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Journal Name	Ecological Research	

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	Received	15 January 2016
Schedule	Revised	
	Accepted	21 February 2016

Abstract

The correct identification of individuals is a requirement of capture-mark-recapture (CMR) methods, and it is commonly achieved by applying artificial marks or by mutilation of study-animals. An alternative, non-invasive method to identify individuals is to utilize the patterns of their natural body markings. However, the use of pattern mapping is not yet widespread, mainly because it is considered time consuming, particularly in large populations and/or long-term CMR studies. Here we explore the use of pattern mapping for the identification of adult individuals in the alpine (*Ichthyosaura alpestris*) and smooth (*Lissotriton vulgaris*) newts (Amphibia, Salamandridae), using the freely available, open-source software

Wild-ID. Our photographic datasets comprised nearly 4000 captured animals' images, taken during a 3-year period. The spot patterns of individual newts of both species did not change through time, and were sufficiently varied to allow their individual identification, even in the larger datasets. The pattern-recognition algorithm of Wild-ID was highly successful in identifying individual newts in both species. Our findings indicate that pattern mapping can be successfully employed for the identification of individuals in large populations of a broad range of animals that exhibit natural markings. The significance of pattern-mapping is accentuated in CMR studies that aim in obtaining long-term information on the demography and population dynamics of species of conservation interest, such as many amphibians facing population declines.

Keywords (separated by '-')	Photo capture-mark-recapture - Computer-assisted photo-identification - Non-invasive individual identification - Photo-id - Wild-ID
Footnote Information	

Ecol Res
DOI 10.1007/s11284-016-1346-y

TECHNICAL REPORT

**Onoufrios Mettouris · George Megremis
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A newt does not change its spots: using pattern mapping for the identification of individuals in large populations of newt species

Received: 15 January 2016 / Accepted: 21 February 2016

Abstract The correct identification of individuals is a requirement of capture-mark-recapture (CMR) methods, and it is commonly achieved by applying artificial marks or by mutilation of study-animals. An alternative, non-invasive method to identify individuals is to utilize the patterns of their natural body markings. However, the use of pattern mapping is not yet widespread, mainly because it is considered time consuming, particularly in large populations and/or long-term CMR studies. Here we explore the use of pattern mapping for the identification of adult individuals in the alpine (*Ichthyosaura alpestris*) and smooth (*Lissotriton vulgaris*) newts (Amphibia, Salamandridae), using the freely available, open-source software Wild-ID. Our photographic datasets comprised nearly 4000 captured animals' images, taken during a 3-year period. The spot patterns of individual newts of both species did not change through time, and were sufficiently varied to allow their individual identification, even in the larger datasets. The pattern-recognition algorithm of Wild-ID was highly successful in identifying individual newts in both species. Our findings indicate that pattern mapping can be successfully employed for the identification of individuals in large populations of a broad range of animals that exhibit natural markings. The significance of pattern-mapping is accentuated in CMR studies that aim in obtaining long-term information on the demography and population dynamics of species of conservation interest, such as many amphibians facing population declines.

Keywords Photo capture-mark-recapture · Computer-assisted photo-identification · Non-invasive individual identification · Photo-id · Wild-ID

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Introduction

Capture-mark-recapture (CMR) methods are widely used in ecological studies to estimate population parameters (e.g., abundance, survival rates) and collect data on the demography, migration and life cycle of animals; the ability to distinguish individuals from other conspecifics in natural populations is fundamental to this process (Lebreton et al. 1992; Williams et al. 2002). Individual identification is most commonly achieved by applying artificial marks (e.g., PIT-tags, visible implant elastomers, bands, tattoos) or by removing small parts (e.g., toe or scale clipping or tipping) of animals. Some of these invasive techniques may induce stress and influence the survival and/or behaviour of the target animal (Gauthier-Clerc et al. 2004; McCarthy and Parris 2004; Langkilde and Shine 2006; Antwis et al. 2014), and are often expensive, particularly in long-term studies (Morrison et al. 2011). In addition, there have been concerns about ethical and animal welfare issues that arise from their broad use (Wilson and McMahon 2006; Parris et al. 2010; Perry et al. 2011).

An alternative, non-invasive method to identify individuals is to utilize their natural body markings, such as stripes, spots or blotches. These natural patterns are photographed and then used to distinguish between individuals. Although pattern mapping is a cheap and non-invasive method, it is considered very time-consuming (Donnelly et al. 1994), particularly in large datasets and/or long-term CMR studies. However, recent technological advances in digital photography and pattern recognition algorithms (Arzoumanian et al. 2005; Van Tienhoven et al. 2007; Gamble et al. 2008; Bolger et al. 2012) have enabled field ecologists to create and analyse large photographic databases quickly and efficiently while minimizing effort and misidentification errors (Bolger et al. 2012), thus allowing for larger sample sizes in CMR studies. In recent years pattern mapping has been employed in a wide and quite varied range of

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animals, including beetles (Caci et al. 2013), seadragons (Martin-Smith 2011), lizards (Sreekar et al. 2013), giraffes (Halloran et al. 2015) and dolphins (Martinho et al. 2014).

The global declines of many amphibian populations (e.g. Blaustein and Wake 1990; Stuart et al. 2004, 2008) stress the need for long-term information on their demography and population dynamics (Blaustein et al. 1994). Long-term monitoring studies employing CMR methods are an important tool for estimating abundances and then identifying negative population-trends. Although most amphibians exhibit some form of natural patterns, the temporal inconsistency of these patterns documented in some species and the fact that it is considered a time-consuming method (Donnelly et al. 1994) prevent the wide-spread use of pattern mapping in amphibian CMR studies. Instead, more traditional methods such as toe-clipping and PIT-tagging are usually preferred (Ferner 2010), despite their invasive nature and the fact that many of the target-species are of conservation interest. Adult newts (Amphibia, Salamandridae) exhibit natural spot patterns that can be utilized for the identification of individuals. Alpine newts, *Ichthyosaura alpestris* (Laurenti, 1768), have numerous spots on their flanks, and smooth newts, *Lissotriton vulgaris* (Linnaeus, 1758), have spots on their belly and throat areas (Figs. 1 and 2). Hagström (1973) and Winkler and Heunisch (1997) used these spot patterns to successfully identify individuals of smooth and alpine newts, albeit using a small number of animals because the method was at the time considered impractical for large populations (Hagström 1973).

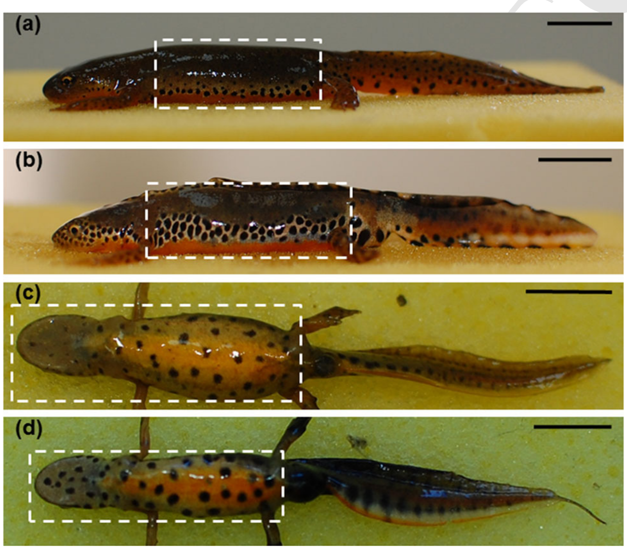

Fig. 1 Spot patterns of male and female alpine and smooth newts. The *dashed white lines in each image* indicate the cropped region that was used for the identification of individuals with Wild-ID. **a** *Ichthyosaura alpestris* female, **b** *I. alpestris* male, **c** *Lissotriton vulgaris* female, **d** *L. vulgaris* male. The *black scale bars on the right* represent 1 cm

The correct identification of individuals is a basic assumption of CMR methods, and violation of this assumption may lead to negatively biased population-parameter estimates (Morrison et al. 2011). Thus, when using the natural patterns of individuals as a marking method, it is vital to ensure that the patterns of the target-species are both sufficiently varied and distinct to allow for individual identification, and remain unchanged at least for the duration of the study (Ferner 2010). Here, we explore the use of pattern mapping for the identification of individuals in the alpine and smooth newts over a 3-year time period using the freely available, open-source software Wild-ID (Bolger et al. 2012). Our aims are: (1) to assess the efficacy of using the spot patterns of alpine and smooth newts for their individual identification in real CMR datasets varying in size, (2) to test the temporal consistency of the spot patterns of these individuals over the 3-year study period and (3) to evaluate the performance and reliability of Wild-ID as a tool for computer-assisted pattern recognition in the two target-species.

Methods

Study site and field work

Our study site is a semi-natural temporary pond in northern Peloponnese, southern Greece (38°01′N, 22°02′E, about 800 m a.s.l.), in which adult alpine (*I. alpestris*) and smooth (*L. vulgaris*) newts gather each year to breed. Sampling was carried out for three consecutive years (2012–2014), approximately twice a month (2012) or once a week (2013–2014) during the newt's breeding season, using dip-nets to capture adults in the water. Because accurate morphometric data (not shown here) on all captured newts were required, it was deemed necessary to anaesthetize them. We note, however, that anaesthesia is not strictly required for taking photographs, and other methods for immobilizing captured newts for a few seconds can be applied (e.g., Baker and Gent 1998). We anaesthetised all captured individuals in a solution of 0.25 g MS222 (Sigma–Aldrich) in 1L water, in which 0.25 g of baking soda (sodium bicarbonate) was added to neutralize the pH level. Each newt was placed on a wet sponge and, after measurement, photographs were taken of the lateral (left) side of alpine newts and the ventral side of smooth newts. All pictures were taken using a hand-held Nikon D40 DSLR camera with a 35 mm f/1.8 Nikkor lens and using the built-in flash when required. The handling time for each anaesthetised individual was approximately five to ten seconds. Depending on the number of captured newts the whole process usually lasted for a few hours. After making sure that all newts recovered successfully from anaesthesia, they were released at the site of capture within the day of capture.

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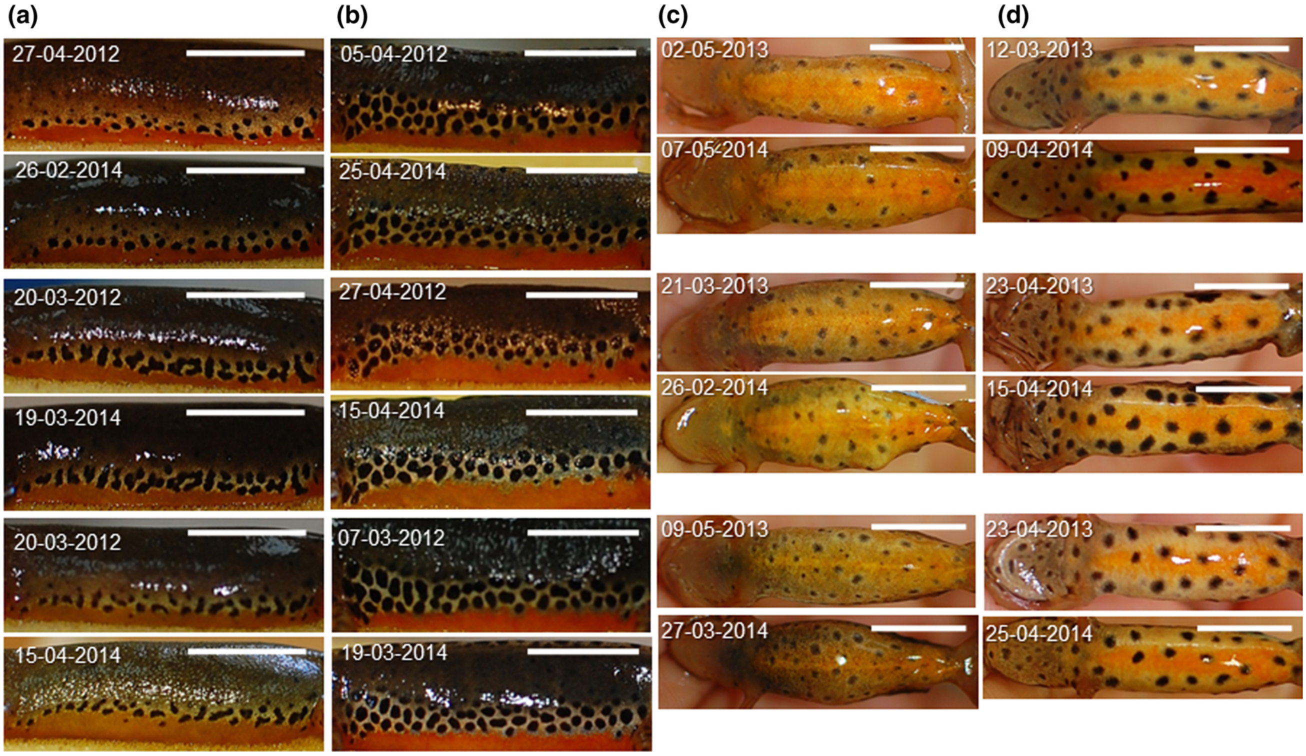

Fig. 2 Example images of alpine and smooth newts recaptured in different years that show virtually no change in their spot patterns. Three individuals per group (**a**–**d**) are shown. Each pair of images in the different columns (**a**–**d**) corresponds to the same individual, captured in different years. The date of capture is shown at the *upper left corner of each image*. **a** *Ichthyosaura alpestris* females, **b** *I. alpestris* males, **c** *Lissotriton vulgaris* females, **d** *L. vulgaris* males. The *white scale bars on the right* represent 1 cm

Manual identification and Wild-ID evaluation

Breeding adult males and females can be easily distinguished from each other in both species (Fig. 1), thus males and females of each species were treated as a different group. After the 2012 sampling was concluded we constructed four photographic datasets, one for each group. These datasets consisted of 298 alpine (162 females, 136 males) and 49 smooth (36 females, 13 males) newts. To establish the identity (i.e., if they are new captures or recaptures) of all newts in the 2012 datasets we followed a manual identification procedure, in which by eye we compared the image of each captured newt to all other images in the same group; individuals captured at a given sampling session were compared to all individuals from all previous sessions. We were thus able to unambiguously identify all captured animals and assign them either as recaptures or new captures (see "Results").

For the computer-assisted individual identification we used the pattern-recognition software Wild-ID (Bolger et al. 2012; available at http://envs.dartmouth.edu/people/douglas-thomas-bolger). Wild-ID processes the images in each dataset sequentially and calculates a similarity score for each pair of images. After all similarity scores are computed, Wild-ID presents the top-20 ranking images for each image for visual confirmation through the user interface. Consequently the final decision for accepting or rejecting a match is made by the user. We analysed the 2012 datasets with Wild-ID, using the results of the manual identification procedure as the known (i.e., correct) matches. The pre-processing stage consisted of the removal of unnecessary background information (Fig. 1). The user of Wild-ID was blind as to the true identity of all individuals and inspected all top-20 images presented for each unknown image by Wild-ID. To evaluate the performance of Wild-ID we estimated the total number of recaptures that were not placed within the top-20 ranking images, as well as the number of images wrongly assigned as recaptures.

Within- and between-years identification

The encouraging results of Wild-ID for the first years (2012) datasets (see "Results") prompted us to use it for the following 2 years datasets. During these years the total number of captured animals (N) was much greater than the first year (2012: N = 347, 2013: N = 2462, 2014: N = 1190). Although Wild-ID ranked all recaptures of the first year well within the top-10 positions (see "Results"), we chose to inspect all top-20 ranking images in these much larger datasets to avoid missing any recaptures and to assess the performance of Wild-ID in large datasets. Again, the images of each years captured newts were partitioned in four groups according to species and sex and were pre-processed to remove background information. We constructed one dataset for each group of each year (i.e., eight within-year datasets), and each dataset was analysed separately with Wild-ID.

To assess whether an individual newt's spot pattern changed over time and to assess the ability of Wild-ID to identify individual newts captured across years, we created three between-years datasets for each group: two datasets for the 1-year time interval (i.e., 2012–2013 and 2013–2014) and one dataset for the 2-year time interval (2012–2014). In each of these datasets we searched for individuals that were last captured in the former year and recaptured in the latter. Again, we inspected all top-20 ranking images presented for each unknown image by Wild-ID.

Results

Field work and temporal consistency of spot patterns

A total of 3333 (1902 females, 1431 males) alpine and 666 (422 females, 244 males) smooth newts were captured during the 3-year period (Table 1). The visual inspection of all within-year and between-years recaptures revealed that the spot patterns of individual newts of both species do not change over time. In Fig. 2 we present some example images of newts of both species that were recaptured across years.

Manual identification and Wild-ID evaluation (2012 dataset)

The spot patterns of individual newts in all four datasets of 2012 were sufficiently varied to allow for the unambiguous identification of all individuals using the manual identification procedure. We were thus able to assign with certainty all captured animals as either recaptures or new captures.

Wild-ID successfully identified all recaptures in all groups and placed them in the top-20 ranking images. Additionally, none of the images were wrongly assigned as recaptures in any group. Of the 25 recaptures, 22 (88 %) ranked first, 24 (96 %) ranked within the top-3 and all 25 recaptures ranked within the top-10 positions.

Within-year and between-years identification with Wild-ID

The pattern-recognition algorithm of Wild-ID was highly successful in all datasets and groups, as indicated by the very high ranking-positions of most recaptures, shown in Table 1 and Fig. 3.

Within-year identification

In the alpine newt, 710 out of 722 (98.3 %) female and 514 out of 517 (99.4 %) male recaptures ranked first. Only four female and one male alpine newt images were positioned below the top-3 ranking images. In the smooth newt, 105 out of 129 (81.4 %) female and 73 out of 79 (92.4 %) male recaptures ranked first. Eleven female and two male smooth newt images ranked below the top-3 positions.

Between-years identification

In both sexes of the alpine newt over 98 % of the recaptured individuals in the 1-year interval dataset ranked within the top-10 positions. In the 2-year interval dataset, two out of 13 females and two out of eight males ranked below the top-3 positions. In the smooth newt all eight males ranked within the top-3 positions, while two females ranked below the 15 top-ranking images in the 1-year interval dataset. Of the few smooth newts captured in 2012 (36 females, 13 males), none were recaptured in 2014 (i.e., in the 2-year interval).

Discussion

In this study we assess the efficacy of using the spot patterns of alpine and smooth newts for their individual identification. Our inspection of nearly 4000 captured animals' images, taken during a 3-year time period, reveals that the spot patterns of alpine and smooth newts are sufficiently varied to allow their individual identification. Even in the larger datasets, containing hundreds or thousands of images, there was virtually no difficulty in assigning unknown individuals as recaptures or new captures based on their spot patterns. However, with the number of images growing, manual inspection of a photographic dataset becomes increasingly harder and potentially more error-prone, and it demands considerably more effort. The employment of pattern mapping in such large datasets without the aid of pattern-recognition software would be forbiddingly time-consuming. Inspection of the four 2012 datasets with Wild-ID took only a fraction of the time required for their manual inspection and yielded the same results, with no misidentification errors. The following 2 years' datasets, although much larger than the first year's, were not harder to inspect, as we only had to visually go through the top-20 closest-matching images presented by Wild-ID. However, as the vast majority of recaptures were ranked within the top-5 positions in all datasets (Fig. 3), Wild-ID greatly reduced the time needed for visual

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Table 1 Ranking positions of recaptures in Wild-ID for each group (i.e., species and sex) and each dataset

Group	Dataset		Total recaptures	Wild-ID ranking position							
				Top 1	Top 2	Top 3	Top 4	Top 5	Top 10	Top 15	Top 20
Ichthyosaura alpestris	Within-year	N_r	722	710	714	718	720	721	721	721	722
females		%		98.34	98.89	99.45	99.72	99.86	99.86	99.86	100
(N = 1902)	1-year interval	N_r	184	166	173	175	178	179	182	184	184
		%		90.22	94.02	95.11	96.74	97.28	98.91	100	100
	2-year interval	N_r	13	8	9	11	12	12	12	12	13
		%		61.54	69.23	84.62	92.31	92.31	92.31	92.31	100
I. alpestris	Within-year	N_r	517	514	514	516	517	517	517	517	517
males		%		99.42	99.42	99.81	100	100	100	100	100
(N = 1431)	1-year interval	N_r	56	46	47	48	48	50	55	56	56
		%		82.14	83.93	85.71	85.71	89.29	98.21	100	100
	2-year interval	N_r	8	4	6	6	8	8	8	8	8
		%		50	75	75	100	100	100	100	100
Lissotriton vulgaris	Within-year	N_r	129	105	115	118	119	120	125	128	129
females		%		81.40	89.15	91.47	92.25	93.02	96.90	99.22	100
(N = 422)	1-year interval	N_r	9	2	3	3	4	5	5	7	9
		%		22.22	33.33	33.33	44.44	55.56	55.56	77.78	100
L. vulgaris	Within-year	N_r	79	73	75	77	77	77	77	77	79
males		%		92.41	94.94	97.47	97.47	97.47	97.47	97.47	100
(N = 244)	1-year interval	N_r	8	5	6	8	8	8	8	8	8
		%		62.50	75	100	100	100	100	100	100

Within-year: animals recaptured in at least 1 year's sampling, pooled for all years (2012, 2013, 2014). One-year interval: animals recaptured 1 year after last been captured, pooled for both intervals (2012–2013 and 2013–2014). Two-year interval: animals recaptured 2 years after last been captured; only *Ichthyosaura alpestris* individuals were recaptured in the 2-year interval. N number of animals captured over the 3-year study period, N_r number of recaptures ranked within each ranking position, % percentage of recaptures ranked within each position over the total number of recaptures

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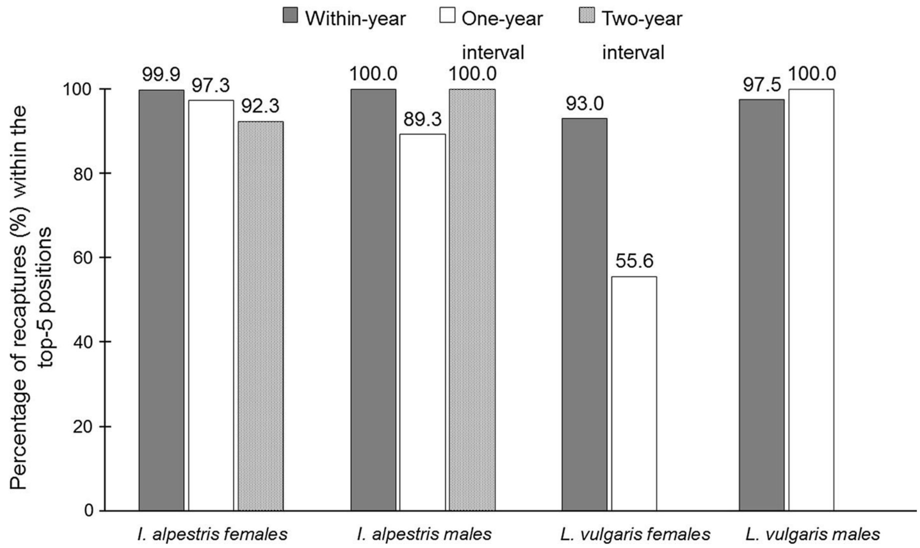

Fig. 3 Percentage of recaptures (%) that were ranked by Wild-ID within the top-5 positions for each group and each dataset

confirmation. Because of the high-ranking success of Wild-ID across all datasets, the likelihood of having missed any recaptures is assumed to be negligible. Up until recently, and compared to other marking methods, pattern mapping was considered appropriate only for short studies of small populations (Hagström 1973; Donnelly et al. 1994; Arntzen et al. 2004); this study clearly shows that this is not the case anymore.

In CMR studies employing pattern mapping for individual identification, it is crucial that the spot patterns of individuals are identifiable throughout the duration of the study (Ferner 2010). The visual comparison of images of individuals recaptured across years (Fig. 2) reveals that the spot patterns of individual newts of both species do not change through time. This is most clearly demonstrated in the case of newts that were recaptured in both the first and last year of the study. Although only alpine newts were recaptured across the 2-year interval, a close inspection of smooth newt images in the 1-year interval datasets confirms the temporal consistency of spot patterns in both newt species.

In the within-year datasets, alpine newts ranked higher than smooth newts, and male newts in both species ranked higher than female newts (Table 1). Alpine newts have a greater number of spots than smooth newts; in addition, male alpine newts have more spots than female alpine newts, while male smooth newts have bigger spots than female smooth newts (Fig. 1 and 2). The larger number and bigger size of spots enabled Wild-ID to distinguish more efficiently among individuals and thus rank them in higher positions. Nonetheless, the high ranking positions in all groups indicate that the performance of Wild-ID is equally satisfactory with spots of different numbers and sizes. In the between-years datasets, the ranking positions were high, although lower than those of the within-year datasets (Table 1). An inspection of the spot patterns of individuals that were recaptured between years (Fig. 2) indicates that the lower ranking positions are not a result of these patterns changing through time. Rather, they may be a consequence of small changes in body shape as a result of weight change. Newts recaptured between years are very unlikely to have the exact same weight. This natural variation in weight, which is likely to be greater between than within years, may lead to slight differences in body shape and size, giving the individual an overall different appearance between years. However, the high ranking positions of the vast majority of between-year images by Wild-ID indicate that these small changes in body shape do not hinder the algorithm's ability to identify individuals recaptured across years.

Wild-ID has already been used with great success in various animal taxa (Morrison and Bolger 2012; Bendik et al. 2013; Cross et al. 2014; Elgue et al. 2014; Halloran et al. 2015). This lack of species-specificity, and the encouraging results reported here and in these previous studies, indicate that Wild-ID can be successfully applied in a wide range of animals that exhibit natural markings, such as many amphibian species. In our study Wild-ID performed equally satisfactory in both newt species, demonstrating that it is capable of handling both ventral/dorsal and lateral patterns. Thus, computer-assisted pattern mapping using pattern-recognition algorithms such as Wild-ID has the potential to become a widely-applied method for individual identification in a broad range of animals. The significance of pattern mapping is further accentuated in CMR studies that aim in obtaining long-term information on the demography and population dynamics of species of conservation interest, such as many amphibians facing population declines.

Acknowledgments We would like to thank Eva Pitta and Christos Papathanasiou for their valuable help during field work, the stuff of the Management Body of Chelmos - Vouraikos National Park for providing their facilities and assistance, Kostas Sotiropoulos for helpful discussions and two anonymous reviewers for helpful comments and suggestions.

Compliance with ethical standards This study complies with the current laws of Greece.

Conflict of interest The authors declare that they have no conflict of interest.

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