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Biology aspects in a population of *Pelobates fuscus insubricus* CORNALIA, 1873
(Anura: Pelobatidae)

Zur Biologie einer Population von *Pelobates fuscus insubricus* CORNALIA, 1873
(Anura: Pelobatidae)

STEFANO SCALI & AUGUSTO GENTILLI

KURZFASSUNG

Pelobates fuscus insubricus CORNALIA, 1873 ist ein endemisches Taxon Norditaliens und der Südschweiz. Die Fortpflanzungsökologie ist recht gut untersucht, während die post-reproduktive Phase bisher kaum studiert wurde. Das Geschlechterverhältnis der untersuchten Population wich nicht signifikant von einem hypothetischen 1:1-Verhältnis ab. Die Art erwies sich als "explosive breeder", wobei die Fortpflanzungsperiode bei speziellen klimatischen Bedingungen (z.B. abwechselnd regnerische und trockene Phasen) verlängert sein konnte oder sogar bei besonders rauhem Klima ganz ausfiel. Frühlingsregen war wichtig zur Beendigung der Überwinterungsphase, bestimmte jedoch das anschließende Aktivitätsmuster nicht. Die Präsenz der Knoblauchkröten an der Oberfläche war nicht mit der Umgebungstemperatur korreliert. Weibchen waren in der Zeit nach der Fortpflanzung aktiver als Männchen, eventuell bedingt durch die Ernährungsbedürfnisse. Fünf Feuchtgebiete mit unterschiedlichen morphologischen Merkmalen sind im Untersuchungsgebiet vorhanden; sie alle wurden von *P. fuscus insubricus* als Laichgewässer genutzt. Diese Population zeigte also keine speziellen Habitatpräferenzen bezüglich Fortpflanzung. Im Gegensatz hierzu waren die Tiere während der Nachlaichzeit nur in Habitaten mit sandigen Böden aktiv.

ABSTRACT

Pelobates fuscus insubricus CORNALIA, 1873 is an endemic taxon of northern Italy and southern Switzerland. Its breeding ecology is quite well known, while that of the post-reproductive period was not studied so much. Sex-ratio of the studied population was not significantly different from the hypothetical 1:1. This species turned out an explosive breeder, but the reproductive period could be prolonged when particular climatic conditions (i.e., short rainy phases alternate to dry ones) occur or it could not occur at all if climatic conditions are particularly harsh. Spring rain was important to end the wintering period, but it did not determine the following activity pattern. The surface presence of the Spadefoot Toads was not correlated to the environmental temperatures. Females were more active than males during the post-breeding period, maybe due to trophic needs. Five damp zones are present in the study area; they have different morphological characteristics, but they are all used by *P. fuscus insubricus* as breeding sites. So this population did not show any particular habitat preference for reproduction. On the contrary, only sites characterised by sandy soils were used during the post-breeding activity.

KEY WORDS

Amphibia: Anura: Pelobatidae, *Pelobates fuscus insubricus*, life history, ecology, activity patterns, habitat selection, Italy

INTRODUCTION

The maintenance of biodiversity is the main object of the global conservation strategies. Endemic taxa are particularly important, because they often are very sensitive to habitat modifications (ANDREONE 1991). Scientific research about life history is the starting point for correct management projects for fauna and habitats (BEEBEE 1996). Amphibians can be used as an indicator group, because they are susceptible to environmental changes and the global amphibian decline has been often underlined by many scientists (BLAUSTEIN & WAKE 1990; BEEBEE 1996; GRIFFITHS 1996).

Pelobates fuscus insubricus CORNALIA, 1873 is an endemic taxon of northern Italy and the province of Tessin in Switzerland, where it became extinct many years ago (HONEGGER 1981; GROSSENBACHER 1988; ANDREONE et al. 1993). The nominate sub-

species *P. fuscus fuscus* LAURENTI, 1768, is present in Central and Eastern Europe, where it is quite rare and protected by law in many countries (NÖLLERT 1997; NYSTRÖM et al. 2002). The subspecies *P. f. insubricus* is now extremely rare in Italy and it is considered endangered by CORBETT (1989), PINCHERA et al. (1997) and ANDREONE & LUISELLI (2000, 2001). Available biological information mainly deals with the breeding period (ANDREONE & FERRI 1987; ANDREONE & PAVIGNANO 1988; CHIMINELLO & GENERANI 1992a, 1992b; ANDREONE et al. 1993), but knowledge on habitat selection and activity patterns after reproduction is still scarce.

MATERIALS AND METHODS

The study area is located in Arsago Seprio and Somma Lombardo (Province of Varese, Lombardy, NW Italy), within the "Parco Lombardo della Valle del Ticino", and represents a typical morainal country. It is 95 ha in size, and its altitude ranges from 280 m to 330 m above sea level. Human disturbance is not important, because neither main roads nor cultivated areas are present. Deciduous woods and grass characterise the main portion of the landscape; some canals, a large pond and a swamp are present. Many parts of the area have sandy soil and are covered with a deep litter (fig. 1). Sandy areas amount to 8 ha (8.4% of the study area). A short description of wet areas follows:

A) a large pond (80 m diameter) with abundant aquatic vegetation on banks and a free central area; the following fish species are present: *Ictalurus melas*, *Carassius carassius*, *Carassius auratus* and *Scardinius erythrophthalmus*;

B) some drainage canals 250 m long with still water, located in a grass field. Maximum depth is one meter. Sometimes the canals dry up completely.

C) two ponds with abundant aquatic vegetation and shallow water, 12 m in diameter each;

D) a marshland of elliptic shape covered with willows and *Phragmites australis*, 500 m x 100 m wide and 50 cm deep;

E) some drainage canals 50 m long with still water, deep litter and without aquatic vegetation. All sites but B are located in woodlands.

The research was conducted from 23 February until 27 October, 1995. Additional inspections were made each year during the breeding period, starting from 1994 and the monitoring is still under way. In 1995 we made one field inspection per week resulting in a total of 34 nocturnal and three diurnal surveys. Out of these, 24 nocturnal inspections were made after the emergence of wintering *P. fuscus insubricus* under different weather conditions (dry, cloud cover 0%: n = 12; dry, cloud cover 50%: n = 1; dry, cloud cover 100%: n = 3; rainy: n = 8). The standard paths included forest trails and borders of wet areas. Trails chosen for standard paths crossed all habitat types present in the study area. The study area was subdivided into 26 zones, each one characterised by a homogeneous habitat (e.g., swamp, or deciduous wood with sandy soil, etc.); the extension of each portion could be different. Animals were caught by hand, by net or by an electro stunner (BERNINI et al. 2000). The following data were collected for each specimen: sex, environmental temperatures immediately after capture (air and ground), weather conditions (dry, cloud cover 0%; dry, cloud cover 50%; dry, cloud cover 100%; rainy), solar time, location, orientation of slopes (N, NW, W, SW, S, SE, E, NE, if present), soil type (sandy, loose, stony), type and percentage of arboreal, shrubby, and herbaceous cover (0%, 50%, 100%). We used a digital thermometer with 0.1°C precision. Daily data of rainfall and air temperature were recorded at the weather station of the Malpensa International Airport, located five kilometres south-west of the study area.

The specimens were marked with an incision of a hind leg inter-digital membrane; they were also photographed for dorsal patterns identification (JEHLE 1997; WIENER 1997a). The statistic data elaboration was conducted with SPSS® rel. 6.1.2 for Windows® . Data were tested for normality with the one-sample Kolmogorov-Smirnov test and for equality of variance with

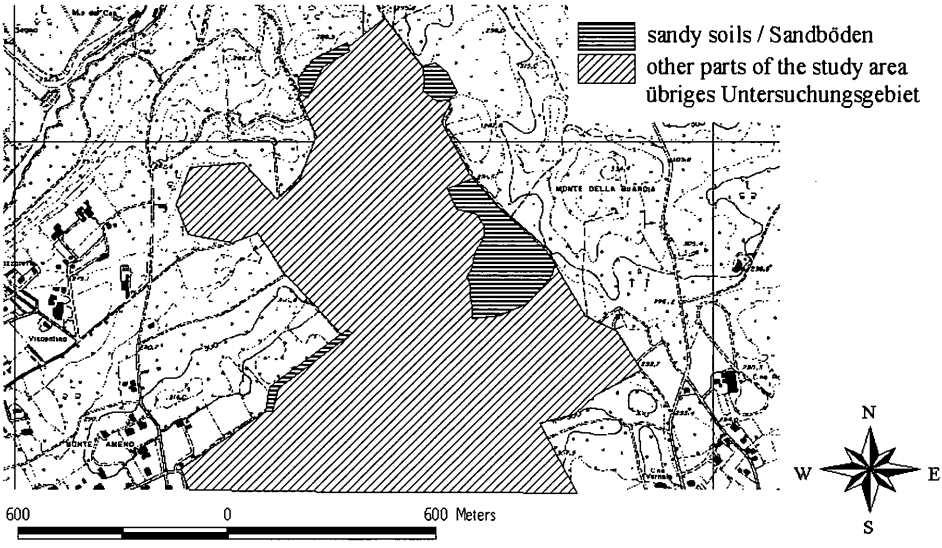

Fig. 1: Distribution of sandy soils (horizontally hatched) in the study area (hatched).
Abb. 1: Verteilung sandiger Böden (waagrecht schraffiert) im Untersuchungsgebiet (schraffiert).

Levene's test. As parametric conditions were not fulfilled, we used non-parametric methods (binomial test, Mann-Whitney U test for two independent samples; Kolmogorov-Smirnov test for two independent samples, χ^2 test). Discriminant analysis was used to analyse habitat choices, considering the following variables (number of character states in parentheses): arboreal cover (3), shrubby cover (3), herbaceous cover (3), soil type (3), and orientation of slopes (9); the direct method was used to test the significance of the canonical discriminant function and the stepwise method was used to highlight which variables determine the presence of Spadefoot Toads (MARNELL 1998).

RESULTS

Sex-ratio

44 adult specimens of *P. f. insubricus* were captured: 18 males (40.9%) and 26 females (59.1%). One female was recaptured and four other specimens were additionally observed. Sex-ratio was 0.69 (♂♂:♀♀); the sex ratio of 29 specimens captured during the reproductive phase (20/4/1995 - 28/4/1995) was 1.07 (15:14 ♂♂:♀♀) (males: n = 15; females: n = 14); in both cases the binomial test result is not significantly different from the hypothetical 1:1 ratio. Hence, sex-specific differences in counts are interpreted as sex-specific differences in activity. The only observed juvenile specimen was found in 1999.

Activity patterns

The first specimens were observed on 20 April and the last (two individuals) were found on 4 October. The breeding period ended at 28 April, when we found the last specimen in the water. Environmental temperatures on 20 April were close to 11°C (air = 10.8 °C; ground = 10.9 °C), but lower temperatures were recorded in the first activity night in 1994 (air = 4.3°C; ground = 6.5°C) (GENTILLI et al. 1996). The analysis of these data by means of the Mann-Whitney U test did not reveal significant differences between the temperatures recorded for males and females. The descriptive statistics of thermal data (their low number of 35 was

Table 1: Thermal data measured immediately after each capture when sampling male and female Spadefoot Toads *Pelobates fuscus insubricus* CORNALIA, 1873, in "Parco Lombardo della Valle del Ticino" (northern Italy) in 1995.

Tab. 1: Temperaturdaten des Jahres 1995 gemessen beim Fang männlicher und weiblicher Knoblauchkröten, *Pelobates fuscus insubricus* CORNALIA, 1873 im "Parco Lombardo della Valle del Ticino" (Norditalien).

Parameter /	Males / Männchen	Females / Weibchen
Number of specimens / Anzahl Tiere	10	25
Air temperature (x ± S. D.) (°C) / Lufttemperatur (x ± Standardabweichung) (°C)	11.27 ± 1.80	11.82 ± 2.94
Ground temperature (x ± S. D.) (°C) / Bodentemperatur (x ± Standardabweichung) (°C)	11.82 ± 2.20	12.44 ± 3.20
Air temperature (Range) (°C) / Lufttemperatur (Bereich) (°C)	8.1 - 14.7	8.1 - 19.8
Ground temperature (Range) (°C) / Bodentemperatur (Bereich) (°C)	9.1 - 15.9	8.2 - 19.2

caused by malfunction of a thermometer) for males and females are reported in table 1.

The number of specimens counted over a ten day period, in relation to the mean minimum and maximum temperature and rain is shown in figures 2 and 3. The annual activity patterns of the two sexes are represented by the numbers of males and females counted in periods of ten days each (fig. 4). The distribution patterns were significantly different (Kolmogorov-Smirnov test: males n = 18, females n = 27; $Z = 0.7368$; $p < 0.001$), with females resulting more active than males after the breeding period. Annual activity of males and females started simultaneously: on 20 April 1995 (first day of activity) we captured five males and eight females, migrating to breeding sites. Activity started earlier in 1994 (1 April: one male and one female) and in 1996 (11 April: two females). Some annual surveys suggested that the beginning of the activity period is quite variable: the first observation, in fact ranged from 26 March (2000) to 14 May (2001). Juvenile and adult specimens started annual activity simultaneously. The maximum distance from the breeding sites covered by a marked female during the post-reproductive dispersion was about 400 m.

χ^2 test was used to analyse possible preferences related to the weather conditions (table 2) The expected frequencies were calculated under the null hypothesis that, in absence of precise weather preferences by Spadefoot Toads, the number of specimens observed under the various weather conditions should have been proportional to the number of inspections made under these above weather conditions. As for the whole sample, Spadefoot Toads chose rainy evenings for their breeding activities (n = 45; $\chi^2 = 14.1671$; $df = 3$; $p < 0.01$). In particular, males did not show any preference (n = 18; $\chi^2 = 3.6193$; $df = 3$; $p > 0.05$) while females preferred rainy nights and avoided fair conditions (n = 27; $\chi^2 = 13.8509$; $df = 3$; $p < 0.01$) (table 3).

Table 2: Weather conditions during the observation. of Spadefoot Toads *Pelobates fuscus insubricus* CORNALIA, 1873, in "Parco Lombardo della Valle del Ticino" (northern Italy) in 1995.

Tab. 2: Wetterbedingungen im Jahr 1995 während der Beobachtung von *Pelobates fuscus insubricus* CORNALIA, 1873 im "Parco Lombardo della Valle del Ticino" (Norditalien). Bewölkung meint Wolkenbedeckungsgrad.

	Weather conditions / Wetterbedingungen			
	dry / trocken clear wolkenlos	dry / trocken cloud cover 50% Bewölkung 50%	dry / trocken cloud cover 100% Bewölkung 100%	rainy regnerisch
No. of inspections / Anzahl Begehungen	12	1	3	8
% of inspections / % Begehungen	50 %	4.2 %	12.5 %	33.3 %
No. of specimens / Anzahl Exemplare	18	0	2	25
(males / females) / (Männchen / Weibchen)	(11 / 7)	(0 / 0)	(0 / 2)	(7 / 18)
% of specimens / % Exemplare	40.0 %	0 %	4.4 %	55.6 %

Habitat preferences

The environmental preferences of the Spadefoot Toads in this area were analysed with a discriminant analysis; the presence or absence of the species in 26 areas was used as a discriminating factor (table 3). We obtained a significant canonical discriminant function with the direct method (Eigenvalue = 0.8752, Wilks' Lambda = 0.5333, χ^2 = 13.517, $df = 5$, $p < 0.02$). The total percentage of correct classification was 84.62% (83.3% for presence and 85.7% for absence). The stepwise method underlined the importance of sandy soils the presence of which is positively correlated with the presence of *P. fuscus insubricus*. No other variable was extracted by the model.

Table 3: Environmental data related to 36 records of male (M) and female (F) individuals of *Pelobates fuscus insubricus* CORNALIA, 1873, in "Parco Lombardo della Valle del Ticino"(northern Italy). Weather: percentages of cloud cover in dry conditions are reported. See Materials and Methods for details.

Tab. 3: Angaben zum Lebensraum bei 36 Nachweisen männlicher (M) und weiblicher (F) Individuen von *Pelobates fuscus insubricus* CORNALIA, 1873, im "Parco Lombardo della Valle del Ticino"(Norditalien). Wetter: Wolkenbedeckungsgrad in Prozent an Tagen ohne Niederschlag.

Sex Geschlecht	Weather Wetter	Orientation Ausrichtung	Soil Boden	Degree of plant coverage (%) / Pflanzenbedeckungsgrad (%) Trees /Bäume	Shrubs /Sträucher	Herbs / Kräuter
M	rainy/regnerisch	SW	stony/steinig	0	50	100
M	rainy/regnerisch	SW	stony/steinig	0	50	100
M	rainy/regnerisch	SW	stony/steinig	0	50	100
M	100%	N	stony/steinig	0	50	100
M	100%	W	loose/locker	0	50	50
M	100%	W	loose/locker	0	50	50
M	0%	NE	sandy/sandig	0	50	100
M	0%	NW	sandy/sandig	50	100	100
M	0%	NW	sandy/sandig	50	100	100
M	0%	NW	sandy/sandig	50	100	100
M	0%	NW	sandy/sandig	50	100	100
M	0%	NW	sandy/sandig	50	100	100
M	0%	NW	sandy/sandig	50	100	100
M	0%	NW	sandy/sandig	50	100	100
M	0%	NW	sandy/sandig	50	100	100
M	0%	SW	stony/steinig	0	50	100
M	rainy/regnerisch	N	stony/steinig	0	50	100
M	0%	E	stony/steinig	0	0	100
F	rainy/regnerisch	SW	stony/steinig	0	50	100
F	100%	N	stony/steinig	0	50	100
F	100%	N	stony/steinig	0	50	100
F	100%	W	loose/locker	0	50	50
F	100%	W	loose/locker	0	50	50
F	100%	W	loose/locker	0	50	50
F	100%	S	sandy/sandig	0	0	100
F	rainy/regnerisch	plain/eben	loose/locker	100	100	0
F	50%	NE	sandy/sandig	0	50	100
F	50%	NE	sandy/sandig	0	50	100
F	50%	NE	sandy/sandig	0	50	100
F	rainy/regnerisch	NE	sandy/sandig	0	50	100
F	0%	NW	sandy/sandig	50	100	100
F	0%	NW	sandy/sandig	50	100	100
F	0%	NE	loose/locker	0	50	100
F	0%	N	loose/locker	0	50	100
F	0%	NE	sandy/sandig	50	0	0
F	0%	NE	sandy/sandig	50	0	0
F	rainy/regnerisch	NW	loose/locker	0	0	100
F	rainy/regnerisch	plain/eben	loose/locker	0	0	100
F	rainy/regnerisch	S	loose/locker	0	0	100
F	100%	E	stony/steinig	100	100	0
F	100%	plain/eben	loose/locker	0	0	100
F	0%	NE	loose/locker	0	50	100
F	rainy/regnerisch	N	loose/locker	0	50	100
F	rainy/regnerisch	plain/eben	loose/locker	100	100	0

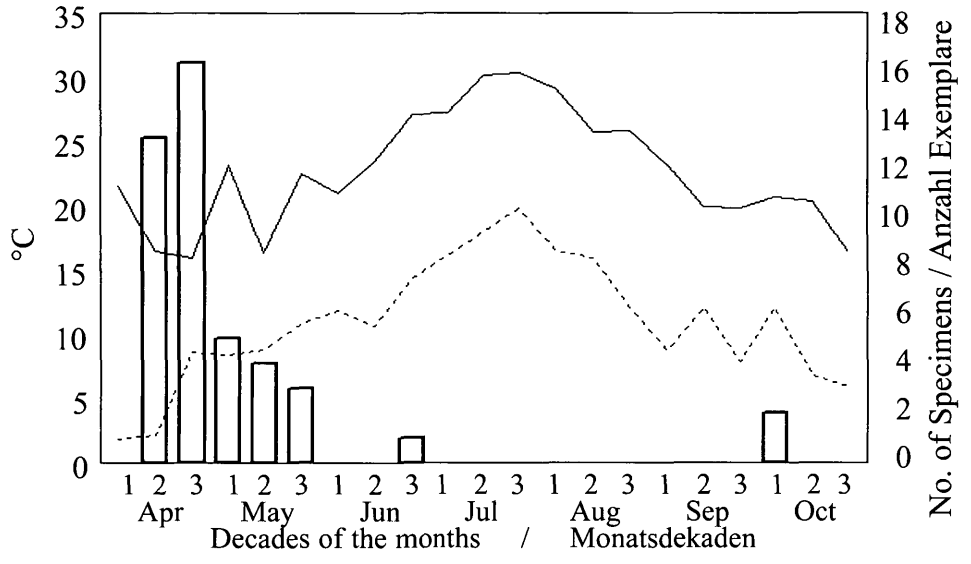

Fig. 2: *Pelobates fuscus insubricus* CORNALIA, 1873, in "Parco Lombardo della Valle del Ticino"(northern Italy). Number of observed specimens (outline bars) every ten days from April through October 1995 in relation to the mean minimum (- - - -) and maximum (———) air temperatures (°C).

Abb. 2: *Pelobates fuscus insubricus* CORNALIA, 1873, im "Parco Lombardo della Valle del Ticino" (Norditalien). Anzahl der von April bis Oktober 1995 in 10-Tages-Abständen beobachteten Tiere (leere Balken) in Relation zu den gemittelten Minimum- (- - - -) und Maximumtemperaturen (———) (°C).

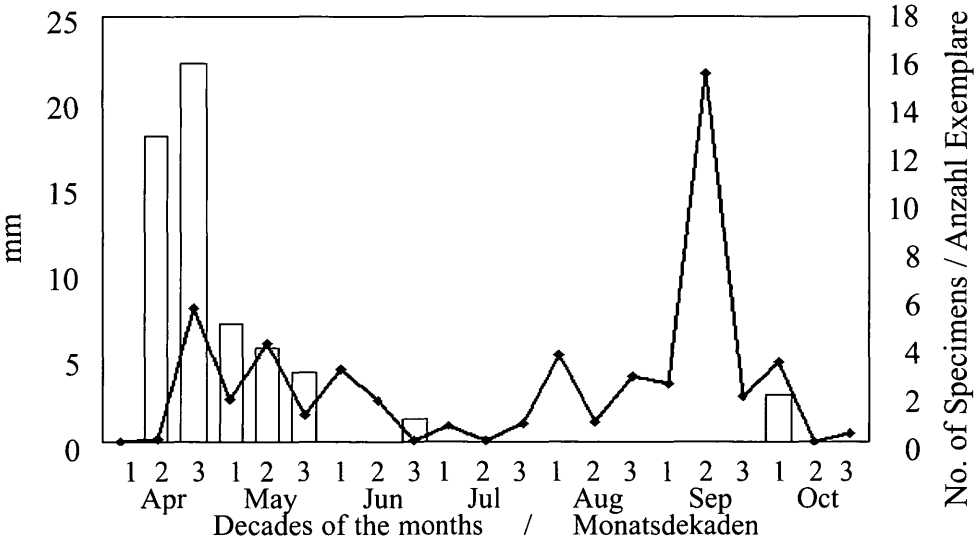

Fig. 3: *Pelobates fuscus insubricus* CORNALIA, 1873, in "Parco Lombardo della Valle del Ticino" (northern Italy). Number of observed specimens (outline bars) every ten days from April through October 1995 in relation to the amount of rainfall (—♦—) (mm).

Abb. 3: *Pelobates fuscus insubricus* CORNALIA, 1873, im "Parco Lombardo della Valle del Ticino" (Norditalien). Anzahl der von April bis Oktober 1995 in 10-Tages-Abständen beobachteten Tiere (leere Balken) in Relation zu den Niederschlagsmengen (—♦—) (mm).

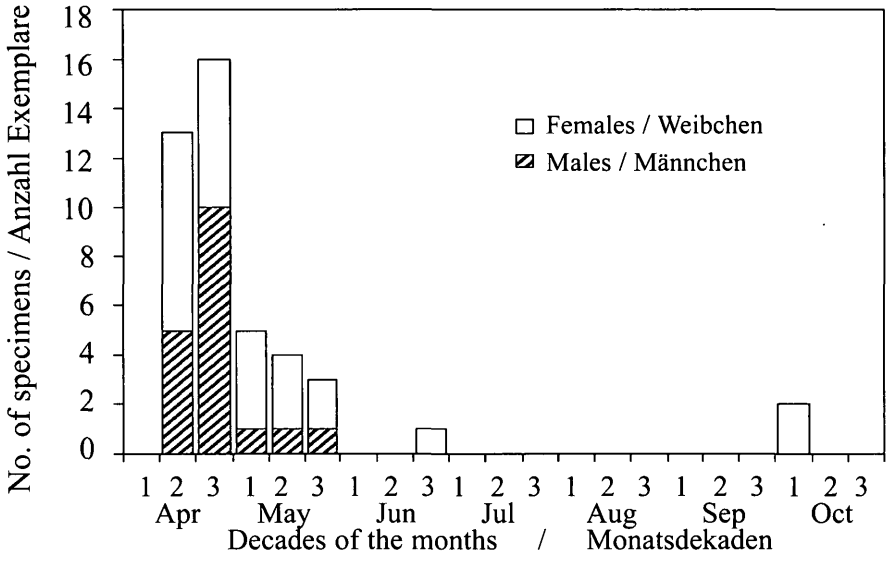

Fig. 4: *Pelobates fuscus insubricus* CORNALIA, 1873, in "Parco Lombardo della Valle del Ticino"(northern Italy). Number of males and females observed every ten days from April through October 1995.

Abb. 4: *Pelobates fuscus insubricus* CORNALIA, 1873, im "Parco Lombardo della Valle del Ticino" (Norditalien). Anzahl der von April bis Oktober 1995 in 10-Tages-Abständen beobachteten Tiere.

DISCUSSION

The observed sex-ratio of 0.69 (♂♂: ♀♀) contrasted with the value of 2.0 calculated by ANDREONE & PAVIGNANO (1988), but agreed with data in ANDRZEJEWSKI et al. (1977) and with MUNK et al. (1995) for the nominal subspecies. Sex-ratio is rather variable in *P. f. fuscus*, as demonstrated by many researches in Central and Northern Europe (NÖLLERT 1990; WIENER 1997b; HELS 2002). Values range from 1.11 (1:0.9) to 0.43 (1:2.3) in relation to different environmental conditions and among different years (WIENER 1997b), even if explosive breeders usually show a skewed sex-ratio in favour of males (DUELLMAN & TRUEB 1994). Calculating the sex-ratio observed in the breeding period (1.07), prevented us from overestimating the number of females due to their increased activity during the post-breeding period.

Pelobates fuscus is considered an explosive breeder by WELLS (1977), ANDREONE (1993), and HELS (2002). Our data seemed to confirm this reproductive strategy, since the breeding period (understood as the time range between the start of breeding migration and the recording of the last specimen in the aquatic sites) lasted only nine days, but observations made during the following years showed a wider mating period (2-3 weeks) with further reproductive peaks (SCALI & GENTILLI unpublished), in accordance with what was stated by KOWALEWSKI (1974) for *P. fuscus fuscus* and by LIZANA et al. (1994) for *P. cultripes* (CUVIER, 1829).

Males and females migrate to the breeding areas in the same period in accordance to what was stated by ANDREONE & PAVIGNANO (1988) and WIENER (1997a). A different pattern of migration was observed for *P. cultripes* in Spain, where males arrive earlier at the breeding ponds than the females (SALVADOR et al. 1986).

The surface activity of the Spadefoot Toads which is strictly nocturnal, set in during the first spring rains (fig. 3), as was already observed in Poland and in Italy (Piedmont region) (KOWALEWSKI 1974; ANDREONE & FERRI 1987; ANDREONE & PAVIGNANO 1988); this factor being important in general for the start of the breeding period of Pelobatinae (LIZANA et al. 1994). Rain was not important to Spadefoot Toads' activity after the reproductive phase. Environmental temperatures, on the contrary, seemed to have little influence on the beginning of the breeding period (fig. 2), as demonstrated by the great variability comparing different years. The minimum air temperature recorded in Arsago Seprio (> 4 °C) was lower than that recorded in Poland (>7°C) (KOWALEWSKI 1974) and higher than that recorded in Austria (-3°C) (WIENER 1997a) for the nominal subspecies. HELS (2002) found that *P. fuscus fuscus* can delay its breeding period owing to extraordinary long winters. Anyway, environmental temperatures below zero prevent the beginning of the breeding activities in *P. fuscus insubricus* as demonstrated by the observations in 2001 and, in some cases, in *P. fuscus fuscus* (WIENER 1997a). In fact a sudden frost at mid-April in this year did not allow for surface activity and, consequently, breeding of Spadefoot Toads did not occur in 2001. These data were confirmed by observations in other sites (F. ANDREONE pers. comm.; SCALI & GENTILLI unpublished). This fact highlights the low adaptability rate of this subspecies and its sensitivity to environmental stress, that could be one of the causes of its numeric reduction in many areas. It is important to underline that all other amphibian taxa [*Triturus carnifex* LAURENTI, 1768, *T. vulgaris* (LINNAEUS, 1758), *Bufo bufo* (LINNAEUS, 1758), *Hyla intermedia* BOULENGER, 1881, *Rana dalmatina* BONAPARTE, 1840, and *R.* synklepton *esculenta* LINNAEUS, 1758] regularly bred in the study area in 2001.

The number of males found on the surface of the ground sharply decreased during the post-reproductive phase, while the females decreased continuously in number and were observed for a longer period, perhaps for their need to increase body reserves. Some females were still active after the summer, maybe for the same reason, but the low number of specimens observed does not allow the drawing of firm conclusions.

Spadefoot Toads show little selection in choosing breeding sites, as they use different types of water bodies for egg laying; in fact they breed in every wet area present in the Arsago Seprio woods. They also use temporary ponds and wetlands containing predatory fish, contrary to what was stated by NYSTRÖM et al. (2002) for the nominate subspecies.

Pelobates fuscus insubricus migrates quite far from breeding sites after reproduction and uses many different habitats; the maximum distance of 400 m observed in our studies is in accordance with data recorded in the northern parts of the species' range (NÖLLERT 1990; MUNK et al. 1995; CHEVALLIER et al. 1997; HELS 2002). Sandy soils seem to be the only important environmental characteristic for the species presence, as underlined by discriminant analysis and in accordance with its fossorial habits (LANZA 1983; ANDREONE et al. 1993; EGGERT & GUYETANT 2002).

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