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Short research contribution

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CO-OCCURRENCE OF AGILE FROG (*RANA DALMATINA* FITZ. IN BONAPARTE) WITH COMMON FROG (*RANA TEMPORARIA* L.) IN BREEDING SITES IN SOUTHERN POLAND

ABSTRACT: A location (area 10 km^2) of the agile frog (*Rana dalmatina* Fitz. in Bonaparte*)* in a southwest part of the Sandomierz Valley (southern Poland), was surveyed during 4 breeding seasons (1999–2002). Eighteen breeding sites of brown frogs were inspected for their characteristics, presence and numbers of egg-batches, and time of egg deposition. Breeding sites were typically temporary, small, and shallow waters (area 5–30 m^2, depth <50 cm). The agile frog was found to co-occur only with the common frog (*Rana temporaria* L.) in most of the sites. Based on the number of egg-batches, the agile frog was more numerous (total of 315 batches in year 2002) than the common frog (163). Over the four years of the study, there were no significant fluctuations in the numbers of eggs. The two species did not differ significantly in their choice of the habitats, except for the sites with low water pH, used predominantly by the agile frog. Habitat niche overlap was high (Pianka's O = 0.86). In the acid peat bogs (water pH about 4.5) most of the eggs degenerated.

KEY WORDS: agile frog, common frog, brown frogs, breeding sites, acidification

Three species belonging to the brown frog group occur in Poland: the common frog *Rana temporaria* (L.), the moor frog *Rana arvalis* (Nilsson), and the agile frog (*Rana dalmatina* Fitz. in Bonaparte). All of them reproduce in early spring, immediately following spring thaw, and they differ in their habitat preferences. The common frog is among them the most ubiquitous species, occurring in a range of habitats throughout the whole country, whereas agile frog is the rarest amphibian in Poland, and its occurrence is still poorly known (e.g. Juszczyk 1987). Mature individuals of agile frog were recorded from southeastern regions: several locations in Carpathian Foothills, and one lowland location in the edge of Sandomierz Valley (20°43´E, 50°00´N; Błachuta and Jabłoński 1986, Rafiński *et al.* 1987, Szymura 1994, Szymura and Rafiński 1997). The species has been classified as endangered (Polish Red List of Vertebrates; Rafiński and Szymura 2001).

Among European anurans, the agile frog exhibits a most disjunctive distribution (Grossenbacher 1997). The species' main range covers large areas of southern Europe. Isolated populations exist in central and northern parts of Europe (Germany, Poland, Denmark, and Sweden). The northernmost location is the Swedish island of Öland (Grossenbacher 1997).

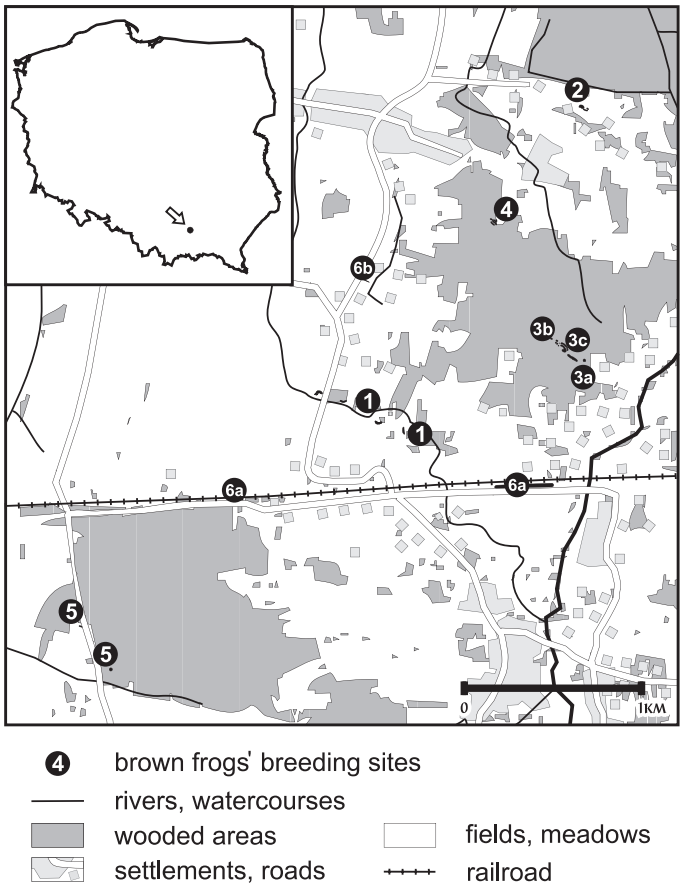

Fig. 1. Location and schematic map of the study site (southwest part of the Sandomierz Valley, southern Poland), with locations of the breeding sites of brown frogs. Numbers correspond to Table 2.

Until now, there were no records of breeding sites of the agile frog from Poland. Therefore, its local breeding preferences, which vary considerably throughout the species range (Grossenbacher 1997), remained unknown. Thus, the first aim of our study was to characterize breeding sites of the agile frog in south-eastern Poland. The second was to assess its co-occurrence with two other species of brown frogs in sympatric populations.

The study was conducted in the south-western part of the Sandomierz Valley (20°43´E, 50°00´N). It is a lowland region (200 m a.s.l.), characterised by a mosaic, human-transformed landscape. The area is covered by fragmented forests composed mainly of pine (*Pinus silvestris*) and birch (*Betula verrucosa*), with admixtures of oak (*Quercus robur*) and alder (*Alnus* sp.) along watercourses. Open habitats include wet meadows, peat bogs, and cultivated fields. The study area covered about 10 km, forests and woodlots made 33% of that area (Fig. 1). In the north, the study site was adjacent to a large managed forest (Radłów Forests, *ca* 80 km^2). The climate of the area is moderate with the mean temperature of January averaging –4.7°C, and that in July 19.3°C. Mean annual precipitation is about 650 mm. Amphibian community includes also the other two species of brown frogs occurring in Poland (i.e. common frog and moor frog), the green frogs (*R. esculenta* L. and *R. lessonae* Camerano), tree frog *Hyla arborea* L., common toad *Bufo bufo* L., and the smooth newt *Triturus vulgaris* L.

Study area was visited several times a year, in March and April 1999–2002. Water bodies were located with the use of a map, informa-

tion from earlier studies (Szymura 1994), and that obtained from local people. In total, 18 breeding sites were found, and surveyed for the presence of egg-batches of brown frogs, but only 7 of them were surveyed each year. As a breeding site, we considered either a single water body, or a group of adjacent, usually small pools, often surrounding larger central pond. The following characteristics of water bodies within the breeding sites were described: size, surroundings, distance from the nearest forest, type of vegetation in the pond, type of bottom, water pH. Egg-batches of brown frogs were counted in each breeding site. Batches were characterized by their distribution (separately or in groups), vertical placement (surface, mid-water, bottom), type of placement (free floating or attached to submersed objects), and appearance of egg-capsules. Developmental stages of embryos (according to Gosner 1960) were determined with a hand lens magnification, in some of the eggs from a group of adjacent batches. From selected batches, samples of 10–20 eggs were collected and transported in plastic bags, to the tanks (filled with water with neutral pH) in laboratory. Shortly after hatching, larvae were observed under a binocular microscope, and their features noted. Grown up tadpoles were identified according to the key of Günther (1996).

Breadth and overlap of the habitat niches of the two species were calculated. Habitat niche breadth was calculated using index *B* (Levins 1968):

$$B = 1 / (n\Sigma pi^2) \quad (1)$$

where p_i is the number of batches in habitat *i* expressed as a fraction of summed batch numbers in all *n* habitats. Value of *B* can range from 1/*n* (use of a single habitat) to 1 (equal usage of habitats).

Overlap of habitat niches was calculated according to Pianka (1973):

$$O_{ij} = (\Sigma p_{ia}\, p_{ja}) \, [(\Sigma p_{ia}^2)\, (\Sigma p_{ja}^2)]^{-½} \quad (2)$$

where O_{ij} is the overlap between species *i* and *j*, p_{ia} is the fraction of *a*-th habitat in the summed numbers of batches of *i*-th species in all habitats, and p_{ja} is the fraction of *a*-th habitat in the numbers of batches of *j*-th species in all habitats. Value of *O* ranges from 0 (exclusive niches) to 1 (complete overlap).

Majority of the breeding sites, consisted of temporary, small (5–30 m^2) and shallow pools (<50 cm). Egg-batches or tadpoles of the agile frog were found in 17 breeding sites, and those of the common frog in 11 breeding sites. Thus, the two species co-occurred in 10 breeding sites, in 8 of them the agile frog bred solely, and in one breeding site only the common frog was found. In 7 sites surveyed throughout the whole study period, the mean total numbers of egg-batches were 45.5 (range 24–62) and 24.3 (range 18–36) for the agile frog and the common frog, respectively. Overall batch numbers throughout 4 breeding seasons did not show significant changes (chi square test, $\chi^2 = 5.57$, df = 3, $P = 0.13$), although they sometimes varied considerably in individual breeding sites (Table 1). The agile frog bred recurrently each year in all the 17 sites, where this species occurred, and the common frog only in old riverbeds. In 2002, in all 18 located breeding sites, agile frog was significantly more abundant than common frog ($\chi^2 = 48.33$, df = 1, $P = 0.00$), 315 egg-batches of the agile frog and 163 of the common frog were found.

Among the breeding sites, six habitat types were distinguished (Table 2). The two species differed in the choice of breeding sites (Wilcoxon test, Z = 2.20, $P = 0.03$), but less in the choice of habitats (Z = 1.89, $P = 0.06$). However, ditches, forest pools, peat ponds, and bogs were used predominantly by the agile frog (comparisons for each habitat, $\chi^2 = 11.0 – 63.6$, df =1, $P << 0.003$ with Holm's correction applied). Using batch numbers in 2002, calculated habitat niche breadth of the agile frog ($B = 0.50$) (equation 1) was wider than that of the common frog ($B = 0.27$). Overlap of habitat niches (*O*) (equation 2) of the two species was 0.86.

The pH of waters, where the frogs bred, ranged from 4.6 to 7.5 for the agile frog, and 6.4–7.5 for the common frog. Mortality of eggs and embryos occurred in dystrophic peat bogs. This breeding site held acid waters with pH 4.6–6.2. Agile frog bred there throughout 4 seasons of the study, while common frog only in one season. Most of the egg-batches observed in that site degenerated. The egg-

Table 1. Numbers of egg batches of the agile frog *Rana dalmatina* (*R. d.*) and the common frog *Rana temporaria* (*R. t.*) found in various habitats (Table 2) during 4 seasons of the study. Qualitative data only are available for peat bogs before 2002. Sand excavation site was found in 2002.

Breeding sites	Number of egg batches found							
	1999		2000		2001		2002	
	R.d.	*R.t.*	*R.d.*	*R.t.*	*R.d.*	*R.t.*	*R.d.*	*R.t.*
Old riverbeds	14	12	12	10	13	14	5	13
Peat ponds	28	4	6	1	39	22	39	8
Peat bogs	+	–	+	–	+	–	103	16
Sand excavations	—	—	—	—	—	—	144	126
Forest pools	13	0	16	8	12	0	13	0
Ditches	7	10	30	2	9	0	11	0
Total number	62	26	64	21	73	36	315	163

masses were milky-green, infested by fungi, and the batches usually lied on the bottom (properly developing eggs float under water surface). However, mortality affected mainly outer eggs of the batch, so often those in the middle developed normally. Despite that mortality, transformed froglets from those ponds were observed (J. M. Szymura, pers. comm.).

The agile frog started breeding in the first half of March, whereas the common frog about a week later (end of March, beginning of April). Egg deposition took place typically after the snow had melted permanently, but in a few cases, egg-batches were found frozen. Single new batches of both species were found up to the end of April. Hatching took place in the first half of April. The larvae of the common frog often started to hatch earlier than those of the agile frog, which were at that time in stages 19–20 (visible heart beat and gill circulation, Gosner 1960).

In the study area, the agile frog shares breeding sites only with the common frog, even though adult moor frogs were also observed (Szymura 1994, K. Bartoń pers. obs.). In the Carpathian Foothill, the agile frog co-occurs only with the common frog (Szymura and Rafiński 1997). In Germany, the species usually occurs also with the common frog, and rarely with the moor frog (Günther *et al*. 1996). Egg-batches of the common frog and the agile frog are often found together, but if the populations are large, the species exclude each other (Rohrbach and Kuhn 1997). In contrast, there is a pronounced lack of sympatry of the two species in northern Europe (Andrén and Nilson 1988). In sites where both species are present, they seem to segregate in their choice of breeding ponds (Riis 1997). Riis (1988) explained such a distribution by competitive exclusion at the tadpole stage.

Most of the breeding sites in the study area were situated within forest, but also far from the forest border. This distance was larger than in Denmark, where frogs mostly used water bodies located 200 m from the forest (Wederkinch 1988). Also in France and south Germany this distance was smaller: 50–100 m, never exceeding 250 m (Rohrbach and Kuhn 1997, Ponsero and Joly 1998). The agile frog is strongly connected with deciduous forests, in Denmark, numbers of egg-batches declined exponentially with increasing distance of a breeding pond

from the forest (Wederkinch 1988). Over the whole range of the agile frog, its habitats consist of forests and wet meadows. In Sweden, the species inhabits scrublands and mosaic of deciduous forest, wetlands, and pastures, the breeding sites were usually found in the wooded areas (Ahlén 1997, Riis 1997). In central Europe, it is found mainly in deciduous and mixed forests (Grossenbacher 1997). In north Germany (Lower Saxony, Podloucky 1997), breeding sites are most often located in mixed oak or beech forests, and varied from small to several-hectare water bodies. In central Europe, the agile frog bred in open, sunny moors, or old riverbeds within forests (Grossenbacher 1997). Likewise, all locations in Poland were found in wooded areas (Szymura 1994, Szymura and Rafiński 1997), and this species was not found in strictly agricultural vicinities of the study site, with scarce woodlots (K. Bartoń unpubl. data).

We observed an evident difference in use of peat bogs by the two species. While agile frog was abundant, common frog avoided that breeding site. Increased mortality and degeneration of eggs was likely to be caused by acidification, a negative factor for eggs and larvae (Andrén *et al.* 1988). Among brown frogs, the agile frog appears to be least tolerant to acidity (Andrén *et al.* 1988). Experiments by Andrén and Nilson (1988) showed that all eggs died at pH 4.0, but at pH 5–6, the hatching success was almost 100%. Low pH exerts a strong harmful effect, causing high mortality of eggs and embryos, la-

Table 2. Description of the breeding sites of the agile frog (*Rana dalmatina*), surveyed in 1999–2002 in the southwest part of the Sandomierz Valley (southern Poland).

No.	Habitat	Location	Number of breeding sites	Size of water bodies (m^2)	Depth (cm)	Characteristics	pH
1	Old riverbeds	Meadows, arable fields, close to forest border	2	20–30	20–40	Former meanders of regulated river, bottom covered with grass and submerse vegetation, surface partially covered by *Lemna minor*.	7.2–7.5
2	Peat ponds	Wet meadow, *ca.* 100 m from edge	2	50, 10	20–30, <100	Shallows covered by grass. Brown water.	5.2–6.2
3a	Peat bogs	Edge of forest and wet meadows	2	1–80	20–30, <50	Group of bogs, former sand excavation. Bottom covered by *Sphagnum* moss and sedge.	4.6–5.5
3c	"	"	1	5	"	Partially covered by sedges, filamentous algae.	6.5
3b	"	"	1	5	"	Layer of dead leaves on bottom.	6.2
4	Sand excavation	Over 100 m within forest	5	1–20	10–30	Reeds and sedges.	6.5
5	Forest pools	Over 100 m within pine-birch forest	2	10	<50	No vegetation, leaves on bottom.	6.0
6a	Ditches	Arable land, over 400 m from wooded area	2	> 200 m long, 1–2 m wide	<30	Overgrown by filamentous algae and *Lemna minor*, partly by reeds and willow.	7.5
6b	"	Arable land	1	50 cm wide	"	Slowly flowing water, growth of grass.	6.4

tent embryonic development, deformations and abnormal behaviour of larvae, affecting size and age at the metamorphosis (Pahkala *et al*. 2001, Räsänen *et al.* 2002). Tolerance to acidification, however, might be higher in populations exposed naturally to low pH (Glos *et al*. 2003).

In the northern part of its range, the agile frog is the earliest breeding amphibian. In Sweden, the species breeds in March and beginning of April, but in early years at the end of February (Ahlén 1997). In Denmark, it starts breeding about a week after the ice has melted, and the common frog two weeks later (Riis 1991). Also in Lower Saxony, agile frogs deposit eggs earlier than common frogs (Podloucky 1997). The agile frog in northern Europe reproduces later than in central Europe, but in the same water temperatures. In Funen, Denmark, the frogs bred on average 2–3 weeks later than in central Germany, but in both localities water temperature was above 6.5°C (Geisselmann *et al.* 1971, Blab 1978, Riis 1991, Riis 1997). Early egg deposition has been explained by the long development of the agile frog (Riis 1991, Ahlén 1997). The total development time of the embryos of the agile frog is twice as long as that of the common frog. However, very early breeding imposes a cost on a population as adults and eggs might be killed by a sudden frost (Riis 1991, K. Bartoń pers. obs.).

In the study area, human activities influenced the condition of amphibian breeding sites in two opposite ways. Drainage of the area and regulation of streams lowered the ground water level and destroyed natural water bodies. On the other hand, the majority of observed breeding sites have been created indirectly as a result of human activities. The existence of the agile frog (*Rana dalmatina*) population in the study area is dependent on a small number of suitable breeding ponds. Nevertheless, numbers of egg-batches did not exhibit noticeable loss during the 4 seasons of the study, therefore the population did not seem to decline.

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