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FRONT COVER: Male midwife toad, Alytes obstetricans, with eggs. (P. Benson)

DISCRIMINANT FUNCTIONS FOR SEX IDENTIFICATION IN TWO MIDWIFE TOADS (ALYTES OBSTETRICANS AND A. CISTERNASII)

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Determining the sex of midwife toads in the field is not easy. Non-pregnant females and males not tending clutches are difficult to sex without dissection. We provide a method to determine the sex of individuals based on the study of linear variables. Fifteen morphological variables were measured from samples of two species of midwife toad in the Iberian Peninsula (*Alytes obstetricans* and *Alytes cisternasii*). Some variables, corrected for the size of the animal, show significant differences between sexes. A discriminant analysis between the sexes in both species shows a high power for discrimination (95% in *A. obstetricans* and 97.6% in *A. cisternasii*). The significant variables in *A. obstetricans* were: snout-urostyle length, distance between the nostrils, distance from elbow to third finger tip. The significant variables in *A. cisternasii* were: head width, jaw bottom length, vertical diameter of the tympanum, distance between the nostrils, and tibia-fibula length.

INTRODUCTION

As Darwin (1871) pointed out: "It is surprising that frogs and toads should not have acquired more strongly-marked sexual differences; for though coldblooded, their passions are strong". Adult midwife toads (genus Alytes) are a good example of similarity between the sexes because they lack secondary sexual characters, either permanent or seasonal. During the mating season males differ from females because they have an advertisement call which is louder than the calls of females, however call intensity is not an adequate discriminating characteristic beacuse it is extremely difficult to observe individuals calling (Heinzmann, 1970; Márquez & Verrell, 1991). Midwife toads have male parental care of the eggs on land. During the brooding period the male carries a string of eggs entwined around his ankles and hence individuals carrying eggs are males. Similarly, females that contain mature oviductal eggs can be identified as such by observing the eggs through the transparent skin of their lower abdomen. However, outside these special situations, it is virtually impossible to tell apart a silent male not tending eggs from a female without oviductal eggs.

Crespo (1982) studied the differences between the sexes for 23 osteological variables and found that in *A. obstetricans boscai* none of the variables were significantly different between the sexes while in *A. cisternasii* only one of 23 variables yielded a significant difference. In general, the larger relative sizes of some segments of the limbs of males and the larger absolute body size of the females (see also Márquez, Esteban & Castanet, 1996) are the only apparent discriminating characters between the sexes. García-París (1992) studied sexual dimorphism in four populations of *Alytes* and found low levels of sexual dimorphism. He found that head width was the only variable that presented relatively marked dimorphism across populations. In a single population of *A. obstetricans*, the variables that presented significant differences between the sexes were head width and minimun distance of the eye to the nostril.

Sexing adult midwife toads in the field may be essential for ethological or ecological studies, and particularly for conservation-related studies (García-París, 1992). Therefore, it is of interest to develop a method that allows the determination of the sex of adult individuals with a high degree of accuracy. Discriminant analysis is a widely used multivariate technique (e.g. Van Vark & Schaafsma, 1992) and is a method of predicting some level of a one-way classification based on known values of the responses. The technique is based on how close a set of measurement variables are to the multivariate means of the levels being predicted. To the best of our knowledge, this technique has not been used for sexing anurans indistinguishable in the field, but it is regularly used to solve taxonomic problems in complex groups (e.g. Heyer, 1978).

MATERIAL AND METHODS

Two populations of midwife toads were studied, one of *A. obstetricans* and one of *A. cisternasii*. The population of *A. obstetricans* occurred near the shores of Peñalara's lake, an alpine lake (2000 m a.s.l) in the Sierra de Guadarrama (province of Madrid). Bioacoustical data from this population (Márquez & Bosch, 1995) suggests that it may be ascribed to the newly described subspecies *A. o. almogavarii* (Arntzen

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SVL	Snout-urostyle length.
HW	Head width (between tympanae).
JL	Jaw bottom length.
ED	Minimum distance between eyes.
HT	Horizontal diameter of tympanum.
VT	Vertical diameter of tympanum.
EW	Eye width.
ND	Distance between nostrils.
END	Minimum distance of between eye and nostril.
TFL	Tibia-fibula length.
HLL	Hind limb length.
TL	Distance between the tarsal tubercle and the tip of
	the third toe.
FTL	Distance between anterior end of middle
	metatarsal tubercle and tip of 3rd finger.
FL	Length of 3rd finger.
EFD	Distance from elbow to 3rd finger tip.

TABLE 1. Morphological variables measured and abbreviations.

& García-París, 1995) and not to *A. o. boscai* as was previously thought. The population of *A. cisternasii* was studied near the Roman dam of Proserpina, a site in a live oak forest, at 235 m a.s.l, located 5 km north of Mérida (Badajoz), the species' *terra typica*.

A total of twenty males and twenty females of A. *obstetricans* and twenty one males and twenty females of A. *cisternasii* were used for the measurements. In all cases, measurements were taken from living individuals that were freed immediately after being measured. Measurements were taken with a digital caliper Mitutoyo CD-15 (precision, 0.01 mm) or with an analog caliper AEMM (precision, 0.05 mm). The sex of all individuals measured was known. Only males observed calling or males captured after being observed releasing their egg masses in the water were used. Similarly, only gravid females with developed eggs observable through the transparent skin of the lower abdomen were measured.

TABLE 2. Mean, standard deviation and ranges for each sex, in the two species studied, and Student *t* test for differences between sexes (M, males and F, females). See Table 1 for abbreviations.

			Alyt	es obst	etrican	8			Alytes cisternasii						
		n	Mean	SD	Min.	Max.	t	Р	n	Mean	SD	Min.	Max.	t	P
SVL	F M	20 20	51.60 46.15	3.04 3.89	43.40 38.00	58.00 52.00	4.94	0.0001	20 21	39.83 37.75	2.82 2.08	36.00 33.00	46.00 41.00	2.69	0.0105
ΗW	F M	20 20	15.77 14.61	0.74 1.10	14.40 12.95	17.00 16.55	3.91	0.0004	20 21	13.68 13.40	0.65 0.46	13.00 12.45	15.10 14.25	1.63	0.1112
JL	F M	20 20	15.06 13.81	0.87 1.17	12.60 11.67	16.70 16.15	3.83	0.0005	20 21	10.77 10.17	0.63 0.61	9.55 8.85	11.85 11.00	3.13	0.0033
ED	F M	20 20	4.11 4.10	0.28 0.31	3.60 3.54	4.65 4.60	0.13	0.8972	20 21	4.08 4.11	0.23 0.22	3.70 3.70	4.50 4.50	0.35	0.7287
ΗT	F M	20 20	3.74 3.65	0.38 0.36	2.60 2.73	4.35 4.30	0.78	0.4426	20 21	3.15 3.30	0.25 0.33	2.70 2.65	3.80 4.00	1.62	0.1130
VT	F M	20 20	3.81 3.67	0.30 0.41	2.90 2.62	4.10 4.30	1.27	0.2114	20 21	2.99 3.22	0.30 0.25	2.40 2.65	3.45 3.55	2.67	0.0111
EW	F M	20 20	5.04 4.61	0.29 0.47	4.40 3.75	5.82 5.55	3.49	0.0013	20 21	3.97 4.01	0.21 0.28	3.60 3.45	4.55 4.45	0.54	0.5946
ND	F M	20 20	3.99 3.88	0.33 0.34	3.30 3.40	4.87 4.44	1.04	0.3068	20 21	3.44 3.26	0.16 0.19	3.15 2.95	3.75 3.80	3.11	0.0035
END	F M	20 20	5.01 4.53	0.28 0.44	4.40 3.64	5.50 5.10	4.15	0.0002	20 21	3.87 3.75	0.21 0.21	3.50 3.45	4.20 4.25	1.81	0.0787
TFL	F M	20 20	19.02 17.99	0.97 1.58	17.70 14.84	20.85 19.85	2.49	0.0171	20 21	13.16 13.44	0.75 0.66	12.20 12.25	14.85 14.70	1.27	0.2135
HLL	F M	20 20	64.64 60.56	3.38 5.17	59.00 51.21	70.80 67.30	2.95	0.0005	20 21	46.09 46.54	3.30 2.62	40.90 40.45	53.45 51.60	0.49	0.6279
TL	F M	20 20	15.71 14.84	1.17 1.58	13.40 11.54	17.65 17.80	1.98	0.0054	20 21	12.07 11.92	0.97 0.93	10.30 10.50	13.70 13.85	0.48	0.6370
FTL	F M	20 20	7.86 7.65	0.40 0.65	6.90 6.34	8.49 9.00	1.23	0.2273	20 21	6.03 5.91	0.37 0.44	5.45 5.25	6.80 6.90	0.89	0.3782
FL	F M	20 20	6.11 5.93	0.51 0.51	5.20 4.59	7.01 6.80	1.07	0.2929	20 21	3.73 3.86	0.29 0.38	3.35 3.15	4.45 4.60	1.27	0.2105
EFD	F M	20 20	20.71 19.69	1.24 1.62	17.50 16.64	22.40 22.30	2.26	0.0299	20 21	15.44 15.84	0.87 1.03	13.60 14.10	16.95 18.05	1.34	0.1897

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FIG. 1. Principal component analysis for *A. obstetricans* (top) and *A. cisternasii* (bottom). Females are represented by open squares, males by solid circles.

A total of 15 morphological variables were measured (Table 1). SVL was measured by pressing the animals flat against a ruler (ventral side). All the variables of bilaterally symmetric features were measured on the right side. Statistical analyses were performed with software SPSS 5.0, Statistica 4.1 and Statview 4.1. Stepwise discriminant analyses were performed with Pin 0.05, and P out 0.10.

RESULTS

Sex specific mean, standard deviation and ranges of the variables measured are shown in Table 2 for both species. These uncorrected data show that, in most *A. obstetricans* measurements (9/15, 60%) females are significantly larger than males, while only four out of 15 variables (36%) showed significant differences in *A. cisternasii.* The data were log-transformed to correct for the allometric effect of continuous growth, and to minimize the differences between variances. For each species every one of these log-transformed variables was used in a linear regression with snout-vent length (SVL), and the residuals of these regressions were used to compare values between sexes.

After transformation, five out of 14 variables (36%) showed significant differences between sexes in A.



FIG. 2. Graphical representation of scores of discriminant analysis of sexes in *A. obstetricans* (top) and *A. cisternasii* (bottom). Females are represented by open bars, males by solid bars.

obstetricans (HT, t = -2.74, P = 0.0094; ND, t = -2.20, P = 0.0341; TL, t = -2.35, P = 0.0238; FTL, t = -3.06, P = 0.004; EFD, t = -3.69, P = 0.0007), and six out of 14 variables (43%) in *A. cisternasii* (HT, t = -3.20, P = 0.0027; VT, t = -3.59, P = 0.0009; EW, t = -2.31, P = 0.0263; TFL, t = -3.55, P = 0.001; HLL, t = -2.69, P = 0.0105; EFD, t = -3.01, P = 0.0045). Only two of these variables (HT and EFD) showed significant differences in both species. A principal component analysis (Fig. 1) shows great similarity between the sexes in both species.

To obtain a system for classification of individuals of unknown sex, discriminant analyses were used. A stepwise discriminant analysis was applied to the data for each species to obtain discriminant functions that maximize the correct classification of individuals. The histograms of the scores of the discriminant functions are shown in Fig. 2. The discriminant function for sex in *A. obstetricans* is: 0.8782 x SVL - 2.0082 x ND -1.4129 x FTL - 1.0611 x EFD - 2.6330, and the cut-off point is 0.0000. For *A. cisternasiii* the discriminant function is: 1.5344 x HW + 1.4455 x JL - 1.9878 x VT + 3.7914 x ND - 2.1746 TFL - 13.4771, and the cut-off point is 0.0368. If the value obtained in the function is above the cut-off point, the diagnosis will be female, and if it is below the cut-off point, the diagnosis will be



Alytes cisternasii



FIG. 3. Diagnostic measurement for each species.

male. For *A. obstetricans* only four variables were included in the function (SVL, ND, FTL, EFD), while for *A. cisternasii* five variables were included (HW, JL, VT, ND, TFL), Fig. 3. The discriminant function obtained for *A. obstetricans* classified correctly 95% of the individuals (100% of the females and 90% of the males), while the function obtained for *A. cisternasii* classifies correctly 97.56% of the individuals (95% of the females and 100% of the males).

Other discriminant analyses were performed with all the variables taken one at a time. In *A. obstetricans* the variable JL showed the highest discriminant power, classifying correctly 80% of the individuals (90% of the females and 70% of the males). For *A. cisternasii*, the variable with highest discriminant power was ND which correctly classified 83% of the cases (80% of the females and 86% of the males). For *A. obstetricans*, if $14.132 \times JL - 107.131 > 12.959 \times JL - 90.199$, then the individual is classified as female; for *A. cisternasii*, if $111.062 \times ND - 191.467 > 105.543 \times ND - 172.930$, then the individual is classified as female.

DISCUSSION

In A. obstetricans, females appear to be consistently larger than males, while in A. cisternasii the differences are less marked. It appears that measures reflecting relative tympanum size (HT, VT) are proportionately larger in males although the differences were not significant in three of four cases, in absolute values. This could be the result of the selective pressure imposed by the vocalisations on the auditory structure. Both males and females are supposed to have their auditory system tuned to the same frequency ranges (determined by the ranges of the advertisement calls of the males in their populations) and, given that tympanum size may have an effect on frequency tuning (Shofner & Feng, 1984), it follows that tympanae of males and females should have similar sizes. Since males have smaller body sizes, this would explain the difference observed in relative tympanum sizes. Similarly, relative forelimb lengths (EFD) are larger in males in both species, possibly reflecting selective pressures for amplexus ability (forelimbs). On the other hand, relative hindlimb lengths (TFL, HLL) are only significantly larger in males of A. cisternasii. Such differences could be the result of selection for improved manipulation and transport of eggs, although the trend may not be as clear in A. obstetricans because, on average, males of this species transport egg masses which are proportionally lower in weight in relation to male body mass (R. Márquez, unpublished data).

The discriminant functions obtained from our data allow for the determination of sexes based on a reasonable number of morphological variables in each species, at least for adult individuals of the populations studied. Actually, the percentage of correct classifications for a single variable (80% in A. obstetricans and 83% in A. cisternasii, may also be sufficient for some studies. Caution should be used in extending the results to individuals from populations of different taxonomic status or with samples that include sizes beyond the range used in our study. Arntzen & García París (1995) demonstrated a marked level of geographical variation within and between Alytes species, but this study did not consider sexual dimorphism within populations and some of the differences observed may well be attributable to differences in sample sex ratios between populations or species. We present our study as a methodological tool that could be of use in such instances, but data from individuals of known sex from the specific populations studied have to be used to generate each discriminant function. We suggest that the methodology used in our paper may be applied in studies of other populations of Alytes or other anurans with little sexual dimorphism, where determination of the sex ratio is a central aim. All measurements should be taken from an increasing number of individuals until enough individuals of confirmed sex are measured and a desired percentage of correct classification is reached. Then the discriminant functions may be obtained and

the sex of the undetermined individuals observed previously can be determined a posteriori. Thereafter, only the variables included in the discriminant function should be measured on any new observed individuals of undetermined sex. Such a technique can be of use for the study of relict populations of Alytes such as the Mallorcan midwife toad, A. muletensis, whose recovery plan is currently being undertaken, and possibly to other threatened species of midwife toads, such as the newly-described A. dickhilleni (Arntzen & García-París, 1995), with highly isolated and reduced populations (Márquez, García-París & Tejedo, 1994). Similarly, the procedure can be of use for year-round field studies of populations of other species of anurans that may present secondary sexual characters only during the breeding season, such as species in the genera Discoglossus and Rana.

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DIETS OF CAIMAN CROCODILUS YACARE FROM DIFFERENT HABITATS IN THE BRAZILIAN PANTANAL

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The diet of *Caiman crocodilus yacare* was investigated during the dry season in different habitats of the Pantanal, Brazil. Stomach contents of 196 animals were analysed by prey class, the total number of prey, and the number of caimans eating each prey type. *C. c. yacare* ate mostly insects and fish. The caimans'diets differed significantly among habitats but not among caiman size classes.

INTRODUCTION

Caiman crocodilus yacare has an extensive South America distribution and occurs in high densities in the Pantanal region of Brazil (Mourao *et al.*, 1994). It is extensively harvested for its skin, illegally through direct hunting, and legally through ranching: collecting wild eggs, incubating them and raising the young in captivity. Several studies have been conducted in order to manage caimans on rangelands on a sustainable basis (Mourao *et al.*, 1994; Campos, 1993).

The Pantanal region contains a high diversity of aquatic habitats and species, and it seems likely that *C. c. yacare* is an important component of such systems. Information on food and feeding strategies is a first step towards evaluating their role, and it may also shed light on their management in captivity, where diet is known to influence growth rate and reproduction (Delany & Abercrombie, 1986).

Studies of the diet of *C. c. yacare* to date have been based on casual observations (Alvaro, 1945; Crawshaw & Shaller, 1980), or analyses of stomach contents (Aquino-Ortiz, 1988; Uetanabaro, 1989). Variation in the diet of crocodilians is thought to be related to both habitat and body size (Taylor, 1979; Webb *et al.*, 1982; Magnusson *et al.*, 1987; Thorbjarnarson, 1993; Allsteadt & Vaughan, 1994).

The present study reports the summer diet of C. c. *yacare* in relation to habitat and size.

MATERIAL AND METHODS

The Pantanal is situated in central western Brazil, between 16 and 22° and 55 and 58° (Fig. 1). It is a relatively flat region of alluvial accumulation, with an average altitude of 120 m above sea level (Brasil, 1974). Inundation is common and can be pluvial or fluvial in origin. Drainage occurs by rivers, streams and temporary channels and there are a great number of ponds. The extent of inundated areas varies seasonally (Carvalho, 1986; Vila da Silva & Kux, 1992). For this study, 196 caimans were caught during the summer dry season, in brackish ponds, freshwater ponds, temporary channels and the Negro river in the Nhecolândia region, freshwater ponds and artificial roadside ponds in the Abobral region and the Miranda river in the Miranda region (Fig. 1).

In October 1987, 21 animals were caught in ponds in Nhecolândia. Between September and October 1989, 46, 45 and 27 caimans were caught in brackish ponds, temporary channels and the Miranda river, respec-



FIG. 1. The study area. Numbers indicate sampling sites. 1, temporary channels; 2, ponds of Nhecolândia; 3, brackish ponds; 4, ponds of Abobral; 5, artificial roadside ponds; 6, Miranda river; 7, Negro river.

tively. Between October and November 1990, 24, 13 and four caimans were collected in brackish ponds, ponds of Abobral and artificial roadside ponds, respectively, and in October 1990, six and 10 animals were caught in artificial roadside ponds and the Negro river, respectively.

All animals were caught at night (between 20.00 hr and 23.00 hr). They were weighed, their snout-vent lengths (SVL) were measured and their stomach contents were removed from live caimans by the method of Taylor et al. (1977) with modifications suggested by Webb et al. (1982). This method enabled removal of stomach contents without harming the animal. Caimans were classified into three size classes: juveniles = < 50cm SVL; sub-adults = 50.1-70.0 cm SVL; and adults = > 70.1 cm SVL. This set consisted of 196 animals, juveniles ranging from 19.2-50.0 cm SVL (mean = 33.1 cm, n = 79) and from 0.1-3.5 kg in weight (mean = 1.1 kg, n = 79); sub-adult ranging from 51.0-70.0 cm SVL (mean = 60.5 cm, n = 52) and from 1.8-11.5 kg in weight (mean = 5.4 kg, n = 52) and adults ranging from 71.0-111.0 cm SVL (mean = 86.7 cm, n = 65) and from 7.0-45.0 kg in weight (mean = 16.8 kg, n = 65). Stomach contents were preserved by formalin. After removal of vegetation and parasites from the stomach contents, all items were grouped into one of nine major classes: (1) Gastropoda; (2) Arachnida; (3) Crustacea; (4) Insecta; (5) Diplopoda; (6) Pisces; (7) Amphibia; (8) Aves and (9) Mammalia. Because of the wide variation in degree of food digestion, data are presented by numerical and occurrence percentages, rather than volumetric and weight measurements. The numerical method (%N) describes the number of items in a given prey class expressed as a percentage of the total number of items across all stomachs, while the frequency occurrence method describes the number of stomachs containing a particular item expressed as a percentage of all stomachs (Hyslop, 1980).

Samples of the main food items were collected for identification and chemical analysis. Fish were sampled in all habitats, except for brackish ponds ,with a seine of 15 m long and with a 0.5 inch bar mesh. Aquatic insects were sampled with a screen (80×50 cm) only in brackish ponds at different depths (20, 30 and 40 cm).

Samples of the foods items were dried in the laboratory at 60°C and ground in a wiley mill. A portion of each sample was then dried at 105°C to determine mineral content. Crude protein was determined using the Kjeldahl method (AOAC, 1970), and crude energy was determined by a calorimetric bomb apparatus. Percentage concentration of phosphorus (P) was determined by colorimetry (Fick *et al.*, 1976) and calcium (Ca) by atomic absorption (Harris & Popat, 1954). Similarities among diet were quantified using the Bray-Curtis (Czekanowski) index of similarity.

Analyses of diets of generalist carnivores present many statistical problems. Firstly, eating one type of prey excludes the eating of another, introducing spurious negative correlations. Secondly, 'diet' is a composite variable. In a comparison of 20 diet categories between two habitats the probability of finding at least one apparently significant difference approaches one, even if data are distributed randomly between the habitats. When many habitats are compared, the probability of a Type I error increases, unless some form of multiple comparison procedure is used. With many prey categories and many habitats to be compared, multiple comparison methods are almost guaranteed to result in Type II errors.

One alternative is to reduce the diet to one or two dimensions and subsequently apply conventional statistical or Monte Carlo procedures. Many multivariate methods are available (James & McCulloch, 1990), and often, though not always, they produce similar results. All are based on a matrix of similarities, or dissimilarities, between the objects to be ordinated. In this paper, the objects were size classes within habitats and distances among them were based on diet category. All indices of similarity or dissimilarity have advantages and disadvantages. The Bray-Curtis (Czekanowski) index of similarity has been shown to be useful in a variety of ecological studies (Faith et al., 1987), it is not affected by joint absences (Belbin, 1995) and is less affected by aliasing than most similarity indices (MacNally, 1994). However, several others could as easily be justified.

In this study, semi-strong multidimensional scaling (MDS - Belbin, 1995) was used to produce axes that reflect similarity among diets because the method is robust and essentially non-parametric. However, some other ordination techniques might also be applicable. Objects that are closer together on those axes are closer in terms of overall diet, taking into account all prey categories simultaneously.

The ordination techniques were chosen for reasons that are totally independent of the comparisons among data sets. The range of similarity indices and ordination techniques available is such that *post-hoc* selection from among them will almost certainly result in relationships concordant with any *a priori* prejudice. Therefore, to maintain the validity of the inferential statistics, only *a priori* selection of the similarity index and ordination technique was used. Use of an inappropriate similarity index or ordination might increase the probability of a Type II error but would not affect the probability of a Type I error.

Conventional statistics have assumptions that could not be met by these data. To test for the effects of size and habitat on each MDS axis, the sum of squares was calculated using the formula for analyses of variance with two factors and no replication (Zar, 1974). The significance of differences was tested with a Monte Carlo simulation devised by David Willians (100 random runs) on the RANUNI random number functions from SAS Inst., Inc. (1985) TABLE 1. Summary of foods categories in the diet of 196 C. c. yacare from different habitats, expressed as percent by number (N) and frequency of occurrence (O).

	Brackish ponds		Mir ri	anda ver	Ne ri	egro iver	Temp chai	orary nnel	Pon Abo	ds of obral	Pon Nhece	ds of olândia	Art roadsie	ificial de ponds
	N%	0%	N%	0%	N%	0%	N%	0%	N%	0%	N%	0%	N%	0%
						JUVENI	LE S (< 5	0 cm SV	′L)					
	n=	32	n=	=3	n	=5	n=	=20	n	=7	n	=8	n-	-4
Gastropoda	0	0	0	0	55.0	20.0	1.6	10.0	0	0	85.5	75.5	2.4	50.0
Arachnida	0	0	0	0	0	0	0.4	5.0	5.5	14.3	0	0	1.9	25.0
Crustacea	0	0	0	0	5.0	20.0	1.2	15.0	30.5	14.3	0	0	0	0
Insecta	99.4	96.8	93.7	100.0	25.0	40.0	95.6	90.0	27.8	100.0	14.5	100.0	64.2	100.0
Diplopoda	0	0	0	0	0	0	0.4	5.0	16.8	71.4	0	0	0	0
Pisces	0	0	6.3	66.6	15.0	60.0	0.4	5.0	19.4	100.0	0	0	9.4	75.0
Amphibia	0.2	6.2	0	0	0	0	0.4	5.0	0	0	0	0	0	0
Aves	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mammalia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
					SUB-	ADULTS	(50.1 cm	n - 70.0	cm SVL))				
	n=	11	n=	10	n	=2	n=	=13	n=	=3	n	=2	n=	11
Gastropoda	0	0	4.8	10.0	14.3	50.0	9.1	7.7	0	0	88.0	100.0	52.2	50.0
Arachnida	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crustacea	0	0	9.5	20.0	0	0	9.1	7.7	0	0	0	0	0	0
Insecta	99.5	100.0	66.7	50.0	71.4	50.0	9.1	7.7	33.4	33.4	12.0	50.0	21.7	100.0
Diplopoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pisces	0	0	19.0	40.0	14.3	100.0	63.6	53.8	66.6	66.6	6.0	0.8	26.1	100.0
Amphibia	0.5	36.4	0	0	0	0	9.1	7.7	0	0	0	0	0	0
Aves	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mammalia	0	0	0	0	0	0	0	0	0	0	0	0	0	0
				×		ADULT	S (> 70.1	cm SVI	L)					
	n=	24	n=	15	n	=3	n=	=7	n=	=3	n=	11	n=	⊧2
Gastropoda	4.2	7.9	0	0	0	0	0	0	0	0	92.3	81.8	0	0
Arachnida	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crustacea	0	0	24.0	40.0	0	0	0	0	0	0	0.4	9.0	0	0
Insecta	88.9	87.5	16.0	20.0	60.0	100.0	61.1	28.6	40.0	66.0	6.5	45.5	20.0	50.0
Diplopoda	0	0	24.0	40.0	0	0	0	0	0	0	0	0	0	0
Pisces	0	0	48.0	73.3	40.0	66.6	38.9	61.1	40.0	66.0	0.4	9.0	80.0	100.0
Amphibia	3.2	58.3	0	0	0	0	0	0	0	0	0	0	0	0
Aves	0	0	0	0	0	0	0	0	0	0	0.4	9.0	0	0

	Brackish ponds	Miranda river (<i>n</i> =70)	Negro river (<i>n</i> =176)	Temporary channel (<i>n</i> =312)	Ponds of Abobral (<i>n</i> =635)	Ponds of Nhecolânda (n=402)	Artificial roadside ponds (n=431)
FISH							
Characidae	0.0	12.8	39.8	42.0	0.0	74.4	55.0
Cichlidae	0.0	28.6	21.0	0.0	69.3	1.7	16.9
Curimatidae	0.0	14.3	21.6	45.8	0.0	0.0	0.0
Erythrinidae	0.0	10.0	0.0	11.5	0.0	1.2	22.7
Loricariidae	0.0	21.4	0.0	0.3	30.3	0.0	0.7
Pimelodidae	0.0	12.8	16.5	0.3	0.2	0.0	3.9
Doradidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Others INSECTS	0.0	0.1	1.1	0.1	0.2	22.7	0.8
Belostomatidae	25.0	 ,	-	-	-	-	-
Hydrophilidae	62.0	-	-	-	-	-	_
Odonata larvae	13.0	-	-	-	-	-	-

TABLE 2. Number and numerical percentage of the main families of fish encountered in different habitats in the Pantanal in October 1991.

RESULTS

DIET COMPOSITION

Table 1 summarizes the diet composition of different sized *C. c. yacare* from different habitats, as represented by nine classes of prey. The most commonly encountered prey in stomachs were insects and fish.

Insects were encountered in all sized caimans in all sites and were most frequent in the diet of juveniles. Insects were mainly of Coleoptera, Diptera and Hemiptera. Insects were the major foods of caimans in brackish ponds which consisted mainly of Belostomatidae and Naucoridae (Hemiptera -Heteroptera), Anisoptera (Odonata) and Hidrofilidae (Coleoptera). Insects were the only food available in brackish ponds during time that the collections were made (Table 2).

Fish was an important food item for all size classes and habitats, except in brackish ponds and ponds of Nhecolândia. Few fish remains could be identified because of their degree of digestion, but these that could were from the family Loricariidae, Erythrinidae (*Hoplias malabaricus*) and Doradidae (*Trachydoras* sp). Table 2 shows the number and percentage occurrence of fish, by family, in different habitats of the Pantanal.

Snails (*Pomacea* sp.) were common in the diets of caimans caught in the ponds of Nhecolândia, artificial roadside ponds and the Negro river. Snails were not found in the stomachs of caimans caught in ponds of Abobral, an only one adult caiman from brackish ponds had snails in its stomach.

Amphibians were found in the stomach of caimans from temporary channels and brackish ponds. *Pseudis paradoxus* (Anura - Pseudidae), especially tadpoles were eaten in one of three brackish ponds sampled in 1989. Crabs (*Dilocarcinus pagei* and *Trichodacytylus borellianus*) were encountered in the stomachs of caimans from the Miranda river, temporary channel ponds of Abobral and the ponds of Nhecolândia. Arachnida, Diplopoda, Aves and Mammalia were found in small numbers in the diets of caimans from most areas. Parasites (Nematoda) were encountered in the stomach of all caimans.

TABLE 3. Composition and energy values of some food items ingested by C. c. yacare in the Pantanal, based on dry matter.

	Dry matter	Crude protein	Gross energy	Calcium	Phosphorus	Ca:P
	(%)	(%)	(Kcal/kg)	(%)	(%)	ratios
Hemiptera (Belostomatidae)	29.6	64.1	5.4	0.25	0.60	1:2.4
Coleoptera (Hydrophilidae)	37.9	65.4	5.1	0.25	0.5	1:2.0
Odonata (Larvas)	19.6	61.1	5.8	0.30	0.45	1:1.5
Snail (Pomacea sp.)	39.2	18.8	1.3	24.90	0.06	415:1
Crustacea (Trychodactylidae)	22.7	65.9	4.5	0.30	1.20	1:4.0
Fish (Loricariidae)	25.6	61.0	4.2	14.2	3.4	4.2:1
Amphibia (Pseudidae)	15.3	67.1	4.5	4.6	3.2	1.4:1



FIG. 2. Multidimensional scaling (MDS) analyses representing variation in diet among three size classes (1, juveniles; 2, subadults; 3, adults) and seven sites (A, brackish ponds; B, Miranda river; C, Negro river; D, temporary channels; E, ponds of Abobral; F, ponds of Nhecolândia; G, artificial roadside ponds)

Composition and energy values of some food items ingested by caimans are listed in Table 3. Mean crude protein and crude energy were generally high, except for snails (*Pomacea* sp.). Insects showed low levels of calcium and phosphorus. Ca to P ratios were 1:2.4, 1:2.0 and 1:1.5 for Belostomatidae, Hydrophilidae and Odonate larvae, respectively. Molluscs, crabs, fish and amphibians had Ca to P ratios of 415:1, 1:4, 4.2:1 and 1.4:1, respectively.

HABITAT AND CAIMAN SIZE VS. DIET

The diets of caimans from the same habitat tended to occupy similar regions of multidimensional space but there was no tendency for similar-sized caimans to be grouped together (Fig. 2). This was reflected in the Monte Carlo analyses which indicated significant grouping by habitats (MDS1, P=0.02; MDS2, P=0.095) but no significant grouping by size (MDS1, P=0.992; MDS2, P=0.33).

DISCUSSION

The diet of crocodilians has been shown to shift ontogenetically (e.g. Jackson *et al.*, 1974; Blomberg, 1977; McNease & Joanen, 1977; Seijas & Ramos, 1980; Webb *et al.*, 1982; Delany & Abercrombie, 1986; Magnusson, 1987; Uetanabaro, 1989). In this study, diet did not differ statistically between the size classes, but this was partly because of the strong habitat effects. As in previous studies, juveniles ate mainly insects while adults ate mostly fish: perhaps large samples from any one habitat would show significant differences.

Diet did differ significantly among habitats. Santos et al. (1994) found that the condition also differed be-

tween habitats, but not among size classes. Juvenile caimans were in the best condition in brackish ponds, while adults caimans were in the best condition in the Miranda river and artificial roadside ponds. These differences may be related to the interaction of food availability and ontogenetic trends in the diet. Juveniles consumed mainly insects which were most abundant in brackish ponds. Adults consumed mainly fish, which were available in all habitats, except brackish ponds.

Caimans caught in the ponds of Nhecolândia ate a diversity of foods such as small fish, crabs, snails and insects. Similar result were found by Uetanabaro (1989). He observed qualitative and quantitative differences in the diet of *C. c. yacare*. This may be explained by larger animals selecting items with larger net energy/feeding time ratios to satisfy energy requeriments (Schoener, 1971). Depending on the habitat, larger animals use less time to cach prey than smaller animals, because they can eat food items of larger nutritive value, such as fish.

Caimans ate mainly aquatic animals despite abundant terrestrial fauna (mammals and birds). However, in Caño Negro, Costa Rica, *Caiman crocodilus fuscus* frequently ate aquatic birds (Allsteadt & Vaughan, 1994). Mammals were frequently encountered in the diet of *Caiman crocodilus crocodilus* in the Central Venezuelan Llanos (Thorbjarnarson, 1993).

Adult caimans probably eat fish throughout the year, except in brackish ponds. The fish community is not stable with time because of changes in water volume (Lowe-McConnel, 1975), but they are available throughout the year. Most of the fish that could be identified are slow moving bottom dwellers such as *Hoplias malabaricus*. This fish has a wide distribution and it generally lives in black water and marginally vegetated sites, where water flows slowly (Ueida, 1984; Kirovsky, 1994). According to Thorbjarnarson (1993), these fish are facultative air breathers that can tolerate the low levels of oxygen found during the dry season in most llanos water bodies.

Insects were the major food item during the dry season in brackish ponds, although caimans captured in brackish ponds in 1989 had eaten large number of amphibians. In that year, hundreds of caimans congregated in one of the three brackish ponds sampled, and it coincided with a bloom of algae and tadpoles. However, tadpoles were not encountered in significant number in other brackish ponds and habitats in other years. High occurence and numeric frequency of insects for all sizes of animals caught in brackish ponds suggest the population feeds mainly on this form of prey.

Items such as fish and amphibians are difficult to identify due to rapid digestion. Delany & Abercrombie (1986) studied digestion rate of *Alligator mississipien*sis and observed that fish and amphibians are quickly digested. Magnusson (1987) suggested that when interpreting data on crocodilians stomach contents the following points should be considered: different prey types are digested at different rates; larger crocodilians digest equivalent sized prey faster than small crocodilians; some prey have indigestible parts that accumulate in the crocodilian's stomach (e.g. operculum of snails); different passage rates may occur for various kinds of food, and different prey of equivalent mass, volume or area may have different nutritive value.

Caimans have a low and variable metabolic rate. Therefore, they may survive longs periods of low food availability. It is common to observe hundreds of caimans concentrated in small natural and artificial ponds during the dry season. Therefore, other factors besides food availability may effect the habitat use by caimans. Similar observations were reported by Allsteadt & Vaughan (1994).

In overview, caimans appear to be opportunistic predators, that ingest the prey which is most abundant. Because of this, habitat is more important in determining prey type than caiman size. However, larger samples may indicate that significant ontogenetic or seasonality variation does exist in some if not all habitats.

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LONG TERM DECLINE AND POTENTIAL FOR RECOVERY IN A SMALL, ISOLATED POPULATION OF NATTERJACK TOADS *BUFO CALAMITA*

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Changes through time in a small, isolated population of natterjack toads on the Solway Firth are reported. The area and linear length of coastline occupied by the colony have declined since it was first described in 1849. The colony now occupies less than 20% of its former range. The rate of decline has increased since the 1960s and has continued into the present decade. This decline has been accompanied by losses of breeding pools and habitat which can be directly attributed to anthropogenic changes in land-use. Comparison of aerial photographs between 1946 and 1988 show, amongst other changes, a major loss (>60%) of coastal sandy grassland, favoured by natterjacks, to agricultural intensification and to the recreational development which now surrounds the colony. Recent population monitoring (1986-1995) shows a failure of recruitment in five of the six years before 1991. During this period the annual peak counts fluctuated between nine and thirty adult males, well below a previous estimate in 1976 of 100 males. Artificial excavation of breeding pools in 1991 resulted in the subsequent production annually of large numbers of toadlets. A peak count of 79 males in 1995 probably reflects the recruitment to the breeding population of these cohorts and gives the first indication of recovery in this population. Re-occupation of the former range is no longer possible. The expansion of the colony to areas of suitable habitat away from the native site, but with no previous records of natterjacks, is discussed.

INTRODUCTION

Within Britain there has been a significant contraction in the range and number of populations of natterjack toads Bufo calamita Laur. This decline has been most severe on the heaths of south-east England (Beebee, 1976; Banks, Beebee & Cooke, 1994; Arnold, 1995). Populations elsewhere, especially those occupying the sand-dune and saltmarsh habitats of Britain's Irish Sea coast, have been affected less severely. These now represent the stronghold for natterjacks in Britain (Banks et al., 1994). Yet even here colonies have become extinct in recent decades, with small isolated populations being at greatest risk (Beebee, 1992; Banks et al., 1994). However, there have been few published long-term studies on these Irish Sea colonies compared to those on heathlands (e.g. Banks, Beebee & Denton, 1993).

The colonies of natterjacks on the north shore of the Solway Firth, in south-west Scotland, are at the northwestern limit of the species' range in Europe (Beebee, 1979; Bridson, 1977). Yet they are important in conservation terms. Collectively, the Solway colonies account for up to an estimated 23% of the total British population (Banks *et al.*, 1994). Southerness represents the furthest point on the Solway to which natterjacks have spread. It is thus of some biogeographical significance (Beebee, 1979, 1989). There are no substantiated records of natterjacks further north or west along the coast from this point (Beebee, 1989). This colony is, however, isolated from the other Solway populations by the tidal mudflats and estuary of the river Nith (Fig. 1), being 11km in a linear distance from its nearest neighbour at Caerlaverock. It is also small in size with, in 1976, an estimated population of less than 100 males (Bridson, 1977). This paper reports on (a) changes in this colony through time, based on historical and contemporary records, (b) associated changes in land cover assessed from aerial photographs, and (c) recent monitoring to assess population trends, especially in response to recovery initiatives.



FIG. 1. Outline map of the Southerness area, showing locations referred to in the text.

METHODS

PREVIOUS RECORDS

We have searched the literature for references to this colony, and have also contacted local naturalists, record centres and others for information that would enable us to assess any changes through time (Mearns, 1994). The British Museum (Natural History) and seven museums in northern Britain (namely The Observatory, Dumfries; the Stewartry Museum, Kirkcudbright; the Kelvingrove Museum, Glasgow; Royal Museum of Scotland, Edinburgh; Tullie House Museum, Carlisle; the Hancock Museum, Newcastle; and Aberdeen University Museum) were also contacted to see if they had any natterjack specimens, originating from Southerness, in their collections. Additional information was obtained from the files of Scottish Natural Heritage (formerly the Nature Conservancy Council) and from the Biological Records Centre, Monks Wood.

HABITAT CHANGE

In order to assess changes in habitats or land-use that may have affected use of the area by natterjacks, we compared aerial photographs taken in 1946 (1:9900) and 1988 (1:24000). The study area was defined as land between Carsethorn and Mersehead (Fig. 1) within 500 m distance of mean high water mark of spring tides (MHWS), modified to the nearest field boundary which remained constant between both sets of air photographs. This area encompassed all known records for natterjacks and represented a realistic area to assess the impacts of changes in land management. For the purpose of the analysis, the study area was split into two using the minor road to Southerness as the division (Fig. 1). Details of land-cover from aerial photographs were transcribed on to 1:10000 scale maps for subsequent measurement and analysis. Features of less than 0.25 ha were not included in the analysis. The following land cover categories were recognised:

Improved agricultural land: either arable land or intensively managed grassland.

- *Coastal grassland*: unimproved or semi-improved pasture, typically very sandy with extensive rabbit activity, also including sand-dune.
- *Heath*: dominated by heather or other dwarf shrubs but often merging into sandy grassland; also including golf course fairways.
- Marsh: including open water, fresh marshland and saltmarsh.
- Urban: including farmsteads and caravan/chalet parks with areas of amenity grassland.
- *Woodland*: both deciduous and coniferous.

Bare sand: areas of bare sand above MHWS.

Land-cover categories were validated ("ground truthed") by comparing land cover types, mapped from 1988 aerial photographs, with a field assessment. Maps, ranging from the 1st Edition Ordnance Survey (OS) maps of 1896 (1:57600) to the most recent Pathfinder range (1:25000), with revisions up to 1985, were compared to record gross changes in features, such as erosion and accretion of the coastline.

MONITORING

From 1986 to 1995 the colony was monitored annually by us in order to assess numbers of natterjacks and to record parameters of breeding success. Visits were timed to coincide with key stages in natterjack breeding activity. The frequency of monitoring visits varied between years: during 1986-91, five to eight visits were made per annum, but from one to three visits per annum were made thereafter. At least one visit, but usually more, was made at night. Numbers of adults, especially calling males, were recorded. Any toads captured were sexed and a snout to vent measurement (mm) taken with a hand-held ruler before their immediate release (Smith, 1990). To avoid double-counting within years, only the highest single peak count of natterjacks and only the mean length of the largest single sample of captured toads are presented. Searches were made for spawn strings, tadpoles and emerging toadlets, and their numbers counted or estimated. All identifications were made in the field though not all Bufo tadpoles could be identified specifically. Other sites to the west of Southerness, to Mersehead (Fig. 1), were also surveyed periodically for evidence of natterjack activity.

RESULTS

RECORDS

The first record known to us of this colony, and for Scotland, was made by Sir William Jardine in an account in Bell (1849), in which he describes natterjacks as being "very abundant" and occurring for several miles along the coast between Southerness and Carsethorn (Fig. 1). Subsequently Service (1895) described the colony at Gillfoot Bay, then thought to be one of only two localities in Scotland. Later, he noted (Service, 1896) the wider distribution of natterjacks on



FIG. 2. Number of 1 km Ordnance Survey grid squares and linear length of coastline occupied through time by a declining population of natterjack toads.

the Solway and described the extent of the colony on the west side of the Nith.

We have found few records of this colony in the earlier part of the present century and we were not able to locate any specimens in the collections of likely museums (Mearns, 1994). However, natterjacks were recorded at Southerness in the 1930s and about this time pre-emergent natterjack toadlets were observed in a pool at the House on the Shore (OS grid reference: NX994572), to the north of Powillimount (A. Truckell, personal communication). Between 1939 and 1941, J. Donnan found natterjacks to be common to the west of Southerness lighthouse in an area where these were later recorded by G. Clarke in 1964 (personal communications). There are, to our knowledge, no confirmed records of natterjacks further west along Preston Merse. Taylor (1963) records the presence of natterjacks at Southerness by the indication of a dot on his distribution maps. This record, for 1961-62, represents the earliest record of natterjacks at Southerness held at the Biological Records Centre.

Bridson (1977) subsequently surveyed this colony comprehensively in 1976 and described the location of breeding pools thought then to be confined entirely to Gillfoot Bay (UK grid reference NX95; 3° 35' W, 54° 53' N). Subsequently, in 1988, we discovered two pools with calling natterjacks at Powillimount, 1 km to the north of Gillfoot Bay and 200 m inland, and a further breeding pool 300 m inland from Gillfoot Bay. We have not recorded any natterjacks to the west of the minor road to Southerness.

CHANGES IN DISTRIBUTION AND LAND-COVER

Whilst it is not possible to be certain of the exact area occupied by natterjacks in the past, we have attempted a quantitative estimate of the changes in distribution through time. To do so, we plotted all records of location, with dates, on to a 1:25000 scale map of the area. We assumed that natterjack occupancy was confined to a strip within 100 m of MHWS unless we had information to the contrary. For each 10-year period with records, we were then able to count the number of 1 km squares of the OS grid, and to measure the linear length of coastline, then occupied by the colony (Fig. 2). In doing so, we also assumed (a) that any breeding sites known to us now were occupied by natterjacks in earlier years; (b) that occupancy of an area terminated at the latest date for which we have records; (c) that range occupation was uniform for any given period; and (d) that any contraction in range was towards localities with later records. We have taken Carsethorn as the northern limit of this colony's distribution despite the vague reference by Service (1896) to natterjacks occurring up to Kirkconnell (some 8 km to the north of Carsethorn).

All the extant breeding pools now fall within a single 1 km square of the OS grid. Despite the relative crudity of these estimates, this colony clearly now occupies less than 20% of its former range (Fig. 2). Moreover,

TABLE 1. Changes between 1946 and 1988 in area (ha) of different land cover types, derived from aerial photographs, in two study areas at Southerness (for definition of types and study area see text).

	West o	f road	East of	f road
Land-cover	1946	1988	1946	1988
Improved agricultural	78	134	241	245
Coastal grassland	208	137	63	25
Heath	57	74	0	0
Marsh	11	36	6	7
Urban	2	3	8	39
Woodland	22	10	16	17
Bare sand	32	3	0	0
TOTAL	410	397	334	333

the rate of contraction in range has increased since 1960 and has continued into the present decade.

Examination and comparison of aerial photographs suggests significant quantitative and qualitative changes in land cover between 1946 and 1988 (Table 1). It is apparent that land between Southerness and Carsethorn ("east of the road" in Table 1) was already intensively farmed by 1946. Even so, significant areas of heavily grazed coastal grassland remained, concentrated around Gillfoot Bay. By 1988, the area of this habitat had had been reduced by over 60% with losses to agricultural intensification and to recreational developments (caravan and chalet parks) at Southerness. Indeed, two years after the 1988 aerial photographs, a further area (7 ha) of sandy coastal pasture at Powillimount, used by natterjacks, was lost to agricultural improvements. Differences in total area between 1946 and 1988, in both parts of the study area, result from net changes in erosion and accretion of the coastline.

Between Mersehead and Southerness ("west of the road" in Table 1), changes were more dynamic. Improved agricultural land increased at the expense of dune heath and coastal grassland. Despite these losses, significant areas of sandy pasture remain. Although the total area of heathland increased, it is now restricted to a golf course. The net increase in the total area of marsh is due to considerable accretion of saltmarsh at Mersehead. However, this obscures significant erosion of the coastline overall. At least 130 m depth of land along Preston Merse was lost to marine erosion during the period between air photographs. Large areas of bare sand in 1946 give the impression then of a dynamic and eroding habitat. Comparison of maps indicate that, at the western end of Preston Merse, the MHWS mark may have receded by up to 700 m since 1896. Up to 480 m of this loss is marked as being vegetated and presumably represented saltmarsh or sand dune.

BREEDING AND TERRESTRIAL HABITAT

The colony is now focused on a narrow strip of ungrazed coastal marshland, 1.4 km in length and up to

TABLE 2. Results of natterjack population monitoring at Southerness from 1986-1995 inclusive (*n* for SE of mean length = *n* for size range column unless otherwise stated). +, 10's of tadpoles/toadlets; ++, 100's tadpoles/toadlets; +++, 100's tadpoles/toadlets; F, failed; *n=20; **n=54; nd, no data.

		Maximur	n number				
Year	toads (males)	spawn strings	tadpoles emergent toadlets		Mean length of toads (±SE)	Size range (mm) (pooled data)	No. visits
1986	23 (20)	1	++	F	55±7.4	46-76 (<i>n</i> =3)	6
1987	36 (30)	7	+++	+	56±0.5*	42-72 (n=32)	8
1988	18 (16)	2	++	F	53±0.3	48-56 (<i>n</i> =9)	5
1989	9 (9)	nd	++	F	59±0.6	54-65 (n=7)	7
1990	23 (23)	nd	+	F	56±0.8	45-62 (n=8)	7
1991	6 (6)	2	+++	+++	52±2.5	50-55(n=2)	7
1992	6 (6)	nd	+++	++	nd	nd	3
1993	2 (1)	16	+++	++	nd	nd	3
1994	6 (6)	nd	++	nd	41±2.9	22-51 (<i>n</i> =5)	1
1995	90 (79)	10	++	+	53±0.1**	31-64 (<i>n</i> =57)	3

100 m wide, at Gillfoot Bay, bordered to the landward by a recreational caravan site. Vegetation in the marshland is dominated by dense stands of *Phragmites australis* and *Bolboschoenus maritimus*. This area is within the Upper Solway Flats & Marshes Site of Special Scientific Interest (SSSI) designated under the Wildlife & Countryside Act in June 1988. Mown amenity grassland dominates within the caravan site.

Natterjack breeding activity has consistently been associated with two pools at Gillfoot Bay. The main pool, although relatively large (100×50 m), is now half its original size as reported by Bridson (1977). The second pool is much smaller, 5 m by 10 m, and with spreading *P. communis*. Both pools may desiccate in dry summers and may be subject to tidal inundation in winter (Beebee, Fleming & Race, 1993). Pools at Powillimount were lost to agricultural drainage and improvement work over the winter of 1989-1990. We have no subsequent records of use of the area by natterjacks. Three other pools have been subject to drainage attempts or have also been lost.

CONSERVATION MANAGEMENT

To ensure the availability of suitable breeding habitat, three new pools were mechanically excavated in September 1990 within the Gillfoot Bay marshland. In addition, the two existing pools were cleared out and re-profiled. Not all the new pools have been used by natterjacks: two have been avoided. Despite clearance work, invasion by *P. communis* has intensified in the minor original pool such that little open water remains and recently use by natterjacks has ceased.

POPULATION MONITORING

In the years between 1979 and 1985 for which we have records (no data from 1983), toadlet emergence was recorded as poor or failed in all but 1979 and 1980 (Beebee, 1989). Combined results of population monitoring from 1986-1995 are presented in Table 2. Despite the presence annually of tadpoles, no emergence of toadlets was recorded in four of the five years

before 1991. In each case, pool desiccation was responsible for breeding failure. Even in 1987 the emergence of toadlets was inferred rather than observed because there were no obvious factors (such as drought) to have prevented emergence of the few hundred tadpoles present that year. Following the creation of breeding pools, the production of tadpoles has been consistently high. Large numbers of toadlets were recorded as emerging in 1991 and 1992, and similar numbers were likely from 1993 to 1995.

Before 1990, peak nightime counts of adult natterjacks fluctuated between nine and 30 males, suggesting a total population not exceeding 60-80 individuals (Table 2). The fluctuations between years may be due to monitoring visits missing, by chance, the peak of breeding activity. Counts from Powillimount, between its discovery and destruction, suggest that although no more than 10 adult natterjacks were recorded there, these may have represented then a significant proportion of the total population. Despite the apparent lack of recruitment between 1986 and 1991, there is no evidence of any progressive increase in mean length that might be expected with an ageing population (Smith, 1990). However, sample sizes are small and factors other than age may influence body size (Denton & Beebee, 1993; Halliday & Verrell, 1988). Very low counts from 1991-94 may be an artefact of reduced recording effort. This view is supported by a high single count of spawn strings in 1993, indicating at least 16 females in the population. Indeed, it is more likely that the population was increasing over this period, resulting ultimately in a peak count in 1995. This single count is the largest number of adults recorded at this site and suggests, assuming an equal sex ratio (Banks et al., 1993), an adult population now approaching 200 individuals.

Further evidence of successful recruitment is suggested by the occurrence since 1994 of juvenile toads much smaller than 43 mm in length (Table 2), the apparent minimum size threshold for breeding (Denton & Beebee, 1993). Counts of spawn strings also increased after 1991, but these results must be interpreted with more caution. In particular, searching for spawn is difficult in the densely vegetated and silty pools characteristic of the site. Pool excavation may also have made the discovery of spawn more easy.

Common toad *Bufo bufo* and common frogs *Rana temporaria*, adults and juveniles, were recorded almost annually. There is no evidence so far of any significant increase in their numbers.

DISCUSSION

The long term, and continuing, decline in this natterjack population is probably typical of many other, but less well documented, colonies. Indeed, it is remarkable in many respects that it has persisted at all. Losses of populations in north Wales have been attributed to extensive recreational developments (Beebee, 1976) not unlike those now surrounding the colony at Southerness. The persistence of the colony is almost certainly due to the survival of the marshland at Gillfoot Bay. It is evident from the earliest descriptions of the colony (Bell, 1849; Service, 1895) that, even then, this was a focus of natterjack activity. The contraction in range that we have documented has been towards this core habitat feature. Paradoxically, it is possible that the caravan site, juxtaposed with the marshland, may have contributed to the survival of the colony. Mown amenity grassland, within the site, may have provided natterjacks with more open foraging conditions (Denton & Beebee, 1994) that might not have been available if the area had been, for example, agriculturally improved.

The decline of this colony has been accompanied by changes in, and intensification of, land-use, principally for agriculture and recreation, typical of losses in most coastal natterjack sites (Beebee, 1992). Most importantly at Southerness, the area of sandy coastal grassland, that seems to have been associated with natterjack use, has been significantly reduced. This has been combined with continuing loss and attrition of breeding pools. Of greatest concern, perhaps, is that these losses have continued, and indeed accelerated, into the present decade despite legal protection given to natterjacks, and elements of their habitat, since 1975. Habitat destruction, however, has taken place almost exclusively outside the boundary of the Upper Solway Flats & Marshes SSSI, since its re-notification (and therefore enhanced protection) under the 1981 Wildlife & Countryside Act. These results reflect the relative benefits of site versus species protection recently reviewed by Banks et al. (1994).

Results of population monitoring indicate that, in the 1980s, the outlook for this colony was far from promising. Reductions in the extent or quality of habitat continued. Recorded breeding success had also been continually poor with toadlet production being recorded in only three of the twelve years before 1991. Although, the count of 30 males in 1987 is below the 100 individuals formerly estimated by Bridson (1977), it is within the range of counts (30-40 males) actually reported from 1976. Whilst this might suggest some stability between the two periods, the counts from this study were typically much lower and included those from a number of pools apparently not known to Bridson (1977), suggesting a genuine decline in population.

It is clear that the artificial excavation of breeding pools in late 1990 was both necessary and timely. These recovery attempts were immediately successful, with large numbers of toadlets produced almost annually thereafter. From a nadir in the late 1980s, the adult population now seems to have recovered to, or exceeded, 1976 levels. However, there are few grounds for optimism in the long term. Some of the pools, and certainly the rank vegetation at Gillfoot Bay, may now favour common toads and common frogs, successful competitors with natterjacks (Banks & Beebee, 1987; Denton & Beebee, 1994; Griffiths, Edgar & Wong, 1991). Management of the vegetation by grazing is unlikely to be a realistic option. Equally, if the population is increasing in size, and presumably density, individual toads may be ranging more widely (Denton & Beebee, 1993) yet there is little other suitable habitat available for them to colonise. The former documented range towards Carsethorn is now entirely unsuitable.

Along Preston Merse (Fig. 1), however, there is potentially suitable habitat for natterjacks (Table 1). Immediately west of Southerness, sandy coastal grassland and heath occur within a distance feasible for natural recolonisation (Langton & Beckett, 1995). There are records of natterjacks from the easternmost part of this area as recently as 1964. Establishment would, however, be limited by lack of breeding pools. Further west, at Mersehead, there is also suitable habitat, consisting of grazed dune pasture and saltmarsh, dune slack, sandy flood banks and relic sand-dunes, now in conservation management. Natterjacks are not likely to be able to colonise here unless by translocation. There is scope, therefore, by positive conservation measures, to enable some recovery of the colony's former abundance by expansion into new habitat.

Despite the apparent present and former suitability of Preston Merse (Table 1), however, there are no historic records. Yet, there may once have been considerably more habitat than is present today. Much of the land lost to coastal erosion was likely to have consisted of saltmarsh and sand-dune. By extrapolation from other Irish Sea colonies (Beebee, 1989), this would have been suitable for natterjacks. Another threatened inhabitant of ephemeral pools, Triops cancriformis, the tadpole shrimp, was recorded from Mersehead in 1907 and 1948 (Balfour-Browne, 1948) suggesting, perhaps, the presence of pools suitable for breeding by natterjacks. It has also been suggested that the original sites for this species have now been lost to coastal erosion (Bratton, 1991). Since there are no obvious obstacles to natterjacks having occupied this area in the past, it is not unreasonable to consider Preston

Merse within the "presumed natural range" of natterjacks (Nature Conservancy Council, 1983). Beyond Mersehead a long stretch of rocky cliffs would have been an obstacle to the spread of natterjacks. Any proposals to establish natterjacks further west beyond this biogeographical barrier would be unwarranted and unjustified.

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LIFE-HISTORY VARIATION IN A COMMUNITY OF LACERTID LIZARDS FROM THE LAKE SKADAR REGION (MONTENEGRO)

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We compared life-history attributes of Algyroides nigropunctatus, Lacerta oxycephala, Podarcis melisellensis and Podarcis muralis populations in the Lake Skadar region (mainland and islands). These lacertids are moderately sized with differing morphology (flattened vs. cylindrical). They have similar duration of egg incubation, size at onset of sexual maturity, size of hatchlings, and rate of juvenile growth. Clutch size is variable between populations and species: P. muralis produced larger clutches than the other species, especially A. nigropunctatus and L. oxycephala. A significant difference in egg size was apparent at the population level only. Smaller clutch size and elongated eggs were associated with a flattened body in L. oxycephala. A canonical discriminant analysis of reproductive variables showed that species similarities in ecological characteristics reflect phylogenetic relationships.

INTRODUCTION

Components of an organism's life history (reproductive characteristics, behaviour, longevity, growth rate, etc.) may be viewed as co-adapted traits that in concert produce the best strategy for survival in a particular environment (e.g. Stearns, 1976). Most studies on life-histories of lizards show large interspecific differences and considerable intraspecific temporal and geographic variations of the traits (reviewed in Dunham et al., 1988). These variations have been attributed primarily to the local environment (e.g. Tinkle and Dunham, 1986; Abts, 1987). However, foraging mode, phylogenetic inertia and morphological constraints (body size and shape) were also evoked as factors potentially influencing variation of life-history traits (e.g. Vitt & Congdon, 1978; Vitt, 1981; Vitt & Price, 1982). In order to obtain empirical data essential to discern the relative importance of all these determinants for the evolution of life-history traits in lizards, simultaneous studies on a set of syntopic populations of species differing in the degree of their phylogenetic relatedness, ecological traits and morphology, are apparently needed (Vitt, 1986; Tinkle & Dunham, 1986; Henle, 1990). Studies on life-history traits of lacertids (Lacertidae) in comparison with other lizard families are still scarce (see Dunham et al., 1988). This is particularly true for the Balkan lacertids (Podarcis spp., Lacerta spp., Algyroides spp., Ophisops spp.). Also, almost all previous studies of these lizard populations have been conducted in locations lacking diverse lizard communities. Out of 19 species that can be encountered throughout the Balkans, nine species compose the

lacertid lizard community of the Lake Skadar region (Crnobrnja-Isailovic & Dzukic, 1995). In this report we compare the life-history attributes of four sympatric (in some cases syntopic) species, both in a coastal area and on islands in Lake Skadar. In addition to differences in the genetic background and meaning of the species, these lacertids also differ in morphology, distribution and ecological traits.

MATERIALS AND METHODS

SPECIES STUDIED

The species studied here represent the closest genera within Old-World lacertids, with affinities as follows: (Podarcis-Lacerta) (Algyroides) (Arnold, 1989). The Dalmatian Algyroides, Algyroides nigropunctatus, ranges along the coastal region of the Adriatic and Ionian seas, from Italian and Slovenian karst regions to the Akarnanien region (Greece), including some northern Adriatic islands. There are a few significant range penetrations inland. This species is usually encountered in degraded scrub, bushes between fields and rocky cliff areas, usually near water. The sharp-snouted rock lizard, Lacerta oxycephala, is a steno-endemic Balkan species, restricted in its range to a narrow zone along the Adriatic coast, from the river Krka to the northwestern part of Albania. L. oxycephala differs distinctly in morphology from all other lacertids by having a flattened body, with a very pointed snout. In an ecological sense, this species is peculiar among the Balkan's lacertids because it is the most specialist petricole, inhabiting mostly sunny cliff faces, rock-pavements,

stone piles and screes. The Dalmatian wall lizard, *Podarcis melisellensis*, is a polymorphic species, with a considerable abundance in diverse habitats, but it is mainly encountered in densely vegetated places. It ranges from Monfalcone (Italy) to Skadar (Albania) along the coastal zone of the Dinaric Alps, extending inland in Lika, Hercegovina, Montenegro and Albania (Tiedemann & Henle, 1986). The common wall lizard, *Podarcis muralis*, has a wide geographic distribution (central and southern Europe), with immense geographic variation in morphology and habitat characteristics (Gruschwitz & Bohme, 1986). Thus, this lizard is thought to be more opportunistic with regard to microhabitat preference than its relatives.

HABITAT AND SPECIES COMPOSITION

Lake Skadar is the largest of the Balkan lakes. The lake is located in a karst terrain in the outer part of the south-eastern Dinaric Alps. Mountains rise steeply from the lake's south-western shore along which an archipelago of about forty large and small islands exists. Vegetation of this part, including the islands, is characterized by maquis and guarigue. Scattered xeromorphic trees are separated by patches of dry grass and herbs. Larger islands usually have a water's-edge rocky zone with scattered trees only, and a central grassy zone with bushy vegetation. The northern and north-eastern shores are flat, providing an extensive semi-littoral zone. This formerly inundated terrain with recent alluvial deposits is now mostly under cultivated soil, with fragments of common oak forests. The climate of the region is characterized by a large amount of precipitation and high summer temperatures (Lasca et al., 1981). The shore region of the lake has two distinct zones with respect to lizard distribution, but on the islands there are species in various combinations of allotopic and syntopic occurrence (Crnobrnja-Isailovic & Dzukic, 1995). P. muralis and L. oxycephala appear to be the most common species, probably due to their better colonizing abilities. They are the only species with allotopical distributions. L. oxycephala occurs as the only lizard on 19, and P. muralis on 10, of the 40 islands. These islands are the most densely populated (up to one individual per 2 m², unpublished data.). When species are syntopic, spatial partitioning occurs; in such cases they generally follow their prefered habitats. Thus, P. melisellensis is mostly encountered in vegetated areas, P. muralis and L. oxycephala incline to shoreline rocky zones, while A. nigropunctatus show habitat preferences between these two groups. Data on morphological traits relevant to this study came from the complete lizard samples taken from the overall Lake Skadar region. Specimens included in the analysis of life-history traits were collected from the following six localities. Bistrica is located on the northern lowland bank. Lizards were collected there from the grassy sides of railroad tracks and nearby road (Podgorica-Bar). P. melisellensis shares this habitat with P. muralis. Malo Starcevo is a small island (0.9 ha) with dense populations of P. muralis only. Beska Velja is one of the biggest islands (15.9 ha), with L. oxycephala as the only lizard species. The island Malo Besko (1.7 ha) is characterized by a dense tree vegetation and grassy patches with boulders of various sizes, inhabited by a relatively robust population of P. melisellensis. In nearby waters, mainly on rocks, many P. muralis individuals can be encountered. Mali Moracnik is a moderate-size island (0.7 ha), with a sparse tree vegetation and grassy fields among large stony patches. Here the large P. melisellensis population inhabits grassy areas syntopically with L. oxycephala. A few individuals of P. muralis were noticed on this island as well. Bisage is an island of 2.8 ha, with P. melisellensis and A. nigropunctatus as the lizard inhabitants.

DATA COLLECTION AND STATISTICAL ANALYSES

Details about field and laboratory methods (body and egg measurements, egg-laying process, egg incubation period, hatchlings raised), including data on clutch and egg size, size at sexual maturity for females of L. oxycephala, P. melisellensis and P. muralis, can be found in our previous papers on reproductive characteristics of the lacertids from the Lake Skadar region (Aleksic & Tucic, 1994; Bejakovic et al., 1995, 1996). Snout-vent length (SVL) was taken as a measure of overall size. Head dimensions (length, height, width), relative to SVL, expressed the body shape. The length of hind limb relative to SVL was considered as a morphological indicator of mobility capacities of the lizard species (see Pianka, 1986). Egg volume (V) was taken as the overall measure of size of eggs laid. RCM values were calculated as the ratio of clutch mass to body mass after egg laying (see Shine, 1980) and were used as a measure of reproductive investment per clutch. The size of the smallest female with enlarged vitellogenic follicles was considered the minimum size at maturity.

Female reproductive characteristics were studied on a number of individuals, except for *A. nigropunctatus* where only ten sexually matured females were available, of which three laid eggs. Nevertheless, we included this species in this study, though we were aware that the paucity of sample size precluded wellsupported conclusions.

Statistics were calculated with SAS package (SAS Institute, 1989). Mean values and standard errors of life-history traits were weighted for population sizes. SVL, egg volume and clutch size were logarithmically transformed. Life-history traits (estimated on females) were analysed with populations nested within species. For each species, one-way ANOVA's were also performed. Pair-wise comparisons between species and populations were done by Scheffe test which controls an experimental type I error. Morphological traits were

SVL Species HL/SVL HW/SVL HH/SWL n HLL/SVL A. nigropunctatus 27 61.27±1.06 0.26 ± 0.00 0.16 ± 0.00 Males 0.10+0.00 0.56 ± 0.01 Females 10 56.71±1.30 0.23 ± 0.00 0.14 ± 0.00 0.08 ± 0.00 0.51 ± 0.01 L. oxycephala 63.01±0.32 0.27 ± 0.00 0.16 ± 0.00 0.09 ± 0.00 Males 292 0.59 ± 0.00 0.25±0.00 0.14 ± 0.00 Females 196 61.21±0.37 0.08 ± 0.00 0.55 ± 0.00 P. meliselensis Males 92 59.76±1.20 0.24 ± 0.00 0.14 ± 0.00 0.10 ± 0.00 0.54 ± 0.00 Females 77 59.70±0.80 0.21±0.00 0.12 ± 0.00 0.09 ± 0.00 0.48 ± 0.00 P. muralis 396 70.74±0.35 0.26 ± 0.00 0.15 ± 0.00 Males 0.12 ± 0.00 0.57 ± 0.00 67.93±0.32 0.22 ± 0.00 0.13 ± 0.00 Females 367 0.09 ± 0.00 0.52 ± 0.00 Significance levels from ANOVA 0.0003 0.0001 0.0001 0.0001 Sex 0.0001 0.0039 0.0002 0.0079 0.0005 0.0080 Species 0.2183 0.0011 0.0961 0.1331 0.0554 Locality (Species) 0.0001 0.0001 0.0001 0.0001 0.0001 Sex x species Sex x locality (species) 0.0136 0.0001 0.0001 0.0001 0.0027

TABLE 1.Comparison of male and female morphology among lacertid species from the Lake Skadar region. HL (head length), HW (head width), HH (head height), HLL (hind limb length) are presented as a proportion of snout-vent length (SVL). Means (\pm S.E.) and sample sizes (*n*) are given. ANOVA for HL,HW, HH and HLL was performed on residuals from regression on SVL.

estimated on both males and females and analysed by partial hierarchical ANOVA on residuals from regression of each trait on SVL (both logarithmically transformed). Sex and species were considered as fixed main factors and populations as random factors nested within species. Effect of sex was tested over error mean square (MS), effect of species over population MS, and sex x species interaction over sex x population interaction. Growth rates were estimated by regressing juvenile length on months. Comparison of growth rates was done by analysis of covariance (ANCOVA). Month x species interaction (measure of slope's heterogeneity) was tested over month x female interaction. Since product-moment correlations estimated on population means were insignificant due to the small number of populations, correlations between life-history traits were estimated on the whole sample. As we dealt here with structured data sets, a canonical discriminant analysis, which maximized variation between groups, was used for joint analysis of reproductive traits instead of a principal component analysis (James & McCulloch 1990). We turned to populations as groups rather than species because of the small number of species used in this study.

RESULTS

MORPHOLOGY

Significant differences in SVL were found among species (P. muralis vs. P. melisellensis and L. oxycephala), and between sexes (males are larger than females; Table 1). Males and females of all species differ significantly in head dimensions relative to body size (SVL), males having larger heads (Table 1). Species differ in head height (L. oxycephala vs. Podarcis species). Head dimensions correlated with the size of prey items (length and width) are considerably different between species. Relative head length discriminates long-headed L. oxycephala and A. nigropunctatus vs. Podarcis species. Differences in the relative head width are less apparent; only L. oxycephala has a significantly wider head than P. melisellensis. Males of all species have statistically longer hindlimbs (relative to SVL) than females (Table 1), which indicates their higher locomotor capabilities. For one morphological trait, locality effect (within species) is statistically highly significant.

Egg shape also varies between the species. L. oxycephala has the most elongated eggs (width/length:

TABLE 2. Comparison	of reproductive tr	aits among	lacertid species	from Sk	kadar Lake	region. Me	an values	(±S.E.) and
significance levels from	nested ANOVA's ar	e given. Spec	cies (population	s) that sha	are the same	letter do no	t differ sign	ificantly. n-
sample size.								

Species	Population	Female SVL (mm)	Number of eggs per female	Egg volume (mm ³)	RCM
A. nigropunctatus	Bisage	57.84	3.33 AB	360.64	0.29
	<i>n</i> =3	±2.94	±0.33	30.08	±0.08
L. oxycephala		62.76	3.40 A	272.38	0.22
		±0.27	±0.10	± 38.45	± 0.02
	Beska	62.49	3.50	310.83 a	0.24
	<i>n</i> =10	±0.62	±0.27	±16.09	±0.01
	Mali Moracnik	63.03	3.30	233.94 b	0.20
	<i>n</i> =30	±0.40	±0.11	±10.00	±0.01
				0.0004	
P between population	ons	0.4838	0.6323	0.0024	0.0672
P. melisellensis		62.91	4.05 AB	257.02	0.22
		±2.52	±0.43	± 23.78	±0.01
	Mali Moracnik	63.38 a	4.90 a	217.12 a	0.23
	n=20	±0.67	±0.26	± 15.50	± 0.02
	Mala Beska	67.02 a	3.60 b	299.38 b	0.21
	<i>n</i> =10	±0.88	±0.16	± 15.18	± 0.02
	Bistrica	58.33 c	3.64 b	254.56 ab	0.23
	<i>n</i> =14	±0.84	±0.25	±22.19	± 0.02
P between populati	ons	0.0001	0.0007	0.0285	0.7836
P. muralis		70.71	5.51B	266.89	0.24
		±2.76	±0.49	±39.56	± 0.02
	M. Starcevo	74.59 a	4.60	206.67 ab	0.20
	n=5	±1.03	±0.24	± 54.53	± 0.01
	Mala Beska	72.18 a	5.67	341.44 a	0.25
	n=18	±0.97	±0.41	±19.66	± 0.02
	Bistrica	65.36 b	6.26	252.56 b	0.26
	<i>n</i> =19	±0.82	±0.42	±7.99	±0.01
		0.0001	0.1642	0.0010	0.2240
P between population	ons	0.0001	0.1642	0.0010	0.3340
P between species		0.2031	0.0711	0.7867	0.5080

 0.51 ± 0.01), followed by *P. melisellensis* (0.53 ± 0.01), *P. muralis* (0.57 ± 0.04) and *A. nigropunctatus* (0.59 ± 0.02) with the most rounded eggs. Differences among species appeared non-significant (*P*=0.260), but differences withing species (interpopulation variability) were significant (*P*<0.05).

LIFE-HISTORY TRAITS

Size of "laid-egg" females did not differ between species, but there was significant variation in interpopulation comparisons in *P. melisellensis* and *P. muralis* (Table 2). Females mature at minimum SVL of 51.0 mm (*P. melisellensis*), 53.3 mm (*A. nigropunctatus*), 53.3 mm (*L. oxycephala*) and 55.0 mm (*P. muralis*). Thus, females at the onset of reproductive life have already reached more than 80% of the average laid-egg female sizes in all species studied. (For the sake of comparison, most female squamates grow between 15 and 30% after maturation; Andrews, 1982.) P. muralis produced the largest clutches and A. nigropunctatus the smallest clutches (Table 2). The clutch size of P. muralis and L. oxycephala were statistically different, though overall interspecies differences in clutch sizes were not. A significant intraspecies difference in clutch size was found only in P. melisellensis. The number of females in clutch size classes showed quite different distribution patterns, where P. muralis was distinguished by the largest variation in clutch size (Fig. 1). Egg size did not differ significantly among species, but there were remarkable differences within species (Table 2). The measure of the reproductive investment per clutch, RCM, was nearly uniform for the Skadar Lake lacertids ranging



FIG. 1. Clutch size distribution in four lacertid species from Skadar lake region.

from 0.29 in A. nigropunctatus to 0.22 in L. oxycephala and P. melisellensis. Time to hatching and size of hatchlings were studied in three populations of P. melisellensis, and in one population of P. muralis (Malo Starcevo) and L. oxycephala (Beska). Minimum and maximum time necessary for newborns to hatch from eggs in incubators were found in P. melisellensis and ranged from 33.4 ± 2.8 days (Bistrica) to 38.0 ± 0.4 (Mali Moracnik). The smallest newborns were those of P. melisellensis (Bistrica, 65.5 ± 3.3 mm) and the biggest those of P. muralis (Malo Starcevo, 71.2 ± 3.0 mm).

We found no significant differences between species for these life history traits, nor between populations of *P. melisellensis*. The somewhat faster juvenile growth rate of *P. muralis* (Malo Starcevo population) compared to *L. oxycephala* (Beska) was insignificant, too (ANCOVA, P=0.124). TABLE 3. Between population canonical structure from canonical discriminant analysis performed on reproductive traits measured in four lizard species from Skadar Lake region.

	CAN 1	CAN 2
Number of eggs per female	0.911	-0.400
Egg volume	0.551	0.800
RCM	0.685	0.015
Eigenvalue	1.5317	0.4979
Proportion	0.7256	0.2359
Cumulative	0.7256	0.9615
Р	0.0001	0.0001

CORRELATION BETWEEN LIFE-HISTORY TRAITS

As expected, RCM was significantly correlated with the number of eggs per brood, and with the volume of eggs (r=0.471, P<0.001, and r=0.593, P< 0.001, respectively). The body size (SVL values) also was significantly correlated with these variables of reproductive potential (r=0.424, P<0.0001, r=0.288, P < 0.01; respectively), as was found in most reptiles (Fitch, 1970; Dunham et al., 1988). There was no relationship between the number of eggs and the size of hatchlings (r=-0.24, P=0.439), or between RCM and the size of hatchlings (r=-0.167, P=0.585). It is possible that small sample size precluded confirming such relationships. Egg shape (width/length ratio) was significantly positively correlated with the number of eggs per female, egg volume and RCM (r = 0.47, 0.35and 0.39, respectively), and significantly negatively correlated with total length of hatchling (r=-0.70). Therefore, more round egg shape associates with larger brood, bigger egg size and, concomitantly, higher reproductive investment (RCM), while newborn lizards are bigger when they hatch from more elongated eggs.

MULTIVARIATE COMPARISONS

Reproductive variables were estimated for populations of P. muralis, P. melisellensis and L. oxycephala, and for one population of A. nigropunctatus. A canonical discriminant analysis was performed on this data set. CAN1 axis took 72.6% of variation in the original variables while 23.6% of that variation could be described by CAN2 axis (Table 3). CAN1 described a positive relationship among all three variables, while the egg number was negatively correlated with CAN2 axis and, at the same time, contrasted strongly with the egg volume. Species were mostly separated by the egg number on CAN1 axis and by the egg volume on CAN2 axis. In the CAN1 and CAN2 scatter diagram, populations separated well by their species designations (Fig. 2). Species were differently distributed along the first axis. P. muralis populations had positive values of CAN1 axis, while the populations of L. oxycephala and A. nigropunctatus



FIG. 2. Mean population scores on first two cannonical axes for four lacertid species from Skadar lake region. *Algyroides nigropunctatus*-solid circles; *Lacerta oxycephala*-solid squares; *Podarcis melisellensis*-empty squares; *Podarcis muralis* - empty circles.

had the most negative values along this axis reflecting their tendency for the most dissimilar clutch size. *P. melisellensis* populations took the middle position between these species. Two pairs of closely associated *L. oxycephala* and *P. melisellensis* populations (Mali Moracnik - Bistrica, Beska - Malo Besko) emerged due to close similarities in the egg size and the egg volume. Syntopic populations (Mali Moracnik - *P. melisellensis* and *L. oxycephala*; Bistrica - *P. muralis* and *P. melisellensis*; Malo Besko - *P. muralis* and *P. melisellensis*) differed considerably in their position in-

dicating species-specific effect on reproductive characteristics, rather than site effect.

DISCUSSION

The following discussion is centered around the main question in this paper: How do these variations in life-history traits correlate with the species body shape, phylogenetic relatedness, distribution areas and ecological demands? Individuals of *L. oxycephala*, and to a lesser extent of *A. nigropunctatus*, are dorsoventrally flattened, showing striking dissimilarities to stream-lined morphology of *Podarcis* species. Such a morphology is adaptive as it allows individuals to seek refuge in narrow crevices when disturbed or inactive.

Cylindrical body morphology, on the other hand, enhances locomotor capabilities in more open, usually vegetated habitats. Predator pressures on surface-active lizards (e.g. when basking) in the Skadar Lake region are presumably similar. However, potential predators of these species once they are within crevices may be quite different. Their known predators in the Skadar Lake region include snakes (Malpolon monspessulanus, Coluber laurenti, Natrix tessellata), some birds (e.g. Ardea purpurea, Larus spp.) and rats (*Rattus rattus*). Because of the cylindrical morphology of snakes, they are not capable of predating lizards once they are in a rock crevice: the flattened morphology decreases the vulnerability to predation. Thus one may expect lower predator pressure on L. oxycephala and A. nigropunctatus than on those Podarcis species studied and, concomitantly, their higher lifetime reproductive success. Concomitant reproductive adaptations, associated with the effect of habitat selection on flattened morphology, usually include a reduced clutch size and more elongated eggs compared to other relatives (Vitt, 1981; 1993). The consequence is that non-reproductive females are as flat as females carrying eggs. In L. oxycephala, and in A. nigropunctatus to a lesser extent, reproductive adaptations that appear to be consequences of the exclusive use of rock outcrops are just as noted above. However, reduced clutch size in this species was not associated with reduced clutch volume, as was found in some flat lizards (Vitt, 1981). Also, contrary to other lizards in which morphological adaptations associated with the use of crevices in rocks for predator escape constrain RCM value (Vitt, 1981), the reproductive investment per clutch of L. oxycephala and A. nigropunctatus is about the same as in other lacertids from the Skadar Lake region.

Analyses made to date have suggested that phylogenetic relationships account for a large proportion of the variation observed in life-history variables of squamata (e.g. Dunham & Miles, 1985). This seems to be proven in our study as well. When reproductive characteristics, which determinate the coevolutionary framework for other life-history features (see Stearns, 1976; 1977) are compared, populations of P. muralis and P. melisellensis appeared to be closer to each other than populations of other the two species (L. oxycephala and A. nigropunctatus). The most common species (P. muralis and L. oxycephala) are on the opposite sides in reproductive traits similarity. Both tactics seem to work well in the Skadar Lake lizard community. Apparently, species level effects here are superseded by genus level effects as proposed by Stearns (1984) for the squamate reptiles.

Species distribution patterns in CAN1 and CAN2 reproductive-dependent space correspond to their general ecological relatedness. Both *A. nigropunctatus* and *L. oxycephala* are highly specialized with respect to their habitat choice. Contrary, *P. muralis* is more generalist with regard to microhabitat preference. It seems that *P.*

melisellensis is probably, in this respect, somewhere between these two groups. As stated above, intraspecific variations in reproductive traits are apparent, but well within species multivariate framework. Such variation is due to adaptations to specific environmental conditions and plasticity in characteristics that reflect immediate responses to proximate conditions. In this respect insular populations, at least those of P. muralis and P. melisellensis species, differ from continental populations in body size and clutch size. Island lizards are larger than conspecifics on the nearby continental area. It seems that lizards on islands, under high intraspecific competition (islands in Skadar Lake have much denser populations than the mainland; unpublished data), allocate more energy to somatic growth than to reproduction. In contrast, the life-history strategy of the continental populations could be to invest more in the number of offspring than in growth.

In conclusion, our analysis substantiates the claim that much of the pattern of covariation in life-history data can be explained by lineage-specific effects, either constrained by or coadapted with morphological differences, even if phylogenetically close taxa are compared.

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TEMPERATURE SELECTION FOR EGG INCUBATION BY THE LIZARD PODARCIS HISPANICA ATRATA

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This paper reports on the selection of substrate temperatures at oviposition sites by female lizards *Podarcis hispanica atrata* under laboratory conditions. Seven females deposited 11 clutches (a total of 34 eggs) in very small areas, representing 20 % of the available surface. The mean temperature of the sites used for egg-laying was 25.7 °C. Results are compared with incubation temperatures of the same species under natural conditions (Columbretes islands, Mediterranean, Spain).

INTRODUCTION

Temperature has an important and instantaneous effect on the rate of embryo development, and hence on the duration of egg incubation, and can have long-lasting effects on the morphology, physiology, behaviour, and survival of hatchling reptiles (e.g., Packard & Packard, 1988; Van Damme et al., 1992; Janzen, 1993; Overall, 1994; Castilla & Swallow, 1996). Thus, the decisions that female reptiles make while selecting an appropriate egg deposition site have important conseauences on their reproductive success. Notwithstanding their importance, the factors that influence the choice of egg-laying sites are a neglected part of lizard biology. This is hardly surprising, given that lizard clutches are extremely difficult to locate in the field. Consequently, little information is available about the environmental conditions in natural nesting sites (but see Bock & Rand, 1989; Christian, Lawrence & Snell, 1991; Christian & Lawrence, 1991; Burger, 1993; Castilla & Bauwens, 1996). Therefore, laboratory studies should provide a fruitful alternative, because eggs are easier to locate and environmental factors can be controlled more readily and precisely.

In this study, selection of substrate temperatures for egg deposition by Podarcis hispanica atrata are examined in a laboratory photo-thermal gradient. Confinement to a terrarium undoubtedly produces unnatural stress. However, individuals of this species have repeatedly shown (e.g., during this study) to tolerate captive conditions well enough to perform normal activities (e.g., thermoregulation, foraging, social interactions, copulation). Hence, egg-laying behaviour by P. hispanica atrata is considered to be largely unaffected by captivity. In addition, captive conditions enabled lizards to select the substrate temperature for egg deposition from a wide range of available temperatures. Thus, although the results do not necessarily apply to field conditions, they do indicate temperature selection in a laboratory situation.

MATERIALS AND METHODS

P. hispanica atrata is a heliothermic lizard endemic to 20 ha of the Columbretes archipelago (Mediterranean Sea, Spain; Castilla & Bauwens, 1991a, 1991b, 1996). In their natural habitat, lizard density is high (up to ca. 800 ind/ha; Castilla & Bauwens, 1991b). Females attain sexual maturity within their first year of life at a snout-vent length (SVL) of 50 mm and, within a single reproductive season (April-June), they may produce up to at least three clutches composed of 1 - 5 eggs (Castilla & Bauwens, 1996). During the egg-laying period, dense concentrations of pregnant females were observed in several very restricted areas (Castilla & Bauwens, 1996). In addition, the only nesting site found in the Columbretes islands is a communal nest where 240 eggs were found over two consecutive years (Castilla & Bauwens, 1996). Together, these data indicate that communal laying behaviour could be a common behavioural characteristic of this species. As Podarcis hispanica possesses sex chromosomes (Odierna, Kupriyanova, Capriglione & Olmo, 1993), the thermal conditions during incubation do not affect sex determination.

During June-July 1995, seven females (mean SVL ± 1SE: 60.8 ± 1.9 mm) and four males (mean SVL \pm 1SE: 62.8 ± 3.0 mm) were kept in two terraria (100 x 40 cm) at a laboratory in Hasselt (Belgium). Several lizards were intentionally kept in the same cage (3 females + 2 males, and 4 females + 2 males), considering the high population densities and aggregations of gravid females in the field. Lizards in terraria were provided with water (supplemented with vitamins) and food (meal-worms and crickets) ad libitum. The substrate of the terraria consisted of a 7 cm thick layer of eolic sand of very fine grain. The entire sand surface was covered with litter, and flat stones and pieces of wood were scattered over the entire surface. Illumination and heat were provided by a 150 W light bulb suspended 25 cm above the substrate near one end of the terrarium (Fig. 1). The



FIG. 1. Thermogradient (100 x 40 cm) designed to examine temperature selection for egg incubation by the lizard *Podarcis hispanica atrata*. Shown are the different sections where substrate temperature was measured (n = 6 records/section). L1 and L2 are the sections where all clutches were found; H is a circle of 15 cm diameter at 25 cm below a 150 W bulb.

light bulb was switched on daily from 0900 to 1800 h; within this interval, the bulb was disconnected for 15 min periods at 1015, 1130, 1345, 1500 and 1600 h. As the substrate was heated by the light bulb only, substrate temperatures followed a cyclic regime. I deliberately chose a cyclic temperature regime, because it more closely simulates natural conditions. I also chose an interrupted light cycle, because lizards in the field may not be active continuously.

During a single day, substrate temperatures were measured at a depth of 7 cm, using a thermocouple connected to an electronic thermometer (Digitap Therma 3; to the nearest 0.1 °C), during nine hourly intervals (0700-0800, 0900-1000, 1000-1100, 1200-1300, 1300-1400, 1500-1600, 1700-1800, 1900-2000, 2000-2100 h). During each hourly interval, temperature was measured atsix different sites within each of seven sections of the terrarium (Fig. 1). The daily average substrate temperature was estimated in each section by calculating the mean of the readings obtained at 0700-0800 h and 1700-1800 h, which correspond to the minimum and maximum daily temperatures. This approach is considered reliable on biophysical grounds (W. P. Porter, pers. comm.). In addition, in a previous study of artificial egg laying sites for P. hispanica atrata (Castilla & Swallow, 1996), a significant correlation was found between the average of the two extreme temperatures and the mean value obtained from measurements made at 3 h intervals throughout a day. At the very least, the approach provides an index of the

TABLE 1. Descriptive statistics of substrate (7 cm deep) temperatures (in $^{\circ}$ C) in the different sections of the thermogradient (see Fig. 1 for section codes).

Section	Mean	Variance	Minimum	Maximum	n
LI	26.11	4.92	22.1	30.0	54
L2	25.77	3.75	22.1	28.8	54
В	26.61	5.43	22.1	29.9	54
Н	29.02	13.05	22.1	33.7	54
Α	24.60	2.01	22.1	27.1	54
С	24.37	1.37	22.1	26.0	54
D	23.63	0.58	22.1	24.6	54



FIG. 2. Diel variation of substrate temperatures (depth: 7 cm) recorded on 27 July 1995 in different sections within a thermogradient (see Fig. 1). Shaded areas indicate the time intervals when the lightbulb was switched off. L1 and L2 denote the sections when all clutches were laid.

relative temperature differences among various sections within the terrarium. Note that "daily average temperature", i.e., the mean of the records obtained at 0700-0800 h and 1700-1800 h, differs slightly from the arithmetic mean temperature, i.e., calculated by averaging the readings obtained during all seven hourly intervals (Table 1).

Humidity potentially influences the females' searching behaviour for laying sites, because extreme dry and extreme wet conditions affect egg development in some lizard species (Muth, 1980; Packard, 1991). Water was sprayed uniformly over the entire surface of the terraria every other day. Despite its apparent importance, humidity could not be measured in this study. Only the soil water content was estimated (0.6 g of water per 10 g of dry sand) by weighing wet sand, drying it in an oven (90°C) for 1 h and reweighing it.

To avoid disturbance of the terraria, no attempt was made to locate the clutches until all females had deposited their eggs. To confirm that all clutches were laid, both terraria were regularly inspected for the presence of post-reproductive females.

RESULTS

A total of 11 clutches (one to three per female), comprising a total of 34 eggs, were found. All were discovered at a depth of ca. 7 cm (the maximum depth of the substrate) in two very narrow (10 x 40 cm each) sections near the bulb (L1 + L2, Fig. 1). The null hypothesis was examined, that females used different zones in proportion to their availability. As all clutches were found in 20% of the available surface, and none in the other areas of the terrarium, the observed distribution differed significantly from the expected one ($\chi^2 =$ 44.0; 1 df; P < 0.001). Overall substrate temperatures fluctuated between 22.1 and 33.7 °C (Table 1, Fig. 2). Hourly variation of substrate temperature was significant in all sections of the thermogradient (ANOVA, P < 0.001 in all cases; Fig. 2). In the two sections where all clutches were located, substrate temperatures varied between 22.1 and 30.0 °C (L1) and 22.1 and 28.8 °C (L2). *P. hispanica atrata* avoided the lowest and highest substrate temperatures available in the thermogradient (Fig. 2) and laid their eggs at sites that experienced a daily average substrate temperature of 25.7 °C.

DISCUSSION

Because several females were housed together in single terraria, the first ovipositing female could have guided the others to lay at the same site. However, this cannot explain why the same areas were chosen in both terraria. Therefore, the deposition of all clutches in a restricted area, corresponding to a narrow range of substrate temperatures, was interpreted as evidence for the existence of very precise cues of temperature selection for egg incubation.

The precise selection of substrate temperatures must be seen in the light of the pervasive effects of temperature on the rate of embryo development and characteristics of hatchlings in lizards. Very low (20 °C) and very high (> 32 °C) mean constant incubation temperatures have lethal effects on the eggs of some closely related lacertid lizards (Van Damme et al. 1992; D. Bauwens, pers. comm.). It is evident that lizards will avoid laying their eggs at such extreme temperatures. However, the daily mean substrate temperatures in all sections of the thermogradient, as well as the minimum and maximum values (Table 1), were well within the range of non-lethal incubation temperatures for the mainland subspecies P. h. hispanica (Van Damme et al. 1992, D. Bauwens, pers. comm.). Thus, the present observations suggest that very precise mechanisms for the selection of substrate temperatures exist, even within the non-lethal range for incubation.

The daily average substrate temperatures at the laying sites in the thermogradient (25.7 °C) corresponds closely to the mean temperature recorded near a clutch deposited by a field-active female in an artificial nesting site (25.3 °C; Castilla & Swallow, 1995), and was somewhat lower than the temperature recorded in a natural communal nesting site (28.3 °C; Castilla & Bauwens, 1996). Relatively low substrate temperatures prolong the duration of incubation in this and other lizard species (e.g., Packard & Packard, 1988; Van Damme et al., 1992; Castilla & Swallow, 1996). However, eggs incubated at relatively low temperatures produce larger-sized hatchlings than those kept at higher temperatures (e.g., Beuchat, 1988; Phillips et al., 1990; Van Damme et al., 1992; Phillips & Packard, 1994). In the closely related P. h. hispanica, a constant incubation temperature of 26 °C maximises hatchling size as compared to temperatures of 29 °C and 32 °C (Van Dijck, 1993). Being big at hatching may have positive fitness consequences (Van Damme et al., 1992 and references therein), especially in the populations of P. hispanica. atrata where cannibalism appears to constitute an important cause of egg and juvenile mortality (Castilla, 1995; Castilla & Van Damme, in press).

Several factors may influence a female's decision to deposit its eggs at a given site (i.e., a variety of physical factors, protection of eggs and females from predation, etc.), but the design of the present study did not allow identification of these. The results only suggest that substrate temperature is an important physical factor guiding females in the selection of a laying site. Nevertheless, more research is needed in this field. In particular, integrated field and laboratory studies that examine the effect of both substrate temperature and soil water content on the duration of incubation, hatching success, morphological characteristics and physiological performances of juveniles are needed to enhance our understanding of the relevance of incubation environments and female parental care. In addition, the distribution of some lizard species is apparently limited by the combinations of soil temperature and water content that allow successful egg incubation (Porter & Tracy, 1983). Thus, studies on the characteristics of nest environments will also help to understand the geographical distribution of lizards.

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REPRODUCTIVE BIOLOGY OF THE VIVIPAROUS LIZARD, LIOLAEMUS PICTUS (*TROPIDURIDAE*): BIENNIAL FEMALE REPRODUCTIVE CYCLE?

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Viviparity has been reached independently by different genera of reptiles. Synchronization of the reproductive style with the environment facilitates offspring birth and survival. Several authors postulate a close relationship between reproductive mode and environmental conditions in these species. *Liolaemus pictus* is a viviparous medium-sized lizard that lives in temperate habitats up to moderate altitudes in the Andean-Patagonian forest. Sexual dimorphism, follicle number and size in relation to body size, date and locality, reproductive cycle and litter size were studied. In the light of our results we reject an annual reproductive style among viviparous lizards, biennial (or triennial) reproduction enlarges the known diversity of reptilian reproductive responses to short and cold activity seasons, such as those typical of the highlands of southern South America.

INTRODUCTION

Most lizards are oviparous, but squamate viviparity (Callard, Fileti, Pérez, Sorbera, Giannoukus, Klosterman, Tsang & McCracken, 1992; Guillette, 1993; Stewart, 1993) has evolved at a multitude of taxonomic levels, and within *Liolaemus* itself (Blackburn, 1982, 1985).

The relationship between reptilian reproductive styles (sensu Balon, 1990) and environment is complex (Vitt, 1992). In addition, it may be the consequence of inherited traits that evolved in the past (Guillette & Méndez de la Cruz, 1993). Optimization of offspring production implies not only present reproductive success but also parental survival and future reproductive success. Thus, we can expect that species will find an adequate balance between the age of first breeding, the annual number of broods, clutch size and duration of embryonic life, considering only the most important reproductive characteristics (Shine & Schwarzkopf, 1992). An appropriate co-ordination of the reproductive style with the environment (e.g. temperature, photoperiod, rainfall) allows offspring birth and survival

The cold climate hypothesis (Shine, 1985, 1989) considers viviparity evolved in cold climates and this evolution is an adaptation to low temperature conditions, among other factors. Guillette (1993) contrasts this hypothesis with that of Tinkle & Gibbons (1977), who considered viviparity as a pre-adaptation to such conditions. Irrespective of the causal order of the events, there is a relationship between reproductive mode and environmental conditions in squamate reptiles. Viviparity is more common among cool-climate reptiles than among species living in warmer climates (Shine, 1995).

Probably, the strong relationship between embryonic developmental rate and temperature (Heulin, Osenegg & Lebouvier, 1991) establishes the main constraint to reproduction in relation to the shortening of the activity season under cold climates. Recently, Shine (1995) proposed and tested successfully the idea that prolonged uterine retention directly enhances hatchling viability, because eggs incubated at maternal body temperatures produce 'better' hatchlings than do eggs incubated at normal nest temperatures. The phenotypic plasticity hypothesis (Shine, 1995) predicts that prolonged uterine retention might enhance offspring fitness in any environment in which maternal temperatures differ from nest temperatures.

Many of the extant reptiles of southern South America belong to the family *Tropiduridae* (sensu Frost & Etheridge, 1989), with three subfamilies. One of them, *Liolaeminae*, has three genera. The genus *Liolaemus*, with at least 50 known species in the centre and south of Argentina, is broadly distributed from the highlands of Perú and Bolivia to Tierra del Fuego and from the Pacific islands to Brazil (Cei, 1986). *Liolaemus pictus* (Duméril & Bibron) is a viviparous medium-sized lizard (23 to 75 mm snout-vent length) that lives in temperate habitats up to moderate altitudes (520 to 1600 m), in leaf-mould and under logs (Donoso-Barros, 1966). It is the most common lizard in the Andean-Patagonian forest of Nahuel Huapi National Park (Christie, 1984).

Several annual reproductive cycles have been described in the genus *Liolaemus* (Pearson, 1954; Ramirez Pinilla, 1991, 1992*a*, 1995; Leyton & Valencia, 1992; Duarte Rocha, 1992), but this genus has great variations in the timing of reproductive processes. Within viviparous *Liolaemus* species we can see two different ways of coping with high altitude conditions. As do most autumn/winter-breeding viviparous lizards (Flemming & van Wyk, 1992), *L. multiformis multiformis* (4600 m) and *L. huacahuasicus* (3700 m) exhibits autumn vitellogenesis and ovulation, followed by pregnancy over winter and parturition during spring (Pearson, 1954; Ramirez Pinilla, 1991). Three viviparous montane species (2500 m), *L. altissimus*, *L. leopardinus*, and *L. nigroviridis* (Leyton & Valencia, 1992), exhibit vitellogenesis from late summer to early spring, followed by pregnancy during spring and summer, and the same situation occurs in *L. gravenhosti*, a mid-elevation (700 m) viviparous lizard (Leyton & Valencia, 1992).

The aim of this work is to study the reproductive biology of *Liolaemus pictus*, in order to augment knowledge of the existing reproductive styles of the genus *Liolaemus*, and understanding of the relationship between environment and reproduction in reptiles.

MATERIALS AND METHODS

MATERIAL USED

Specimens preserved in 70% ethanol (Sample A, n=186), collected from November to April 1982, 1983, and 1984, during an extensive survey of Nahuel Huapi and Lanín National Parks (Christie, 1984) were used. Additional specimens (Sample B, n=30) were collected from October 1993 to April 1994 in the rain forest and at the shore of the Nahuel Huapi lake. As a result of weather, *L. pictus* was inactive during the winter and capture was not possible several months of the year.

AUTOPSY PROCEDURES

Lizards were killed by intraperitoneal administration of sodic thiopental, fixed in 4% formaldehyde (Sample A) or Bouin's solution (Sample B) for 24 h and preserved in 70% ethanol. Small lizards, undoubtedly juvenile, were not dissected.

Ovarian follicles, corpora lutea and uteri were characterized by morphological and histological observation. Follicular categories were defined on the basis of the follicular size and the absence (previtellogenic follicles), presence (vitellogenic follicles) or anomalous distribution (atretic follicles) of yolk.

Estimation of ovarian size was based on the diameter of the largest follicle (Duarte Rocha, 1992), recorded with a vernier calliper on camera lucida schemes. For testicular size the antero-posterior diameter was used. Litter size was estimated by counting the number of embryos in the uterus.

DATA RECORDED

Capture dates (DATE) were considered as the number of days after 21 September. Longitude and latitude of sampling sites were noted, and the following data were recorded for each lizard: sex, reproductive stage, gonadal size, snout-vent length (SVL), head length (HL), head width (HW) and width at vent (WAV). When needed, all original measures were transformed to natural logarithms to approximate multivariate normality and linear relationships, and corrected for SVL according to the following equation (Reichow, Largiadèr, Kligerberg, Clemmesen, Froese & Ueberschär, 1991; Battini, Alonso & Cussac, 1995):

(a) $AM = OML - (RC \times (\ln SVL - Mean(\ln SVL))),$

where AM = adjusted measurement, OML = originalmeasurement logarithm, RC = regression coefficient between the logarithm of the character and the logarithm of SVL, Mean(ln SVL) = overall mean of SVL logarithm.

STATISTICAL ANALYSIS

Data were analysed using analysis of variance (ANOVA), regression analysis (REGR), Kruskal-Wallis (KW), paired *t*-test (T-TEST) and discriminant analysis (DISCR). Normality and variance homogeneity assumptions were tested, comparing predicted and observed frequencies, by means of Kolmogorov - Smirnov (KS) and Runs test (RUN), and by means of Bartlett test, respectively (Sokal & Rohlf, 1969; Norusis, 1986).

RESULTS

JUVENILE PERIOD, SEXUAL DIMORPHISM, AND SEX RATIO

In agreement with Pflanz, Cusumano & Powell (1991), the maximum juvenile size was considered less than the size of the smallest vitellogenic female (49 mm, Fig. 1).

Temporal distribution of juveniles collected is presented in Fig. 2. Capture date was bimodal (KS, Z=1.398. n=54, P<0.04), with groups either side of



FIG. 1. Maximum juvenile size (vertical line) of male (filled circles) and female (hollow circles) *L. pictus* in relation to gonadal diameter and SVL. The triangle indicates the smallest vitellogenic female.



FIG. 2. Frequency (histogram) and SVL (filled circles) of juveniles against DATE (number of days after September 21).

DATE 140 (RUN, Z=-2.30, n=54, P< 0.0001), but DATE groups did not show different SVL (KW, n=54, $X^2=0.036$, df=1 P>0.84). On the other hand SVL was also bimodal (KS, Z=1.51, n=54, P<0.02) with groups of SVL (Fig. 2), less than and more than 35 mm (RUN, Z=-7.14, n=54, P<0.0001).

Liolaemus pictus does not show notable differences between the sexes. Only males had pre-anal glands (sensu Antoniatzzi, Jared, Pellegrini, & Macha, 1993), which were two to four in number. The SVL did not differ between males and females (ANOVA, F=0.25, df=150, P>0.61) but body shape did (DISCR, Wilks' Lambda=0.487, n=147, P<0.0001, AM were used). The relative importance of the morphometric variables was WAV > HW > HL. Analysed separately, each of these variables was significantly larger in males (Table 1).

TABLE 1. (a) Standardised canonical discriminant function coefficients P<0.0001; grouped cases correctly classified: 87.76%), (b) Pooled-within-groups Pearson correlations between discriminating variables and canonical discriminant function and (c) significance of the ANOVA between male and female lizards.

Variable*	Canonical coefficient	Correlation coefficient	Р
	(a)	(b)	(c)
HW	0.3310	0.70509	0.0001
HL	0.2846	0.46990	0.0001
WAV	0.6875	0.92044	0.0001

* Adjusted measurements



FIG. 3. Juvenile (J), male (M) and female (F) allometric relationship of HL, HW and WAV with SVL. Regression lines are indicated. Vertical dotted lines indicate SVL=46.6 mm and SVL=49 mm.

Comparison of 95% confidence intervals, for slopes and intercepts regression lines for adults and juveniles, showed that HL grew faster in adult males (n=54) than in juveniles (n=52). Other significant differences of slopes and intercepts between adults and juveniles were not found (Fig. 3).

Scatterplots of allometric relationships between HL, HW and WAV with SVL did not show a clear cut-off point between juvenile and adult lizards, but the intersection between juvenile and male HL lines (SVL=46.6 mm) is consistent with the assumed maximum juvenile size (SVL=49 mm, Fig. 3).

Amongst specimens collected (Sample A), the ratio of adult males to adult females was 0.86:1.

GONADS AND DUCTS

Male. The epididymis in adults males appeared convoluted and white, while in juveniles it was thinner and less conspicuous. Slight but significant differences were found between right and left testicular diameter (t=6.68, df=58, P<0.001).

Female. Previtellogenic follicles were present in the ovaries of all examined juvenile females (smallest SVL=41 mm), while previtellogenic, vitellogenic or atretic follicles were found in adult females. Corpora lutea were seen only in gravid females, together with previtellogenic follicles. Large vitellogenic follicles were never recorded in gravid females.

The uterus of vitellogenic and postgravid female *L. pictus* was folded and pale white. Folds grow, in preovulatory individuals, with a notable thickening of the mucosa and proliferation of secretory glands. Juveniles had a transparent uterus without folds.

No significant differences were found between right and left follicle diameter (t=0.30, df=69, P>0.76) nor

TABLE 2. Multiple regression analysis of standardised gonadal diameter versus locality coordinates, SVL and DATE. Latitude and Longitude are expressed in the Gauss-Kruger system.

	Variable	Slope	n	Р
Males	Longitude	0.002033	50	0.5101
	Latitude	-0.001345	50	0.1179
	Altitude	-0.000073	38	0.7748
	SVL	0.002915	50	0.5952
	DATE	-0.000208	50	0.8716
Females	Longitude	0.005302	46	0.3342
	Latitude	-0.003325	46	0.0126
	Altitude	0.000203	36	0.7323
	SVL	0.003591	46	0.7531
	DATE	0.001182	46	0.6632
Vit. Fem.	Longitude	-0.001025	30	0.7907
	Latitude	-0.001027	30	0.2527
	Altitude	0.000181	25	0.6615
	SVL	-0.009281	30	0.4052
	DATE	0.003821	30	0.0448

follicle number (t=0.00, df=14, P=1). Follicle growth (REGR multiplicative model, F=5.63, df=42, P<0.023) and follicle number (REGR, multiplicative model, F=4.75, df=14, P<0.049) in vitellogenic females showed a significant relationship with SVL. Thus, female gonadal diameters were adjusted according to equation (a) for further studies.

REPRODUCTIVE CYCLE

Male. No significant regression of male testicular diameter, adjusted to equation (a), ° latitude of localities was found (Table 2, n=50, P<0.12), even when relationships with SVL and DATE were considered into the analysis. In the same way, adjusted testicular diameter did not show variation in relation to DATE in the period considered (REGR, n=60, $\alpha=0.64$).

Female. A significant regression (n=46, P<0.01) of adjusted follicle diameter of all females ° latitude of localities was found (Table 2), but not for vitellogenic females only (n=30, P<0.25), even when relationships with SVL and DATE were considered in the analysis.

The number of non-reproductive adult females captured during summer and early autumn (Fig. 5) represented 20% of the total number of adult females.

The adjusted follicle diameter, from late spring to early autumn, keeps a linear and significative relationship with DATE (Fig. 4 and 5, REGR, n=43, $\alpha=0.0003$), (estimate \pm S.E.).

(b)
$$Y = 0.0049 (\pm 0.0012) X + 1.0 (\pm 0.2)$$

Time span from the onset of vitellogenesis until peak vitellogenic activity occurred in preovulatory females, was 129 days.



FIG. 4. Adjusted follicular diameter of vitellogenic females (filled triangles) and frequency of gravid females (histogram) against DATE (number of days after September 21).

Gravid females (n=21) were captured from late spring (December) to late summer (March, Figs. 4 and 5). The four females caught in spring contained in the uterus ovulated oocites only, with no embryonic development apparent. Gravid females caught in summer (January to March) all contained advanced embryos. Litter size (LS) ranged between three and six foetuses in 62 to 74 mm SVL females (LS_{media} = 4.08, n=12).



FIG. 5. SVL of non-vitellogenic females (small squares), vitellogenic females (hollow circles; sizes are proportional to the diameter of the largest ovarian follicles, ranging from 2.27 to 11.02 mm) and gravid females (triangles) against DATE (number of days after September 21).

DISCUSSION

The juvenile populations of *L. pictus* clearly show two cohorts. Modal grouping of juvenile by DATE or by SVL, as Christie (pers. com.) proposed, points to the unanswered question of the magnitude of juvenile growth from autumn to spring. In any case, sexual maturity would not be reached in less than one and a half years from birth. This is in agreement with observations of Sinervo & Adolph (1994) for mid-elevation *Sceloporus occidentalis* lizards from southern California (2200 m) and central Oregon (750 m).

Although brood size is associated with female body size, and a selective advantage to large female body size could be expected because of its immediate reproductive consequences (Vitt & Blackburn, 1991), we failed to record differences in SVL between male and female *L. pictus*.

Donoso-Barros (1966) noted a narrower female head in *L. pictus*. We noted a larger male head and, in addition, two to four pre-anal glands (three to four according to Cei, 1986) and a greater width at vent in males. Among the differences here observed, the last was found to be the best variable to identify the sex in the field, even before capture.

Sexual differences in head size could reflect divergence resulting from resource partitioning, or differential energy allocations for reproduction, or could be a response to selective pressures due to the social structure (Mouton & van Wyk, 1993). The observed sexual differences in the head size of *L. pictus*, however, are due to an allometric increase in male head size relative to juvenile head size. This phenomenon, differing from reports for other lizard species (Mouton & van Wyk, 1993), could be the result of sexual selection, given the sex ratio and that less than half of adult females seem to be ready to ovulate each year (see below).

Morphology and histology of male and female reproductive organs agree with the descriptions for *Iguana* (Cei, 1986) and *Liolaemus* Ramírez Pinilla, 1991, 1992b; Leyton & Valencia, 1992).

Data about the male cycle suggest a prolonged spermatogenetic period, from late spring to early autumn. An exhaustive histological study will be necessary to relate male and female cycles, particularly in relation to the possible storing of sperm in the epididymides (Pudney, 1995) or in the uterus-vagina transition (Ramírez Pinilla, 1991, 1992b).

Similar to other squamates (Schwarzkopf, 1992; Shine & Schwarzkopf, 1992; Méndez de la Cruz, Guillette & Villagran-Santa Cruz, 1993), female *L. pictus* showed positive relationships between the size and number of follicles and SVL, contrary to Leyton & Valencia (1992) who considered the follicle population to be stable in six *Liolaemus* species.

Females coming from southern localities showed a larger mean follicle size, when all females were considered in the analysis. Vitellogenic females did not show this variation. Thus, an earlier onset of ovary growth in southern populations may be indicated, rather than a different energy allocation to reproduction as can be seen in other reptilian and vertebrate groups (Iverson, Balgooyen, Byrd & Lyddan, 1993).

The statements of Callard *et al.* (1992), that hepatic vitellogenin synthesis is down-regulated by progesterone and that the corpus luteum is responsible for the prolonged pattern of progesterone secretion common to viviparous species, point out vitellogenesis and pregnancy as two phenomena mutually exclusive in viviparous squamates.

If vitellogenesis and pregnancy cannot coexist, we would expect capture dates of vitellogenic and pregnant females to show a successive pattern. The almost complete temporal overlapping found between vitellogenesis and pregnancy (Figs. 4 and 5) is inconsistent with the hypothesis of an annual reproductive cycle in *L. pictus*. Plotting data as Pearson (1954) did shows that the presence of advanced vitellogenic females in almost all the range of DATE and SVL of pregnant females (Fig. 5) discards an artefact due to dependence with SVL.

The minimum time required by *L. pictus* to complete the reproductive cycle appears to be two years, as was also observed in *Cordylus giganteus* (van Wyk, 1991). However, the criterion used by van Wyk (1991) and previously by Aldridge (1979 cited in van Wyk, 1991) to determine biennial reproduction was, as we interpreted, the presence of ca. 50% of non-reproductive adult females during the breeding season. No temporal overlapping between vitellogenesis and pregnancy was described in *C. giganteus*; most females skip a year before starting the next vitellogenic cycle, probably due to the fact that parturition take place in autumn at the time when vitellogenesis starts. Therefore, the skipping of a cycle may be related to environmentally constrained access to energy.

We postulate that L. pictus vitellogenesis begins in late spring and is continued through the summer and autumn, being completed after a period of latency or low follicle growth over winter. Ovulation and mating would happen in the next spring, when the male gonads are already active. Such a lengthening of the ovarian cycle beyond the annual cycle was also shown by Cree, Cockrem & Guillette (1992) in Sphenodon. In the female tuatara, vitellogenesis of a single clutch usually begins in the first year after nesting, but often continues for several years before ovulation occurs. As well as having a prolonged period of vitellogenesis, female tuatara have an exceptionally long period of gravidity (6-8 months) compared with other oviparous reptiles (Cree et al., 1992). In Hoplodactylus maculatus (from the Macraes-Middlemarch population) a biennial cycle is reached by means of a prolonged vitellogenesis of 10 months and a gestation of 14 months (Cree & Guillette, 1995). In this species, and in Barisia monticola (Vial & Stewart, 1985 cited in Cree & Guillette, 1995), the biennial cycles do not skip a year (Cree & Guillette, 1995). In *L. pictus* pregnancy occurs during late spring and summer. The advanced development of foetuses seen in gravid females of *L. pictus* caught in summer and the juvenile capture dates lead us to propose that parturition takes place from late summer to early autumn.

At the time of ovulation in a female group, we expect that another group of females starts a new vitellogenic cycle. However, we found many (20%) non-reproductive adult females during summer and early autumn (Fig. 5). This suggests that, after parturition, at least some females skip a year before starting the next vitellogenic cycle. These females will undergo vitellogenesis every third year.

Multiannual female reproductive cycles have also been observed in several snakes (Bonnet, Naulleau & Mauget, 1992), but the cause seems to be the time necessary to store lipids for future vitellogenesis. The evolution of viviparity in lizards has been related to low temperature conditions (Tinkle, Wilbur & Tilley, 1970; Tinkle & Gibbons, 1977; Shine, 1985, 1989), particularly in association with the time and the optimum temperature needed for development (Shine & Harlow, 1993; Shine, 1995). It seems that L. pictus responds to short and cold activity seasons in a new way, allocating vitellogenesis and development to separate years, and sometimes combining the lengthening of the ovarian cycle with the skipping of a year. The reproductive cycle of L. pictus enlarges the spectrum of reptilian responses to climate, and can be seen as a means of access to new niches, particularly in the cold highlands of southern South America.

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EFFECT OF INTRODUCED FISH ON AMPHIBIAN ASSEMBLAGES IN MOUNTAIN LAKES OF NORTHERN SPAIN

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We have analyzed the effect of introduced fish on amphibian assemblages in mountain lakes of the Cantabrian range (Asturias and León; northern Spain), by comparing amphibian species richness, abundance and diversity in lakes occupied by introduced fish and in those without fish. Amphibian species numbers were significantly lower in lakes inhabited by fish, both considering the absolute mumber and the values corrected for the effect of the main correlates of the species richness (shore extent and altitude). Amphibian abundance (all species and stages pooled) and diversity also tended to be lower in lakes inhabited by fish as compared with the fishless ones. Direct predation by fish, for which we present evidence in our studied lakes, is likely to be the main cause for the reported amphibian depletion, although recognition by adult amphibians of chemical cues from predatory fish and subsequent avoidance of habitats with fish for reproduction could also be contributory.

INTRODUCTION

The herpetological literature of the past decade provides evidence that amphibian populations are undergoing a rapid decline. Changes in environmental factors such as ultraviolet radiation or pH of breeding sites, infectious outbreaks, severe droughts and pollution, among others, were implicated as possible factors in explaining the "amphibian decline" (Barinaga, 1990; Wake, 1991; Dunson, Wyman & Corbett, 1992; Crump, Hensley & Clark, 1992; Blaustein, 1994; Márquez, Olmo & Bosch, 1995). However, there is still an open question about whether the decline actually represents a particular phenomenon in amphibians or whether it follows the general biodiversity crisis related to anthropogenic causes (Pechmann, Scott, Semlitsch, Caldwell, Vitt & Gibbons, 1991; Beebee, 1992; Pechmann & Wilbur, 1994; Travis, 1994). Further, population sizes of some amphibians are known to fluctuate dramatically, so the evaluation of population changes was difficult in most cases because of the lack of long-term census data (Pechmann et al., 1991; Crump et al., 1992; Dunson et al., 1992; Travis, 1994). Despite a number of uncertain issues, the recent spread of studies on amphibian population dynamics and conservation has provided evidence that complex life cycles and especially the vulnerability of larval stages make amphibians very sensitive to environmental stress (Sadinski & Dunson, 1992; Blaustein, 1994), so their conservation requires increased effort in studying particular populations and identifying potential threats.

Predation by introduced fish and perhaps by other aquatic vertebrates (e.g. *Rana catesbeiana*, Hayes & Jennings, 1986) has been reported as a certain cause for reduction in local amphibian populations (Macan, 1966; Honegger, 1978; Bradford, 1989; Aronsson & Stenson, 1995), even leading to virtual extinction (Bradford, 1991). This effect can be particularly risky in the case of metapopulations comprising a number of

local populations, each associated with isolated breeding ponds, as this situation makes the balance between local extinction and recolonization critical for amphibian population persistence (Bradford, 1991; Pechmann & Wilbur, 1994). Successful reproduction in large pools and lakes is likely to be a key factor, because they can provide the source stocks for recolonization (Pulliam, 1988; Travis, 1994), but such large permanent pools were frequently fish-stocked for commercial or recreational purposes, thus threatening amphibian populations on a wider scale. The purpose of this study was to elucidate whether fish introductions affected amphibian species richness and abundance in a chain of permanent mountain lakes lying within a 150 km west to east strip in the Cantabrian Mountains (northern Spain).

METHODS

We selected 16 large and permanent pools or lakes (listed in Table 1), ranging from 1070 to 1750 m in elevation, 200 to 1850 m in perimeter, and 2.0 to 50.3 m in maximum depth. The occurrence of fish was determined by visual observation of individual specimens or signals, such as waves of surface feeding. Electrofishing from the lake shore and immediately adjacent reaches of tributaries (for lakes 1, 2, 7, 8, 9, as numbered in Table 1), catches with gillnets (7, 8, 9), and examination of fish caught by fishermen (2, 5, 6, 8, 9) allowed species identification. Additional information was provided by the freshwater fisheries authorities of Asturias and León. Fish presence was determined in nine out of 16 lakes (species in Table 1), mainly originating from repeated stocking since the earliest decades of the present century (Terrero, 1951). Lake Ercina was categorized as having fish because of reports of repeated stocking with several species, mainly rainbow trout (Muñoz, 1967). As this lake had a

TABLE 1. Occurence of fish and amphibians in lakes of the Cantabrian Mountains range (northern Spain). Numbers in parentheses indicate species location according to the following lake identifications: 1- Ercina, 2- Enol, 3- Lillo, 4- Isoba, 5- Embalse de Valle, 6- Lago del Valle, 7- Cerveriz, 8- La Cuerva, 9- Calabazosa, 10- La Mesa, 11- Arbás, 12- Muniellos (Isla), 13- Muniellos (Candanosa), 14- Ubales, 15- Ausente, 16- Bueno. Asterisks in fish list indicate possible native populations.

Fish species	Amphibian species
(lake number)	(lake number: water)/(surrounding land)
Salmo trutta	Chioglossa lusitanica
(2,3*, 4,5*, 6,8,9)	(13)/
Salvelinus fontinalis	Salamandra salamandra
(9)	/(6)
Oncorhynchus mykiss	Triturus alpestris
(1?,3)	(1,8,10,13,14,16)/(6,7,8,9)
Phoxinus phoxinus	Triturus boscai
(2,6,7,8,9)	(12,13)/
Tinca tinca	Triturus helveticus
(2)	(1,11,12,13,14,15,16)/(2,4,8,9)
Rutilus arcasii	Triturus marmoratus
(4)	(11)/
	Alytes obstetricans (1,11,15,16)/(1,2,5,6,9,10)
	Bufo bufo (1,10,13,14,16)/(1,5,6,7,8,9,10,11,16)
	Rana iberica /(1,7)
	Rana perezi (4)/(3)
	Rana temporaria (6,7,8,9,10,11,12,14,15,16)/ (4,5,6,7,8,9,10,11,12,16)

comparatively rich amphibian fauna, we have maintained the classification, although its status is uncertain at the present time, to make the tests conservative with respect to acceptance of differences in amphibian presence and abundance between lakes with fish and without fish.

The lakes shared the potential amphibian fauna (listed in Table 1), and all the samples were taken in a two week period in early July 1995, to avoid excessive among-lakes heterogeneity in the phases of the reproductive cycle. The presence of amphibians was assessed by searching the shoreline for larvae and metamorphosed individuals, surveys were extended into the surrounding land up to 500 m from the shoreline. In addition, four to six representative sampling points were chosen in each lake to determine and count larval amphibians, and so obtain figures of abundance

and diversity. Abundance relative to shore length was log (x+1) transformed to achieve normality (Kolmogorov-Smirnov, P < 0.05) and to avoid the log 0 indetermination. Diversity of larvae was computed by Simpson's index $(D=1/p_i^2)$; see Pianka, 1973).

RESULTS

Amphibian species richness, including larval and adult stages sampled in the water, was significantly lower in lakes inhabited by fish, both considering absolute species numbers (Fig. 1; range, mean species number, SD, for lakes without fish: 3-5, 4.00, 0.82, n=7; for lakes with fish: 0-4, 1.22, 1.30, n=9; t=4.92, P<0.001). Values were corrected for differences in shore extent (ANCOVA, with lake perimeter as the covariate; $F_{1,15}=26.57$, P<0.001), or altitude ($F_{t,15}=16.44$, P=0.001), the main correlates of amphibian species number. Differences in the numbers of amphibian species within the 500 m surrounding the lakes, although showing the same trend, were not statistically significant (range and mean and SD, for lakes without fish: 3-5, 4.29 0.76; for lakes with fish: 1-6, 3.67 1.58; t=0.95, P=0.358).

Lakes with fish tended to exhibit lower amphibian abundance (all species and stages pooled) than the fishless ones, but this difference was at the limit of statistical significance (t=2.13, P=0.051). Across lakes, amphibian abundance was significantly correlated with the extent of the shallow platform (less than 0.5 m in depth; r=0.659, P=0.006; see Fig. 2). Thus, we used this covariate to further examine the effect of fish presence on amphibian abundance. This resulted in a slight reduction of the effect of fish presence ($F_{1.15}=3.92$,



FIG. 1. Frequency distributions of amphibian species number (all stages considered) within the water for lakes with fish and without fish. The asterisk indicates the position of lake Ercina, whose classification was considered uncertain (see text).



FIG. 2. Plot of the ratio $[\log (y+1) \text{ transformed}]$ between amphibian abundance and lake perimeter versus the mean width of the shallow platform (less than 0.5 m in depth), for lakes without (empty squares) and with (black squares) fish.

P=0.069). Amphibian species diversity was not correlated with other habitat features (altitude, lake perimeter, depth, extent of the platform), but again tended to exhibit higher mean values for lakes without fish (1.40± 0.53 vs. 0.69± 0.76; t=2.06, P=0.059).

DISCUSSION

Choice of oviposition sites by breeding adults, development of chemical defences, rapid growth rates, and behavioural modifications by larval stages to reduce detection risk are common responses of amphibian populations evolved under intense predatory pressure (Petranka, Kats & Sih, 1987; Lawler, 1989; Resetaris & Wilbur, 1989; Holomuzki, 1995; Manteifel, 1995). Although subjected to modification by experience, such anti-predator behaviours have been shown to be inherited in some amphibian species (Semlitsch & Reyer, 1992). However, most amphibian species in our study area have evolved under conditions of limited fish predation. Mature individuals are aquatic only during the reproductive period, juveniles are mainly terrestrial, and egg and larval stages develop in small temporary ponds or isolated lakes of glacial origin not containing fish. Only two species (Triturus boscai and Rana iberica) reproduce regularly, in running waters inhabited by predatory fish, and frequently in small first-order reaches where juvenile brown trout are the only fish present. Therefore, specific avoidance, defence or escape mechanisms towards introduced predators (or towards high general levels of predation) are unlikely to be developed, although some generic predator recognition and avoidance behaviours can occur when amphibians are faced with unknown potential predators (Kats, Petranka & Sih, 1988; Manteifel, 1995; but see Aronsson & Stenson, 1995). In any case, the strong effect of introduced fish on amphibians in our study agrees with that expected in systems where prey and predators had no common evolutionary history (Thorp, 1986; Townsend & Crowl, 1991).

The scarcity of amphibians in habitats with predatory fish can be the result of direct predation on various stages of development, as repeatedly reported for both anurans and salamanders (e.g., Aronsson & Stenson, 1995; Manteifel, 1995; Resetaris, 1995). Analyses of stomach contents of fish from the Calabazosa lake (No. 9 in Table 1) sampled at the time of the amphibian survey provided evidence of amphibian consumption for the two most abundant and ubiquitous fish species in the study area. Despite the extreme scarcity of amphibians in this lake, two out of 12 brown trout examined contained identifiable remains of one larval salamander and three larval anurans, respectively; one out of 65 Phoxinus phoxinus did so; no amphibian remains were found in stomachs of 14 adult Salvelinus fontinalis (A. F. Ojanguren, pers. com.). Further evidence of fish predation on newts (Triturus sp.) and even the detection of a sudden decline of amphibians during the few years following the earliest salmonid introductions (by 1880) was reported for lake Enol (No. 2 in Table 1; Terrero, 1951). However, chemical-mediated recognition and subsequent avoidance by adult amphibians of habitats with fish for reproduction has been demonstrated for several amphibian species (e.g., Resetaris & Wilbur, 1989; Kats & Sih, 1992; Holomuzki, 1995) and could also be contributory to the reported impoverishment. In any case, the amphibian shortage relates to fish presence, and the final outcome is the almost complete disablement of large permanent pools or lakes for amphibian reproduction. Small temporary or unstable breeding-ponds are likely to exhibit high among-years variability and eventual failure in amphibian recruitment and so could be "sink habitats" (Pulliam, 1988), requiring regular immigration from more productive (or more constant in production) neighbouring source stocks. On the contrary, large pools or lakes in our study area are more stable over time and have large peripheral shallow areas suitable for amphibian reproduction (lake perimeter and extent of the shallow platform were positively correlated with species number and abundance), representing potential source stocks for colonization, and so being critical for population persistence.

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We are indebted to B. Elvira, F. G. Reyes-Gavilán and A. F. Ojanguren for help in fish sampling. V. Vázquez provided us with information about fishing in several lakes. This study was supported by grants from the Consejería de Medio Ambiente y Urbanismo (Principado de Asturias).

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BOOK REVIEWS

Ecology and Conservation of the European Tree Frog. Proceedings of the 1st International W 3.orkshop on Hyla arborea, 13-14 February 1992, Potsdam, Germany. A. H. P. Stumpel and Uwe Tester (eds) (1993) 105 pp. DLO-Institute for Forestry and Nature Research, Wageningen, The Netherlands. Hfl. 35.00 (Paper).

Der Laubfrosch. Wolf R. Grosse (1994) 211 pp. Die Neue Brehm-Bücherei Bd. 615. Westarp Wissenschaften, Magdeburg, Germany. DM 44.00. (Paper).

When some hundred years ago Wolterstorff declared the common treefrog, *Hyla arborea*, a species so abundant 'that it is not worthy of special attention', clearly he did not envisage today's dramatic decline of the species and the special measures that have to be taken in several countries to keep it extant.

Ecology and Conservation of the European Tree Frog holds the proceedings of a workshop held in Potsdam, Germany, in 1992 and is edited by Ton Stumpel and Uwe Tester. It contains ten chapters covering many aspects of the biology of Hyla arborea. The book opens with a chapter by U. Tester on methods and definitions in treefrog research. The definitions are clearly presented, but the list is far from complete and does not cover all the concepts referred to in the chapters following. The section on methods is incomplete to the extent of being unhelpful. For example, the individual marking of tadpoles by tail fin clipping or by their immersion in a dye solution is mentioned as a means for estimating tadpole density, but the principle on which such an estimate would be based, namely capture-recapture, is not referred to. Moreover, from my own experience I can ascertain that tadpoles of Hyla arborea are too delicate to stand such treatments with no ill-effects, and some larvae, especially the smaller ones, will not survive the neutral red dye, which is a toxic chemical.

The second chapter by H. Schneider presents an authoritative account on treefrog bioacoustics, including a phenetic classification of the European species and subspecies on the basis of mating call parameters. The next four chapters summarise data on behaviour, development and growth and characteristics of the aquatic and terrestrial habitat. In a brief account P. Edenhamn summarises general metapopulation theory and highlights some aspects that are relevant to amphibians. However, a crucial issue - how to recognize an 'empty patch' (i.e. a potential reproduction site where the species does not occur for reasons other than habitat unsuitability) and how such an empty patch might function in a treefrog metapopulation - is not addressed. In the concluding chapter E. Balletto and C. Giacoma present an attempt to model common treefrog population persistence. It is shown that under a range of population parameter values (including population size, survival rate, dispersal rate) populations and simple metapopulations are likely to go extinct within the decade. Would it be possible that such an anomalous result reflects the properties of the modelling software, rather than the biology of the frog? Vortex (Lacy, 1991) is designed for the individual-based modelling of populations composed of families with small offspring numbers such as found in mammals and birds, whereas amphibians are mostly characterised by large clutch sizes. Additionally, the modelling of genetic heterozygosity of treefrog metapopulations seems rather pointless where real genetic data to compare the results with appear to be unavailable.

The remaining two chapters deal directly with conservation issues. A. Borgula presents a detailed and insightful qualitative account to the threats and causes of decline in Hyla arborea. The highlight of the book is the chapter by K. Fog. It presents a wealth of data on migratory movements in the common treefrog. Such data are highly relevant for conservation and they show that Hyla arborea may well be an almost ideal organism for metapopulation research. The book concludes with a literature list for further documentation. It lists several unpublished theses from various universities and research institutes that might otherwise go unnoticed. Important omissions are the excellent historical account of common treefrog distribution and conservation status in the Netherlands by Bergmans & Zuiderwijk (1986), references to the observed levels of genetic heterozygosity in closely related treefrog species (Nevo & Yang, 1979; Nishioka et al., 1990) and the demonstration that genetic heterozygosity and population survival in treefrogs (Hyla cinerea) may be linked (McAlpine, 1993).

This proceedings volume would have benefited from tighter editing. Its main value is probably in the new data presented in some of the chapters and in showing us the direction treefrog research is taking. Surprisingly, almost all participants to the workshop come from north-western Europe where the common treefrog has become a rare and often endangered asset. The design and implementation of conservation measurements for the common treefrog might benefit more from research in countries where the species is still widespread and abundant than from research in areas where it has almost gone. It would be unfortunate if Wolterstorff's unjust attitude would prevail for much longer.

The book by Wolf Grosse with the simple title *The Treefrog* deals with the down-to-earth natural history of *Hyla arborea*. It opens with an overview of evolutionary descent of amphibians, frogs and treefrogs, the distribution of treefrog species and subspecies in the Palearctic region and with some examples of the local distribution of the common treefrog in central Europe. The next two chapters describe its morphology and anatomy. Two long chapters follow on 'Ecology and behaviour' and on 'Mating, reproduction and development'. The final chapter is on 'Threats and conservation'. Grosse's monograph of the Common treefrog is full of detail, yet to the point - a delightful book worth every *pfennig* of it.

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Fossil Reptiles of Great Britain. M. J. Benton and P. S. Spencer. (1995) 386 pp. Geological Conservation Review Series 10. Chapman and Hall. (Cloth).

This is an extremely valuable reference book for any palaeontologist concerned with the fossil reptiles and amphibians of the British Isles and has a lot to offer anyone with an interest in the history of reptiles (and some amphibians) in Britain as actually documented. It is one of an extended series of 50 volumes being published by the Earth Science Branch of the Joint Nature Conservation Committee with the aim of documenting significant surviving geological and palaeontological localities for designation as SSSI's. The conservation of sites which produce dead animals rather than living ones may seem an unusual perspective for many readers of this journal but as palaeontologists devise new techniques for collecting and analyzing fossils and the sediments in which they occur, it is invaluable to be able to return to the original fossil-producing locality with the confidence that it is the right locality and that it is safe from gratuitous destruction. Designating a site as a geological SSSI is, of course no guarantee of its safety any more than it is for biological SSSI's. Many fossil-producing sites are in defunct quarries which local authorities would like to use as landfill sites for waste disposal, while others are in low coastal cliffs which those who live above, would like to see concreted over so that their houses do not tumble into the sea.

Benton and Spencer not only document in detail the fifty surviving localities which most merit SSSI status but survey their fossil faunas and the history of study. They also document over five hundred other sites, both extant and lost, which have produced fossil reptiles, and the resultant compendium is the most complete ever produced of reptile-bearing localities from the Carboniferous up to the Pleistocene. In relation to its land area, Britain is unusually rich in the diversity of its rocks, resulting from a long history of folding and erosion. The fossil record is also rich because of the relatively long period of collection and study of fossils in western Europe giving us up to a centuries start over much of the rest of the world.

In this book you can find accounts of Slickstones Quarry, Avon, which has produced Triassic sphenodontids (among the earliest Tuataras), Kittlington Quarry, Oxfordshire (Middle Jurassic), which has produced some of the earliest true lizards and salamanders in the world; and the earliest frogs in Europe; Encombe Bay, Dorset (Upper Jurassic) which has produced one of the earliest aquatic turtles in the world; and Hordle Cliff, Hampshire (Upper-Eocene) with its immense range of frogs, salamanders, lizards, snakes and even an amphisbaenian. There are also accounts of sites which have produced dinosaurs, pterosaurs, plesiosaurs and ichthyosaurs for those who like that sort of thing. The museum locations of fossils are given, selected localities are illustrated with photographs and maps and some fossils and reconstructed skeletons are figured. There are no descriptive accounts of the animals, the book being primarily a directory, but there is a detailed literature documentation and the reference list alone is an immensely valuable resource, listing over 1,100 papers on British fossil reptiles and their localities. In summary, essential for the palaeontologist's bookshelf, one for the library for non-palaeontologists.

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Die Amphibien Russlands und angrenzender Gebiete. Sergius L. Kuzmin (1995) 274 pp. Die Neue Brehm-Bücherei; Bd. 627. Westarp-Wissenschaften., Magdeburg, Germany (Paper).

This book has been published in the popular German Neue Brehm Bücherei, a series in which many titles on amphibians and reptiles have appeared. The present monograph describes 'the amphibians of Russia and neighbouring areas', a title which sounds less revisionist-like than 'the former Soviet Union.' But this is the area covered by the book. With a very few exceptions, the distribution maps and the quoted literature only cover the geographical area east of the western boundary of the former Soviet empire. This is a reasonable restriction, but the disadvantage is that important data for species which have their main distributions in more westerly regions are missing.

Forty-one species of amphibians are presented, about half of which do not also occur in central or western Europe. The book is state-of-the-art on the basis of cited publications and PhD dissertations written in languages which are not accessible to most western biologists, such as Russian, Georgian and Azerbaidjani.

Following introductory chapters on geography, climate, the history of herpetology in Russia, and on general amphibian biology, the author devotes a substantial chapter to the problems of endangered species, impoverishment of habitat, population declines and to conservation. The book continues with keys for identification of eggs, larvae and adults. The main part of the book is taken up by the species descriptions. Every chapter contains a wealth of detailed information on distribution, ecology, reproduction, trophology, predators and present status. A useful register of geographical entities, in Russian, German and English concludes the volume. Every species is illustrated by colour and black-and-white photographs; eggs and larvae are rendered in pen-drawings.

When compared to the high standards set by a number of other regional herpetofaunas in the west, there is inevitably room for some criticism on this volume, which may be considered in case a second edition is envisaged:

The distribution maps, although without doubt wellresearched, show less detail than those published in Bannikov *et al.* (1977) which was the Russian standard work of reference for the area until now. The maps would have been more informative if the new distribution data collected since 1977 were plotted onto Bannikov's maps.

The drawings of the caudate larvae are generally good; among the anuran larvae those of the *Bombina* species show a misplaced, lateral spiraculum.

Another criticism concerns the degree of precision in the information on reproductive behaviour. Whereas the author gives very detailed information on amphibian feeding habits and food composition, a subject on which he has worked extensively himself, he occasionally gives too little information on reproduction modes. In his treatment of Ranodon sibiricus, for instance, the author tries to reconcile two different observations of what appear to be mutually exclusive reproduction modes: fertilisation by transfer of a spermatophore and external fertilisation such as is practiced by all hynobiids, namely by shedding milt over the egg-sacs. As I have argued in my review of the author's other recent book on Asian Clawed Salamanders, the latter mode of fertilisation is more likely to be correct than the first (Thorn, 1986) and the observation of a spermatophore, which is going back to one Russian record in 1958, may be a mistake. For Onychodactylus fischeri, the author claims that fertilisation takes place by means of a spermatophore (following Serbinova & Solkin, 1992). According to the same source, however, the male of

this species manoeuvers the egg-sacs with his hind-legs in a way similar to the fertilisation behaviour in other hynobiids. For this and other reasons, I am inclined to believe that the reference to a spermatophore here is based on inaccurate observation or misunderstanding of what a spermatophore is, namely a sperm transport aid consisting of a gelatinous base holding the spermcap. A spermatophore has thus far only been found with certainty in species which practice internal fertilisation (Sparreboom, in press). There exists conflicting evidence for the fertilisation mode in Salamandrella keyserlingii. According to some recent work (Savelev, Kuranova & Besova, 1991) this hynobiid species practices internal instead of external fertilisation as was always assumed. One of several points that remain unclear from that publication, is how the sperm is transferred to the female, but in the meantime, this view has already found its way in a fieldguide and reference book on the amphibians of Europe (Nöllert & Nöllert, 1992). Kuzmin makes no reference to the work of Savelev et al. (1991). I therefore assume that by ignoring the paper he implicitly rejects its conclusions. But a discussion of the matter would have been more informative. Clearly there is a need for a critical appraisal of hynobiid fertilisation modes.

For *Mertensiella caucasica*, it is stated that the caudal spike on the male's tail-base has no function during reproduction. Although the author exceptionally quotes a non-Russian publication here (Schultschik, 1994), he apparently does not integrate Schultschik's results which show clearly that at a certain stage of the courtship the tail-spike actually enters the female cloaca during amplexus. In this way the male stimulates the female or manoeuvers her into a position to pick up the spermatophore. For *Rana arvalis* it is stated that 'pairs are mating up incidentally', which is probably an inaccurate or over-simplified description of the actual process.

References are given at the end of each chapter. This may on one hand facilitate a cursory reading of the text, but has the disadvantage that valuable information is lost. The specialist reader will have difficulty in tracing specific pieces of information for which he needs the original source.

Despite these criticisms, we must be grateful to the author for making available to us a great amount of hitherto inaccessible ecological work that no doubt will be new to many researchers. It is understandable that this is done in the most widely spoken west-European language. But the book was originally written in English and there is no Russian version. I wonder if the Russian colleagues and the non-German-speaking part of the western market might not have preferred publication in English.

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Turtles of the United States and Canada. Ernst, E. H., Lovich, J. E., and Barbour, R. W. (1994). 578 pp. Smithsonian Institution Press, Washington, D.C. (Cloth).

Certain books seem to have a life of their own. On my "books about turtles" shelf sits a worn copy of the *defacto* handbook of turtles, terrapins and tortoises of North America. Published in1972, *Turtles of the United States* by Carl Ernst and Roger Barbour presented 49 species accounts and a wealth of information on those most fascinating of vertebrates. Much more than a field guide, *Turtles of the United States* was a thorough and rigorous review of the state of biological knowledge of American turtles.

Ernst and Barbour (1972) was the perfect introduction to the study of turtles for a young herpetologist. I picked up my copy at a herpetological meeting in Florida in 1985, the first such meeting I attended. Having spent a couple of years learning the ropes of "turtlology", Ernst and Barbour was indispensable from the moment I went to the library to find out the scientific name of the common snapping turtle. The format was well thought out, and well illustrated - a veritable "Encyclopedia Turtlogica".

The revision of *Turtles of the United States* has been 22 years in coming - perhaps not a complete generation of most of the species described - and makes some substantial improvements on the original. Inclusion of 57 colour plates certainly makes the volume more lively. Gone are the sections on care of living turtles: there are now plenty of sources for that. The 56 species accounts

of *Turtles of the United States and Canada* retain the organized format of the original, and their increased detail reflect the vigour of the field twenty years on. A detailed glossary and extensive bibliography help fill out the 578 pages - 231 pages more than the original.

The contributions of Jeff Lovich as second author on the revision are reflected throughout the text (the book is dedicated to co-author Roger Barbour, who sadly died in 1993 as the manuscript was being completed). Lovich is a productive and eloquent scientist who has had a significant effect on North American herpetology during the past decade.

Like the original, Turtles of the United States and Canada is a gold mine of information for anyone interested in the biology of turtles. Each species account begins with a section on recognizing the species, and then presents what is known about its karyotype, fossil record, distribution, and geographic variation. Further sections detail behavioural ecology and autecology: behaviour, habitat use, reproductive biology, demography, food habits, and predators. Finally, information on the status of specific populations (especially for endangered species) and remarks round out each account. Each species account is rounded out by photographs and a range map. When considered with the dichotomous key and an introduction that encapsulates the evolutionary history of turtles, the principle points of their morphology and distribution, and the state of concern over their conservation, this volume is indeed encyclopedic. As J. Whitfield Gibbons is quoted on the dust jacket, "This will be a major contribution as a reference book for wildlife biologists and naturalists."

This certainly is an important reference work, but I think it has a significance that goes beyond its use as a "turtle database". The careful assembly and review of our knowledge that Ernst, Lovich and Barbour present reveals that the knowledge base is far from complete. Significant gaps in our basic empirical understanding exist for some of the better-known species. Filling those gaps is important for the present generation of turtle biologists.

During that first herp meeting I attended in 1985 I happened to meet Carl Ernst and asked him to autograph my copy of *Turtles of the United States*. He wrote "Never lose your interest in turtles!" in that used book, and I certainly haven't. I hope that future herpetologists will find *Turtles of the United States and Canada* as useful and inspirational as the original. This book belongs on the shelves, and in the hands, of everyone interested in turtles.

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THE HERPETOLOGICAL JOURNAL

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(revised January 1992)

- 1. The *Herpetological Journal* publishes a range of features concerned with reptile and amphibian biology. These include: full papers (no length limit); reviews and mini-reviews (generally solicited by a member of the editorial board); short notes; controversies, under 'Forum' (details available from the Editor); and book reviews. Faunistic lists, letters and results of general surveys are not published unless they shed light on herpetological problems of wider significance.
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- 9. Final acceptance of a paper will depend upon the production by the author of a typescript and illustrations ready for the press. However, every assistance will be given to amateur herpetologists to prepare papers for publication.
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