	82**
Tail width	6.6	6.9	6.5	4.3	6.7	6.2
Tail depth	5.8	6.3	5.1	3.0	5.6	5.8
Head length	13.0	12.8	12.5	9.3	12.9	12.4
Head width	7.4	7.7	7.6	6.2	7.9	7.5
Head depth	5.4	5.6	5.3	3.6	5.0	5.0
Snout length	??	8.9	8.5	6.8	8.6	8.4
Internarial distance	2.0	1.9	2.0	1.6	1.9	1.9
Interocular distance	4.7	5.2	5.5	4.1	5.2	5.0
Orbital length	2.7	2.7	2.9	2.3	3.0	2.9
Eye-naris distance	3.2	3.1	2.9	2.4	3.2	2.9
Eye-ear distance	4.0	3.3	3.7	3.0	3.8	3.4
Frontal length	2.8	2.9	–***	2.3	3.2	3.0
Interparietal length	1.8	1.7	–***	1.2	1.7	1.8
Axilla-groin length	23	23	20	18	25	20
Forelimb length	15	13	14	11	13	13
Hindlimb length	20	21	21	15	20	18
4th finger length	3.9	4.1	3.7	3.2	3.9	4.4
4th toe length	6.9	6.9	6.6	5.1	6.5	6.4

dorsolateral rows on each side, beginning above and behind the ear opening and extending along body; another row begins on the shoulder behind the neck between the vertebral and dorsolateral rows on each side, extends along body, and converges with other rows at base of tail; and a weakly expressed, ventrolateral row of three spots is present in front of each forelimb. White spots become narrow on posterior dorsal and dorsolateral half of body and tail, occupying only the medial one-third of each scale and joining in places to form a faint white stripe.

Colour in preservative is only slightly changed from colour in life. The yellowish brown ground colour is more subdued in preservative, and the white spots are less distinctive, but the pattern remains.

Variation. Morphometric and meristic variation is summarized in Tables 1-4. Five of the seven known specimens are mature males; only one of the two females is immature. There is no obvious sexual nor ontogenetic morphometric and meristic variation in this small sample.

Males generally have more, and more strongly expressed, white spots on the head and body than females. White spots are present in front of the hindlimbs of males but not females. The single juvenile has a slightly darker ground colour than the adults, and its white spots are more vividly expressed.

Individual measurements are rather homogeneous. All specimens, except the juvenile female, have either broken or regenerated tails. One specimen (UADBA 1), 51 mm SVL, has an original tail broken (and lost) at 57 mm from cloaca. The original tail of the juvenile specimen is 1.6 times the SVL.

Individual meristic variation (Tables 2 and 4) is slight, with the notable exception of the presence of seven keels on the middorsal scales of one paratype (UADBA 1), in contrast to five on the remaining five.

Similarly, there is little individual variation in coloration. However, one specimen (UADBA 1) has very faint white spots on the posterior half of the body, although the white spots still extend onto the tail. Variation of head spots is restricted to differences in spot size.

Etymology. The name "vezo" (pronounced "vayzoo") refers to the Vezo ethnic group of Malagasy who occupy Lavenombato village and protect the type locality through their fady (taboo) system.

Habitat. All specimens collected and others that were observed were active on rocks with abundant crevices, either on the slope above Lavenombato or on the plateau of this village. The site contains tombs and is, therefore, "fady", or taboo, which affords the site protection from human disturbance. The area includes patches of degraded spiny forest, but the lizards were

TABLE 4. Meristic variation in *Mabuya vezo* paratypes. [1] (l-r) = left-right; [2] number of ventral scales counted longitudinally from postmentals to cloaca; [3] supralabial(s) contacting first loreal; [4] supralabial(s) contacting second loreal; [5] subdigital scales on fingers I-V of manus, left and right; [6] subdigital scales on toes I-V of pes, left and right; * damaged.

	UADBA	UMMZ				
	1	217101	217102	217103	217104	217105
Frontoparietals	2	2	2	2	2	2
Supraoculars (l-r)[1]	4-4	4-4	4-4	4-4	4-4	4-4
Superciliaries (l-r)	4-4	5-5	5-5	4-4	4-4	4-4
Supralabials (l-r)	4-4	4-5	4-4	4-4	4-4	4-4
Infralabials (l-r)	5-5	6-6	6-6	6-6	6-6	6-6
Scale rows around midbody	33	34	33*	31	32	33
Ventral scale rows[2]	51	50	51	49	53	51
First loreal/supralabial[3]	1,2	2,3	1,2	2	2	2
Second loreal/supralabial[4]	2,3	3,4	2,3	2,3	3	3
Keels on middorsal scales	7	5	5	5	5	5
Sdm I (l-r)[5]	6-6	5-6	5-5	6-6	6-5	6-6
Sdm II (l-r)	10-10	10-10	10-12	11-10	10-10	10-10
Sdm III (l-r)	14-14	14-15	12-13	14-13	13-13	14-14
Sdm IV (l-r)	15-14	14-15	15-15	14-14	15-15	14-14
Sdm V (l-r)	9-10	8-8	9-8	8-8	8-8	8-8
Sdp I (l-r)[6]	6-6	6-6	6-6	7-6	6-6	6-6
Sdp II (l-r)	11-11	12-10	11-11	10-10	11-11	11-11
Sdp III (l-r)	15-16	14-16	14-16	12-15	14-14	15-15
Sdp IV (l-r)	19-20	19-18	19-*	18-17	18-18	19-18
Sdp V (l-r)	12-13	13-14	13-12	14-12	11-12	10-11
Scales on upper eyelid (l-r)	13-12	12-13	12-12	13-12	11-11	13-14
Scales on lower eyelid (l-r)	14-14	14-16	16-15	14-15	15-16	15-14

always in forest openings between 0.5-2.0 km from the Onilahy River. *Mabuya vezo* is similar to *Mabuya vato* in that both are relatively small, rock-dwelling species. *M. vato*, however, was not found in microsympatry with *M. vezo*, and neither *M. vato* nor *M. dumasi* appear to occur on the south bank of the Onilahy River in the region of Lavenombato and St. Augustin. *M. vato* and *M. dumasi* were observed on the other (northern) side of the Onilahy River, near Sept Lacs, in gallery forest. This kind of forest is highly disturbed near Lavenombato and is used by local people to make charcoal. *M. vezo* is broadly sympatric with *M. aureopunctata*, *M. elegans*, and *M. gravenhorstii* on the south side of the Onilahy River, but it has a niche distinct from those of the latter three species. *M. elegans* is a relatively small ground dweller, usually observed in open areas with patches of grass, weeds, and bushes. It also occurs in open dry forests, and only rarely climbs onto tree trunks or rocks, and then only to escape capture. *M. aureopunctata* and *M. gravenhorstii* are larger species with apparently broader niches. They are occasionally found on open ground, but more often at sites with complex three-dimensional structures, such as piles of rocks or logs, that offer many refuges in crevices, root holes, and under sloughed bark and rotten wood. Frequently, there is dense cover of brush associated with these latter two species. *M. gravenhorstii* often basks on logs and rocks, whereas *M. aureopunctata* is more likely to be seen closer to ground level, although on one occasion a large adult of the latter species was observed on a narrow branch two meters up in a small tree.

Distribution. Mabuya vezo is known only from the type locality near the mouth of the Onilahy River in south-western Madagascar.

Breeding. The testes of three of the four adult males collected in March were not active; testes of the fourth were slightly enlarged. The testes and ovaries of the mature male and female collected in October are well developed and seemed to be enlarging at the time of capture. The right-side testis of the male (UMMZ 217105) is white and measures 4.7 x 2.3 mm. The right ovary of the mature female (UMMZ 217104) caught in October contains eight yolking oocytes of various sizes, the largest measuring 1.6 mm; the left ovary contains six yolking oocytes, the largest 1.3 mm. Only a few of these 14 oocytes are likely to develop to maturity during the ensuing reproductive season. The juvenile female (UMMZ 217103) caught in October has four very small oocytes of uniform size on each side. It appears that the breeding season of *Mabuya vezo* is such that hatchlings will appear early in the southern summer at the peak of the wet season.

DISCUSSION

Geographic variation in some of the other white-spotted species of the *aureopunctata*-group complicates the identification of *Mabuya vezo*. However, the differences between *M. vezo* and the other white-spotted species exceed the variation within each of the latter species, and the pattern of geographic variation within the white-spotted species does not support the argument that *M. vezo* is a geographic variant of one of them.

Mabuya vezo differs from *M. vato*, *M. dumasi*, and *M. aureopunctata* by having regular rows of white spots on the posterior half of body and tail, but some individuals of *M. aureopunctata* from Ampanihy and Beloza (near Tulear) also have white spots posteriorly. However, in these populations, which are not adjacent to the type locality of *M. vezo*, the posterior dorsal spots are arranged irregularly. A single specimen of *M. aureopunctata* was collected at Lavenombato, and it lacks white spots on the dorsal and dorsolateral surfaces of the posterior body and tail. *M. aureopunctata* from Ampanihy and Beloza are larger than *M. vezo*, which is consistent with their relatively large body size throughout their range.

The population of *Mabuya vato* closest to the type locality of *M. vezo* (Sept Lacs, 40 linear km distant on the other side of the Onilahy River) has, like *M. vezo*, seven rows of white spots anteriorly on the neck instead of the usual nine. However, *M. vato* differs consistently from *M. vezo* by having fewer rows of spots posteriorly on the neck and in the shoulder region, in lacking white spots posteriorly on the body and tail, and also by having reddish posterior coloration. The intensity of the reddish posterior coloration of *M. vato* varies geographically, but never approaches the yellowish-brown posterior coloration of *M. vezo*. Populations of *M. vato* that are most similar to *M. vezo* in posterior dorsal coloration (dull, reddish brown) occur at Mt. Ibity south of Antsirabe, which is a high-elevation site 525 linear km NE of the type locality of *M. vezo* at Lavenombato. Low-elevation populations of *M. vato* nearer to Lavenombato have the typical, bright, reddish posterior coloration.

Occasional individuals of *Mabuya dumasi* have small, irregular white spots on the dorsum of the neck and above the forelimbs, and others have tiny, posterior, dorsal, white spots confined to the posterior edge of dorsal scales. However, the distinctive row of white spots on each side of the neck, bordered above by a black stripe, readily identifies these individuals as *M. dumasi*.

The relationships among the white-spotted members of the *aureopunctata*-group are complex, and continuing surveys are revealing unusual distribution patterns and geographic variation that is difficult to assess. For example, *Mabuya vato* was previously believed to be a species confined to relatively low elevations in the south-west (Nussbaum & Raxworthy, 1994). However, the species has subsequently been found further north on the high plateau near Ihosy and Antsirabe at elevations up to 1650 m (Nussbaum & Raxworthy, unpublished). Distinctive populations of *M.* "*vato*" at other places may be only geographic variants, but they may also be undescribed species. *M. aureopunctata* is

highly variable throughout its range, and some local populations contain a bewildering variety of colour types, some of which are almost certainly hybrids. Chromosomal and molecular studies will be needed to fully comprehend the relationships between individuals and populations of white-spotted species of the *aureopunctata*-group.

The habitats of *Mabuya vezo* and other white-spotted Madagascan mabuyas are relatively immune to the type of human destruction that is likely to cause extinction in other species. Although the irrational burning and deforestation of Madagascar continues with increasing intensity, these species survive in marginal and degraded habitats, and their immediate future seems secure.

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ACUTE TOXICITY TESTS ON JAPANESE AMPHIBIAN LARVAE USING THIOBENCARB, A COMPONENT OF RICE PADDY HERBICIDES

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Acute toxicity tests were carried out on five species of Japanese amphibian larvae, at different developmental stages, to assess the risk posed by thiobencarb, a component of rice paddy herbicides. Test substances were four types of commercially formulated herbicide containing mainly thiobencarb, and the 24 h, 48 h, 72 h and 96 h LC_{50} (median lethal concentration) values of these herbicides were calculated by probit analysis. These values ranged from 0.9 to 6.5 mg/l of thiobencarb. Newly hatched larvae seemed to be slightly more resistant to the herbicides than well-developed larvae in all test species. There were no clear interspecific differences in responses. The actual thiobencarb concentration in paddy water was measured with indoor models for two weeks, and it ranged from <0.005 to 3.1 mg/l. Some of the measured concentrations exceeded the LC_{50} values. Thiobencarb residue in paddy water can therefore be lethal to amphibians throughout larval development. Tests with *Xenopus laevis* produced approximately the same LC_{50} values as those of Japanese amphibians. This indicates that experimental frogs such as *Xenopus laevis* can act as a model for these native and wild amphibians when toxicity tests are conducted.

Key words: Japanese amphibians, herbicide, thiobencarb, acute toxicity, risk assessment

INTRODUCTION

One of the most serious problems in wildlife conservation concerns the reasons underlying the decline of amphibians in different parts of the world (Barinaga, 1990; Blaustein & Wake, 1990, 1995; Blaustein, Wake & Sousa, 1994; Tyler, 1994; Stebbins & Cohen, 1995). Some amphibian declines may be caused by environmental contaminants, such as agricultural chemicals and heavy metals (e.g. Power, Clark, Harfenist & Peakall, 1989). In the study by Corn, Stolzenberg & Bury (1989), the effects of acid precipitation were related to the declines of several species of amphibian in the Rocky Mountains. However, there is little evidence that acid precipitation is a widespread cause of amphibian declines (Dunson, Wyman & Corbett, 1992). Recently, increased ultraviolet radiation resulting from ozone layer depletion has been highlighted as a possible cause of amphibian declines (Licht & Grant, 1997).

In Japan, common amphibians such as *Cynops pyrrhogaster*, *Rana nigromaculata* and *Hyla japonica*, as well as hynobiid salamanders have decreased in number (Matsui, 1996). The area of paddy fields has been reduced because of the overproduction of rice. This may be related to the decline of amphibians which inhabit or breed in paddy fields, as described by Matsui (1996). However, agricultural chemicals, in particular those herbicides frequently used in rice paddies, also seem to contribute to the declines of Japanese amphibians, because the recent amount of chemicals used on agricultural land in Japan is much higher (1.77 t/km^2) than that of most other western developed countries (0.09–0.58 t/km^2; Organization for Economic Co-operation and Development - OECD, 1991). Nevertheless, the risk from and the harmful effects of herbicides on Japanese amphibians have been little studied. Recent research by the Japan Ministry of Agriculture, Forestry and Fisheries (1994, 1995, 1996) shows that the production of thiobencarb (*S*-4-chlorobenzyl *N*,*N*-diethylthiocarbamate) has been the highest of all herbicidal chemicals, and herbicides containing thiobencarb have been generally used in rice paddies.

Consequently, this study investigated the toxicity of and the risk from thiobencarb to Japanese amphibians, focusing on the aquatic larval stages which seem to be most vulnerable to contaminants in water. Several species of amphibian were selected which differ phylogenetically from one another, and acute toxicity tests were conducted with larvae of these amphibians at several developmental stages. The actual thiobencarb concentration in paddy water was measured with indoor paddy models. The potential risk from thiobencarb to Japanese amphibians is discussed by comparing lethal concentrations with the actual concentrations in paddy water. The differences in susceptibility to thiobencarb among developmental stages and among species are also described.

MATERIALS AND METHODS

TEST SPECIES

Five species of Japanese amphibian belonging to different families were selected as test species. They were the Japanese fire-bellied newt, *Cynops pyrrhogaster* (Salamandridae), the eastern Japanese common toad, *Bufo japonicus formosus* (Bufonidae), the Japanese tree frog, *Hyla japonica* (Hylidae), the black-spotted pond

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TABLE 1. Developmental stages of amphibian larvae to which acute toxicity tests were applied. [1] Stage no. of test species was based on the tables of normal stages by Kajishima & Eguchi (1989), Ichikawa & Tahara (1989), Iwasawa & Futagami (1992), Iwasawa & Kawasaki (1979) and Nieuwkoop & Faber (1975) for *Cynops pyrrhogaster*, *Bufo japonicus formosus*, *Hyla japonica*, *Rhacophorus arboreus* and *Xenopus laevis*, respectively. Because the table of normal stages of *Rana nigromaculata* has not been published, that of *Rana porosa porosa* (Iwasawa & Morita, 1980), a related species to *Rana nigromaculata*, was used. [2] Each value is a mean of 20–50 individuals from the test populations.

Species	Developmental stage[1]	Time after hatching (day)	Total length[2] (mm)	Body weight[2] (mg)	Morphological characteristics
Cynops pyrrhogaster	Early (41–43)	<1	11	13	Balancers remaining.
	Middle (50–52)	14	16	25	Almost completely developed forelimbs.
	Late (57–59)	42	27	160	Almost completely developed hindlimbs and degenerating external gills.
Bufo japonicus formosus	Early (30)	<1	15	25	External gills remaining.
	Middle (36)	14	26	110	Developed opercula.
	Late (40, 41)	28	28	170	Almost completely developed hindlimbs but forelimbs still invisible.
Hyla japonica	Early (22, 23)	<1	6	2	External gills remaining.
	Middle (27–29)	14	16	61	Developed opercula.
	Mid.-late (31, 32)	28	26	190	Limbs still only buds.
Rana nigromaculata	Early (23, 24)	<1	10	11	External gills remaining.
	Middle (26, 27)	14	18	60	Developed opercula.
	Mid.-late (28, 29)	28	28	180	Limbs still only buds.
Rhacophorus arboreus	Early (30, 31)	<1	19	52	External gills remaining.
	Middle (36, 37)	14	27	160	Limbs still only buds.
Xenopus laevis	Early (35–38)	<1	6	5	External gills remaining.
	Middle (46, 47)	14	16	29	Developed opercula.
	Mid.-late (49)	32	35	210	Appearance of sensory tentacles but limbs still only buds.

frog, *Rana nigromaculata* (Ranidae) and the forest green tree frog, *Rhacophorus arboreus* (Rhacophoridae). These amphibians were collected in and around the paddy fields of mountainous areas in the northern part of Kyoto Prefecture, from April to June. *Bufo japonicus formosus*, *Rana nigromaculata* and *Rhacophorus arboreus* were obtained as egg masses. For *Hyla japonica*, amplectant pairs were captured and allowed to spawn in the laboratory. For *Cynops pyrrhogaster*, only adult females were collected and induced to lay eggs in the laboratory by injection of human chorionic gonadotropin (HCG) (Wako Pure Chemical Industries Ltd., Osaka, Japan). In addition to these Japanese amphibians, *Xenopus laevis* (Pipidae), a common experimental frog, was also used as a test animal. Adult *Xenopus laevis* were obtained from a commercial dealer (Shimizu Laboratory Supplies Co., Kyoto, Japan). Amplexus and egg-laying were induced by HCG injection. After hatching, larvae of the six species were maintained at 20±1°C, in polypropylene aquaria filled to a depth of 10 cm with dechlorinated tap water. Feeding began at one week after hatching. Larval newts were fed brine shrimp (larvae of *Artemia salina*) and frog tadpoles were fed homogenate of boiled spinach daily. The daily diet amount was determined by larval size so as to avoid water deterioration from excess food and cannibalism from insufficient food. The six species of larvae were divided into two or three stage groups on the basis of larval size and morphological characteristics (Table 1). Test species were examined at each developmental stage, except for the late stages of *Hyla japonica*, *Rana nigromaculata*, *Rhacophorus arboreus* and *Xenopus laevis* because the larvae were too large to be examined under proper conditions with the limited experimental facilities. Each test was conducted using larvae from three egg masses (*Bufo japonicus formosus*, *Hyla japonica*, *Rana nigromaculata*, *Rhacophorus arboreus* and *Xenopus laevis*) or those from twenty-four adult females (*Cynops pyrrhogaster*).

The numbers of amphibians collected and larvae used for toxicity tests were minimized in the interest of conservation and ethics. With the exception of *Xenopus laevis*, adult amphibians after egg-laying and the larvae not used for toxicity tests were released at the sites of capture.

TABLE 2. The components of the four types of commercially formulated herbicide based on the data on the herbicide packages, and the recent annual amount of each herbicide on the market published by the Japan Ministry of Agriculture, Forestry and Fisheries (1994, 1995, 1996). [1] Oct. 1993–Sept. 1994; [2]Oct. 1994–Sept. 1995; [3]Oct. 1995–Sept. 1996.

Type	Formulation and thiobencarb content	Herbicidal chemicals other than thiobencarb	Other ingredients	Recent annual amount of each herbicide on the market in Japan		
				1994[1]	1995[2]	1996[3]
Type A	Granules (5%)	Mefenacet (1%) Bensulfuron-methyl (0.17%)	Mineral powder etc.	10 052 t	10 335 t	9167 t
Type B	Granules (7%)	Simetryn (1.5%)	Mineral powder etc.	1093 t	1220 t	668 t
Type C	Granules (10%)	MCPB-ethyl (0.8%) Simetryn (1.5%)	Mineral powder etc.	2231 t	2207 t	1595 t
Type D	Emulsifiable concentrate (50%)	None	Organic solvents (xylene etc.) and emulsifiers etc.	38 kl	45 kl	42 kl

TEST SUBSTANCES

The primary test substance for acute toxicity tests was not standard thiobencarb but four types of commercially formulated herbicide whose main ingredient was thiobencarb (Table 2), because the latter are actually used in rice paddies and the former is used only for chemical analysis. To confirm whether the lethality of the formulated herbicides was caused mainly by thiobencarb, tests of standard thiobencarb (purity 99 %, Wako Pure Chemical Industries Ltd., Osaka, Japan) were conducted with middle stage larvae of *Cynops pyrrhogaster* and *Xenopus laevis* - these two species of amphibians can be easily induced to lay eggs by hormone injection. (If there are large differences in lethality values of the four formulated herbicides among species and among developmental stages, standard thiobencarb tests should be conducted with all test species and all developmental stages of larvae.)

In addition to the tests with four types of herbicide and standard thiobencarb, tests with pentachlorophenol sodium salt (PCP-Na; purity 90 %, Wako Pure Chemical Industries Ltd., Osaka, Japan), a reference substance, were also conducted for two reasons: (1) the toxicity of PCP-Na has been studied well and it is recommended as a reference substance for acute toxicity tests with aquatic animals, in order to confirm whether or not experimental conditions are appropriate and the response of test animals is normal (the Japan Environment Agency, 1990); and (2) past effects of herbicides on Japanese amphibians can be also estimated, as pentachlorophenol (PCP) and its salts were generally used as herbicides in Japanese rice paddies in the 1960's until thiobencarb replaced them (Kobayashi, 1979).

ASSAY PROCEDURE

Various toxicity tests on aquatic organisms other than amphibians have been validated by OECD to evaluate the effects of chemicals on biotic systems. In the present study, toxicity tests with amphibians were performed in accordance with OECD *Guidelines for Testing of Chemicals* No. 203: "Fish, acute toxicity test" (OECD, 1993).

Dechlorinated tap water (hardness: 40 mg/l as $CaCO_3$) was used for exposure water and control water. For one test substance, one blank (control) and at least five concentrations in a geometric series with a factor of $10^{1/4}$ (=1.8) were prepared. The test solutions of each concentration including the control were each poured into a set of ten glass beakers (200 ml-beakers for early stage larvae of all test species and middle stage larvae of *Cynops pyrrhogaster*, *Hyla japonica*, *Rana nigromaculata* and *Xenopus laevis*, and 500 ml-beakers for the others). Solution volume was 100 ml per 200 ml-beaker and 300 ml per 500 ml-beaker. Standard thiobencarb was first dissolved in a small amount of acetone, because it is nearly insoluble in water. Accordingly, in the test of standard thiobencarb, all of the test solutions were adjusted to contain the same volume of acetone (0.01 %). Each beaker held one individual to prevent cannibalism and was kept in an environmental chamber maintained at 20±1°C on a 12 h L: 12 h D photoperiod with white fluorescent lamps. Handling of larvae was performed with a pipette or a net of 0.2 mm mesh depending on larval size. Exposure period was 96 h. All tests were conducted without feeding or aeration. The test solutions were renewed every day to prevent degradation of water quality. Both used and renewed

solutions were examined for pH and dissolved oxygen (DO) with a pH meter and a DO meter.

In the tests with PCP-Na and standard thiobencarb, three beakers of each concentration were picked out at random and the used solutions in them were examined for the concentration of the test substance. PCP-Na is easily absorbed by aquatic animals (Kobayashi, 1979) and the PCP-Na concentration of the test solutions may drift during the test. Thiobencarb may also be absorbed by larvae or broken down during the test period, and the thiobencarb concentration of used solutions may be significantly decreased. However, used solutions of the four formulated herbicides, types A–D, were not examined for the thiobencarb concentration because of low recoveries (<60 %) of standard thiobencarb from the test solutions of types A–D (The test solutions of types A–D were suspensions or emulsions of the formulated herbicides, and this may account for the low recoveries). The PCP-Na concentration was measured with a spectrophotometer by the chloroform extraction method (American Public Health Association, American Water Works Association and Water Pollution Control Federation, 1975). The thiobencarb concentration was measured with a gas chromatograph connected to a mass spectrometer (GC/MS) after extraction with dichloromethane, as described in the subsequent section.

Dead larvae were counted at the time of daily changes of the test solutions. Larvae were considered dead if there was no visible movement and if touching the caudal peduncle produced no reaction. The mortality at each test concentration was calculated, and the 24 h, 48 h, 72 h and 96 h LC_{50} (median lethal concentration) values were calculated by probit analysis following Finney (1952) and Yoshimura & Ohashi (1992).

To choose the appropriate test concentration range, a range-finding test was properly conducted before the definitive test. There was no replication in the definitive test unless its results contradicted those of the range-finding test.

Measuring Thiobencarb in Paddy Water

The actual thiobencarb concentration in paddy water was measured with simple indoor paddy models. Although measuring in the field may be a better indication of exposure levels in natural amphibian populations, the results would vary according to prevailing weather conditions and be difficult to standardize. Thiobencarb concentration was therefore measured using the following indoor models.

Five polypropylene containers (60 cm × 40 cm × 20 cm) were prepared. They were filled with paddy soil to a depth of 10 cm and dechlorinated tap water to a depth of 3 cm from the top of the paddy soil. The paddy soil (clay-based soil; carbon, 1.57 %; nitrogen, 0.12 %) was collected from an experimental paddy area where no herbicides had been used for over a year, in the Kyoto Prefectural Institute of Agriculture. The herbicides of types A–D were spread over four of the containers separately at the rate of 3 kg/10 a (types A–C) or 800 ml/10 a (type D) based on the directions for use of each herbicide printed on its package. The last container was used as a control and no herbicides were applied to it. The containers were put in an environmental chamber under the same conditions as the acute toxicity tests, and analysed after two weeks. When the water level in a container dropped because of evaporation, water was added up to the original level.

TABLE 3. GC/MS operating conditions.

Gas chromatograph	Hewlett Packard 5890
Column	J & W DB-1 (length 30 cm, i.d. 0.25 mm, film 0.25 μm)
Oven temperature	60 °C for 2 min, then rising at 20 °C/min to 180 °C, 4 °C/min to 240 °C, and 10 °C/min to 280 °C
Injection mode	Splitless (purge off 1 min)
Mass spectrometer	JEOL JMS-AX505WA
Monitor ion (m/z)	257

Water from each paddy sample was carefully decanted so as not to disturb the mud, at intervals of 6 h, 24 h, 48 h, 72 h, 96 h, 7 d and 14 d after applying the herbicides. The water samples were extracted by liquid-liquid partitioning with dichloromethane, and the extract was analysed with a GC/MS. The operating conditions are shown in Table 3. The lower detection limit for thiobencarb was 0.005 mg/l. The recovery of standard thiobencarb through the chemical analysis was more than 90 %.

RESULTS AND DISCUSSION

Lethality Values of PCP-Na as a Reference Substance

Throughout all tests of PCP-Na, no abnormal responses of the larvae were observed in the control solutions, and the values of pH and DO in the test solutions were normal (pH, 6.7–7.9; DO, 72–120 % of the air saturation value at 20 °C). Consequently, death of larvae throughout the tests seemed to be caused only by the test substance. The PCP-Na concentration of the used test solutions was more than 80 % of the nominal concentration, even though it was possible that the PCP-Na concentration was somewhat reduced due to absorption by test individuals. Therefore, the results of PCP-Na tests were accepted and the LC_{50} values were calculated.

The LC_{50} values of PCP-Na are shown in Fig. 1. These values ranged from 0.070 to 0.31 mg/l (24 h

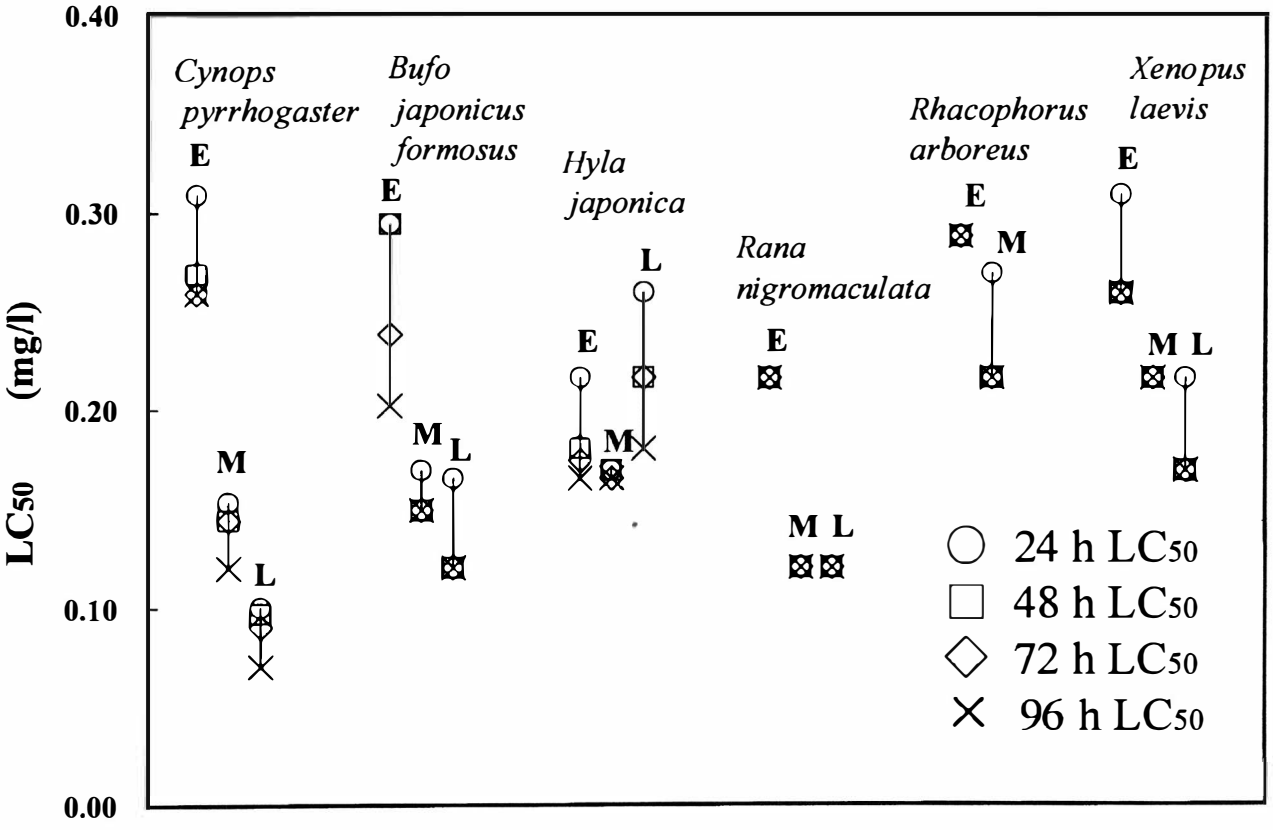

FIG. 1. The LC_{50} values of PCP-Na for the larvae of six species of amphibian in various developmental stages. E, M and L represent early, middle and mid-late or late stages, respectively. Characteristics of each stage are described in Table 1.

LC_{50}, 0.10–0.31 mg/l; 48 h LC_{50}, 0.097–0.29 mg/l; 72 h LC_{50}, 0.090–0.29 mg/l; 96 h LC_{50}, 0.070–0.29 mg/l). All the LC_{50} values, except those of late stage *Cynops pyrrhogaster* larvae, were distributed between 0.1 and 0.4 mg/l. There were no obvious interspecific differences in the LC_{50} values. In all species except *Hyla japonica*, the LC_{50} values became lower as developmental stage proceeded. This tendency was clearest in *Cynops pyrrhogaster*. A similar tendency was observed by Sanders (1970) who reported that susceptibility of *Bufo woodhousii fowleri* to DDT increases with age of the tadpoles. In my tests, late stage larvae of *Cynops pyrrhogaster* seemed to be highly susceptible to PCP-Na. This high susceptibility may be related to developmental changes associated with metamorphosis as suggested by Sanders (1970). Hall & Swineford (1980, 1981), however, reported the opposite trend, i.e. a positive correlation between age and resistance to chemicals, in toxicity tests of toxaphene with the larvae of seven species of amphibian. There may be different patterns in the relationships between age and susceptibility to chemicals in different amphibian larvae.

PCP and its salts were common rice paddy herbicides in the 1960's in Japan, and their application to rice paddies often caused mass mortality of freshwater fishes and shellfish living near paddy fields (Kobayashi, 1979). The 48 h LC_{50} values of PCP for Japanese freshwater fishes such as carp and trout, and shellfish such as setashijimi are 0.056–0.38 mg/l (Kanazawa, 1979). In the six amphibian species, the 48 h LC_{50} values of PCP-Na were 0.097–0.29 mg/l, corresponding to 0.090–0.27 mg/l of PCP. Therefore, PCP is as lethal to amphibians as it is to Japanese freshwater fishes and shellfish, and it is possible that in the past, PCP residue in paddy water had a lethal influence on amphibians as well as on freshwater fishes and shellfish.

ACUTE TOXICITY OF THIOBENCARB

In all tests of thiobencarb (types A–D and standard thiobencarb), no stressed or weakened individuals were observed in the control solutions, and there were no abnormal values of pH or DO in the test solutions (pH, 6.7–7.5; DO, 76–110 % of the air saturation value at 20 °C). In the tests with standard thiobencarb, the thiobencarb concentration of used solutions was more than 80 % of the nominal concentration.

The LC_{50} values of types A–D are shown in Fig. 2. The LC_{50} values of each type did not differ obviously among species. Large decreases in LC_{50} values with increased larval development were not observed, unlike the results of the PCP-Na tests. However, among the 24 h LC_{50} values within each species, the 24 h LC_{50} value of early stage larvae was always the highest. Consequently, early stage larvae seemed to be slightly more resistant to the herbicides than well-developed larvae when they were exposed for only 24 h. In the report by Licht (1985), a jelly coat seems to protect embryos

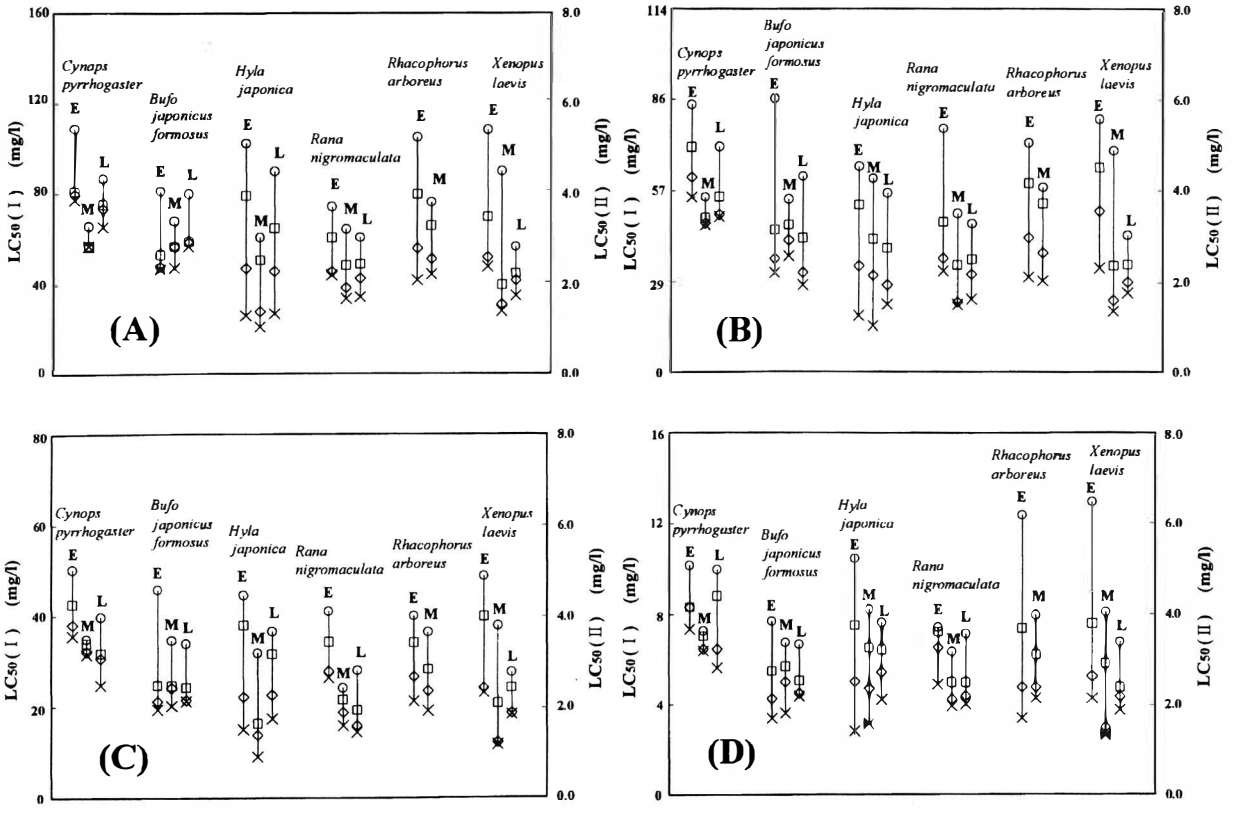

FIG. 2. The LC_{50} values of the four types of herbicide shown in Table 2 for the larvae of six species of amphibian in various developmental stages. (A), (B), (C) and (D) represent the LC_{50} values of types A, B, C and D, respectively. LC_{50} (I) is shown as the concentration of a whole formulated herbicide and LC_{50} (II) is shown as the thiobencarb concentration on the basis of the thiobencarb content. Symbols and abbreviations are the same as in Fig. 1.

from absorbing pesticides. The short term resistance of early stage larvae may be caused by jelly coats remaining around the bodies of newly hatched larvae.

There were apparent differences in lethality values among the four types of herbicides: as the concentration of a whole formulated herbicide, the 24–96 h LC_{50} (I) values of types A, B, C and D were 21–110 mg/l, 14–86 mg/l, 9.0–50 mg/l and 2.6–13 mg/l, respectively. These values decreased in alphabetical order corresponding to the increase of the thiobencarb content. When these values were expressed as thiobencarb concentration on the basis of the thiobencarb content, there were no distinct differences among the four types: the 24–96 h LC_{50} (II) values of types A, B, C and D were 1.0–5.4 mg/l, 1.0–6.0 mg/l, 0.9–5.0 mg/l, and 1.3–6.5 mg/l, respectively. These were approximately the same as the LC_{50} values of standard thiobencarb for middle stage larvae of *Cynops pyrrhogaster* and *Xenopus laevis* (Fig. 3). The results suggest that the lethal effects of the four types of herbicide were caused mainly by

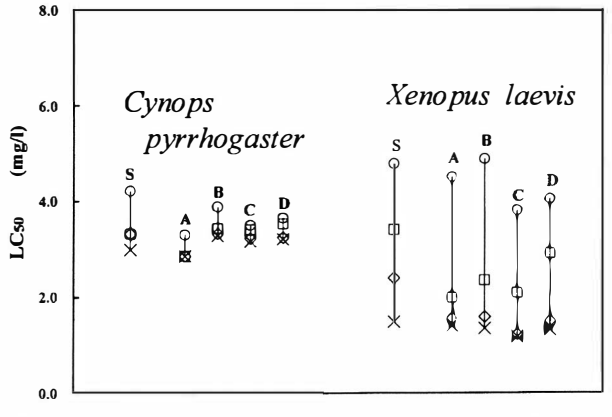

FIG. 3. Comparison of the LC_{50} values of types A–D with those of standard thiobencarb for middle stage larvae of *Cynops pyrrhogaster* and *Xenopus laevis*. The LC_{50} values of types A–D are shown as the thiobencarb concentration. S, A, B, C and D represent standard thiobencarb, type A, type B, type C and type D, respectively. Symbols are the same as in Fig. 1.

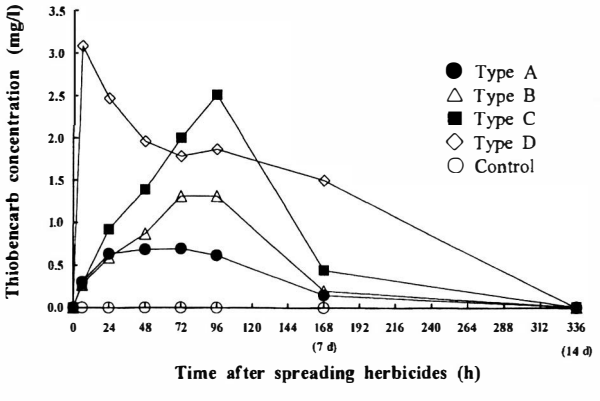

FIG. 4. Temporal changes in the measured thiobencarb concentration in model paddy water for two weeks. Each paddy model was treated with types A–D herbicides separately.

thiobencarb, although these herbicides contain other chemicals such as mefenacet, bensulfuron-methyl, simetryn, MCPB-ethyl and xylene.

THIOBENCARB CONCENTRATION IN PADDY WATER AND RISK ASSESSMENT OF THIOBENCARB FOR JAPANESE AMPHIBIANS

Thiobencarb concentrations in paddy water of the indoor models are shown in Fig. 4. In types A–C, the thiobencarb concentration increased gradually in the beginning, and attained maximum levels after 72 or 96 h. This was because they were in granular form and took a long time to dissolve in water. The thiobencarb concentration then decreased rapidly and dropped to approximately 0.005 mg/l, the lower limit of detectable thiobencarb, two weeks after applying the herbicides. In type D, the thiobencarb concentration increased rapidly and reached its maximum soon after applying the herbicide, because type D was an emulsifiable concentrate which mixed with water easily. Subsequently, the thiobencarb concentration decreased gradually and dropped to lower than 0.005 mg/l at the end of the experiment. In the control paddy, thiobencarb was undetectable during the experiment. The maximum thiobencarb concentrations were 0.70 mg/l, 1.3 mg/l, 2.5 mg/l and 3.1 mg/l for types A, B, C and D, respectively, and they increased in proportion to the increase of the thiobencarb content.

In the field, thiobencarb concentrations may be lower than these results because thiobencarb can be absorbed by soil or vegetation in rice paddy, and can be decomposed by microorganisms or ultraviolet radiation. According to Kanazawa (1992), in Japan, thiobencarb concentrations in paddy water in the field attained a peak of 0.908 mg/l on the day following the herbicide application and dropped to 0.26 mg/l after seven days. Ross & Sava (1986) reported that when thiobencarb was applied in granular form to a rice field in California, the thiobencarb concentration in paddy water was highest (0.576 mg/l) four days after the herbicide application and dropped to 0.056 mg/l after sixteen days. There were no great differences in the pattern of temporal change of the thiobencarb concentration and its maximum level between the results of these two field experiments and those of the indoor experiment, considering the differences in experimental conditions such as temperature and water depth of rice paddies. The results of the indoor experiment were therefore used for the risk assessment of thiobencarb for Japanese amphibians.

As described above, the 24–96 h LC_{50} values for amphibian larvae ranged from 0.9 to 6.5 mg/l. In all four types, the thiobencarb concentrations remained lower than 6.5 mg/l for two weeks after the herbicides were applied. However, in types B–D, there was a period when the thiobencarb concentration exceeded 0.9 mg/l. In type A, although the thiobencarb concentration did not exceed 0.9 mg/l, there was a period when it came very close to this level. Thus, the thiobencarb concentration in paddy water can be lethal to amphibian larvae for two weeks after applying herbicides.

Although there were no clear differences in the LC_{50} values of thiobencarb among the amphibian species, the risk of thiobencarb to them in nature should be discussed with due regard to not only the LC_{50} values but also to their natural histories. The risk of thiobencarb to Japanese amphibians was assessed with reference to the reports by Nakamura & Ueno (1963) and Maeda & Matsui (1989). Herbicides are spread within a month of rice-planting, between April to June in Japan (Japan Plant Protection Association, 1994). Because several species of Japanese amphibian such as *Cynops pyrrhogaster*, *Hyla japonica*, *Rana nigromaculata* and *Rhacophorus arboreus* spawn in rice paddies in the rice-planting season, spawning of these amphibians can coincide with herbicide applications. As Fig. 4 shows, thiobencarb concentrations in paddy water were detected for less than two weeks after applying herbicides. Therefore, amphibians such as *Rana nigromaculata*, which usually spawn in rice paddies for a comparatively short period, would be affected by thiobencarb. If their spawning occurs when the thiobencarb concentration in paddy water attains its peak, many individuals would be affected by thiobencarb. On the other hand, although *Cynops pyrrhogaster*, *Hyla japonica* and *Rhacophorus arboreus* also spawn in rice paddies, entire local populations would not be damaged by thiobencarb as much as *Rana nigromaculata*, because their breeding seasons continue for a long period and their risk of encountering the peak of the thiobencarb concentration in paddy water would be attenuated overall. In comparison with the above amphibians, *Bufo japonicus formosus* would be hardly affected at all by thiobencarb, because it usually spawns in mountain roadside ditches or temporary pools in mountainous areas, rather than in rice paddies.

In the present study, all five species of Japanese amphibian used to investigate interspecific differences in susceptibility to herbicides showed approximately the same lethality values to PCP-Na and thiobencarb. Moreover, there was no remarkable difference in lethality values between these Japanese amphibians and *Xenopus laevis*, a common experimental frog. Because there was no replication in each definitive test, the result of each test is not entirely representative of the species and the developmental stages which were tested. However, the consistency in susceptibility among species and among developmental stages suggests that the results are representative of amphibians in general. Hall & Swineford (1981) also observed that acute lethality values of endrin and toxaphene tested on the larvae of six species of amphibian differed from those of *Rana sphenocephala*, a standard test species, by less than one order of magnitude. They concluded that a water quality standard based on tests with *Rana*

sphenocephala would protect many species of amphibian if a safety factor of 0.1 is used. Therefore, a variety of amphibian species are not always necessary for toxicity tests. When toxicity tests are conducted, an experimental frog such as *Xenopus laevis* can be used as a substitute for many species of amphibian, including both widespread and locally restricted species.

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SHORT NOTE

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STATUS OF THE EXTINCT GIANT LACERTID LIZARD *GALLOTIA SIMONYI SIMONYI* (REPTILIA: LACERTIDAE) ASSESSED USING mtDNA SEQUENCES FROM MUSEUM SPECIMENS

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Gallotia simonyi (Steindachner, 1889) is a member of an endemic Canary Island genus and is among the largest of the approximately 250 recent species of lacertid lizards. Although Steindachner gave the locality of the types as the Roques de Salmor, off El Hierro island, previous workers (Urusaustegui, 1983; Manrique & Saavedra, 1873; Viera & Clavijo, 1983) and subsequent ones (Machado, 1985) regard the more western of the two rocks in the group, the Roque Chico de Salmor, as the actual source of material. The Roque Chico is 37 m high with a surface area of less than 10,000 m^2 and lies 830 m from the northern coast of El Hierro (Machado, 1985). Several specimens were collected from the rock after *G. simonyi* was described but none were encountered later than about 1940 (Salvador, 1971; Machado, 1985). The species was consequently considered extinct by the scientific community. In fact, residents on El Hierro were aware of a surviving population on an almost inaccessible cliff, the Fuga de Gorreta, on El Hierro itself (Salvador, 1971). This was formally reported in 1975 (Böhme & Bings, 1975; see also 1977; Böhme *et al.*, 1981). Some morphological differences from the extinct population of *Gallotia simonyi* from the Roque Chico de Salmor, were noted by Machado (1985), who referred to the El Hierro population as *Gallotia* affinis *simonyi*. This population was subsequently given formal subspecies status as *Gallotia simonyi machadoi* López-Jurado, 1989. Morphological differences from the Roques de Salmor subspecies, *G. s. simonyi*, are said to include: smaller size, less robust build, a less triangular and more oval pileus, a less depressed head, and smaller more numerous temporal scales surrounding a clearly defined enlarged masseteric scale (Machado, 1985; López-Jurado, 1989). The temporal and masseteric characters have been confirmed by Rodríguez-Domínguez *et al.* (1998), who also note that *G. s. machadoi* has relatively shorter hind legs than *G. s. simonyi*. It should, however, be borne in mind that the samples available of *G. s. simonyi* were small and that those of *G. s. machadoi* came from a captive population derived from few individuals (Pérez-Mellado *et al.*, 1997).

It is possible that the differences between the two populations of *G. simonyi*, one living and one extinct, are merely part of an original pattern of complex minor geographical variation which has been accentuated by the restriction of a once widespread species to two very small and different localities. *G. simonyi* was certainly once far more widely distributed and recent fossil remains are known from many localities on El Hierro (Böhme *et al.*, 1981; Bings, 1985; Izquierdo *et al.*, 1989; López-Jurado *et al.*, 1998; Fabiola Barahona *pers. com.*). Alternatively, given the apparent degree of morphological difference between them, the two populations could represent lineages that have been separate for a long time. We distinguished between these two hypotheses by examining mitochondrial DNA gene sequences.

Although suitable fresh tissue samples were available for *G. s. machadoi*, they were inevitably lacking for *G. s. simonyi*, which is now only known from about ten specimens in museum collections, mainly preserved in alcohol. Those most easily available to us, in the Natural History Museum, London (formerly the British Museum (Natural History)), are kept in industrial methylated spirit (IMS), a commercial preparation containing ethanol but also methanol and a wide range of other chemicals, some of which are known to be damaging to DNA. As might be expected, the DNA extracted from these specimens, which are 68 years old, was very degraded, the largest fragments consisting of about 500 bp. Nevertheless, it has been possible to compare it with homologous regions of sequence from *Gallotia simonyi machadoi*.

Data for the animals used are as follows.

Gallotia s. simonyi, BMNH 1967.1736-1737, male, female; labelled 'Roques Zalmor'; collected by H. B. Cott in 1931.

Gallotia s. machadoi. Six captive animals from the "Centro de Reproducción e Investigación del Lagarto Gigante de El Hierro 38911, Frontera"

To reduce the possibility of contamination, all the molecular work on the *Gallotia s. simonyi* tissue was completed before the six *Gallotia s. machadoi* samples were received. Total genomic DNA was extracted either from 0.5-1 cm^3 of thigh muscle (*G. s. simonyi*) or 2-3 mm^3 of tail tissue (*G. s. machadoi*). The material was air dried for two minutes, finely diced with a sterile scalpel blade and transferred to a tube containing 800 µl of proteinase K digestion solution (100mM Tris-HCL pH8; 100mM NaCl; 10 mM EDTA pH8; 0.5%

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TABLE 1. List of the primers used to amplify the three mitochondrial genes.* represents the position of the 3' end of the primer in reference to the human mitochondrial complete genome (Anderson *et al.* 1981). # it is not possible to homologise these positions with the human reference but both are internal with respect to 16SL(F) and 16SH1(R).

Primer	Gene	Sequence (5'-3')	Position*	Reference
GLUD-5'(F)	Cyt b	TGA TAT GAA AAA CCA TCG TTG	14724	Martin *et al.* (1992)
CB107(F)	Cyt b	CAC ATY CAY CGT GAY GTY CAA	14968	This study
CB144(R)	Cyt b	CGR ATT ART CAR CCR TGT TG	14966	This study
CB1(F)	Cyt b	CCA TCC AAC ATC TCA GCA TGA TGA AA	14841	Kocher *et al.* (1989)
CB2(R)	Cyt b	CCC TCA GAA TGA TAT TTG TCC TCA	15149	Kocher *et al.* (1989)
CB2(F)	Cyt b	TGA GGA CAA ATA TCA TTC TGA GGG	15172	This study
CBSim(F)	Cyt b	CCT TCT ACC ATT TAT AAT YTT AGG YAC	15321	This study
CBSim(R)	Cyt b	GTC TGA GTT TGA GTT YAR TCC GGT T	15369	This study
CB3(R)	Cyt b	GGC AAA TAG GAA RTA TCA TTC	15556	Martin *et al.* (1992)
COI(F)	COI	CCT GCA GGA GGA GGA GAY CC	6586	Palumbi (1996)
COI2(F)	COI	TGA CTT GCA ACR CTT CAC GGA GG	6892	This study
COI2(R)	COI	CCT CCG TGA AGY GTT GCA AGT CA	6870	This study
COI(R)	COI	CCA GAG ATT AGA GGG AAT CAG TG	7086	Palumbi (1996)
16SL1(F)	16S	CCG TGC AAA GGT AGC ATA ATC AC	2605	This study
16SSI(F)	16S	TAG TTG GGG CGA CTT CGG AGY A	####	This study
16SSI(R)	16S	GTT GGC CRT GAT ATG GTA GGG	####	This study
16SH1(R)	16S	CCG GTC TGA ACT CAG ATC ACG T	3059	This study

SDS and 0.75 mg/ml of proteinase K); the tube was then incubated at 37°C overnight in an orbital shaker. Purification was by phenol/chloroform extraction (Sambrook *et al.*, 1989), followed by centrifugal dialysis though a Centricon 30000 MW membrane (Amicon). The Polymerase Chain Reaction (PCR) technique was used to amplify and directly sequence 813 bp of the cytochrome-*b* (cyt-*b*), 501 bp of the cytochrome oxidase I (COI) and 411 bp of the 16S rDNA mitochondrial genes from the eight *Gallotia simonyi*. The primers used in both the amplification and the sequencing of these three mitochondrial fragments are listed in Table 1. Thermocycling consisted of an initial 90 s at 94°C followed by 35 cycles of 94°C for 30 s, 45°C for 45 s, and 72°C for 1 min, and then by a single cycle at 72°C for 10 min. Successful PCR bands were cut out and purified using a silica-based method (Boyle & Lew, 1995). The PCR products was sequenced using an ABI 377 automated sequencer, following the manufacturer's protocols.

Fragments of three different mitochondrial genes (cyt-*b*, COI and 16S rDNA) comprising a total of 1725 bp were sequenced for two specimens of the extinct *G. s. simonyi* and six of the extant *G. s. machadoi*. Results showed that the sequences are identical at all sites, suggesting that the two subspecies are more similar than previously thought and represent an example of morphological variation without molecular divergence, at least in the studied sequences. The eight cyt-*b* sequences analysed here differ in three positions (positions 15142, 15144-45 of the human mitochondrial genome; Anderson *et al.*, 1981) from the three *G. s. machadoi* cyt-*b* partial sequences cited by González *et al.* (1996). These apparent nucleotide differences are situated in a short region spanning the last seven nucleotides of the sequence analysed by González *et al.* (1996) and, if real, would cause an amino acid substitution. The amino acid concerned is a leucine encoded by nucleotides 15143-45 of the human mitochondrial genome (Anderson *et al.*, 1981) which is conserved in insects, mammals and reptiles (including the *Gallotia simonyi* material investigated here and all the other species of *Gallotia*). In contrast, it would be replaced by a proline in the *G. s. machadoi* sequence of González *et al.* (1996). A comparison of all available *Gallotia* sequences point towards a mistakenly inserted G at position 15142 of the González *et al.* (1996) *G. simonyi* sequences, causing a displacement of the reading frame and, consequently, the other two nucleotide changes.

The identity of 1725 bp of their mitochondrial DNA sequence indicates that *G. s. simonyi* and *G. s. machadoi* are likely to have been part of the same basic population until quite recently. When the cyt-*b* sequences of *G. simonyi* are compared with those of other species of *Gallotia*, the lack of genetic variability between the two subspecies *of G. simonyi* contrasts strongly with both the mean cyt-*b* genetic divergence for other subspecies (5.5%), and the within subspecies mean variability (0.6%) (González *et al.*, 1996). These results do not necessarily indicate that *G. s. machadoi* and *G. s. simonyi* are genetically identical overall, and faster evolving genetic material, such as micro satellites, may possibly still show differences between them. Nevertheless, any such differences are likely to have developed over a very short time span, given that the total of 438 third codon positions (cyt-*b* and COI genes), in principle selectively near-neutral and free to vary, show no differentiation at all between the two

populations. Similar insular studies with the lizard *Podarcis atrata* (Castilla *et al.*, 1998*a,b*) using 306 bp of the cyt-*b* (106 third codon positions) exhibit a medium to high degree of genetic variability between different island populations, again contrasting with the genetic identity of *G. s. simonyi* and *G. s. machadoi.*

Disparity in amount of difference within morphological and molecular data sets for the same taxa is quite common. Thus, molecular differences may sometimes be more marked than morphological ones, for example in the Pacific scincid lizard genus, *Emoia.* (Bruna *et al.*, 1996), *Thropeus* fishes of Lake Tanganyika (Sturmbauer & Meyer, 1992), and the "living fossil" horseshoe crabs, *Limulus*, in which morphology has remained essentially unchanged for many millions of years, even though they exhibit normal levels of molecular evolution (Selander *et al.*, 1970). On the other hand, the reverse is true in the species flocks of cichlid fishes in the rift lakes of East Africa, that originated extremely rapidly through adaptative radiation and are genetically exceptionally similar (Meyer *et al.*, 1990). In the scincid lizard *Gongylomorphus bojeri* of Mauritius, some small-island populations are virtually identical in mtDNA but differ markedly in morphology (J. Austin, *pers. com.*), a situation paralleling that in *Gallotia simonyi*. In both the latter cases identity, or near-identity of DNA sequence, together with the fact that the islands concerned probably only became separated a few thousand years ago as a result of sea-level rise at the end of the Pleistocene, suggests that morphological differentiation was rapid. This being so, it is possible that, if examples of *G. s. machadoi* were placed on the Roques de Salmor, the introduced population would soon develop the morphological characteristics of the original inhabitants. A recovery plan for *G. simonyi* is already in progress (Pérez-Mellado *et al.*, 1997)

The nucleotide sequences in this paper are available from GenBank: *G. s. simonyi* (BMH 1967-1736) AF101217, AF101209 and AF101201; *G. s. simonyi* (BMH 1967-1737) AF101218, AF101210 and AF101202; *G. s. machadoi* (number 16) AF101219, AF101211 and AF101203; *G. s. machadoi* (number 25) AF101220, AF101212 and AF101204; *G. s. machadoi* (number 26) AF101221, AF101213 and AF101205; *G. s. machadoi* (number 46) AF101222, AF101214 and AF101206; *G. s. machadoi* (number 160) AF101223, AF101215 and AF101207; *G. s. machadoi* (number 161) AF101224, AF101216, AF101208; for cyt-*b*, COI and 16S rDNA respectively.

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BOOK REVIEWS

Biologie und Ökologie der Kreuzkröte. Ulrich Sinsch (1998). 222 pp. Laurenti Verlag, Bochum. DM 44.00 (paper).

This attractive little book is a monograph on the natterjack toad, *Bufo calamita.* It deals mainly with the ecology of this species throughout its range and summarizes the knowledge accumulated in the scientific literature. The natterjack toad is probably the most thoroughly researched anuran species in Europe. It owes this status to the long term interest it has attracted from a number of very good biologists, who have written authoritative papers about this species over a number of years (Anthony Arak, Trevor Beebee, Ulrich Sinsch, Miguel Tejedo, to mention but a few). All this knowledge is now synthesized in an exemplary manner by Ulrich Sinsch, who also presents much first-hand information and unpublished data from recent research carried out in Germany.

The book starts with a comprehensive introductory chapter on the evolution and taxonomy of toads, and then sets out to describe and explain the prehistoric and recent distribution of *Bufo calamita.* These introductory chapters are followed by chapters on behavioural physiology (including paragraphs on activity patterns, reproduction, phenology, individual reproductive behaviour in males and females, spawning and tadpole behaviour); behavioural ecology (including such topics as migration, orientation, aestivation and hibernation); and ecology of the aquatic and the terrestrial stages of life (biotic factors, feeding ecology, competition, predation, abiotic factors, temperature, preferred water bodies). This is followed by a chapter on population ecology, and the book ends with an extensive chapter on conservation issues, dealing with–among other things–the question of whether *Bufo calamita* is to be considered an endangered species. The book closes with a valuable bibliography (minor addition: ecological work on *Bufo calamita*, the 'Strandtudse', was carried out in Denmark by John Frisenvaenge in 1994, and referred to in 'Bevarelsen af Danmarks padder og krybdyr', 1995, ed. by H. Bringsoe and H. Graff, Aage V. Jensens Fonde, pp. 57-63). The text is illuminated by a great number of maps, figures and photographs, many of which are in colour, showing the animals and their habitats.

The last monograph on *Bufo calamita* was published in 1983 by Trevor Beebee. Much has been published since, much by Beebee himself. Sinsch began publishing on the species in 1988. Beebee's book was intended for a more general (British) audience of interested naturalists and is written as an excellent and readable narrative. Sinsch's book is also a good narrative, but is different in style: it includes much more scientific detail, is somewhat more demanding to work through, and addresses a more specialized audience. However, I believe that a reader who purchased Beebee's book at the time and enjoyed it (as your reviewer did) will find a treasure trove in Sinsch's book, which is packed with new information. The great merit of the book is that it does not merely make a series of statements and justify these with reference to the literature–a style often used to maintain brevity in similar treatises. Instead it explains mechanisms and theories, and discusses the data in the light of these. This is particularly valuable, since the life history, ecology and habitat choice of the natterjack toad are so different in the areas where the animal has been studied (British Isles, Central Europe, Iberia, Northern Europe), that generalizations are often difficult to make. *Bufo calamita* is a species with a very plastic way of life that can adapt to very different local situations, where it may act as a pioneer species. Any statement about habitat use, migration or breeding site fidelity must take into account that there exists enormous regional variation. The author takes account of this variation in a very careful manner, giving a thoughtful survey and discussion of recent work carried out in areas both central and peripheral to the toad's range.

The book was published by Burkhard Thiesmeier in Laurenti Verlag, and if I am not mistaken, this new publishing house will accommodate what looks like the successor series to the Neue Brehm Bücherei of Westarp Wissenschaften, where Thiesmeier used to work. In this series many interesting volumes were published on specialist herpetological topics. In my view, the present treatise is one of the best ecological monographs on European anurans produced to date. Author and publisher are to be congratulated on this publication, which should become compulsory reading for any student who plans to work on *Bufo* species. In addition, the book serves as a very useful introduction to the ecology of European anurans in general.

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Ecophysiology of Amphibians Inhabiting Xeric Environments. M. R. Warburg. (1997). 182 pp. Springer-Verlag, Berlin. £69.00 (cloth).

This book represents the fifteenth so far published in a series devoted to the *Adaptations of Desert Organisms* (edited by J. L. Cloudsley-Thompson). The volume aims to assemble information about terrestrial amphibians, especially those inhabiting xeric, semi-arid and and environments, but it takes a broad view, frequently extending its scope to include details for species typical of mesic conditions. Although most previous studies concerning amphibians in arid-zone environments have emphasized the adaptations of desert-inhabiting anurans, this book is distinctive for its prominent consideration of urodeles, reflecting the author's career-long research interest in *Salamandra* and in epidermal structure and function.

The first two chapters review very briefly the scope of the book and the xeric habitats in which amphibians occur; this introduction is accompanied by an extensive list of species found in these environments world-wide. Chapter 3 considers the structure and function of organs that play an important role in survival, specifically the integument, respiratory organs, kidney, urinary bladder and, briefly, the female reproductive system. The section on the integument is the most detailed, with an in-depth account of the structure (especially the ultrastructure) of epidermal and dermal cell types, and water and ion movements across the skin. As elsewhere, the primary focus - particularly in the sections on respiratory and female reproductive systems - is on urodele examples. Chapter 4 presents brief accounts of sensory perception (photoreception, acoustic and olfactory senses) and behaviour, and this is followed in Chapter 5 by an extensive review of the physiological adaptations of amphibians in xeric environments. This, appropriately, is the longest section of the book (about one-third of the text); it considers selected aspects of physiology, concentrating specifically on water, nitrogen and thermal balance, including detailed consideration of the respective endocrine controls. Chapter 6 considers ecological adaptations, dividing these between the aquatic and terrestrial environments in which amphibians live. The former is concerned particularly with reproduction and larval development, together with brief accounts of larval feeding and competition, and factors affecting larval survival and metamorphosis. In considering the terrestrial phase of amphibians, the author again deals briefly with breeding followed by short accounts of aspects of population ecology. The final chapter presents a five page review and discussion of the topics previously highlighted, reinforcing the scarcity of published information on key aspects of ecophysiology and identifying the need for more research.

Inevitably, a review such as this may leave aspects of the subject area unexplored, and individual readers may judge for themselves whether some omissions are important. Thus, there is little emphasis on the constraints imposed by arid environments in terms of the extreme restriction on feeding opportunities and prey availability: a very brief section provides information on feeding behaviour (primarily for *Salamandra*), but this is relevant to all amphibians, rather than to those adapted to arid environments. A corollary of the ability of desert anurans to exploit briefly-available prey populations is their exceptional ability to tolerate long periods of starvation; there is no consideration of this nor of the essential role of the coelomic fat bodies in starvation survival and in reproduction. Readers interested in the response of arid-adapted anurans to ephemeral breeding sites will find little discussion of the relatively extensive literature on explosive breeding and mate choice. I noted in the bibliography the pioneering papers of Dimmitt and Ruibal who documented for *Scaphiopus* both the environmental cues for emergence from dormancy and the remarkable ability of these toads to exploit prey, but I could not find accounts of these exciting findings in the appropriate sections of the text.

A key feature of this book is the truly encyclopaedic reference list: a total of over 1000 references are listed in the bibliography reflecting the author's intimate knowledge of his fields of interest. As an important source of reference material, this book contains a vast review of primary literature. The organisation of the book achieves maximum coverage by listing relevant information and references in the form of extensive tables throughout the text. One table alone cites information from over 140 publications (which are referenced in the bibliography). Chapter 5, dealing with "Physiological adaptations", has ten very extensive tables recording, for instance, data for nitrogen excretion for 39 amphibian species, evaporative water loss for 35 species, and so on (all with appropriate references). A compilation of studies of water economy in anuran species lists over 110 references (Table 5.1). This is a rich condensation of published information and will be absolutely invaluable to researchers wishing to become familiar with the literature in this field. However, these readers may find it frustrating that the book does not provide a list of Tables (with their titles and subject matter). There is also no index to the species referred to in the text; which means a laborious search is necessary to locate information for specific taxa. Further, it seems a great pity that the subject index is exceedingly brief: given the enormous detail in the text about a comprehensive series of adaptations, there is a relatively limited selection of key words and a far from comprehensive selection of page references to the given topics. This book has enormous value for researchers interested in amphibians, on the one hand, and arid environments on the other. However, these readers may find that the lack of adequate indices greatly hampers the practical utility of this book as a reference work for which the author's enormous effort deserves very high praise.

The quality of production is high; the text is illustrated by very good line drawings and black and white photographs. As mentioned above, a particularly valuable feature of the author's approach is the summarizing of much detailed information in a series of very comprehensive tables (18 in total) and the enormous bibliography. However, it must be a regret to the author, following the completion of such an obvious labour of love, that this slim volume is relatively expensive: it will be a pity if this limits its appearance on the shelves not only of interested researchers but also of financially-limited libraries.

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THE HERPETOLOGICAL JOURNAL

INSTRUCTIONS TO AUTHORS

(revised January 1999)

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. Authors should bear in mind that the *Herpetological Journal* is read by a wide range of herpetologists from different scientific disciplines. The work should therefore appeal to a general herpetological audience and have a solid grounding in natural history.

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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. The journal is typeset direct from the author's computer diskette, so all manuscripts should be prepared using a wordprocessor (preferably on a PC-compatible microcomputer). It is not necessary to submit a computer diskette with the initial manuscript, but this will be required in the event of the manuscript being accepted for publication.

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5. The usual rules of zoological nomenclature apply.

6. Tables are numbered in arabic numerals, e.g. TABLE 1; they should be typed double spaced on separate sheets with a title/short explanatory paragraph above the table. Horizontal and vertical lines should be avoided.

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. Illustrations produced using other types of computer printer are not usually of suitable quality. 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. Articles 'submitted' or 'in prep' may not be cited in the text or reference list. 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. (1969*a*). 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. (1969*b*). 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, illustrations and computer diskette ready for the press. However, every assistance will be given to amateur herpetologists to prepare papers for publication.

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THE HERPETOLOGICAL JOURNAL

Volume 9, Number 2 1999

CONTENTS

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