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LONG-TERM SURVIVAL AND GROWTH OF FREE-LIVING GREAT CRESTED NEWTS (*TRITURUS CRISTATUS*) PIT-TAGGED AT METAMORPHOSIS

CLIVE P. CUMMINS[1] AND MARY J. S. SWAN[2]

[1]*Centre for Ecology & Hydrology, Monks Wood, Abbots Ripton, Huntingdon PE28 2LS, UK*

[2]*Amphibian Habitat Advisory Services, Huntingdon, UK*

Twenty-one late-larval or newly-metamorphosed great crested newts (*Triturus cristatus*) were implanted with passive transponders (PIT tags) and released into the wild at their natal pond, in England, in 1996. Body mass at the time of release ranged from 0.69 g to 1.57 g, excluding the tag. Eight of the tagged newts were recaptured as breeding adults in 1999 (5 males, 3 females). Seven of the eight were recaptured again in 2000 (5 males, 2 females), along with four more tagged animals (3 males, 1 female). The mean annual survival over the four years from tagging and release in 1996 to the breeding season in 2000 was at least 85%. The tagged newts recaptured in 1999 were among the smallest in the breeding population that year, which was consistent with their being the youngest; this interpretation was supported by the appearance of a new group of similar-sized newts the following year. The growth of tagged newts between 1999 and 2000 was consistent with that of the population at large. Overall, we found high mean annual survival and normal rates of growth among great crested newts PIT-tagged at metamorphosis, indicating that the tagging procedure we used was benign. This use of PIT tags, which allow long-term and instant identification of individual animals, provides a means for investigating dispersal, colonization and metapopulation dynamics during the hitherto little-studied juvenile phase of the life cycle of this protected species.

Key words: PIT tags, newt larvae, marking methods, survival, growth

INTRODUCTION

There have been numerous studies of the survival, growth and reproduction of adult great crested newts, *Triturus cristatus* (e.g. Hagström, 1979; Verrell & Halliday, 1985; Francillon-Vieillot *et al*., 1990; Miaud, 1991; Arntzen & Teunis, 1993; Baker, 1999). Such studies have been facilitated by the annual movement of adults into breeding ponds, which makes them susceptible to trapping by various methods, and by the fact that each adult can be identified by its unique, black-and-yellow belly pattern (Hagström, 1973). Although such studies have contributed much to our knowledge of the population dynamics of great crested newts, the juvenile period – between the times when newly-metamorphosed newts leave their natal pond and first appear as adults in breeding condition – remains very largely unstudied. This is because no satisfactory, long-term means of marking juveniles individually has been developed.

Practical considerations in choosing methods for marking newts and salamanders have been discussed recently by Henle *et al*. (1997) and Ott & Scott (1999), including the time taken to mark each animal, the cost of equipment, reliability and specificity of marks and the objectives of the study. Toe-clipping may be effective in the short term, but it is not reliable in the medium- to long term because of newts' capacity to regenerate lost digits, and because digits or limbs may be lost in other ways. Attempts to identify individuals or particular groups of animals (e.g. size-classes) by clipping combinations of toes are particularly prone to uncertainty, owing to the greater probability that at least one of the clipped digits will re-grow to the extent that it is no longer recognizable.

Correspondence: C. P. Cummins, Centre for Ecology & Hydrology, Monks Wood, Abbots Ripton, Huntingdon PE28 2LS, UK. *E-mail*: cc@ceh.ac.uk; M. Swan: mjss@ahas.co.uk

The unique belly pattern of a great crested newt provides a potentially harmless means of "marking", requiring only that the animal be captured and photographed to record its identity. However, two limitations on the use of this method are (1) the instability of the pattern in juveniles (Arntzen & Teunis, 1993); and (2) the ever-growing magnitude of the task of comparing new records with previous ones, and the associated probability of making mistakes (Jehle & Hödl, 1998; Oldham & Humphries, 2000).

The use of passive integrated transponders (PIT tags) offers a tractable and increasingly affordable means of identifying individual newts quickly and reliably (Fasola *et al*., 1993; Faber, 1997; Jehle & Hödl, 1998; Ott & Scott, 1999), and is particularly attractive for long-term studies. PIT tags are also particularly suitable for studies of dispersal, because tagged immigrants can be recognized without the need to catalogue members of any resident population. As with any means of marking wild animals, however, the potential long-term and sub-lethal consequences of implanting PIT tags in newts must be considered, both from an ethical standpoint and as a possible source of bias. Fasola *et al*. (1993) injected PIT tags into larval and post-metamorphic alpine newts (*T. alpestris*) and adult Italian crested newts (*T. carnifex*), using the metal cannula supplied for routine use with larger animals.

Although the smallest larva (1.3 g body mass) died, Fasola *et al.* (1993) found no adverse effects on growth or survival of the remaining tagged animals kept in captivity over the following six months; they recommended a minimum body mass of 2 g for newts to be PIT-tagged. Faber (1997) tagged and released juvenile and adult *T. alpestris* weighing 1.5-6.7 g, using the injection technique. He recaptured many tagged newts during the same season but did not test for adverse effects. Jehle & Hödl (1998) tagged juvenile and adult *Triturus dobrogicus* weighing more than 2 g, also by injection. They compared the recapture rate of tagged newts with the recapture rate of newts recognized using photographs of belly patterns, but recapture rates in all cases were too low and erratic to discount the possibility of adverse effects of tagging. Ott & Scott (1999) implanted PIT tags surgically in recently-metamorphosed marbled salamanders (*Ambystoma opacum*) weighing 1.7–4.1 g, and found no significant effect of tagging on subsequent growth or survival over a five month period in outdoor enclosures. Here, we describe PIT-tagging of late-larval and newly-metamorphosed great crested newts (*T. cristatus*), their release into the wild, and their survival and growth up to four years later.

METHODS

The study was carried out at a pond in Cambridgeshire, UK (national grid reference TL1878). The pond measures approximately 12 m x 3 m x 1 m deep when full and was created in 1994, within 100 m of a breeding pond that was subsequently destroyed during the widening of the A1 highway. Since 1995 an area of rabbit-grazed turf and scrub around the pond, maximum dimensions approximately 70 m x 40 m, has been largely enclosed by a fence of polythene sheeting about 1 m high. The fence was intended to confine the newt population to a safe area around the pond while construction work was under way, and until surrounding vegetation had re-grown, especially along a ditch which connects the site to nearby gardens and arable land. The fence has not presented a complete barrier to newts, but the site is now almost completely surrounded by roads and there is no other known breeding site within 1 km, so the population was effectively isolated during the period of study. The management plan for the site provides for the removal of the perimeter fence in 2001, when newts will be free to disperse. The fenced area includes two man-made refugia comprising heaps of rubble, capped with soil, within 5 m of the pond.

In the summer of 1996, 21 larval *T. cristatus* were rescued from the pond as it dried out (temporarily). Subsequently, as late-larval or newly-metamorphosed newts, they were tagged by one of us (CPC) using passive integrated transponders (PIT tags). The PIT tags used consist of a transponder encased in a glass capsule 11 mm long x 2.1 mm in diameter, total mass approximately 100 mg (Trovan system, AEG, supplied by UKID Systems Ltd, Preston, UK). Each tag was supplied in a sterile, metal cannula which was not suitable for use with such small animals. Therefore, the tag was removed from the cannula and washed, then manipulated using forceps. Each newt was anaesthetized in a buffered solution of MS-222, a small incision was made in the side of the abdominal wall, behind a foreleg, and the tag was inserted gently into the peritoneal cavity. The wound was then closed with a single suture. No antiseptic was used. Tagged newts were placed in a tank of shallow water (larvae) or a box lined with damp paper tissue (metamorphs) and were kept under observation for 2-3 days, during which time the wound healed over. The suture was removed without anaesthesia on the third day if it had not already dropped out. In larval newts, metamorphosis began immediately after tagging. Experience has shown that both larval and post-metamorphic newts will usually accept food as soon as the effect of the anaesthetic has worn off. However, to reduce the risk of disturbing the tag or the wound, food was not offered until the day after tagging. It was considered important to establish that each newt was able to feed and pass faeces before release. Immediately prior to release, each newt was weighed and its belly pattern was recorded. The tagged newts were released at the edge of their natal pond, shortly after dusk on three occasions when the weather was mild and wet: 20 August (n=7), 28 August (n=2) and 25 September (n=12), in 1996. By the time of their release, all the newts had completed metamorphosis.

We sampled the pond on four occasions between March and July in 1997, on five occasions between April and June in 1999, and on six occasions between February and June in 2000; we did not sample the pond in 1998. In 1997 we used bottle traps, but subsequent sampling was carried out by searching the pond margins after dusk and catching newts by hand or with a small, hand-held net. All newts encountered were photographed alongside a ruler and scanned for the presence of a PIT tag, and most were weighed to 0.01 g precision. We measured the lengths of the newts from photographs, using the ruler in the photograph for calibration (1 mm precision). We measured from the tip of the snout to the posterior end of the cloaca, which we found to be the most easily definable point in the cloacal region in males. We termed this measurement snout-vent length (SVL), although others may have used the same term differently (e.g. Arntzen & Teunis, 1993). We made allowances as best we could for curvature of the body and when more than one photograph was available in a breeding season (e.g. multiple recaptures), we used whichever showed the newt in the straightest posture, i.e. we assumed no growth during the breeding season.

In 2000, we estimated the proportion of adult newts we had encountered, and hence the number of tagged animals that we might have missed. To do this, we estimated the numbers of adult males and adult females in the pond at the height of the breeding season, in mid-April, using belly patterns as individual marks. We were dealing with a closed population in which the proportion of animals susceptible to sampling (i.e. in the pond) increased during the early part of the breeding season, as animals arrived from their winter refuges on land; therefore, we did not use a mean estimate of population size derived from multiple-recapture data. Instead, we calculated simple Petersen estimates (Begon 1979) from our mid-April sample, assuming that all newts encountered previously in 2000 were still present in the pond at that time; this seems to be a reasonable assumption, given that courtship and egg-laying were still going on and that mean annual survival of adults was high. We based confidence intervals on the estimate of standard error given by Begon (1979), after Bailey (1951). As in many mark-recapture exercises, we do not know what proportion of the adult population was actually at risk of being captured.

RESULTS

TAGGING

All the newts accepted food readily on the day after they were tagged, and no adverse effect of tagging was apparent. Upon release, the mass of the newts, excluding the tag, ranged from 0.69 g to 1.57 g (mean±SD = 1.05±0.23 g).

SURVIVAL

None of the tagged animals described above was encountered in 1997. The first recaptures of tagged newts were made in 1999, when five males and three females were encountered among the breeding population. Seven of the eight tagged newts encountered in 1999 (five males, two females) were recaptured during the 2000 breeding season, along with another four of the tagged animals (three males, one female). Thus, at least 12 of the 21 animals tagged in 1996 were still alive three years later, in 1999 – a mean annual survival of at least 83%. Furthermore, at least 11 of the 12 tagged newts known to be alive in 1999 were still alive and breeding in 2000 – a mean annual survival of at least 85% over the four years. These are minimum values for survival, based on the numbers known to be alive. It is possible that other survivors went undetected.

We estimated the number of adult males in the pond in mid-April 2000 to be 104; the upper boundary on the 95% confidence interval was 116. After the mid-April sampling exercise we had encountered 96 different males, and by the final sampling exercise, on 21 June 2000, the total had risen to 98 males. Assuming that the great majority of breeding newts were in the pond in mid-April, it is likely that we missed about six males over the season (104-98=6), but unlikely that we missed more than 18 ($P\cong0.025$, assuming 95% confidence intervals symmetrical about the mean estimate). Eight of the 98 males encountered were tagged, so if tagged newts were neither more nor less likely to be captured than non-tagged newts, it is not unlikely that we missed one tagged male.

TABLE 1. Initial body mass (excluding tag) and snout-vent length (SVL) at recapture of great crested newts implanted with PIT tags at metamorphosis and released into the wild in 1996, then recaptured in 1999 and/or 2000. n.c., not caught.

Ref. no.	Sex	1996 Body mass (g)	1999 SVL (mm)	2000 SVL (mm)
A1F4	F	1.00	68	73
D246	F	1.00	66	n.c.
D66E	F	0.88	64	69
DBA5	F	1.56	n.c.	76
6179	M	1.01	n.c.	71
9812	M	1.31	64	69
BF1E	M	0.81	65	72
C3D7	M	1.03	62	68
E450	M	0.85	64	72
E40B	M	1.27	n.c.	69
EBC6	M	0.92	65	72
F5B4	M	1.19	n.c.	71

We estimated the number of adult females in the pond in mid-April to be 72, with an upper 95% confidence limit of 88. By 21 June we had encountered no females that had not already been encountered by mid-April, the total remaining at 61. Thus, we are likely to have missed about 11 females, but unlikely to have missed more than 27. Three of the 61 adult females encountered (i.e. 5%) were tagged. Extrapolating from that percentage, which is based on a sample of about 85% of adult females, and a best estimate of 11 females missed, it is not unlikely that one tagged female was missed over the season.

There was no significant difference between the means of initial mass of the tagged newts recaptured as adults and those of newts not recaptured: 1.07 (SD±0.22 g) and 1.02 (SD±0.25) g respectively, excluding the mass of the tag. The body mass at release in 1996 and snout-vent length (SVL) at recapture in 1999 and/or 2000 are listed for recaptured newts in Table 1. The body mass of recaptured newts at the time of their release was not correlated significantly with body length at recapture in either 1999 (r=0.09, P>0.10) or 2000 (r=0.24, P>0.10).

GROWTH

Without evacuating the gut contents of newts, we found that short-term fluctuations in body mass were too large for body mass to be a useful indicator of individual growth. Therefore, our analyses of growth are based on snout-vent length (SVL). To investigate the possibility that tags had an adverse effect on adults' growth, we used SVL data from all newts captured in both 1999 and 2000. We calculated separately for males and females a linear regression of SVL in 2000

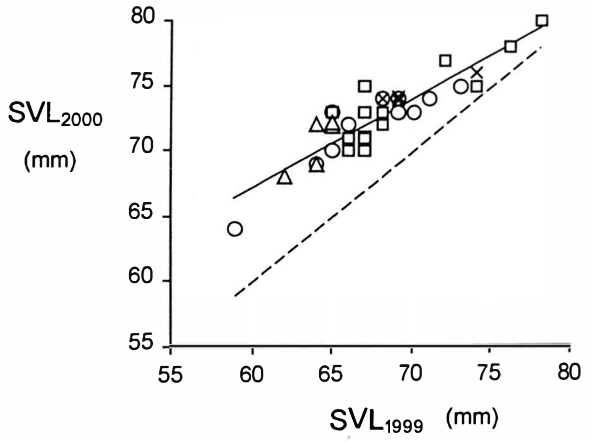

FIG. 1. The snout-vent length (SVL) of adult male great crested newts (*Triturus cristatus*) captured in 1999 and 2000 at a pond in Cambridgeshire, UK. Open triangles represent newts of the 1996 cohort, which were implanted with PIT tags at the time of metamorphosis. Other newts were recognized by their unique belly patterns and are labelled according to the year in which they were first encountered at the site: circles, 1999; crosses, 1997; squares, 1996. The solid line shows a least-squares regression of SVL_{2000} on SVL_{1999} for non-tagged newts; the broken line indicates $SVL_{2000} = SVL_{1999}$, i.e. no growth.

on SVL in 1999, excluding data from newts tagged as metamorphs in 1996. Figures 1 and 2 show that the increase in SVL of tagged newts between 1999 and 2000 was entirely consistent with the trends among other adult males and females during the same period. Because the tagged newts were at the (lower) end of the range of sizes of adults, it is important to note that the regressions shown in Figs 1 and 2 are not influenced by

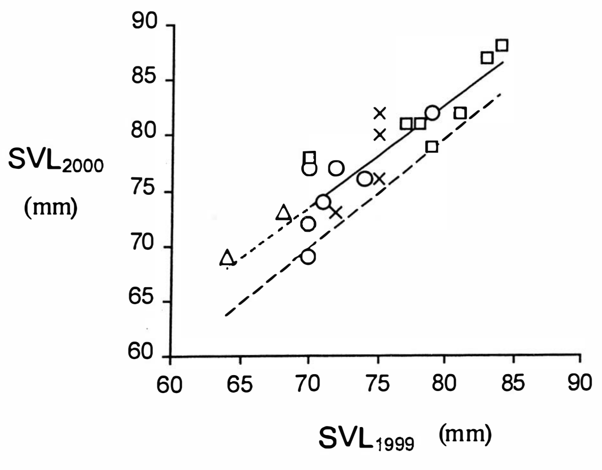

FIG. 2. The snout-vent length (SVL) of adult female great crested newts (*Triturus cristatus*) captured in 1999 and 2000 at a pond in Cambridgeshire, UK. Open triangles represent newts of the 1996 cohort, which were implanted with PIT tags at the time of metamorphosis. Other newts were recognized by their unique belly patterns and are labelled according to the year in which they were first encountered at the site: circles, 1999; crosses, 1997; squares, 1996. The solid line shows a least-squares regression of SVL_{2000} on SVL_{1999} for non-tagged newts and is extrapolated as a dashed line; the broken line indicates $SVL_{2000} = SVL_{1999}$, i.e. no growth.

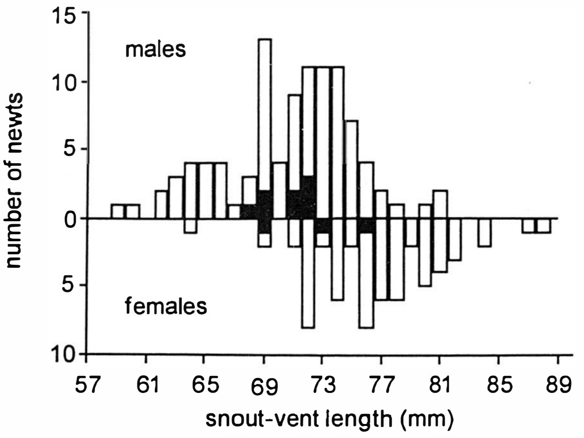

FIG. 3. The length-frequency distribution of great crested newts (*Triturus cristatus*) captured at a pond in Cambridgeshire, UK, in the year 2000. Filled bars represent newts of the 1996 cohort, which were implanted with PIT tags at the time of metamorphosis. Open bars represent newts of other cohorts, not tagged as metamorphs.

data from the tagged newts. The single instance of a newt's measured length decreasing between 1999 and 2000 may be attributed to the "error" inherent in measuring newts that are not at maximum extension. Other data points may be assumed to be subject to such errors, but we have no reason to suspect any bias.

The lengths of tagged males when captured in 1999 (62-65 mm, Table 1), aged three, corresponded well with the modal length of a group of males that were captured for the first time in 2000 and which were smaller than the tagged newts recaptured in that year (Fig. 3). In fact, 21 of the 22 non-tagged newts of SVL <69 mm in 2000 were encountered for the first time that year; the other one was first seen in 1999 (SVL_{1999}=59 mm, SVL_{2000}=64 mm; Fig. 1). Because the pond dried out before any newt larvae had reached metamorphosis in 1996, albeit temporarily, we know that the tagged newts were the sole representatives of the 1996 cohort. Our current interpretation of the data is that the males of SVL 62-66 mm in 2000 were from the 1997 cohort and that the smaller males were probably from the 1998 cohort. Accordingly, we think that most males in this population matured at three years of age, with a minority maturing after two years (e.g. the male first encountered in 1999 with SVL=59 mm). In that case, our observations would suggest that tagged males were not unusually small by the age of three. There are fewer data from which to make a similar comparison for females. In 2000, some of the females first encountered in 1999 were similar in SVL to the tagged females (Fig. 2) and it is pertinent to ask whether they were younger or older than the latter. We know that the former were already at least 69 mm long in 1999 (Fig. 2) and it seems unlikely that they could have reached that size in two years; accordingly, we think it more likely that those newts were from the 1995 cohort or earlier and that the 64-mm female encountered in 2000

(Fig. 3) was probably the first representative of the 1997 cohort. An alternative interpretation of the data is that the 1997 cohort was poorly represented in the adult population and that the smaller newts seen for the first time in 2000 were mostly from the 1998 cohort. Although we cannot refute that interpretation, we think it unlikely for the reasons given above.

DISCUSSION

The mean annual survival of the tagged newts in our study was higher than values reported elsewhere for free-living, non-tagged *T. cristatus* (e.g. Hagström, 1979; Dolmen, 1982; Francillon-Vieillot *et al.*, 1990; Miaud, 1991; Arntzen & Teunis, 1993; Baker, 1999). Even if the 12 recaptured newts were the only survivors in 1998, their mean annual survival in the first two years of terrestrial life would have been 75%, whereas other estimates of juvenile survival, from six- and eight-year studies, respectively, have been 17% (Arntzen & Teunis, 1993) and 59% (Baker, 1999). It may be that our study site was inherently less hazardous than those studied by others, though it was certainly visited by avian predators and grass snakes (*Natrix natrix*) and was disturbed by a variety of people erecting and removing fences, digging pitfall traps and removing vegetation from the pond. Also, if the urge to disperse is relatively short-lived, that may have contributed to the high rate of recovery of tagged animals in our study, as several days elapsed between tagging and release. The important point is not so much that annual survival was higher in our study than in others, but that it was as high as 85% over a four-year period – which is strong evidence that our tagging had little, if any, adverse effect on the newts' survival.

We cannot quantify any effect that tagging may have had on the newts' growth as juveniles. The tagged newts were among the smallest in the breeding population in 1999, but that was consistent with their being the youngest, aged three years, apart from one small male that may have been a two-year-old. We found no evidence that tags affected adult growth.

Arntzen & Teunis (1993) were able to recognize individual, juvenile *T. cristatus* in subsequent years from their belly patterns, as long as they were recaptured each year. However, the changes in belly pattern in the first few years of life make this method of recognition unreliable without such a complete record. In our study, tagged newts were first recaptured at three years of age and we could not have identified members of a free-living, unmarked, control group unequivocally. Therefore, we could not have made a direct comparison of survival or growth between tagged and non-tagged newts in the same cohort. In these circumstances, we believe that our results demonstrate as clearly as is practicable that judicious use of PIT tags in late-larval or newly-metamorphosed great crested newts is essentially benign.

Perhaps the most useful application of PIT-tagging great crested newts at metamorphosis is in the study of dispersal, colonization and metapopulation dynamics. Little is known of the dispersal behaviour of great crested newts when they first leave their natal pond (cf. Kupfer & Kneitz, 2000; Hayward *et al.*, 2000), but in studying this behaviour it may be prudent to tag and release newts prior to, or soon after, the completion of metamorphosis. Although the range of detection of PIT tags is limited to about 15 cm or less, the tags can be used to locate and identify animals in dense vegetation or other inaccessible refuges (Faber, 1997). The ability to identify an individual quickly and unequivocally allows its displacement from previous, known locations to be determined reliably and with minimal disturbance. Furthermore, tagged animals that have dispersed from their natal pond and joined other populations can be identified as such simply by scanning members of the receiving population. PIT-tagging at metamorphosis also provides a means of ageing newts unequivocally, and is therefore an improvement on skeletochronology (Francillon-Vieillot *et al.*, 1990) and inference based on body size or fluctuations in recruitment (Arntzen & Teunis, 1993; Baker, 1999), particularly during the early years of life.

In theory, PIT-tagging should be permanent, but several authors have reported loss of tags (Faber, 1997; Jehle & Hödl, 1998; Ott & Scott, 1999). Usually, loss occurred shortly after implantation and may have resulted from incomplete closure of the wound or subsequent infection, allowing the tag to drop out. In this study, all the tagged newts were intact at the time of release. Another possible source of error is a failure of a tag to respond to the signal from the scanner. All the tags in our newts were working at the time of release, but of 40 unused tags from the same batch, two did not work when scanned in 2000. We do not know why those tags failed to respond, but it appears to cast some doubt on their reliability over the potential lifespan of a great crested newt.

Overall, we have demonstrated that PIT tags can be used to mark great crested newts individually at the time of metamorphosis, with little or no adverse effect on growth or survival. This methodology provides a means of investigating aspects of the hitherto little-studied, juvenile phase of the life cycle, particularly dispersal and metapopulation dynamics. In the UK a Home Office licence is needed to carry out the tagging procedure and this methodology is not available for routine monitoring.

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