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PITS VERSUS PATTERNS: EFFECTS OF TRANSPONDERS ON RECAPTURE RATE AND BODY CONDITION OF DANUBE CRESTED NEWTS (*TRITURUS DOBROGICUS*) AND COMMON SPADEFOOT TOADS (*PELOBATES FUSCUS*)

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During a long-term study (1987-1996) near Vienna (Austria), individual Danube crested newts (*Triturus dobrogicus*) and common spadefoot toads (*Pelobates fuscus*) were registered by photographs of highly variable skin patterns, and the implantation of Passive Integrated Transponders (PIT tags). To test for potential detrimental effects of the electronic tags, we compared within-year (for *P. fuscus*) and between-year (for *T. dobrogicus*) recapture rates and body condition index (CI) of individuals marked with the two alternative techniques. No significant negative effects of PIT tags on CI and recapture rate could be demonstrated for either species. There was a decrease in body condition and recapture rates in the study years 1995 and 1996, but can be attributed to population trends rather than to the different marking methods. For recaptured *T. dobrogicus* a 10.5% loss of PIT tags was observed.

INTRODUCTION

One of the major difficulties in conducting studies on free-ranging amphibian populations is the selection of a suitable marking technique. Compared to other vertebrates, amphibians are especially difficult to mark permanently on an individual level (for reviews see Ferner, 1979; Donnelly, Guyer, Juterbock & Alford, 1994; Henle, Kuhn, Podloucky, Schmid-Loske & Bender, 1997). Toe clipping is the most commonly applied technique, but may pose some problems: the number of individual codes is limited, and for European newts, this technique is applicable only for a limited time scale due to their regeneration ability (Andreone, 1986). In some cases, a negative effect of toe clipping on recapture rates was reported (Clarke, 1972; Golay & Durrer, 1994). Being a putatively non-invasive method, pictures of individual spot patterns have been used successfully for identification of appropriate species (Hagström, 1973; Gill, 1978; Arntzen & Teunis, 1993; Jehle, Hödl & Thonke, 1995). In recent years, the implantation of Passive Integrated Transponders (PIT tags) was introduced as a marking method for reptiles and amphibians (Camper & Dixon, 1988). PIT tags are glass-encased electromagnetic coils (approx. size: 10 x 2.1 mm) bearing a unique alphanumeric code that is read by generating a low-frequency electromagnetic signal with an external reader. Several field studies have been conducted to test for potential harmful effects of implanted transponders on snakes and lizards (Germano & Williams, 1993; Keck, 1994; Jemison, Bishop, May & Farrell, 1995). For amphibians, the application of this technique has been thoroughly described (Sinsch, 1992; Faber, 1997), and some laboratory and field-enclosure studies exist comparing PIT-tagged with untagged toads and newts over periods of up to a few months (Corn, 1992; Fasola, Barbieri & Canova, 1993). So far, only a single investigation on free-ranging amphibian populations has been conducted to test for detrimental effects of implanted transponders by calibrating the results with alternative techniques [Brown (1997), on *Bufo bufo* and *Rana temporaria*]. For most species on which this method has already been applied, long-term field data are lacking.

During a long-term study near Vienna (Austria), two out of eleven investigated species (*Pelobates fuscus*, the common spadefoot toad, and *Triturus dobrogicus*, the Danube crested newt) were recognizable individually (Thonke, Jehle & Hödl, 1994; Jehle *et al.*, 1995; Hödl, Jehle & Gollmann 1997). Individuals were registered using photographs of ventral spot patterns over six study years (for *T. dobrogicus*), and using dorsal patterns over seven years (for *P. fuscus*). As the workload for comparing pictures increased with study duration, the method of pattern mapping was replaced by the implantation of PIT tags in the 8th year (Hödl *et al.*, 1997). This enabled us to test possible detrimental effects of implanted transponders on these two species. In this paper we present a comparison of within-year and year-to-year recapture rates and body condition of two amphibian species in the field, emphasizing differences between individuals marked with the two alternative techniques. Although Faber (1997) presents field results on recapture rates of PIT-tagged alpine newts (*T. alpestris*), data derived from free-ranging urodeles are for the first time compared with those from individuals recognizable by other methods.

MATERIALS AND METHODS

The study was conducted from 1986 to 1997 (with the exception of 1988). Data presented here encompass the years 1987 and 1989-1995 for the spadefoot toad; and 1987 and 1989-1996 for the crested newt. Study animals were collected using a drift fence and pitfall traps completely encircling the breeding pond (Dodd & Scott, 1994; Arntzen, Oldham & Latham, 1995). A detailed description of the study site and the fence system is given in Hödl *et al.* (1997). The traps were checked daily between 0700 and 0900 hrs, and the animals were processed immediately after capture. For measuring, weighing, photography and PIT tag implantation, individuals were transported to a laboratory about 1 km away from the study pond. As amphibians can substantially lose mass due to water loss in a dry environment (pers. obs.), all study animals were immersed in water for at least 10 min and then weighed to the nearest 0.1g using an electronic balance. Snout-vent length (SVL) was measured with a vernier calliper to the nearest 1 mm (1987, 1989) or 0.1 mm (from 1990 onwards). In *T. dobrogicus* this measurement was taken from the tip of the head to the end of the cloaca, while the same in *P. fuscus* was from the tip of the head to the posterior end of the body. For *T. dobrogicus*, several attempts to straighten the wriggling animal were necessary before taking the measurement. After processing, the study animals were released on the opposite side of the drift fence to the point of capture, the presumed direction of migration. Transponders were applied from August 1994 onwards; prior to this date individuals were identified by photography only. Due to their small size, *T. dobrogicus* weighing less than 2 g (after Fasola *et al.*, 1992) and *P. fuscus* metamorphs were photographed but not PIT tagged. Once a PIT-tagged individual was recaptured, no further photographs were taken. In order to test whether an individual had lost its tag, adult catches without transponders were both PIT tagged and photographed, and the obtained pictures were compared with previous photographs.

Prior to implantation, the study specimens were anaesthesized using a 1:500-1:1000 solution of MS 222 (Ethyl-m-Aminobenzoate-Methanosulfonate, Sandoz). After 10-20 minutes, the muscular system was relaxed and the animals stopped moving. For *T. dobrogicus*, the transponders [Trovan (Germany), supplied by Datatronic (Wr. Neustadt, Austria)] were implanted in the abdominal body cavity using a sterile hypodermal syringe supplied by the vendor. The needle was inserted laterally in the posterior one-third of the body, oriented with its beleved surface away from the venter and directed towards the anterior part of the body. To prevent injury to the intestines, the piece of skin where the needle was positioned was lifted with two fingers, enabling an insertion almost parallel to the anterior-posterior body axis. Visible bleeding occurred only in rare cases. After the implantation, the wound was closed using the tissue glue VET SEAL (after Faber, 1997). For *P. fuscus*, PIT tags were injected into the subcutaneous lateral lymph sacs. Basically, the same technique was used but without the need to penetrate the body cavity. No obvious complications occurred during the implantation procedure. The animals were released as soon as they had recovered from anaesthesia.

Amphibians entering a breeding pond are in a different physiological condition than animals leaving a reproductive site (Verrell, Halliday & Griffiths, 1986). Therefore, apart from separating the sexes, immigrants and emigrants were treated differently during the statistical analysis. For female *P. fuscus*, we observed a substantial mass loss during the aquatic phase, most probably due to oviposition (unpublished data). *Pelobates fuscus* stay for a relatively short time inside the fenced area [average 1987, 1989-1995: 22.9 days (females), 37.3 days (males), data from Wiener, 1997]. They were PIT tagged for the first time when entering the pond in 1995 and re-registered when leaving the breeding site in the same season. Therefore, within-year recapture rates were compared for this species.

In 1994, 35 (48%) of the registered *T. dobrogicus* (13% of the total population including juveniles) were captured after August and PIT tagged. A comparison of between-year recapture rates between 1994/95 and other study years is therefore possible, although it has to be considered that individuals from 1994 were only PIT tagged rather late in that year, whereas recaptures in 1995 were PIT tagged over the whole season.

Chi-square tests with Yates' correction were used, for samples with both categories exceeding zero, to test for deviations from even distributions of recapture rates of pattern-mapped individuals and individuals with transponders. A condition index (CI) was calculated for both species with the formula CI = (mass [g])/(SVL [cm])^3 x 100 (Weatherley, 1972). The Mann-Whitney *U*-test was used to test for significant differences in CI in the same year; for significant differences between study years, analysis of variance (ANOVA) was applied.

RESULTS

Adult individuals without transponders, which were captured after the introduction of PIT tags, were both photographed and PIT-tagged, and they were compared with the photographs of the captures from previous

TABLE 1. Between-year recapture rates for *T. dobrogicus*. Data from the first five rows were obtained using pattern mapping. See text for juveniles and subadults. * Data from Jehle *et al.* (1995) and Ellinger & Jehle (1997).

Triturus dobrogicus	Females	Males
1989/90*	15/44 (34%)	20/43 (47%)
1990/91*	11/32 (34%)	19/36 (53%)
1991/92*	18/45 (40%)	15/44 (34%)
1992/93*	18/40 (45%)	9/41 (22%)
1993/94*	10/41 (24%)	7/32 (22%)
Weighted mean 1989/90-93/94	36%	36%
1994/95		
PIT-tagged	7/25 (28%)	4/10 (40%)
Pattern-mapped	8/36 (22%)	6/23 (26%)
χ^2	$P>0.05$	$P>0.05$
1995/96		
PIT-tagged	5/39 (13%)	0/20 (0%)
Pattern-mapped	-	-

TABLE 2. Within-year recapture rates of *P. fuscus*. Data without special indications refer to pattern-mapped individuals.

	Females	Males
1989	60/78 (77%)	77/138 (57%)
1990	31/58 (53%)	34/55 (62%)
1991	19/30 (63%)	15/27 (56%)
1992	22/32 (69%)	28/48 (58%)
1993	48/66 (73%)	101/137 (74%)
1994	117/146 (80%)	95/155 (61%)
Weighted mean 1989-1994	72%	62%
1995 (PIT-tagged)	67/111 (60%)	106/139 (76%)

years. With this procedure, two lost transponders (0.8% of the recaptured individuals) were recorded for *P. fuscus*, and six for *T. dobrogicus* (10.5% of all within and between-year recaptures).

In 1994/95, PIT-tagged male and female *T. dobrogicus* had higher (but not significantly higher) year-to-year recapture rates than individuals which were only photographed (Table 1). Adult recapture rates between 1995 and 1996 were very low. Between 1994 and 1995, none of the 40 juveniles with transponders was recaptured, whereas 7% (n_{total} = 645) non-PIT-tagged *T. dobrogicus* were recaptured. For juveniles from 1995, 18% (n_{total} = 11) of PIT-tagged individuals and 9% (n_{total} = 877) of pattern-mapped indi-

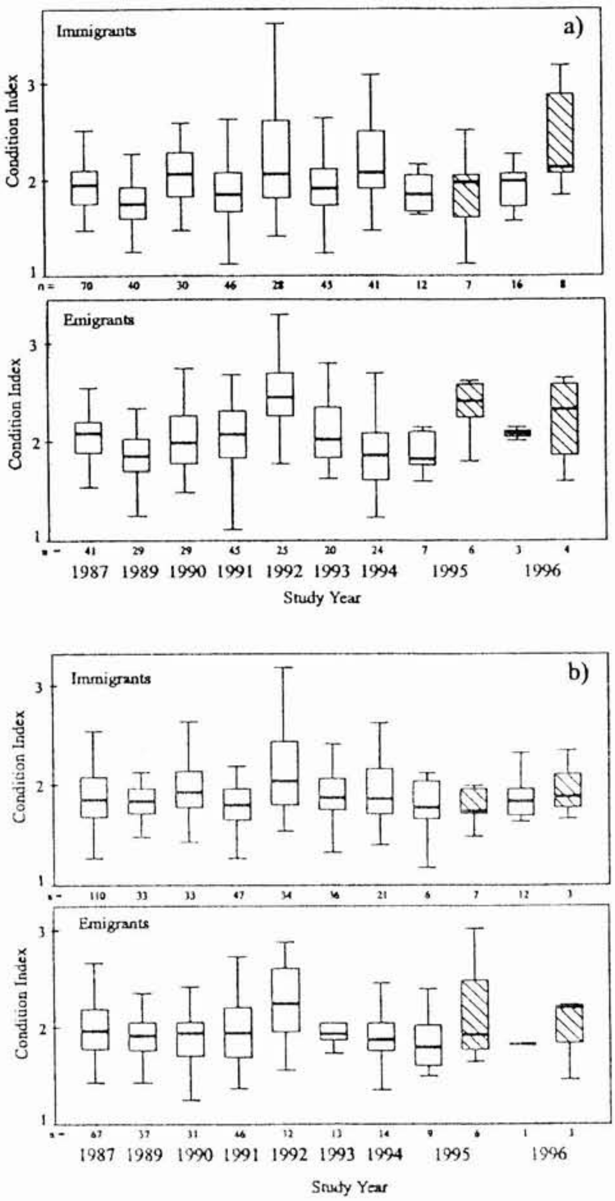

FIG. 1. Condition index of *T. dobrogicus*. Boxplots without special indications represent data obtained from pattern-mapped individuals, hatched boxplots represent data obtained from PIT-tagged individuals. (a) Females, (b) Males.

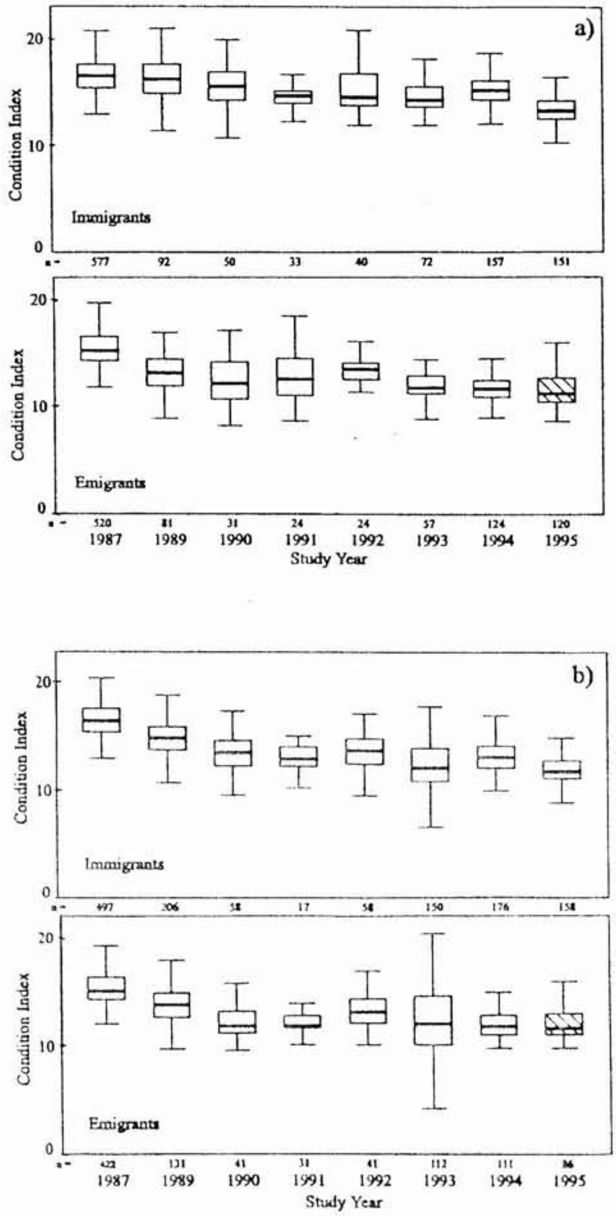

FIG. 2. Condition index of *P. fuscus*. Boxplots without special indications represent data obtained from pattern-mapped individuals, hatched boxplots (emigrants from 1995) represent data obtained from PIT-tagged individuals. (a) Females, (b) Males.

viduals were recaptured at the pond in 1996. Recapture rates for PIT-tagged female *P. fuscus* were low, but within the range of values obtained from previous years (Table 2). PIT-tagged male *P. fuscus* showed the highest value recorded.

When photographs of skin patterns were used exclusively for both species, CI values differed significantly between the study years (ANOVA: *F*-values ranging from 3.48 for *T. dobrogicus* male emigrants to 108.84 for *P. fuscus* female emigrants, $P<0.01$ in all cases). Therefore, statistical analysis between study years to test the effect of the identification method is not appropriate. In general, *T. dobrogicus* had a higher condition index when leaving the pond (Fig. 1), whereas *P. fuscus* had higher values when entering the breeding site (Fig. 2). For both species, the study years 1995 and 1996 were characterized by low CI values compared to previous years. In seven out of eight cases, PIT-tagged *T. dobrogicus* had higher CI values than pattern mapped individuals (Fig. 1); in one case this difference is actually significant (female emigrants of 1995, $P<0.05$). PIT-tagged *P. fuscus* showed the lowest condition index recorded (Fig. 2). However, equally low CI values of the non-PIT-tagged immigrants of the same year were observed, suggesting a year-specific rather than a methodological effect for both sexes.

DISCUSSION

For both scientific and conservation purposes, marking techniques should have no negative influence on the animals under investigation. No significant long-term detrimental effects of transponders on recapture rate and body condition could be demonstrated in this study. However, the influence of habitat-related factors and/or climatic conditions on yearly variation in recapture rates and especially CI values seemed to be substantial, making statistical tests between study years impossible. Nevertheless, CI was used for analysis of somatic condition. The observed values of individuals marked with transponders could be calibrated against variability of CI in general, and, in the case of *P. fuscus*, PIT-tagged emigrants could be compared to non-PIT-tagged immigrants in 1995. Individual growth rates were very variable in different seasons, and were therefore less useful as the animals were captured only at irregular intervals. The data presented in Brown (1997) on *B. bufo* and *R. temporaria*, showing no differences in mass-length relationships and recapture rates between PIT-tagged and dye-marked individuals, are statistically more meaningful, as both methods were applied simultaneously. However, Brown (1997) had sufficient data only for male *B. bufo*, whereas we present a data set for two species and both sexes, divided into immigrants and emigrants.

This study depends upon the assumption that pattern mapping has no effect on the animal and is error-free. Error levels of the photographic method were not included in the analysis. Several field workers independently checked the photographs, and the proportion of individuals which were registered twice at the same side of the fence (i.e. erroneously matched captures or individuals which trespassed the fence without registration) was as low as 3.51% (Hödl *et al.* 1997). Thus, the error level for pattern-matching is assumed to be low (<3%), although in another study, a value of 14% was recorded (cited in Sweeney, Oldham, Brown & Jones, 1995).

The *T. dobrogicus* recapture rate between 1995 and 1996 was only 9% compared to an average of 41% between 1989 and 1994. Only one newt, PIT tagged in 1994, was re-registered in 1996. The decrease of CI values in 1995 and 1996 for both species is consistent with a reduction of the adult population size (especially for *T. dobrogicus*, unpublished data). However, the non-PIT tagged juvenile *T. dobrogicus* emerging from the pond in 1996 showed a 30.0% lower mass and a 19.8% lower total length compared to the mean of 1987 and 1989-1995. Recapture rates of pattern-mapped juveniles from 1995/96 were reduced to 57% of the mean value from 1989/90 - 1993/94. Non-PIT tagged emigrating toads from 1995 also had very low CI values (Fig. 2). Thus, year-specific population traits seem to be responsible for the observed difference in the measured parameters rather than effects of transponders.

Higher recapture rates of PIT-tagged newts in 1994/95 (Table 1) can be attributed to the fact that they were exclusively registered in the second half of 1994, whereas pattern-mapped individuals were last seen when entering the pond in spring. Therefore, they were traced back over a longer time span, and were exposed to the risk of mortality for longer. The fact that PIT-tagged *T. dobrogicus* had (in one case, significantly) higher CI values when recaptured in 1995 may be due to the fact that most of the pattern-mapped individuals (i.e. individuals which were registered before, but not after the breeding season of 1994, and again in 1995) spent the winter inside the fenced area or even in the pond. Only two adults were PIT tagged in the autumn when entering the pond. Thus, the body condition mainly seems to reflect this difference in their life history.

No loss of transponders has been reported previously, neither for studies on captive amphibians (Fasola *et al.*, 1993; Brown, 1997), nor for field studies (Faber, 1997). To detect transponder losses in the field, animals additionally need to be registered with an alternative method when being PIT tagged. In our investigations, one tenth of *T. dobrogicus* but less than 1% of *P. fuscus* lost their tag. One possible explanation for this bias is that the healing of an opening in the newt's body cavity takes more time than a wound of the rather thin anuran skin, and that the tissue glue was shed during ecdysis, resulting in an opening through which the transponder could exit during the first few days after implantation. One individual was found in the field with an open wound (Tamnig, pers. comm.). A 10.5% loss is a rather high rate, even when recapture rates do not seem to be affected. A closing of the wound with sutures does not seem to be appropriate, as the incision is only approx. 2 mm in diameter. However, we suggest that this problem can be overcome by more thoroughly closing the body opening with tissue glue, and by inserting the PIT tag more deeply in the body cavity (at least 5 mm away from the opening) before closing the wound.

In sum, the variation of recapture rate and condition index is mainly caused by differences in population traits. However, it is very important to state that being unable to prove significant harmful effects is completely different from proving that a marking technique definitely is not detrimental. For example, although six *T. dobrogicus* were re-registered after having lost their transponder, deaths of a few individuals caused by an opened body cavity cannot be completely excluded. Juveniles, the life stage on which currently the most urgent need for studies exists, can be identified with pictures, but as a rule are too small for transponders and microtags may be the only option (e.g. Sinsch 1997*a,b*). Although recommendations are very difficult, our data provide evidence that PIT tags, when applied very carefully, can be used successfully for field studies on adult *T. dobrogicus*, *P. fuscus*, and on closely related species of similar size.

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REFERENCES

Andreone, F. (1986). Considerations on marking methods of newts, with particular reference to a variation of the belly pattern technique. *Brit. Herp. Soc. Bull.* **16**, 36-37.

Arntzen, J. W. & Teunis, S. F. M. (1993). A six year study on the population dynamics of the crested newt (*Triturus cristatus*) following the colonisation of a newly created pond. *Herp. J.* **3**, 99-110.

Arntzen, J. W., Oldham, R. S. & Latham, D. M. (1995). Cost effective drift fences for toads and newts. *Amphibia-Reptilia* **16**, 137-145.

Brown, L. J. (1997). An evaluation of some marking and trapping techniques currently used in the study of anuran population dynamics. *J. Herpetol.* **31**, 410-419.

Camper, J. D. & Dixon, J. R. (1988). Evaluation of a microchip marking system for amhibians and reptiles. Texas Parks and Wildlife Department, Research Publication 7100-159: 1-22.

Clarke, R. D. (1972). The effect of toe clipping on survival in Fowler's toad (*Bufo woodhousei fowleri*). *Copeia* **1972**, 182-185.

Corn, P. S. (1992). Laboratory and field evaluation of effects of PIT tags. *Froglog* **4**, 2.

Dodd, C. K. & Scott, D. E. (1994). Drift fences encircling breeding sites. In *Measuring and Monitoring Biological Diversity - Standard Methods for Amphibians*, 125-130. Heyer, W. R., Donelly, M. A., McDiarmid, R. W., Hayek, L. and Foster, M. S. (Eds.) Washington: Smithsonian Institution Press.

Donnelly, M. A., Guyer, C., Juterbock, E. & Alford, R. A. (1994). Techniques for marking amphibians. In *Measuring and Monitoring Biological Diversity - Standard Methods for Amphibians*, 277-284. Heyer, W. R., Donelly, M. A., McDiarmid, R. W., Hayek, L. and Foster, M. S. (Eds.) Washington: Smithsonian Institution Press.

Ellinger, N. & Jehle, R. (1997). Struktur und Dynamik einer Donaukammolch-Population (*Triturus dobrogicus* Kiritzescu 1903) am Endelteich bei Wien: Ein Überblick über neun Untersuchungsjahre. In *Populationsbiologie von Amphibien: eine Langzeitstudie auf der Wiener Donauinsel*, 133-150. Hödl, W., Jehle, R. and Gollmann, G. (Eds.) Linz: Stapfia 51.

Faber, H. (1997). Der Einsatz von passiven integrierten Transpondern zur individuellen Markierung von Bergmolchen (*Triturus alpestris*) im Freiland. *Mertensiella* **7**, 121-132.

Fasola , M., Barbieri, F. & Canova, L. (1993). Test of an electronic individual tag for newts. *Herp. J.* **3**, 149-150.

Ferner, J. W. (1979). A review of marking techniques for amphibians and reptiles. *Herp. Circular* **9**, 1-41.

Germano, D. J. & Williams, D. F. (1993). Field evaluations of using passive integrated transponder (PIT) tags to permanently mark lizards. *Herp. Rev.* **24**, 54-56.

Gill, D. E. (1978). The metapopulation ecology of the red-spotted newt, *Notophthalmus viridescens* (Rafinesque). *Ecol. Monogr.* **48**, 145-166.

Golay, N. & Durrer, H. (1994). Inflammation due to toe-clipping in natterjack toads (*Bufo calamita*). *Amphibia-Reptilia* **15**, 81-83.

Hagström, T. (1973). Identification of newt specimens (Urodela, *Triturus*) by recording the belly pattern and a description of photographic equipment for such registrations. *Brit. J. Herpetol.* **4**, 321-26.

Henle, K., Kuhn, J., Podloucky, R., Schmid-Loske, K. & Bender, C. (1997). Individualerkennung und Markierung mitteleuropäischer Amphibien und Reptilien: Übersicht und Bewertung der Methoden; Empfehlungen aus Natur- und Tierschutzsicht. *Mertensiella* **7**, 133-184.

Hödl, W., Jehle, R. & Gollmann, G. (Eds.) (1997). *Populationsbiologie von Amphibien: eine Langzeitstudie auf der Wiener Donauinsel*. Linz: Stapfia 51.

Jehle, R., Hödl, W. & Thonke, A. (1995). Structure and dynamics of central European amphibian populations: a comparison between *Triturus dobrogicus*

(Amphibia, Urodela) and *Pelobates fuscus* (Amphibia, Anura). *Aust. J. Ecol.* **20**, 362-366.

Jemison, S. C., Bishop, L. A., May, P. G. & Farrell, T. M. (1995). The impact of PIT-tags on growth and movement of the rattlesnake, *Sisturus miliaris*. *J. Herpetol.* **29**, 129-32.

Keck, M. B. (1994). Test for detrimental effects of PIT tags in neonatal snakes. *Copeia* **1994**, 226-228.

Sinsch, U. (1992). Zwei neue Markierunsmethoden zur individuellen Identifikation von Amphibien in langfristigen Freilanduntersuchungen: erste Erfahrungen bei Kreuzkröten. *Salamandra* **24**, 161-174.

Sinsch, U. (1997*a*). Postmetamorphic dispersal and recruitment of first breeders in a *Bufo calamita* metapopulation. *Oecologia* **112**, 42-47.

Sinsch, U. (1997*b*). Effects of larval history and microtags on growth and survival of natterjack (*Bufo calamita*) metamorphs. *Herp. J.* **7**, 163-168.

Sweeney, M., Oldham, R. S., Brown, M. & Jones, J. (1995). Analysis of belly pattern for individual newt recognition. In *Conservation and management of great crested newts*, 75-77. Gent, T. and Bray, R. (Eds.) Report No. 20, English Nature, Peterborough.

Thonke, A., Jehle, R. & Hödl, W. (1994). Structure, dynamics and phenology of a population of the Danube warty newt (*Triturus dobrogicus*) on the Danube island near Vienna: a preliminary report. *Abh. Ber. f. Naturk.* **17**, 127-134.

Verrell, P. A., Halliday, T. R. & Griffiths, M. L. (1986). The annual reproductive cycle of the smooth newt (*Triturus vulgaris*) in England. *J. Zool.* **210**, 101-119.

Weatherley, A. H. (1972). *Growth and ecology of fish populations*. London: Academic Press.

Wiener, A. K. (1997). Phänologie und Wanderverhalten einer Knoblauchkröten-Population (*Pelobates fuscus fuscus*) nördlich von Wien: ein Vergleich der Untersuchungsjahre 1986, 1987 und 1989-1995. In *Populationsbiologie von Amphibien: eine Langzeitstudie auf der Wiener Donauinsel*, 151-164. Hödl, W., Jehle, R. and Gollmann, G. (Eds.) Linz: Stapfia 51.

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