

## A long term study on *Bombina variegata* (Anura: Bombinatoridae) in the “Parco dei Colli di Bergamo” (North-western Lombardy)

Maurizio DINO<sup>1</sup>, Stefano MILESI<sup>2</sup>, Anna Rita DI CERBO<sup>3\*</sup>

<sup>1</sup> Via della Libertà 39/a 24046 Osio Sotto (BG)

<sup>2</sup> Parco Regionale dei Colli di Bergamo, via Valmarina, 25, 24123 Bergamo (BG)

<sup>3</sup> Centro Studi Faunistica dei Vertebrati - Società Italiana di Scienze Naturali, C.so Venezia 55, 20121 Milano (MI)

\* Corresponding author: bombinatoridae@gmail.com

**Riassunto.** La presente ricerca si colloca nell'ambito di un progetto a lungo termine finalizzato alla salvaguardia delle popolazioni di *Bombina variegata* e dei suoi habitat riproduttivi nel Parco Regionale dei Colli di Bergamo. Nel periodo 1988-2009, sono state effettuate sessioni periodiche di catture che prevedevano l'identificazione individuale e la misurazione di ciascun animale, per un totale di 94 individui catturati e 412 tra catture e ricatture. La sex ratio complessiva (M:F) è risultata pari a 1.73:1. Rispetto ai dati annuali, è emerso che il rapporto sessi è stato per lo più (17 su 19 anni considerati) a favore dei maschi, con una differenza tra i due sessi altamente significativa nei diversi anni. I dati raccolti hanno permesso, inoltre, di effettuare una stima dell'età degli ululoni e ottenere dati in merito alla longevità della specie. Il 2,1% degli individui ha certamente raggiunto e probabilmente superato i 20 anni di età. I maschi hanno mostrato una maggiore longevità, ma la differenza tra i due sessi non risulta significativa. La mortalità osservata è stata pari al 2.1% della popolazione. Sono stati inoltre registrati spostamenti individuali fino a 960 m in linea d'aria dal sito principale. I risultati ottenuti in questo studio forniranno indicazioni utili per piani di conservazione, di protezione per le specie e per l'ampliamento delle aree riproduttive in Lombardia.

**Abstract.** The present work is part of a long term project on yellow-bellied toad conservation in the Parco dei Colli di Bergamo. During the 1988-2009 period a total of 94 yellow bellied toads (46 males, 34 females, 14 immature) were captured and identified. The frequency of recaptures did not significantly differ in males and females (Mann-Whitney Test:  $p > 0.05$ ). Overall sex ratio (M:F) was 1.73:1. Sex ratio differs significantly over the years (paired *t*-test:  $p < 0.001$ ). In the majority of the years (17) sex ratio was in favour of males,

in seven years (39%) it was greater than 2:1 (up to 3:1). The collected data of individual recaptures were considered in order to estimate the longevity of the species. 2.1% of adults have surely reached and probably exceeded 20 years of age. Males were on average older than the females, but the differences among the age classes are not statistically significant (Wilcoxon:  $p > 0.05$ ). The observed mortality in the studied population was 2.1%. At last, we recorded individual movement of toads during recaptures. The maximum distance, measured as the crow fly, was covered by a male (960 m). This study surveyed a *B. variegata* population living at the limit of its Italian geographic range. Moreover, our results were used to develop conservation plans for the species and to safeguard and enlarge the reproductive areas in Lombardy.

**Keywords.** *Bombina variegata*, population structure, sex-ratio, longevity.

The distribution of yellow-bellied toad, *Bombina variegata*, in Lombardy is fairly discontinuous and localized to the middle and northern part of the regional territory, specifically from Lecco to Brescia Prealps, with the majority of records in Bergamo province (Di Cerbo, 2001a; Di Cerbo and Milesi, 2004). In this area, the abundance of toads appeared to decrease in the last two decades (Di Cerbo and Ferri, 2000; Di Cerbo and Milesi, 2004; Giovine *et al.*, in this volume; Di Cerbo and Biancardi, pers. comm). At regional level, the species is protected (D.G.R. VIII/7736, 24 July 2008) and programs to monitor its *status* are advised (D.G.R. VII/4345, 20 April 2001).

The present work is part of a long term project on yellow-bellied toad conservation in the Parco dei Colli di Bergamo (north-western Lombardy). Thus, a *B. variegata* populations monitor program, extended also to areas around the Park, started in 1988 (Milesi S. and collaborators) and is still in progress (Dino, 2009).

The Park territory includes about 10 municipalities located in the north of Bergamo (total surface: 4800 ha; altitudinal range: 244-1148 m a.s.l.). It represents one of the most important areas of the Bergamo province in terms of herpetological diversity, including regional high priority of conservation species as well as species listed in annexed II, IV and V of the Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora.

Monitor program carried out since more than twenty years allowing the authors to collect a huge amount of data. Some of the studied topics were the structure and dynamics of the yellow-bellied toad population (numbers of animals, sex ratio, longevity, survival rate). Data have been also used in the purpose to develop conservation plans for the species and to protect and enlarge the reproductive areas (Dino, 2009).

During monitoring activity, the yellow-bellied toads have been captured, photographed, sexed, and measured (Fellers *et al.*, 1994; Scott and Woodward, 1994). Photos of the ventral pattern have been used for individual identification during recaptures (Donnelly *et al.*, 1994).

During the period 1988-2009 a total of 94 yellow bellied toads (46 males, 34 females, 14 immature) were captured and identified, 19 of them (20.2%) were identified only during the first capture, while the other 75 (79.8%) were recaptured at least once, to a maximum of 18 times (Table 1). The majority were recaptured only once (mode: 2). The frequency of recaptures did not significantly differ in males and females (Mann-Whitney Test:  $U=652.0$ ;  $p>0.05$ ).

Individual years of recaptures data (Table 1) are lower in females, however the difference is not statistically significant (Mann-Whitney Test:  $U=648.0$ ;  $p>0.05$ ).

		<b>Males (n=46)</b>	<b>Females (n=34)</b>	<b>Total (n=80)</b>	<b>Mann-Whitney</b>
<b>Recapture years</b>	Min-Max (median)	1-13 (4)	1-10 (3)	1-13 (3)	n.s.
	mean± SD	5,5±3,6	4,1±2,7	4,4±3,3	
<b>Recapture numbers</b>	Min-Max (median)	1-18 (4)	1-17 (4)	1-18 (4)	n.s.
	mean ± SD	6,6±5,0	4,9±3,8	5,3±4,5	

Tab.1. Summary statistics of yellow bellied toads recaptures in the Park.

Overall sex ratio (M:F) was 1.73:1. Difference to an expected sex ratio of 1:1 is highly significant ( $\chi^2= 28.37$ ; d.f.= 1;  $p < 0.001$ ).

Sex ratio significantly differs over the years (paired  $t$ -test;  $t = -7.08$ ; d.f. = 18;  $p < 0.001$ ). In the majority of years (17) sex ratio was in favour of males, in seven years (39%) it was greater than 2:1 (up to 3:1). The male-female ratio was balanced only during 2005, and slightly in favour of females in 2009.

Our results on annual sex ratio are similar to those reported by Di Cerbo and Biancardi (2004) on another Lombard population. But the authors also reported that seasonal ratio varied within the activity season (1:1 at the beginning of the season, skewed to males during reproductive phase and became in favour of females before hibernation). We didn't collect monthly data with a regular rate however this seasonal pattern seems to be also confirmed in our population.

Data collected over twenty-two years of individual recaptures were considered in order to estimate longevity of yellow bellied toad living in the Park. At this scope, we proceeded as follows:

-first, we calculated the total amount of recapture years of each toad ( $YR_x$ ) by subtracting the date of its last recapture ( $YL_x$ ) to the date of its first capture ( $YF_x$ );

-then, we added 0 to  $YR_x$  if the toad was a juveniles ( $J < 1$  year age), the value 1 if

subadult (S), and 2 if adult (A) at  $YF_x$ . Generally, yellow-bellied toads reach the sexual maturity between the second and the third year of their life (Lanza, 1983), even if, for some individuals, this can occur also at the fourth year (Di Cerbo, 2001b).

As for toads belonging to J and S at the first capture (16% of the total sample), we are sure that the estimated age corresponds to the real age of the individuals, for A at the first capture (84%), we only could estimate the minimum age, and in some cases we are persuaded that it was an underestimation. First, because we decided to add only the minimum age at sexual maturity (2 years), second at their first capture, some of our toads measured more than 40 mm (SVL), while the minimum size (SVL) of *B. variegata* to be considered adult is 27 mm for males and 28.5 mm for females (Lanza, 1983). Therefore we can suppose that at that time they were much older than 2 years. In figure 2, the age pyramid of yellow bellied toads living in the Park. The histogram doesn't represent the population age structure, as individual data are not referred to a single year. However, it shows some useful information about the longevity of *B. variegata*.

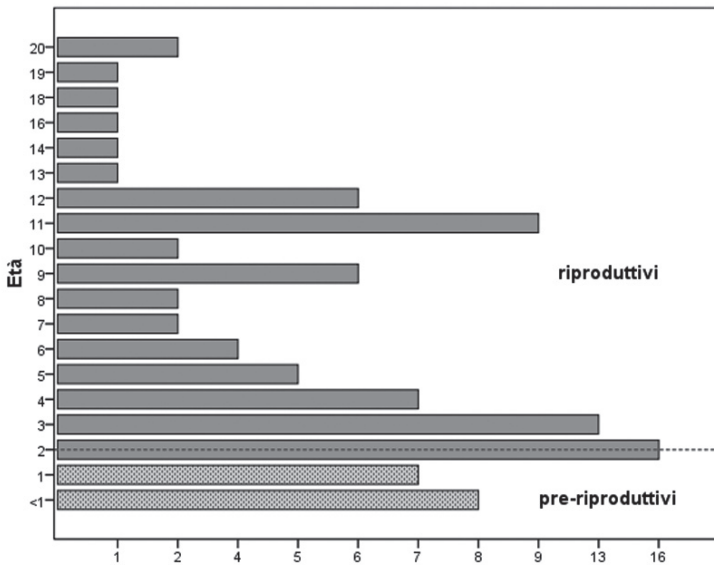


Fig. 2. The age pyramid of yellow bellied toads living in the Park.

58.5% of the adults were at least 2-10 years old, 25.5% were included in the estimated age classes between 10 and 19 years, 2.1% have surely reached and probably exceeded 20 years of age.

Considering the two sexes separately, males were on average older than the females (Table 2), but the differences among the age classes are not significant (Wilcoxon;  $z = -1.18$ ;  $p > 0.05$ ).

	Mean±SD	Min-max	25 <sup>th</sup> percentile	50 <sup>th</sup> percentile	75 <sup>th</sup> percentile
<b>Males</b>	7.6±5.1	2-20	3	6.5	11
<b>Females</b>	5.7±4.3	2-19	2	4	9
<b>All sample</b>	5.8±5	<1 -20	2	4	10

Tab. 2. Descriptive statistic on age classes of *B. variegata*. All samples (both adult and young): N=94; adult males: N=46; adult females N=34. Ages are reported in years.

Overall, the maximum longevity estimated for this species was 19 years (Seidel, 1996). In Italy, only few data on age of *B. variegata* are available and, to our knowledge, they only refer to Lombard populations, where the estimate longevity was at least 20 (this study), 19 and 8 years respectively (Di Cerbo, 2001b; Di Cerbo, pers. comm.)

The observed mortality of the yellow bellied toads of our population was 2.1% (two males of 3 and 4 estimated years old, respectively), but the death causes couldn't be determined. Again, we observed a low number of young individuals (16%). We couldn't investigate the specific causes of that (i.e. predatory pressure, low reproductive success of the population, etc.) and we can only suppose the following possible factors: i) high mortality of individuals during the immature phase; ii) high rate of dispersion of the newly metamorphosed toads; iii) a lower contactability of small toads due to an objective difficulty of detect them (at metamorphosis their size is 12-18 mm and their dorsal pattern is very cryptic) and to their elusive attitude when they are still not reproductive.

Moreover, we assessed the presence of *Natrix natrix* and imago and larvae of macro invertebrates (Odonata, Ditiscidae, Tricoptera, Naepidae) at the ponds inhabited by *B. variegata*. They are all potentially predators of tadpoles and juveniles of yellow-bellied toads (Di Cerbo and Bressi, 2007), and sometimes predations on tadpoles were observed. Anyway, the impact of natural factors with respect to anthropogenic ones was difficult to determine (Dino, pers. comm.).

At last, we could record individual data on movements of yellow bellied toads during recaptures. The maximum distance was covered by a male that moved from the main aquatic site to a new site far-away 960 m as the crow fly. Milesi *et al.* (2004) reported a similar distance for a *B. variegata* population living near the Park.

Summarizing, this long term study allowed us to collect useful information about the structure of the yellow bellied toad population living in the Park. This monitoring program is part of a specific project aimed at protecting and managing amphibians (*B. variegata* included) and their habitat in one of the most relevant protected areas of Lombard Prealps in terms of herpetological diversity (Dino, pers. comm.). The high longevity detected is a positive factor in the general framework of fragmentation of yellow-bellied toad populations. Empowerment of suitable reproductive sites and the creation of ecological corridors to

improve the movements and dispersal of adults and young are the priorities of current conservation projects.

Moreover, the few quantitative data on abundance, sex and age structure of *B. variegata* available in Italy seems to point at higher number of individuals in the eastern region populations (Di Cerbo and Bressi, 2007; Di Cerbo and Milesi, 2004). Thus, our results could help to better understand the composition of those populations living at the limit of its Italian geographic range.

## Aknowledgements

This work is dedicated to the memory of prof. Francesco Barbieri (University of Pavia), we are also grateful to Cristina Crestani (Park “Colli di Bergamo”) for her invaluable help in the field, prof. Paolo Tremolada (University of Milan) for his useful suggestion, Dr. Carlo Biancardi (Centro Studi Faunistica dei Vertebrati, Società Italiana di Scienze Naturali) for the manuscript revision.

## References

- Di Cerbo, A. R. (2001a): Ecological studies on *Bombina v. variegata* (Linnaeus, 1758) in Alpine habitats. (Anura: Bombinatoridae). 1st International Scientific Meeting The biology and ecology of alpine amphibians and reptiles. 1 - 3 September 2000. *Biota* 2 (1): 17-28.
- Di Cerbo, A. R. (2001b): Accrescimento e struttura dell'età in una popolazione di *Bombina v. variegata* (Linnaeus, 1758) (Anura: Bombinatoridae). In: Atti 3° Congresso Nazionale S.H.I. (Pavia, 2000), *Pianura*, 13: 255-258.
- Di Cerbo, A. R., Biancardi, C.M. (2004): Seasonal activity and thermobiology of *Bombina v. variegata* (Linnaeus, 1758) in Lombardy (Seriana Valley, Northern Italy). *It. J. Zool.* (2004) suppl. 2: 143-146.
- Di Cerbo, A.R., Bressi, N. (2007). *Bombina variegata* (Linnaeus, 1758). In: Fauna d'Italia, vol. XLII, Amphibia, p. 280-287. Lanza, B., Andreone, F., Bologna, M.A., Corti, C., Razzetti, E., Eds, Calderini, Bologna.
- Di Cerbo, A.R., Ferri, V. (2000). La conservazione di *Bombina variegata variegata* (Linnaeus, 1758) in Lombardia. In: Atti del I Congresso della Societas Herpetologica Italica (Torino, 2-6 Ottobre 1996), 713-720. Giacomina, C., Ed, Mus. Reg. Sci. nat. Torino.
- Di Cerbo, A.R., Milesi, S. (2004). Ululone dal ventre giallo (*Bombina variegata*). In: Atlante degli Anfibi e Rettili della Lombardia, p. 81-83. Bernini, F., Bonini, L., Ferri, V. Gentilli, A., Razzetti, E., Scali, S., Eds, Monografie di Pianura 5. Provincia di Cremona, Cremona.
- Dino, M. (2009). Gli Anfibi nel Parco Regionale dei Colli di Bergamo: indagini per la gestione e il potenziamento degli habitat riproduttivi di *Bombina variegata* e delle altre specie prioritarie. Unpublished master thesis, University of Milan.

- Donnelly, M. A., Guyer, C., Juterbock, J. E., Alford, R. A., (1994): Techniques for Marking Amphibians. In: Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians, p. 277-284. Heyer, W. R., Donnelly, M. A., Mcdiarmid, R. W., Hayek, L. A. C., Foster, M. S., Eds., Smithsonian Institution Press, Washington.
- Fellers, G.M., Drost, C.A., Heyer, W.R. (1994): Handling Live Amphibians. In: Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians, p. 277. Heyer, W. R., Donnelly, M. A., Mcdiarmid, R. W., Hayek, L. A. C., Foster, M. S., Eds., Smithsonian Institution Press, Washington.
- Giovine, G., Di Cerbo, A.R., Corbetta, A., Dino, M. (2010). alvanguardia degli anfibi e dei loro habitat riproduttivi nel Parco Regionale delle Orobie Bergamasche (Lombardia): criticità e indicazioni per il recupero degli habitat acquatici. In: Atti VIII Congresso Nazionale S.H.I. (Chieti, 22-26 settembre 2010), p. 493-502. Di Tizio L., Di Cerbo A.R., Di Francesco N., Cameli A. (Eds), Ianieri Edizioni, Pescara.
- Lanza, B. (1983): Guide per il riconoscimento delle specie animali delle acque interne. 27. Anfibi, Rettili. Collana del Progetto finalizzato "Promozione della Qualità dell'ambiente AQ/I/205. Consiglio Nazionale delle Ricerche.
- Scott, N.J. Jr, Woodward, B.D. (1994): Surveys at breeding sites. In: Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians, p. 118-125. Heyer, W. R., Donnelly, M. A., Mcdiarmid, R. W., Hayek, L. A. C., Foster, M. S., Eds, Smithsonian Institution Press, Washington.
- Seidel, B. (1996): Streifzug durch die Verhaltens-und Populationsbiologie von Gelbbauchunken, *Bombina variegata* (L., 1758) (Anura: Bombinatoridae), in einem Habitat mit temporären Gewässern. Naturschutzreport 11: 16-31.