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ASSESSING THE EFFECT OF TOE CLIPPING ON THE YELLOW BELLIED TOADS

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In this paper we analyze/investigate the effect of toe clipping on *Bombina variegata,* by comparing the body conditions of marked and unmarked individuals. We marked 395 adult *B. variegata* in 2003 and 413 in 2004. In 2005 we measured the SVL and wet body weight of 27 recaptured individuals and compared this with unmarked individuals from the population. We found no significant effect of toe clipping on the body condition. These results, together with examination of the health of recaptured toads and behavioural data indicates that toe clipping has no negative effect. Nevertheless, caution is needed in using the toe clipping method because the recently discovered and spreading chytrid fungus that could infect the marked amphibians and cause chytridiomicosis.

Key words: toe clipping, body condition, amphibians.

INTRODUCTION

The mark-recapture approach is considered to be the only reliable method for the analysis of demography, population size, and survival rate (SCHMIDT 2003, BEAUSOLEIL *et al.* 2004).

Toe clipping is the most frequently used marking technique in amphibians (PARRIS & MCCARTHY 2001, MCCARTHY & PARRIS 2004). Since toe clipping involves some level of tissue damage, pain and stress may have a (negative) effect on the movement or feeding, and can increase the risk of infection to the individual. The scientific and ethical aspects of its use are currently under debate (MAY 2004, FUNK *et al*. 2005). However, it has to be pointed out that clipping toes is a suitable method for age determination (YILMAZ *et al.* 2005), an aspect which enhances the benefits of the toe clipping method over its costs.

An animal's fitness, survival and reproductive success are in direct relationship with its energetic state, that is, body condition. Body condition is therewith an important parameter in ecological studies. It allows comparison of the state of populations that face different environmental conditions (COGĂLNICEANU 1997), and allows us to assess the effect of techniques applied to the model organism required for the data collection process, such as marking or tracking methodologies (JEHLE & HÖDL 1998, ARNTZEN *et al.* 1999, PERRET & JOLY 2002).

In 1997 we begin a long term study of an amphibian community that reproduces in a permanent seminatural pond in the middle section of the Târnava Mare Valley (HARTEL 2004). Being closely situated to the town of Sighişoara, the whole area is threatened due to an urbanistic plan (HARTEL & MOGA 2003, unpubl. report to EPA). It was an urgent need to extend the monitoring on an amphibian community that uses a number of temporary ponds in a deciduous forest; at about 800–1500 m from the constant seminatural pond. Beginning with 2003, we monitored the number of temporary ponds through the different seasons (and years) and the reproductive success of the amphibian community that use them. To understand the role of ponds with different hydroperiod in the life of amphibians living here data about the factors affecting temporary pond use and movement intensity was needed. To study this, we chose a typical temporary pond breeder, the yellow bellied toad. Due to the multiple breeding periods within a season (BARANDUN & REYER 1997) and the intense movement of individuals between the ponds (BARANDUN & REYER 1998) the yellow bellied toad is a perfect candidate for both habitat use and movement pattern studies. We used toe clipping to mark toads.

In this paper we examine the effect of toe clipping on *Bombina variegata* by comparing the body condition of marked and recaptured individuals with unmarked ones.

MATERIALS AND METHODS

The study area is a section in the middle of the Târnava Mare Valley, and has approximately 1.5 km^2 area. The temporary pond network is formed by a number of 75 ponds (Fig. 1), with a high variety of hydroperiods, ranging from 4 days to two years.

The average pond area is 22 m (range: 1–250 m, SD = 44), and the depth averages 23 cm (range 3–100 cm, SD = 21).

A number of six ponds, with the longest hydroperiod, were used for reproduction every year (2003–2005) (black circles in the Fig. 1), and the number of toads in these ponds was the largest. Consequently the largest number of toads was marked in these ponds.

In 2003, we spent 21 days, and in 2004, 24 days marking/recapturing toads and monitoring the number of the temporary ponds. In 2005 we spent 8 days in the field recapturing and measuring marked toads (the second part of July–first part of August).

A total of 395 toads were individually marked in 2003, using the methodology presented by HERO (1989). This marking methodology allows the first 99 individuals to be marked with as much as 2 toes clipped and up to 736 individuals to be marked with clipping three digits. In 2004 pond specific marks were used to mark 413 individuals, with one toe removed per individual.

The snout vent length (SVL) (0.1 mm precision) and wet body mass (0.01 g precision) were measured in 41 unmarked and 27 marked and recaptured toads from this population.

In both years we carefully examined all the recaptured individuals to identify signs of infection in the injured toes.

Factorial ANOVA's were run to test differences in means at both body length (SVL) and body mass (M). A General Linear Model (GLM) procedure was used as an exploratory tool to link the response variable body mass to the categorical variables gender and marking status and the continuous variable snout-vent length (SVL) predictors (see GARCÍA-BERTHOU 2001, DARLINGTON & SMULDERS 2001). Both M and SVL have a normal distribution (χ^2 Goodness of Fit; W: $\chi^2 = 2.52$, df = 3, $P > 0.05$; SVL: $\chi^2 = 4.65$, df = 5, $p > 0.05$) and show homogeneity of variance (Hartley F-max, all $P > 0.3$) and consequently, no data transformations had been applied prior to significance testing.

RESULTS

In 2003 we recaptured 17% of the marked toads. In 2004, 10% of the toads marked in 2004, and 14 % of the toads that were marked in 2003 were recaptured. In both years 157 individuals (=19.2%) were recaptured once, and five individuals more than once. No sign of infection was found in the recaptured individuals. Reproductive behaviour of many recaptured toads apparently was not affected by the toe clipping (we found paired marked toads and singing males).

Body length does not differ significantly between the sexes ($F_{[1, 64]} = 0.01$; $P = 0.908$), and between marked or unmarked toads ($F_{[1, 64]} = 1.623$; $P = 0.207$) (Table 1). Significant interaction between gender and marking is not found ($F_{[1, 64]} = 0.156$; $P = 0.694$). As expected, body mass co-varies significantly with size

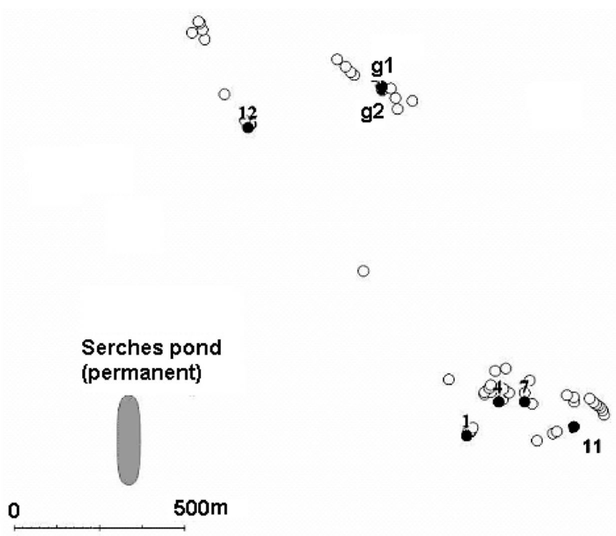

Fig. 1. The map of the study area. The permanent pond (Şercheş) and the temporary pond network (open and black circles) are showed. Black circles represent those temporary ponds that where used for breeding by toads in all three years.

Table 1. Descriptive statistics for body mass and snout vent length at different levels of factors.

Effect	Level of factors	Level of factors	N	Mean	SD	L95%CI	U95%CI
Body mass							
Marking	Unmarked		41	6.60	1.61	6.09	7.11
Marking	Marked		27	6.89	1.74	6.20	7.58
Sex	Females		49	6.77	1.76	6.27	7.28
Sex	Males		19	6.56	1.37	5.90	7.22
Marking*Sex	Unmarked	Females	32	6.64	1.64	6.05	7.23
Marking*Sex	Unmarked	Males	9	6.46	1.59	5.23	7.68
Marking*Sex	Marked	Females	17	7.02	2.01	5.99	8.05
Marking*Sex	Marked	Males	10	6.66	1.22	5.79	7.53
Body length							
Marking	Unmarked		41	40.95	0.75	39.45	42.45
Marking	Marked		27	42.47	0.92	40.62	44.31
Sex	Females		49	41.45	0.69	40.06	42.84
Sex	Males		19	41.84	1.12	39.61	44.07
Marking*Sex	Unmarked	Females	32	41.04	0.86	39.31	42.76
Marking*Sex	Unmarked	Males	9	40.66	1.62	37.41	43.91
Marking*Sex	Marked	Females	17	42.21	1.18	39.85	44.58
Marking*Sex	Marked	Males	10	42.90	1.54	39.81	45.98

($F [1, 63] = 150.6$; $P < 0.0001$), but neither gender ($F [1, 63] = 1.57$; $P = 0.213$), marking ($F [1, 63] = 0.60$; $P = 0.440$) or the interaction of gender and marking ($F[1,63] = 0.92$; $P = 0.338$) have significant effect on body weight. The two categorical and the continuous predictor variables together explain 70.89% of the variations observed in body weight. SVL alone explains 69.2% of variability in body mass, whereas gender and marking together explains less than 2% of the variations in body mass.

DISCUSSION

The negative effects of toe clipping in amphibians has been observed, such as inflammation and infection of feet and limbs, and a reduction in the return rate of marked animals (CLARKE 1972, REASER & DEXTER 1996). PARRIS and MCCARTHY (2001) show that the absence of a statistically significant effect of toe clipping on amphibians found in different studies (i.e. LEMCKERT 1996, WILLIAMSON & BULL 1996) could be attributed to a lack of statistical power rather than the absence of an actual effect. Based on fitted regression lines, they estimated that return rates declined by 4–11% for each toe removed (MCCARTHY & PARRIS 2004).

The effect of toe clipping on body condition in amphibians is not yet clear. We supposed that the body condition of the marked individuals before marking did not differ significantly from the body condition of other individuals from the population. This was based on measurements of SVL and body mass of several toads from four different populations along the Valley, during the field trips in July–August (HARTEL & NEMES, unpubl. data). Thus, we supposed that any difference in the body condition of the marked individuals from those that were not marked is the result of the effects of toe clipping. The absence of a statistically significant difference for body condition in marked and unmarked toads may reflect a minimal effect of toe clipping.

The prediction of a lower body condition due to e.g. the energy demands of wound recovery or possible post traumatic infections was not confirmed by the present study. This might have at least two reasons:

(i) zero or minor effect of toe clipping on the toads;

(ii) differential survival rates of marked and unmarked toads.

A careful examination of all the recaptured individuals during the seasons shows no signs of infection. From this we conclude that the toe clipping had no negative effect in the short term on the recaptured individuals. The reproductive behaviour of many recaptured toads apparently was not affected by the toe clipping; we found paired marked toads and singing males.

The recapture rate in this study (10–17% within years and 19% between years) is larger than that found by SANDS (2005) and MARA (unpubl. data) in a hybrid population in Transylvanian Heath (Romania): 6.2–10.7% within years and 1.5–8.2% between years. Similarly, a very low recapture rate was found in *Rana sylvatica* by BERVEN and GRUDZIEN (1990): 2.49–7.4% recaptures within years and 5% recaptures overall. In all three studies the individuals toes were clipped. However, the results of all these studies are in sharp contrast with those found by GOLLMANN and GOLLMANN (2000) in a yellow bellied toad population in Austria: a recapture rate of 78–81%. In this study belly pattern photography was used to identify the recaptured toads. However this extremely high recapture rate might not stand in all natural populations, future comparative studies are needed.

Differences in the recapture rates in different studies could be related to many factors, such are emigration, mortality, inactivity, sampling design or sampling effort. For example GREENBERG *et al.* (2005) in a long term study carried out on *Scaphiopus holbrookii* used drift fences that encircled 50% of the ponds. Between 1994–2003 they marked 2986 toads using toe clipping, and recaptured 12.7% of them. The sampling effort was large (three times weekly) involving many persons. Moreover they found that 95% of the adult recaptures occurred at the pond where they were first captured. The inter-pond exchange of adults was minimal and at short distances (<130 m and one recaptured at 416 m), so the emigration was not a major cause of the low recapture. A possible explanation of these results was that a great majority of individuals remained in their terrestrial habitats over a long periods of time.

In our case, the sampling effort (one person searching for toads) and design (lack of drift fence) are the main logistical causes with which the low recapture rate could be associated. On the other hand, the absence of the toads from the ponds due to roaming (BARANDUN & REYER 1998) could cause a low recapture rate. We recaptured toads at a distance of about 1300 m outside the breeding area (HARTEL unpubl.), in terrestrial areas. This indicates that both terrestrial habitat use and emigration could cause low recapture rates from the ponds.

Most basic knowledge on amphibian population dynamics has been gathered entirely or in part with the help of toe clipping method (i. e. GILL 1978, BERVEN & GRUDZIEN 1990, READING *et al*. 1991, LIPS 1998; recent papers include GRAFE *et al*. 2004, GREENBERG & TANNER 2005). However, the recent discovery of the quickly spreading chytrid fungus (*Batrachochytrium dendrobatidis*) and its negative effect on amphibian populations worldwide (RACHOWITZ *et al*. 2005) including Europe (GARNER *et al*. 2005), invites a reconsideration. In toads with an outspoken belly pattern such as *B. variegata* individual recognition is possible without physical marking. Habitat use, demography and movements have been studied this way in the short term (BARANDUN & REYER 1998, GOLLMANN *et al*. 1999, 2000), and in long term studies (GOLLMANN & GOLLMANN 2005).

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