HERPETOLOGICAL JOURNAL, Vol. 15, pp. 273-278 (2005)

THE REPRODUCTIVE BIOLOGY OF *SALAMANDRINA TERDIGITATA* (CAUDATA, SALAMANDRIDAE)*

FRANCESCA DELLA ROCCA, LEONARDOVIGNOLI AND MARCO A. BOLOGNA

Dipartimento di Biologia, Università "Roma Tre", Roma, Italy

We studied the reproductive biology of the spectacled salamander, *Salamandrina terdigidata,* in Central Italy by daily sampling over three breeding seasons. Reproduction takes place annually, between February and May. Clutch size varies (1-65), and are usually placed by females on the underside of stones. The total number of eggs deposited varies yearly and is positively related to the period of preceding rainfall. Large females start breeding earlier and show stronger oviposition site fidelity than small ones. For oviposition females choose the portions of the stream that have the highest density of stones. The speed at which embryos develop increased over time, presumably under the influence of rising water temperature. Hatching success is negatively affected by aquatic drift, desiccation, predation by Trichoptera larvae and the absence of hiding sites.

Key words: egg production, Italy, microhabitat selection, salamander

INTRODUCTION

The main limitation of studies on the reproductive biology of species characterised by short individual reproductive periods, is the lack of data due to low sampling frequency. The spectacled salamander, *Salamandrina terdigitata* (Lacépède, 1788), is an elusive species, in which the reproductive biology is rarely studied. Each female has a brief aquatic phase, limited to oviposition activity.

Salamandrina terdigitata belongs to a monotypic genus that is endemic to the Italian peninsula (Lanza, 1988), representing one of the oldest branches within the Salamandridae, with no close Palaearctic relatives (Titus & Larson, 1995). The range of the species extends from central Liguria (Genoa Province) to southern Calabria (Reggio Calabria Province), primarily along the Tyrrhenian slope of the Italian peninsula, with most occurrences at 200-700 m a.s.l. (Societas Herpetologica Italica, 1996; Vanni & Nistri, 1997; Zuffi, 1999; Corsetti & Angelini, 2000). It is considered threatened by the EU Habitats Directive and the Berne Convention. *Salamandrina terdigitata* is a largely terrestrial species adapted to submesic or mesic forests. It can also be found in mixed landscapes composed of pastures and residual woodlands or other derived habitats. For oviposition *S. terdigitata* uses well oxygenated streams with low or laminar flow, stony bottoms and dense riparian vegetation, even if small artificial water basins are utilised (Vanni, 1980; Lanza, 1983; Corsetti, 1999*a*; Barbieri, 2001; pers. obs.).

The aim of the present research is to study several aspects of the reproductive biology of this species in depth, such as microhabitat selection and oviposition phenology in relation to habitat parameters, the number of eggs deposited, the reproductive success and development rate.

Correspondence: M. A. Bologna, Dipartimento di Biologia, Università Roma Tre, Viale Marconi, 446, I-00146 Roma, Italy. *E-mail* : bologna@bio.uniroma3.it

To date only scarce information on this topic, based on occasional records is available and there have been few attempts to investigate the reproductive behaviour and phenology of this species (Ramorino, 1863; Strötgen, 1927; Naviglio, 1971; Barbieri & Tiso, 1993; Corsetti, 1999*a,b;* Angelini *et al.*, 2001; Vignoli *et al.*, 2001*a).*

MATERIAL AND METHODS

STUDY AREA

The population of this study is situated in a natural area inside Rome, in the Insugherata Natural Reserve, at 80 m a.s.l. It is restricted to a very small valley of about three hectares in surface area, with mesic woodland dominated by *Castanea sativa* and *Ostrya carpinifolia*, along a short perennial and sub-rectilinear stream, tributary of the Fosso dell'Acqua Traversa, which flows into the Tiber River. The Reserve, which covers an area of about 700 hectares, is a mosaic of weed cultivations, pastures, thermophilic woodlands, and small marshes with riparian hygrophilic woodlands.

SAMPLING METHODS AND STATISTICAL ANALYSIS

The study period extended from February 1999 and April 2002. Surveys were carried out exclusively within the oviposition site, which is represented by a portion of a stream, about 110 m in length, from the source to a small waterfall (3 m in height). Surveys were extended to the entire stream (about 400 m in length) and to other streams of the Reserve, but no salamanders were found. Although the sexes are indistinguishable morphologically (Brizzi *et al.*, 1989), only females are aquatic during the short oviposition phase (Lanza, 1983), and consequently, all specimens found in the water were considered to be females.

Sampling was carried out every day in spring, less frequently in autumn and winter, as well as in June.

The sampling period was considered to range from the first to the last oviposition found. To define the oviposition period, the study area was checked every year from February to mid-May. Sampling carried out in 1999 was restricted to the identification of each specimen using ventral photography of egg-laying salamanders. The research in 2002 stopped before the end of the oviposition activity, hence we used this data set only for some aims (microhabitat selection, distribution along the stream, correlations between biometry and reproductive biology).

Each substrate available for oviposition was numbered and its position was recorded at every sampling. The oviposition site was divided into 11 sections (A-M), each 10 m in length, starting from the source. Every season, the map was modified according to any morphological variations in the stream, due to rain, flow and erosion. Sections of the stream were divided into two main groups based on topography. The first area, including sections A and B, is characterised by high, sloping (about 70°-90°) banks, mostly shady because of the overhanging vegetation. The second area (sections C-M) has slightly sloping banks, with scattered overhanging vegetation, and it is more exposed to the sun. Multi Response Permutation Procedure test (MRPP), applied to the female size (mouth-cloaca length: MCL), showed a lower intra-group variance (A-B and C-M) than among sections ($P<0.05$), supporting the division of the stream in two homogeneous areas.

Each sampling in the 2000-2002 period included both physical and biological surveys. We recorded the water temperature using a multiparametric probe. We tested the relationship between the number of available substrates for oviposition and the presence of salamanders among the stream sections in which we found specimens. Each captured specimen was recorded by a photograph of the ventral pattern and a photographic database was prepared. Using an electronic calliper (resolution 0.01 mm) we measured the body length, from apex of head to middle of cloaca (MCL) of each specmen. We recorded body measurements when spectacled salamanders were first captured, and repeated the measurements every year, at each first recapture. An analysis of female fidelity to the oviposition site was carried out on individuals recaptured at least once during the study period: consequently we considered only females captured in 2000 and then recaptured in 2001. The daily sampling allowed us to assess in several cases the egg number in a single egg mass laid by a female. The daily variation in the number of eggs and masses in the oviposition area depended on three main factors: egg deposition rate, embryonic mortality and hatching. We also evaluated the embryonic survival rate and number of eggs hatched. Lastly, we recorded the number and phenology of larvae during the sampling period.

Statistical analysis was performed using the package STATISTICA 5.1 G (1997, Statsoft) and the Microsoft Excel 2000 programme. Descriptive statistics, both parametric (*t*-test to compare the size of females) and non-parametric (Mann-Whitney *U*-test, to compare the presence of females and stones in the stream; Chi-square test to examine the female fidelity to the stream sections; Spearman correlation to test if females enter the water in the same sequence each year), and linear regression (to relate the development rate to water temperature), were utilised to evaluate results.

RESULTS

REPRODUCTIVE PHENOLOGY

During the sampling period we recorded 165 female spectacled salamanders: 36 in 1999, 60 in 2000, 57 in 2001, 12 in 2002. The population had a single spring reproductive period, extending from February to mid May, with great variation between years. The oviposition period extended from 20 March to 26 April in 1999; from 1 March to 6 May in 2000; from 13 February to 13 May in 2001; and from 9 March to 18 April in 2002.

Females usually enter the water only once to lay eggs during one reproductive season. Only 13 specimens came back to the stream after their first visit within the same year: 6 out of 74 (8.1%) in 2000; 6 out of 99 (6.1 %) in 2001; 1 on 31 (3.2%) in 2002. Females remain in the water for between one and eight days (2000: mean =2.85; n=74; SD=1.91; 2001: mean=2.47; n=98; SD=1.73).

An analysis based on 32 individuals captured in 2000 and recaptured in 2001 highlighted a significant correlation between years for individual time of entrance at the oviposition site (n=32; r=0.66; $P<0.001$; Spearman correlation; Fig. 1).

MICROHABITAT SELECTION

The number of specimens recorded from 2000 to 2002 in each section (A-M) of the stream is reported in Table 1. The 2000 oviposition area was restricted to the first two sections, A-B, particularly in B. In 2001 the oviposition area was greatly expanded and sections A-C, E-H and M were colonised; sections A and B (particularly A) were utilised more than others. In 2002 the number of ovipositions greatly decreased because of

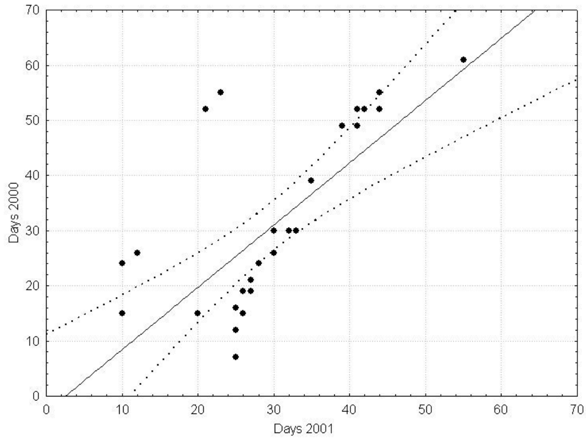

FIG. 1. Spearman correlation of the order of entrance in water of 32 specimens captured in 2000 and recaptured in 2001. Circles represent individuals. For both years the first day of oviposition was indicated as day 1.

TABLE 1. Distribution of specimens and available stones for oviposition in each stream section (2000-2002). Some specimens (8 in 2000 and 11 in 2001) visited both sections A and B during the same reproductive period; hence the sum of the respective columns exceeds the real number of the observed specimens.

Stream sections	2000		2001		2002	
	Specimens	Stones	Specimens	Stones	Specimens	Stones
A	32	24	54	20	16	16
B	50	34	45	21	6	9
C		5	1	1		-
D		-		-		2
E		10	2	14		10
F		7	4	5		3
G		4	1	4	9	3
H		6	2	5		4
I		-		-		-
L		-		-		-
M		2	1	1		-

a reduced water flow, which uniformly affected the entire stream; in this year only sections A-B and G, where submerged objects for oviposition remained available, were utilised.

The 29 females captured in 2000 and recaptured in 2001 tended to lay eggs in the same section (A and B) of the stream as in the previous year (n=29; df=1; χ^2=7.60; P<0.01). The females ovipositing in the two areas differed in size (Table 2): females that oviposited in sections C-M were smaller than those using sections A-B, both in 2001.

Three different substrates were available to oviposit in the stream: stones, leaves or other plant parts, and artificial (plastic or metallic) supports. Water depth was not a significant factor for oviposition: females laid eggs from the water surface to the maximum depth observed in the stream. The salamanders more frequently used stones than other objects for oviposition (Table 3). In 2002 a greater variability in substrate selection was observed, probably due to the reduction of available stones, because of the winter drift. In that year, the salamanders also used some tracts of the stream without stones for oviposition. These contained numerous leaves or tree branches, amassed in small bends, at least 10 cm in depth.

Females oviposited on the underside of stones of variable size, but with a lower surface area of 90-540 cm^2. The number and distribution of stones varied both during the study period and within the same season, because of stream drift due to the intensity of rainfall. There is a significant relationship between the presence of salamanders and stone availability (Table 1): based on data of 2000 and 2001, the ratio between the number of specimens and available stones in the first (A-B sections) and second (C-M sections) areas of the stream 1.91 and 0.6m respectively, differ significantly (Mann-Whitney U-test: $n_{\text{A-B}}$=4, $n_{\text{C-M}}$=6; U=0; P<0.05).

The females laid eggs by sticking them to the underside of the stones or, rarely, on other objects (leaves, tree branches, artificial supports). The percentage of available stones utilised varied between years, from 16 out of 47 (34%) in 2002 to 47 out of 71 (66%) in 2001.

Number of Eggs

Eggs were usually deposited very close to each other, forming an egg mass of variable size (Fig. 2). Each female laid eggs in separate masses under a single or several substrates, as noted by direct observation. The number of eggs oviposited over a single reproductive season varied each year and within the two areas (A-B

TABLE 2. Difference in size (snout-vent length, SVL) between specimens ovipositing within tract A-B (78 in 2001, 21 in 2002) and those ovipositing within tract C-M (13 in 2001, 7 in 2002) (2001-2002).

	Mean $_{\text{A-B}}$	Mean $_{\text{C-M}}$	t	df	P	SD$_{\text{A-B}}$	SD$_{\text{C-M}}$
SVL 2001	3.77	3.44	3.52	89	0.00069	0.32	0.29
SVL 2002	3.76	3.47	2.97	26	0.0063	0.21	0.25

TABLE 3. Number of specimens found under each substrate (2000-2002).

Substrate	No. of specimens (2000)	No. of specimens (2001)	No. of specimens (2002)
Stones	66 (89.19%)	93 (93.94%)	22 (70.97%)
Leaves	8 (10.81%)	5 (5.05%)	7 (22.58%)
Others	-	1 (1.01%)	2 (6.45%)

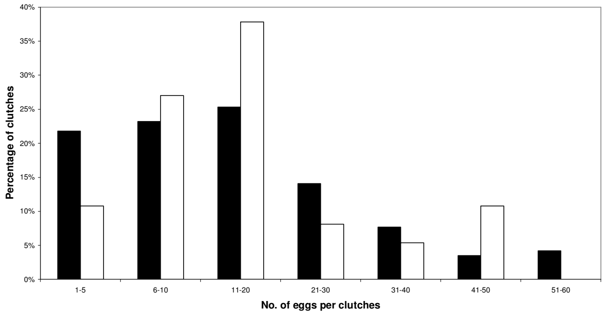

FIG. 2. Percentage distribution of clutches depending on number of eggs per clutch (black, 2000; white, 2001).

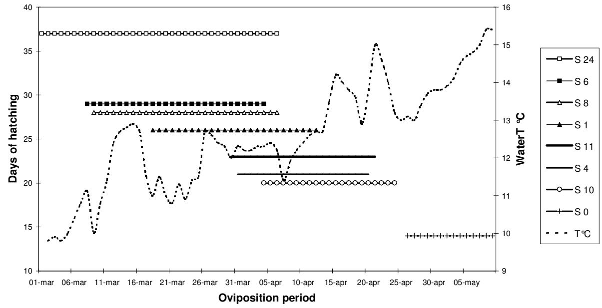

FIG. 4. Relation between hatching period of sampling egg masses (S24-S6-S8-S1-S11-S4-S10-S0) and water temperature.

and C-M): Fig. 3 indicates the number of eggs per day, year and section. In the first area (sections A-B), this number is comparable both in 2000 and 2001 (respectively 2462 and 2495), while the second area (sections C-M) has been utilised for oviposition activity only since 2001 (850).

The peaks of egg-laying activity varied in time and quantitatively each year: e.g., 1 May 2000, 1201 eggs, at the end of the reproductive season; 20 March 2001, 1428 eggs, in the first area, and 10 April 2001, 604 eggs, in the second area; in 2002, the maximum number of eggs observed before the samplings stopped was 320 (17 April) in the first area, and 89 (20 April) in the second area. In 2001 the oviposition activity started in the second area on 2 April, when 1072 eggs were already present in the first area. The decrease in the rate of oviposition of new eggs in the first area almost coincides with the increased oviposition activity in the second area. In 2002, oviposition again started in the first area (9 March) before the second (15 April). The average egg number per egg mass in 2000 was 16.86 (n=140; SD=14.48; range 1-65), and in 2001 was 16.41 (n=36; SD=11.64; range 1-44).

EGG DEVELOPMENT AND SURVIVAL

The embryo development period varies from a maximum of 37 days (eggs oviposited at the beginning of the

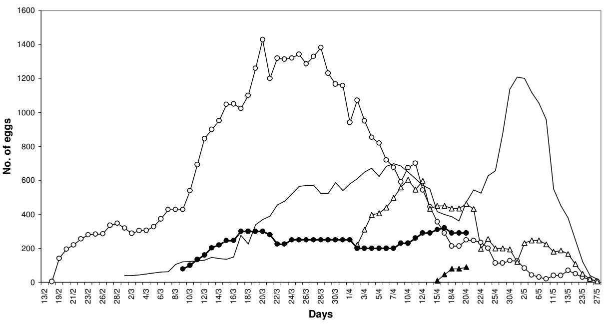

FIG. 3. Egg numbers during three reproductive seasons (2000-2002). Time-course of eggs is determined by the computation of egg deposition rate, embryo mortality and hatching. The A-B area is represented in 2000 by smooth line, in 2001 by empty circles, in 2002 by solid circles. The C-M area is represented in 2001 by empty triangles and in 2002 by solid triangles.

season) to a minimum of 12 days for the latest oviposition. The duration seems to be greatly influenced by water temperature, as higher water temperature reduces the rate of embryonic development. Eight ovipositions from different sites are compared in Fig. 4: S24 hatched after 37 days at a mean temperature of 11.65°C (SD= 0.86); S6 and S8 respectively after 29 and 28 days, at a mean temperature of 11.68°C (SD=0.78) and 11.76°C (SD=0.77); S0 after 14 days at an average temperature of 14.17°C (SD=0.79). A linear relation between water temperature and time of hatching emerged from an analysis based on the data set of eight egg masses (linear regression: y=-6.96x+111.33; r^2=0.81; P<0.005).

The average percentage of hatched eggs in two sampling years (2000 and 2001) was 55.61% (2000: 1286 out of 2462 = 52.23%; 2001: 1973 out of 3345 = 58.98%).

Larvae were only observed in the first days after hatching. They usually drifted to different parts of the stream, or hid under leaves or rubble.

DISCUSSION

The single spring oviposition period characterising this and other studied populations of spectacled salamander (Zuffi, 1999 for a review) is probably due to the perennial condition of the stream. Some Central Italy populations occurring in temporary waters have a second autumnal reproductive season, as noted by Corsetti (1999*a*,*b*) and Angelini *et al*. (2001).

The salamanders used mainly one area of the stream (sections A-B) for oviposition, probably because of the larger number of available substrates and the greater general protection (vegetation, banks structure, etc.). Three main factors concurred to extend the oviposition area from the first area (1999-2000) to the second one (2001-2002): (1) hydromorphological modifications within sections C-M which made this area suitable for oviposition from 2001; (2) winter drift phenomena, which decreased the number of potential substrates for laying eggs in the first area, and increased these substrates in the second area; (3) increased population size, observed from 1999 to 2001 (about 100% each year; triple catch index) (Della Rocca *et al.*, unpublished).

Larger females showed a clear fidelity towards the oviposition site (A-B). Moreover, the tendency of the significantly shorter specimens, presumably younger animals, to reach the stream after the older ones (observed in other populations by Angelini *et al.*, 2001), could force the former individuals to look for available substrates for oviposition out of sections A-B. Our results suggest that the most used substrate for egg laying is the underside of stones, even though not all available stones were utilised. These results differ from those obtained by other authors (e.g. Lanza, 1983; Zuffi, 1999; Barbieri, 2001), who considered other objects as the most used substrate. Personal observations on other populations in Central Italy (e.g. Tolfa Mts., Sabini Mts., Reatini Mts.) indicate that oviposition substrates vary according to local availability. Even though stones are the most suitable substrates for oviposition, females of this population showed great plasticity when the availability of the preferred substrate decreased (e.g. in 2002), using a variety of alternative substrates such as leaves or submerged branches.

Each female oviposited one or more egg masses, consisting of a variable number of eggs, or less frequently a single egg, each glued to the substrate by a peduncle. This observation agrees with much of the literature (e.g. Ramorino, 1863; Thorn, 1968; Angelini *et al.*, 2001), but diverges from Lanza (1983), who considered egg groups to be the result of adhesion of eggs deposited by several females. The number of eggs per mass is comparable with literature records (e.g. Bruno, 1973; Zuffi, 1999).

The number of oviposited eggs is comparable in the first area (A-B) in both 2000 and 2001, while in the second one (C-M, used since 2001) the number varied. The first area seems to reach regularly its carrying capacity for the oviposition substrates, but the second one seems to be utilised only when habitat conditions are suitable.

Embryo development varied between 12 and 37 days, and is positively related to water temperature, as reported in literature (Corsetti, 1999*a*,*b*). Both biotic and abiotic causes influenced the embryo mortality. Abundant rain caused stones and other substrates to drift, either crushing the eggs or exposing them to air. On the other hand, scarcity of rain decreased water levels, producing desiccation or anoxic conditions. Moreover, banks that collapsed caused changes to the stream structure with analogous effects. The main biotic cause of mortality is represented by predation on the eggs by the larva of the trichopteran *Plectrocnemia conspersa* Curtis, 1834 (see Vignoli *et al.*, 2001*b*). The predator crab *Potamon fluviatile* Herbst, 1785, observed feeding on a dead adult specimen (see also Lanza, 1983), may represent a further threat.

ACKNOWLEDGEMENTS

We thank the administration of "RomaNatura", for permission and support our research in the Insugherata Regional Park, Massimo Capula, Alberto Venchi, Marco Zuffi and two anonymous referees for suggestions, Marzio Zapparoli, Riccardo Scalera and students of the Roma Tre University for helping the field. We also thank Maria Lombardi for the English revision, and Tommaso Russo for statistical support.

REFERENCES

Angelini, C., Antonelli, D. & Utzeri, C. (2001). Aspetti della fenologia riproduttiva di *Salamandrina terdigitata* (Lacépède, 1788) in Italia centrale. *Pianura* **13**, 105-108.

Barbieri, F. & Tiso, E. (1993). Nuove stazioni di *Salamandrina terdigitata* nell'Appennino settentrionale (versante padano). *Supplemento alle Ricerche di Biologia della Selvaggina* **21,** 383-385.

Barbieri F. (2001). La salamandrina dagli occhiali (*Salamandrina terdigitata*) nel versante padano dell'Appennino centro-settentrionale (Amphibia: Salamandridae). *Pianura* **13**, 101-104.

Brizzi, R., Delfino, G. & Calloni, C. (1989). Female cloacal anatomy in the spectacled salamander, *Salamandrina terdigitata* (Amphibia: Salamandridae). *Herpetologica* **45**, 310-322.

Bruno, S. (1973). Anfibi d'Italia: Caudata (Studi sulla fauna erpetologica italiana, XVII). *Natura* **64**, 209-450.

Corsetti, L. (1999*a*). Habitat characteristics of the spectacled salamander *Salamandrina terdigitata* (Lacépède, 1788) in southern Latium (Central Italy). *Amphibia-Reptilia* **20,** 77-82.

Corsetti, L. (1999*b*). Reproductive activity and embryo growth of the spectacled salamander *Salamandrina terdigitata* (Lacépède, 1788) in southern Latium (Central Italy). *British Herpetological Society Bulletin* **67**, 13-20.

Corsetti, L. & Angelini, C. (2000). *Salamandrina terdigitata* (Lacépède, 1788). In *Anfibi e rettili del Lazio*, 38-39. Bologna, M. A., Capula, M. & Carpaneto, G. M. (Eds). Roma: Palombi.

Lanza, B. (1983). *Anfibi, Rettili (Amphibia, Reptilia). Guide per il riconoscimento delle specie animali delle acque interne italiane. 27.* Roma: Consiglio Nazionale delle Ricerche.

Lanza, B. (1988). *Salamandrina terdigitata* (Lacépède, 1788): emblem of the Unione Zoologica Italiana. *Bollettino di Zoologia* **55,** 1-4.

Naviglio, L. (1971). Il periodo degli amori nella *Salamandrina terdigitata* (Lacépède, 1788) (Amphibia, Caudata). *Notiziario dell'Unione Erpetologica Italiana* **1**, 39-43

Ramorino, G. (1863). *Appunti sulla storia naturale della* Salamandrina perspicillata. Genova: Tipografia Sordo-Muti.

Societas Herpetologica Italica (1996). Atlante provvisorio degli anfibi e dei rettili italiani. *Annali del Museo Civico di Storia Naturale di Genova "G. Doria"* **91,** 95-178.

Strötgen, F. (1927). Liebesspiele und Begattung bei den Brillensalamnder. *Blätter für Aquarien und Terrarianekunde* **38**, 94-95.

Thorn R. (1968). *Les salamandres*. Paris: P. Lechevalier.

Titus, T. A & Larson, A. (1995). A molecular phylogenetic perspective on the evolutionary radiation of the salamander family Salamandridae. *Systematic Biology* **44**, 125-151.

Vanni, S. (1980). Note sulla salamandrina dagli occhiali [*Salamandrina terdigitata* (Lacépède, 1788)] in Toscana (Amphibia Salamandridae). *Atti della Società Toscana di Scienze Naturali, Pisa, Memorie* (B) **87**, 135-159.

Vanni, S. & Nistri, A. (1997). *Salamandrina terdigitata* (Lacépède, 1788). In *Atlas of Amphibians and Reptiles in* Europe, 70-71. Gasc, J. P., Cabela, A., Crnobrnja-Isailovic, J., Dolmen, D., Grossenbacher, K., Haffner, P., Lescure, J., Martens, H., Martinez Rica, J. P., Maurin, H., Oliveira, M. E., Sofianidou, T., Veith, M. and Zuiderwijk, A. (Eds). Paris: Societas Europaea Herpetologica, Muséum National d'Histoire Naturelle.

Vignoli, L., Della Rocca, F. & Bologna, M. A. (2001*a*). Biologia riproduttiva di una popolazione planiziale tirrenica di salamandrina dagli occhiali. *Pianura* **13,** 109.

Vignoli, L., Della Rocca, F. & Bologna, M. A. (2001*b*). Predazione di uova di salamandrina dagli occhiali da parte di Tricotteri. *Pianura* **13,** 176.

Zuffi, M. A. L. (1999). *Salamandrina terdigitata* (Lacépède, 1788). Brillensalamander. In *Handbuch der Reptilien und Amphibien Europas, 4/I: Schwanzlurche (Urodela) I (Hynobiidae, Proteidae, Plethodontidae, Salamandridae I)*, 229-246. Grossenbacher K. & Thiesmeier B. (Eds.). Wiebelshein: AULA.

Accepted: 21.10.04

* NOTE ADDED IN PROOF

After the acceptance of this manuscript, using mtDNA analysis Mattoccia *et al.* (*Zootaxa* **995**, 1-19, 2005) demonstrated the existence of two species of spectacled salamander. The population studied in this paper belongs to *S. perspicillata* (Savi, 1821).