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Variation in life history traits in *Bombina bombina* from the lower Danube floodplain

Dan Cogălniceanu[1], Claude Miaud[2]

[1] University of Bucharest, Faculty of Biology, Department of Ecology, Splaiul Independenţei 91-95, 76201 Bucharest, Romania
e-mail: danco@bio.bio.unibuc.ro
[2] University of Savoie, UMR CNRS 5553 Laboratory Biology of Alpine populations, 73 376 Le Bourget du Lac, France
e-mail: claude.miaud@univ-savoie.fr

Large river-flooplains are disturbance-dominated landscapes in a constant state of change, characterized by high habitat diversity and biota adapted to exploit the spatial and temporal heterogeneity (Ward et al., 1999). Floods are considered to be major regulators of both aquatic (Jowett and Richardson, 1994) and nearby terrestrial communities (Bosman et al., 1997), since they provide the connection between the river and the rich resources derived from the floodplain (Bayley, 1995).

Perennial species must optimize both the age at maturity and also the division of resources between growth and reproduction in all the years following maturation (Kozłowski and Uchmański, 1987). This involves a trade-off between reproductive effort and reproductive lifespan (Charnov, 1997; Nilsson and Svensson, 1996). In amphibians both the short age of resources and high mortality rates are among the factors promoting low age and small size through life.

The study of the amphibian communities inhabiting an island in the lower Danube floodplain was started in 1994. A negative impact of the prolonged periods of inundation was observed on the body condition of water frogs (Cogălniceanu, 1997), and on the body size and fecundity of smooth newt populations (Cogălniceanu, 1999).

The aim of the present study was to understand how an extremely abundant, small-sized amphibian species, the fire-bellied toad (*Bombina bombina*), has adapted under the environmental constraints prevailing in the floodplain of a large river.

Table 1. Body size and weight (mean value, standard error, and range) in *B. bombina*.

Life stage/sex	n	SVL (mm)	SVL range	Wet weight (g)	Wet weight range
Juveniles	87	22.6 ± 0.30	13.8-29.0	0.87 ± 0.03	0.22-1.85
♀	71	35.2 ± 0.32	29.4-44.0	3.17 ± 0.07	1.94-5.37
♂	34	34.4 ± 0.55	28.9-43.8	2.97 ± 0.18	1.79-8.05

The study site, Insula Mică a Brăilei, is situated in the lower Danube floodplain, south of the town of Brăila (Romania) (45°10′-44°47′N, 27°49′E). With an area of 17.58 km^2 it is one of the largest wetlands upstream the Danube Delta still under a natural inundation regime. It is a protected area at national level and was established as a Ramsar site in 2001 in recognition of its importance. The relief is flat, with an average elevation of 4.5 m and a maximum elevation of 8.2 m. Besides the network of channels that delineate a number of islands, several large, shallow lakes exist in the inner parts. Danube water discharge at Brăila is highly dynamic, ranging up to 10,000 m^3/s between high and low water levels. The biological and ecological processes on the island are controlled by the periodic floods of the Danube (Botnariuc, 1967).

Fire-bellied toads were observed and captured along transects in 1995, or were caught in pitfall traps filled with ethyleneglycol used to survey invertebrate recolonization rates after floods (see Cogălniceanu et al., 2001, for further details).

For fecundity analyses females ($n = 17$) were anaestesized with MS 222 Sandoz, the snout-vent length (SVL) measured with dial-callipers to the nearest 0.5 mm and weighed on a portable Gibertini electronic balance to the nearest 0.01 g. They were sacrificed and the ovaries weighed (fresh weight to the nearest 0.01 g). Two subsamples of each ovary were weighed and preserved in Gilson's solution (Montori, 1989). After the conjunctive tissue was dissociated, ovocytes were counted using a binocular. At least 75 ovocytes were measured from each sample to the nearest 10 μm with a micrometer. The gonadal index was computed as the ratio between ovary weight and body weight, multiplied by 100. Two alternative indexes were also computed as the ratio between the number of ovocytes per gram of body mass or per 0.1 g of ovary weight.

Toes cut from either living (i.e. marked and released individuals) or sacrificed individuals were stored in 80% alcohol and the skeletochronological procedure was done according to previous descriptions (Miaud et al., 1993) to estimate the age of each individual. A number of 42 samples were used in this study of which 38 allowed for age assessment (5 juveniles, 13 males and 20 females).

Normality of body length and weight were tested with Kolmogorov-Smirnov D-test (Siegel, 1956). Body length and weight were normally distributed in males and females of both species (each Kolmogorov D-test, $P < 0.005$) allowing comparisons with parametric tests (t-test). Annual adult survival rate (S), was calculated according to Robson and Chapman's formula (1961, in Miaud et al., 1999): $\mathrm{S} = \mathrm{T}/(\mathrm{R} + \mathrm{T} - 1)$, where $\mathrm{T} = \mathrm{N}_1 + 2\mathrm{N}_2 + 3\mathrm{N}_3 + 4\mathrm{N}_4 + \ldots$, $R = \sum \mathrm{N_i}$, $\mathrm{N_i}$ = number of individuals in age group i. Adult life expectancy (ESP), the expected total longevity of individuals which have reached maturity, was estimated using Seber's (1973) formula: $\mathrm{ESP} = 0.5 + 1/(1 - \mathrm{S})$. ESP is the expected average age and differs from the "longevity" value that is simply the highest recorded age. All tests were performed with the Statistica 5.0/W package (Statsoft Inc., USA).

The two sexes did not differ in either size or weight (t-test, $P > 0.1$) (table 1). Age was also similar in both sexes. The fire-bellied toad reached maturity at two years of age and the observed longevity was five years. The annual survival rate was estimated at 0.59 and adult life expectancy was 2.9 years. The mean age was estimated at 3.4 years (males: $n = 13, s = 0.93$; females: $n = 20, s = 1.04$). SVL was significantly correlated with age in females ($r = 0.56, n = 20, P < 0.01$) but not in males ($r = 0.26, n = 14, P < 0.1$).

The main parameters characterizing reproduction are presented in table 2. The total number of ovocytes was significantly correlated with wet weight ($r = 0.68, n = 16$, $P < 0.01$), SVL ($r = 0.69, n = 16, P < 0.01$), and ovary weight ($r = 0.70, n = 16$,

Table 2. The main parameters characterizing the reproductive effort in the studied fire-bellied toad population. All females were sampled before the start of the reproductive period. Where n represents the sample size and $\bar{x} \pm s_{\bar{x}}$ the average value with standard error.

Parameter	n	$\bar{x} \pm s_{\bar{x}}$	Range
Ovary weight (g)	17	0.32 ± 0.04	0.14-0.68
No. mature ovocytes	16	198 ± 27.2	59-492
Total no. ovocytes	16	306 ± 33.9	148-607
Ovocyte diameter (mm)	17	1.20 ± 0.04	0.92-1.62
No. ovocytes / 1 g body weight	16	102.1 ± 8.5	55.8-189
No. ovocytes / 0.1 g ovary weight	16	101.3 ± 8.5	55.8-189.7
Gonadal index	17	10.2 ± 0.67	7.03-15.08

$P < 0.01$). There was no significant correlation between age and ovary weight or total number of ovocytes ($P > 0.3$, $n = 10$). Taking into account the life expectancy of fire-bellied toad females, the average expected total reproductive output is 576 eggs (range 171-1423). Considering their small size and limited breeding opportunities this imposes a high annual relative reproductive effort. This high annual investment in reproduction of the fire-bellied toad might partly explain its reduced lifespan and limited growth. The larger amount of energy allocated to current reproduction induces lower growth rates and leads to smaller body size, which usually causes decreased future reproductive rates and often lower survivability (Kozłowski and Wiegert, 1987). In *B. bombina* the mean number of eggs deposited reported by Rafińska (1991) in Poland was 363. Fog (1996) reported for Denmark a mean number of 360 eggs deposited annually (ranging between 50 and 450 eggs). These values are higher even than the total number of ovocytes in the ovaries of the females from the floodplain population, in accordance with their smaller body size, since in amphibians, fecundity is related to body size (Jørgensen, 1992). The smaller size of females in the studied population is most probably the cause of the diminished reproductive effort. A similar lower reproductive effort due to smaller body size was reported from a smooth newt population (Cogălniceanu, 1999). Egg limitation imposes a trade-off between egg size versus egg number (Rosenheim, 1996). Since egg size cannot decrease below a certain threshold, to deposit a clutch of equal size, smaller females of *B. bombina* have to invest more in reproduction and resources allocated to somatic maintenance must be kept to a minimum. This finding supports the hypothesis of the negative trade-off between current reproductive effort and future reproductive success.

Stugren (1980) reported that in *B. bombina* from the former Soviet Union, SVL was smallest in the Danube Delta populations (mean value 36.5 mm, $n = 31$), similar to our present study. He suggested a geoclinal pattern of variation in body size starting from this point. Rafińska (1991) reported that females from a population in southern Poland had an average SVL of 47.08 mm, much higher than 35.23 mm in the studied floodplain population. Fog (1996) reported that in Denmark the maximum SVL of about 50 mm was reached at the age of 5-6 years, with a maximum life span of 12 years. Longevity in fire-bellied toads from Russia can reach 11 years (Ananjeva and Borkin, 1979, in

Smirina, 1994). In the related species, *Bombina variegata*, age could reach 12 years in a German population as estimated from successive recaptures data (Sy and Grosse, 1998), and even more than 20 years in Poland based on skeletochronology (Płytycz and Bigaj, 1993). Annual survival rates of *B. bombina* populations in Denmark as estimated from mark-recapture studies range between 0.5 and 0.9, depending on surrounding habitat (Briggs, 1996). Overall the life span of the floodplain population is severely reduced due to the highly variable and unpredictable environment. Skeletochronology proves to be a valuable tool that offers useful insights in the structural parameters of a population whose demography cannot be investigated using traditional methods due to the highly dynamic changes of the habitat.

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Low genetic diversity in Chinese *Hynobius leechii*, with comments on the validity of *Hynobius mantchuricus*

Xiaomao Zeng[1,2], Jinzhong Fu[3]

[1] Department of Biology, Eastern China Normal University, Shanghai 200062, China
[2] Chengdu Institute of Biology, The Chinese Academy of Sciences, Chengdu, Sichuan 610041, China
[3] Department of Zoology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Hynobius leechii Boulenger 1887 is distributed in northeastern China and Korea. Recent studies revealed substantial genetic variation among the Korean populations (Yang et al., 1997; Lee et al., 1998), with two cryptic species being discovered. Among the Chinese populations, Mori (1927) described a second species, *Hynobius mantchuricus*, based on a single specimen. Chang (1936) synonymized it with *H. leechii* without giving reasons, but Zhao and Adler (1993) resurrected the species name. Nevertheless, the Chinese populations have never been evaluated genetically. In this study, we examine genetic variation among the Chinese populations of *H. leechii* using allozyme electrophoresis and DNA sequencing.