SALAMANDRA **58**(1): 52–63
15 February 2022 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

Successful automated photographic identification of larvae of the European Fire Salamander, *Salamandra salamandra*

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Manuscript received: 15 December 2021
Accepted 14 January 2022 by Stefan Lötters

Abstract. Computer-aided individual recognition of animals based on their natural markings has become an indispensable tool in ecology research. However, this is problematic in species with faint patterns. Here we test whether individually reared larvae of the European Fire Salamander (*Salamandra salamandra*) can be reliably recognized from images of their lateral tail patterns. We used Wild-ID software to (1) estimate the recognition uncertainty resulting from pre-processing the images, (2) quantify how pre-contrasting improves recognition, (3) assess the effect of ontogenetic pattern change on recognition until metamorphosis, and (4) test how recognition performs with larger image libraries. Our results show that discrimination of larvae is highly successful. Pre-processing did not lead to a relevant change in the recognition probability, while pre-contrasting even reduced the recognition probability. The shorter the time interval between two photos, the more readily an individual will be recognized. The overall recognition rate was 99.81%, with false rejection rates (FRR, calculated as the number of falsely rejected images divided by the number of matching attempts) amounting to 4.66, 0.77 and 0.20% for FRR_1 (first image provided by Wild-ID does not match), FRR_{10} (none of the first ten images provided by Wild-ID matches) and FRR_{21} (none of the first 20 images provided by Wild-ID matches), respectively. These rates are among the lowest ever reported. The inclusion of images of 130 wild-caught larvae did not negatively affect successful individual recognition. Automated photo-identification may therefore be considered a reliable tool for fieldwork on European Fire Salamander larvae.

Key words. Amphibia, Caudata, false rejection rate, ontogenetic pattern change, photographic capture-recapture, tail pattern, Wild-ID.

Zusammenfassung: Die computergestützte individuelle Erkennung von Tieren anhand ihrer natürlichen Muster ist zu einem unverzichtbaren Instrument in der Ökologie geworden. Bei Arten mit nur schwachen Mustern ist dies jedoch problematisch. Wir untersuchen in dieser Studie, ob individuell aufgezogene Larven des Europäischen Feuersalamanders (*Salamandra salamandra*) anhand von Bildern ihrer seitlichen Schwanzmuster zuverlässig erkannt werden können. Wir verwendeten Wild-ID, um (1) die aus der Vorbearbeitung der Bilder resultierende Erkennungsunsicherheit abzuschätzen, (2) zu quantifizieren, wie unterschiedliche Vorkontrastierungen die Erkennung verbessern, (3) die Auswirkung der ontogenetischen Musterveränderung auf die Erkennung bis hin zur Metamorphose zu beurteilen und (4) zu testen, wie die Erkennung bei größeren Bildbibliotheken funktioniert. Unsere Ergebnisse zeigen, dass die Unterscheidung von Larven sehr erfolgreich ist. Die Vorbearbeitung führte zu keiner relevanten Veränderung der Erkennungswahrscheinlichkeit, während die Vorkontrastierung die Erkennungswahrscheinlichkeit sogar reduzierte. Je kürzer das Zeitintervall zwischen zwei Fotos ist, desto leichter ist die Erkennung. Die Gesamterkennungsrate lag bei 99,81 %, wobei die Raten fälschlich zurückgewiesener Bilder (FRR, berechnet als Anzahl der falsch zurückgewiesenen Bilder geteilt durch die Anzahl der vorgenommenen Tests) zwischen 4,66 %, 0,77 % und 0,20 % für FRR1 (erstes von Wild-ID geliefertes Bild stimmt nicht überein), FRR10 (keines der ersten zehn von Wild-ID gelieferten Bilder stimmt überein) bzw. FRR21 (keines der ersten 20 von Wild-ID gelieferten Bilder stimmt überein) lagen. Diese gehören zu den niedrigsten jemals beobachteten Fehlerraten. Die Einbeziehung von Bildern von 130 wild gefangenen Larven hatte keinen negativen Einfluss auf die erfolgreiche Erkennung von Individuen. Insofern ist die automatische Fotoidentifizierung ein zuverlässiges Instrument für die Feldarbeit mit Larven des Europäischen Feuersalamanders.

Introduction

Reliable estimates of a species' population size are of utmost importance for conservation biology (White 2000). A wide range of estimation techniques exist, of which those for open populations, such as the Cormack-Jolly-Seber method, are considered the most powerful (Jolly 1965, Seber 1965, Pledger et al. 2003). Open population ap-

proaches estimate the population size based on individual capture-recapture histories. Therefore, individual-based recognition of specimens in the wild is a requirement for solid population size estimation, making the choice of the marking method a crucial factor in planning a capture-mark-recapture (CMR) study. There is a wealth of techniques for marking vertebrates in the wild (e.g., Silvy et al. 2012), but only some of them are practicable for amphibians (Ferner 1975, Heyer et al. 1994). Most of these marking techniques are considered invasive and may negatively affect the survival rates of the marked individuals [e.g., May 2004, Lunghi & Veith 2017, Wagner et al. 2020a). This leaves fieldworkers with the necessity to carefully identify the most appropriate method by assessing both ethical responsibility and scientific validity (Perry et al. 2011, Silvy et al. 2012).Therefore, less invasive marking techniques that are at the same time cost-effective and reliable are highly desirable (Beausoleil et al. 2004). The most harmless way of individual identification of wild animals is not to mark them, but to use their natural individual 'markings' via photographical identification (capture-recapture only, CR). Such unique colour patterns are present in many amphibian species (Schlüpmann & Kupfer 2009).

Occasionally, common species become the focus of conservation biology. This is increasingly caused by emerging infectious diseases (Fisher et al. 2012), as in the case of the European Fire Salamander (*S. salamandra*), which is currently threatened by the spread of the chytrid fungus *Batrachochytrium salamandrivorans* (*Bsal*). For more than a decade now, this pathogenic fungus, which is believed to be of Asian origin (Martel et al. 2014, O'Hanlon et al. 2018), has caused dramatic population declines and mass mortality events in The Netherlands, Belgium, Germany and Spain (Martel et al. 2013, Spitzen-van der Sluijs et al. 2013, 2016). Therefore, detailed population studies on the European Fire Salamander are currently underway to monitor population developments in areas where the fungus is expected to expand its range (Lötters et al. 2020).

The terrestrial life stages of the European Fire Salamanders display a unique dorsal pattern with conspicuous, usually yellow, markings on a black background, which, in combination with their toxic skin components, makes them a candidate study object for aposematism in amphibians (Preissler et al. 2019). Various studies have already proven that this pattern can be used for individual identification of adult fire salamanders (Bradfield 2004, Plaiasu et al. 2005, Schlüpmann & Kupfer 2009, Mathé et al. 2017, Speybroeck & Steenhoudt 2017). Ironically, despite this unique pattern, estimating the size of adult fire salamander populations is very time-consuming due to their secretive lifestyle (Jung et al. 2000, Hyde & Simons 2001, Schmidt et al. 2015). Monitoring of larval populations of European Fire Salamanders is therefore increasingly used as a proxy for the adult population when information on population trends is needed (Schlüpmann & Kupfer 2009, Reinhardt et al. 2018), especially in areas where population breakdowns due to *Bsal* have been observed or are expected (Sandvoss et al. 2020, Wagner et al. 2017, 2020b,c). All these studies have used a removal sampling approach as suggested by Schmidt et al. (2015).

Wagner et al. (2020a) tested the performance of the removal sampling approach of Schmidt et al. (2015) using a CMR study based on Visible Implant Alpha tags (VIA), which are widely employed to mark amphibians (e.g., Buckley et al. 1994, Heard et al. 2008, Osbourn et al. 2011), including larvae (Courtois et al. 2013). They demonstrated that removal sampling strongly underestimated population size compared to CMR estimates. Apart from the fact that their recaptured marked larvae had significantly lower body indices compared to newly captured ones (so marking with VIA may be too invasive to be applied to fire salamander larvae), some larvae had lost their tags or their tags were barely legible through the skin in which cases they successfully used photos of lateral tail patterns to re-identify individuals (Wagner et al. 2020a).

Larval fire salamanders possess a unique and characteristic, albeit faint black tail pattern on a more or less greyish background. However, no comprehensive study regarding the use of larval tail patterns for individual recognitions has been conducted as yet. Only in a few instances have tail images been matched visually (e.g., Wagner et al. 2020a), which is time-consuming and, according to Eitam & Blaustein (2002), only feasible when there are few (i.e., ≤ 20) individuals. In addition, when the larvae approach metamorphosis, their tail patterns transform from a greyish ground colour to the species-specific black-yellow pattern (Giesenberg 1991), which makes continued recognition nearly impossible.

Here we test if the lateral tail pattern of larval European Fire Salamanders can be used for computer-aided photographic capture-recapture studies (PhCR; Bolger et al. 2012). If successful, this would pave the way for large-scale application in PhCR studies of larval populations. In particular, we ask if, and to which extent, the ontogenetic alteration of the tail pattern will over time reduce recognition efficiency. We also assess how metamorphosis influences recognition results, and up to which ontogenetic stage recognition is possible. Furthermore, we hypothesise that pre-contrasting of tail images may improve individual recognition. Finally, we test the assumption that increasing the size of the image library by adding images of wild-caught larvae will reduce individual recognition probability and increase the false recognition rate (FRR; Bolger et al. 2012).

Materials and methods

In 2018, we captured 40 newborn larvae of the European Fire Salamander (*S. s. terrestris*) (under license from the 'Struktur- und Genehmigungsdirektion (SGD) Nord' of the Federal State of Rhineland-Palatinate, Germany) in a first-order creek in the Hunsrück Mountains (Rhineland-Palatinate, Germany; 50°11'00" N, 7°37'45" E). Nine larvae were captured on 22 April, and the other ones one week later (see Supplementary document S1). They were kept one by one,

each in 5 litres of tap water, to preclude the possibility of individual mix-ups. The temperature was kept constant in a climate chamber at 15°C, with a 12/12-hour day/night cycle. The water was completely replaced on a weekly basis (aged tap water), and the larvae were fed ad libitum with sludge worms (*Tubifex tubifex*). Depending on their growth and ontogenetic development, the larvae remained in the experiment for up to ten weeks. From the beginning of the second week, each larva was photographed once a week with a specially adapted camera/tripod construction to document the change of their tail patterns. For this purpose, they were each placed in a small rectangular glass cuvette filled with water (lwh = 8 × 3 × 4 cm), which was placed in a special bracket equipped with LEDs. Both sides of the animals were photographed parallel to the camera with a Nikon D70 with standard zoom lens (28–80 mm focal length, aperture 1 : 3.3–5.6 G), but without flash. We consider left and right tail images as two more or less independent datasets of the same individuals, since there is no indication that left and right tail patterns develop equally, apart from the general background colour and dot density. To further test the performance of automated identification, we extended the dataset of the 40 test animals with images of another 130 wild-caught larvae captured in the same creek in May 2018 (photographed and released on site).

Wild-ID (1.0.0)

Wild-ID (1.0.0) is a Java-based matching software that recognises individual patterns in images, compares them with patterns in other images and finds matches (Bolger et al. 2011). Unlike other pixel-based matching software, Wild-ID uses a feature-based 'Scale Invariant Feature Transform' (SIFT) algorithm. To some extent, this algorithm tolerates variations in image scale, rotation, illumination and camera viewpoint (Lowe 2004). The feature-based matching-algorithm proceeds across the following three steps: (1) the image is examined for characteristic, invariant features, so-called key points; (2) the properties of these features are summarised in so-called feature descriptors; (3) the descriptors of an image are compared with those of other images during the matching process (Nischwitz et al. 2011, Bolger et al. 2012).

Based on the similarity of the descriptors, a score ranging from 1.0 (complete concordance) to 0.0 (no concordance) is determined during the matching process of two images (Bendik et al. 2013). The use of Wild-ID is generally divided into two parts: first, the steps outlined above are performed automatically by the software. In a second step, the user manually evaluates the 20 potential matches proposed by the software.

Data processing

To prepare the photos for Wild-ID, they were aligned horizontally alongside the shape of the tail of the larvae using Adobe Photoshop Elements 11. Then the part of the tail from the base of the hind leg to the tip of the tail was cropped at a ratio of 10 : 3.5. This left only the part of the image relevant for further investigation: the tail of the larva, which is characterised by an individual dot pattern (Eitam & Blaustein 2002).

Contrast versions

Wild ID is mainly used for species with conspicuous, high-contrast patterns. We therefore assumed that faint patterns, such as the lateral tail pattern of fire salamander larvae, might cause problems in the automatic recognition of individuals and that enhancing the contrast of an image before analysing it with Wild ID would increase the identification success. We therefore compared three different contrast versions (Fig. 1): (1) the original contrast version (ORG; no additional contrast), (2) a high-contrast colour version (COL), and (3) a binary greyscale version (BGS).

Contrast version 2 (COL) was generated by maximising contrast and brightness (+40%) and acuity (+50%) with Microsoft PowerPoint. The image was then saved and reloaded, again maximising contrast and brightness (+40%). Contrast version 3 (BGS) was also produced by maximising contrast and brightness (+40%) and recolouring (greyscale: 25%). This step was effected with Microsoft PowerPoint 2010 (Eitam & Blaustein 2002). The images were saved as JPEGs with 220 dpi in Microsoft PowerPoint (Eitam & Blaustein 2002).

Editing precision – pre-test

The precision with which a user aligns and crops the images during processing may lead to a background variation of the matching coefficients, the possible impact of which we investigated in a pre-test. One image each of ten randomly selected larvae (from the same randomly selected week) was aligned and cropped ten times each. They were then contrast-enhanced according to their contrast version. The first image of an individual was compared to the respective nine other images, resulting in three datasets (one per contrast version) containing 90 images each. These were analysed separately with Wild-ID to test how image processing itself affects matching results.

Compilation of datasets

Fire salamander larvae change their background colour over time; the density of melanophores increases so that the dot patterns become darker and darker during ontogeny (e.g., Pederzoli et al. 2003). To investigate whether this ontogenetic pattern change causes the recognition probability to decrease over time, the first image of a larva (taken in week 2) was compared with the images of this individu-

al from the following weeks ('change-over-time' test). This was done separately for the left and right tail sides; again we tested the performance of the three different contrast versions.

The ultimate goal of a pattern recognition software like Wild-ID is to detect and record the individual characteristics of captured animals. Therefore, a dataset usually contains all images taken during a field study. We simulated this scenario ('reality test') by compiling a data set containing all images collected from all laboratory individuals over time, as well as 130 images of the wild-caught larvae. This 'reality test' was performed separately for the right (431 photos) and left (429 photos; two images had been lost) tail sides.

Statistics

Automated species identification with Wild-ID is primarily aimed at identifying individuals. If the pattern of the specimen in question is very clear and unique and the pattern has already been stored in the image database, easy recognition can be expected. Ideally, the first image that the software selects from the image database should already match, so that a false rejection of this image as not matching the photos from the database is unlikely. Therefore, the false rejection rate (Bolger et al. 2012) quantifies the ease of finding a matching photo. In addition, the matching scores quantify the degree to which two images match.

Matching scores were tested for normal distribution (Shapiro-Wilk tests). Since normal distribution was rejected in almost all instances (see Supplementary document S2), we consistently applied non-parametric tests to guarantee equal test powers. To compare matching scores between treatments, we used the Friedman test for repeated measurings in cases where the three contrast versions of the same images were compared, including a post hoc test for paired comparisons with sequential Bonferroni correction for multiple testing; in the few cases where images of only two contrast versions were available, we used the Wilcoxon test instead. For the comparison of independent, non-repeated matching scores we used the Kruskal-Wallis test, complemented by a sequential Bonferroni post hoc test for multiple comparisons. All tests were performed using IBM SPSS®.

False rejection rate (FRR) in the 'reality test'

In addition to measuring the matching scores, we also wanted to know how easily Wild-ID correctly identifies individuals already known. Therefore, we estimated the False Rejection Rate (FRR) to assess the overall identification performance of Wild-ID. The FRR represents the probability at which the matching software fails to match two images of the same individual within a given number of best-matching photos divided by the number of identification attempts as a percentage and corresponds to the false negative error (Bolger et al. 2012). We estimated three different FFR versions: FRR_1 refers to cases where Wild-ID was unable to find a true match at first; FRR_{10} refers to cases where no true matches were found among the ten highest ranking images; FRR_{21} refers to cases where Wild-ID was unable to assign a correct image of the same individual to a

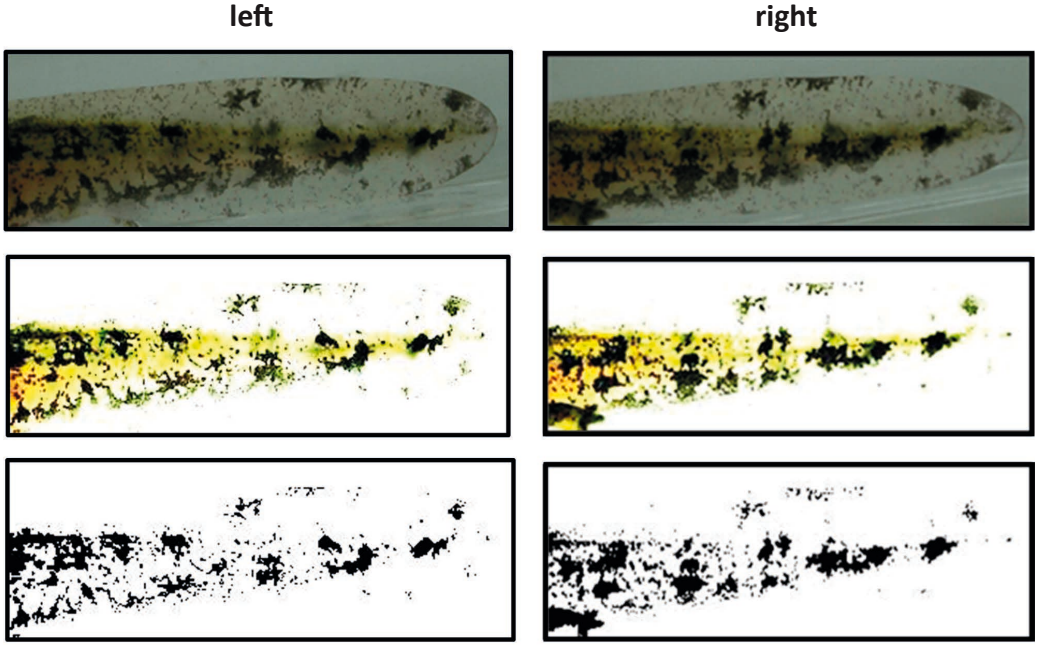

Figure 1. Comparison of contrast versions. Three contrast versions of the left and right tail side of the same individual: original version (ORG; upper), high-contrast colour version (COL; middle), binary greyscale version (BGS; lower).

Table 1. FRR-values of studies using Wild-ID and/or amphibians; [1)] in these studies, FFR was calculated in a slightly different manner: number of failures to match two images of the same individual within a given number of best-matching photos divided by the number of successful identification attempts (as a percentage); [2)] FFR calculated by ourselves based on the data presented in the paper.

Species	FRR-value [%]	Software	Image sublibrary	Reference
FFR21				
European Fire Salamander (*Salamandra salamandra*)	0.19	Wild-ID	original contrast version	this study
Jollyville Plateau Salamander (*Eurycea tonkawae*)	0.76	Wild-ID	high quality photos	Bendik et al (2013)
Jollyville Plateau Salamander (*Eurycea tonkawae*)	15.9	Wild-ID	low quality photos	Bendik et al (2013)
Masai Giraffe (*Giraffa camelopardalis tippelskirchi*)	0.67	Wild-ID		Bolger et al. (2011)
Tuatara (*Sphenodon punctatus*)	2.4	Wild-ID	right side	Sá Rocha Mello et al. (2019)
Pacific Horned Frog (*Ceratophrys stolzmanni*)	5	Wild-ID		Bardier et al. (2020) [1]
FRR10				
European Fire Salamander (*Salamandra salamandra*)	0.77	Wild-ID	original contrast version	this study
Great Crested Newt (*Triturus cristatus*)	2	AmphIdent		Drechsler et al. (2015) [1]
Marbled Salamander (*Ambystoma opacum*)	5	own software		Gamble et al. (2008)
Strinati's Cave Salamander (*Hydromantes strinatii*)	4.2	Wild-ID	cloaca/chest	Renet et al. (2019)
FRR1				
European Fire Salamander (*Salamandra salamandra*)	4.66	Wild-ID	original contrast version	this study
Alpine Newt (*Ichthyosaura alpestris*)	0.58	Wild-ID	males	Mettouris et al. (2016) [2]
Alpine Newt (*Ichthyosaura alpestris*)	1.66	Wild-ID	females	Mettouris et al. (2016) [2]
Smooth Newt (*Lissotriton vulgaris*)	7.59	Wild-ID	males	Mettouris et al. (2016) [2]
Smooth Newt (*Lissotriton vulgaris*)	18.6	Wild-ID	females	Mettouris et al. (2016)
Marbled Salamander (*Ambystoma opacum*)	30	own software		Gamble et al. (2008)
Strinati's Cave Salamander (*Hydromantes strinatii*)	8.2	Wild-ID	cloaca	Renet et al. (2019)
Strinati's Cave Salamander (*Hydromantes strinatii*)	9.3	Wild-ID	chest	Renet et al. (2019)

test image within 20 potential matches (Bolger et al. 2012, Drechsler et al. 2015). FFR values were calculated from the data set we had compiled for the reality test (see above).

Results

Pre-test

Our pre-test produced the best results using the ORG version (Fig. 2), with an average score of 0.974 across all individuals (the maximum value of 1.0 was achieved in 81% of all matches), followed by the COL version (0.909; 71% with a score of 1.0), and the BGS version (0.872; 70% with a score of 1.0). When analysed together, the matching scores differed significantly between the three versions (Friedman test); however, none of the post hoc pairwise comparisons remained significant after Bonferroni correction, with a tendency of ORG producing higher scores than BGS (p=0.063) (Supplementary document S2).

'Change-over-time test'

Wild-ID performed best with ORG over time and worst with the BGS. All contrast versions showed decreasing scores with the more time had elapsed between two images (Fig. 3). The Kruskal-Wallis test with post hoc Bonferroni correction for the comparisons over weeks almost always revealed highly significant differences for ORG ($p < 0.001$); interestingly, the decrease in matching scores between the respective first pair of comparison (e.g., week 2 with 3 versus week 2 with 4) was not significant (Supplementary document S2). The other two contrast versions exhibited less pronounced differences over time (Supplementary document SI 2). Friedman tests of post hoc pairwise comparisons of contrast versions within weekly comparisons always showed higher scores for ORG compared to COL and BGS, but only a slight significance for COL and BGS in week 2 with week 3 comparisons (Supplementary document S2).

Comparison of body sides

Matching scores for the left and right tail side of an individual can vary (e.g., ORG for week 2 with 3 of individual 2: 0.605 and 0.129 for the left and right side, respectively). Their median scores across all individuals and weeks are similar (Fig. 4), with no significant difference in all three contrast versions (all $p > 0.05$; see Supplementary document SI2).

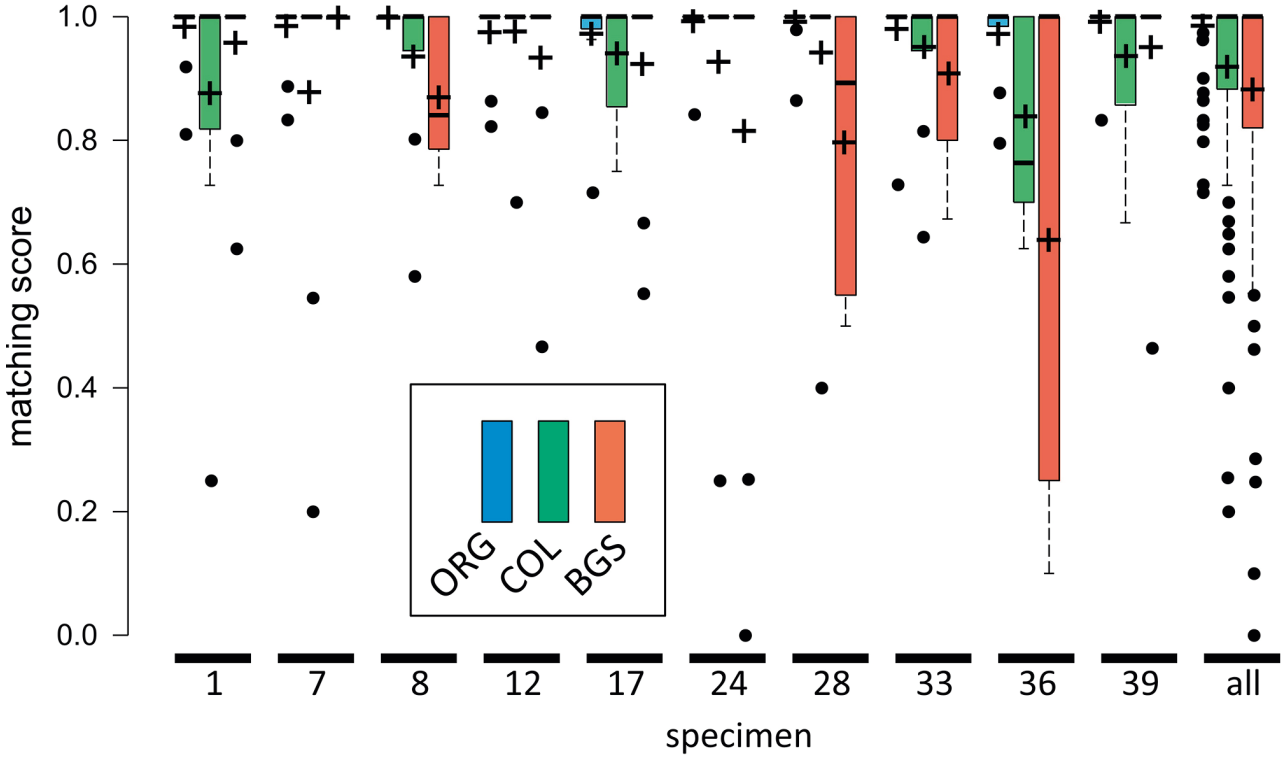

Figure 2. Matching scores of the pre-test (ORG: original version, COL: high-contrast colour version, BGS: binary greyscale version). Scores of ten independently edited images from ten randomly selected individuals (pre-test) are given; average matching scores are indicated by crosses; median (-) and mean (+) matching scores are given.

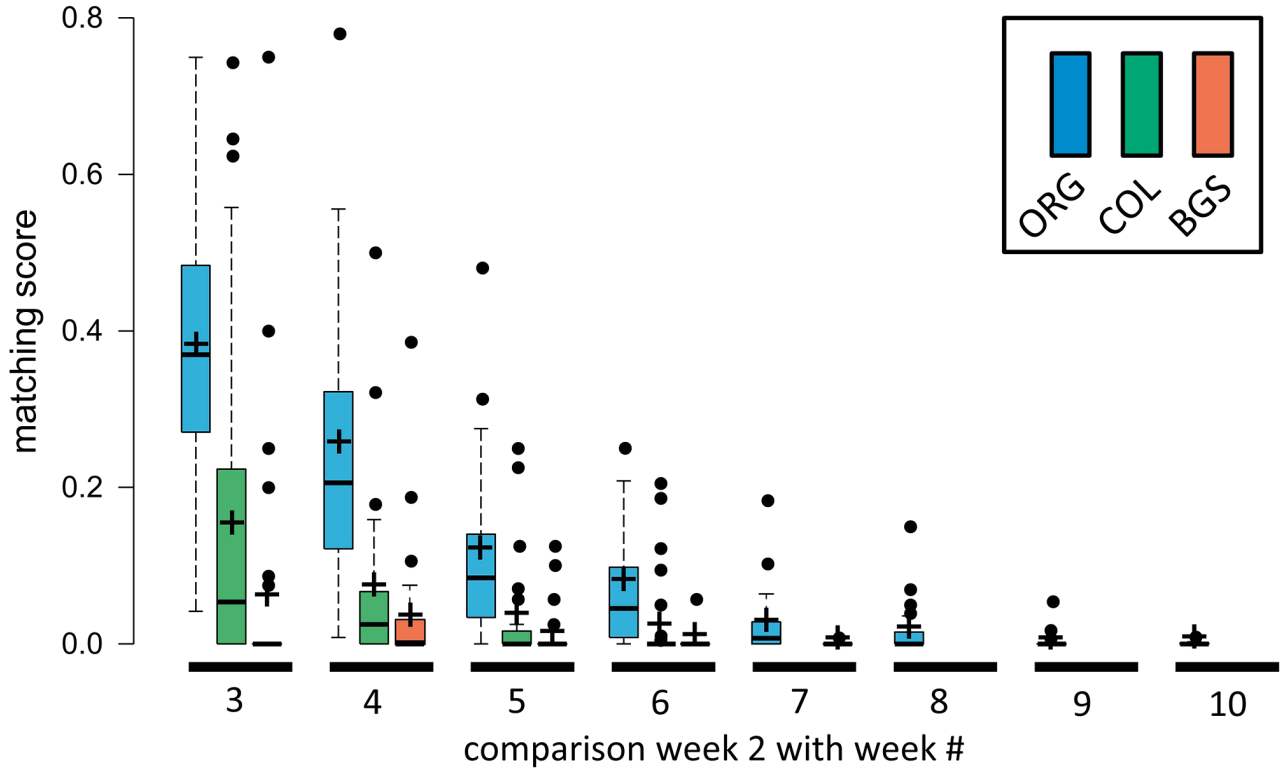

Figure 3. Box-whisker plots with median (-) and mean (+) matching scores of weekly comparisons (ORG: original version, COL: high-contrast colour version, BGS: binary greyscale version). Scores for left and right sides are averaged.

'Reality test' with wild-caught larvae

Only one image of the right tail side could not be matched. For all images of the left tail side, a matching image was identified within the 20 possible matches offered by Wild-ID. For all other images of the 40 individuals, Wild-ID was able to find correct matches, even for those images from later weeks that could not be successfully matched in the 'change over time' test. This resulted in an average success rate of 99.81% for both sides, with FRR_1 at 4.66%, FRR_{10} at 0.77%, and FRR_{21} at 0.20% (Fig. 5). The lowest score of an image that matched correctly within the 20 images suggested by Wild-ID was 0.00002, while the highest score for an image that did not match within the first 20 images was 0.5. Nevertheless, there was almost no overlap in scores between matching and non-matching images (Fig. 6).

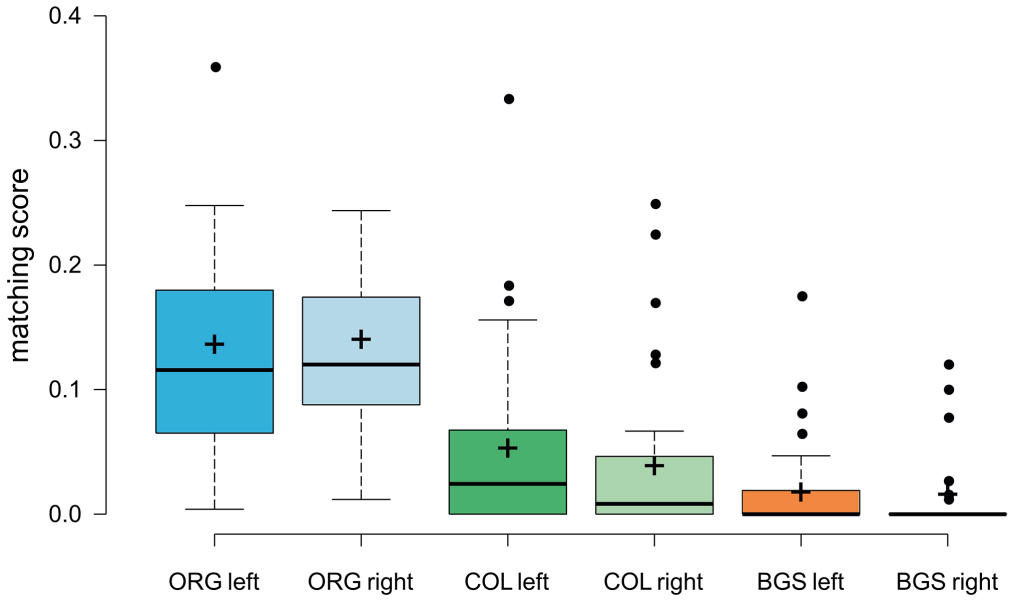

Figure 4. Box-whisker plots with median (-) and mean (+) matching scores for the left and right tail sides (ORG: original version, COL: high-contrast colour version, BGS: binary greyscale version). Scores are given for weekly comparisons.

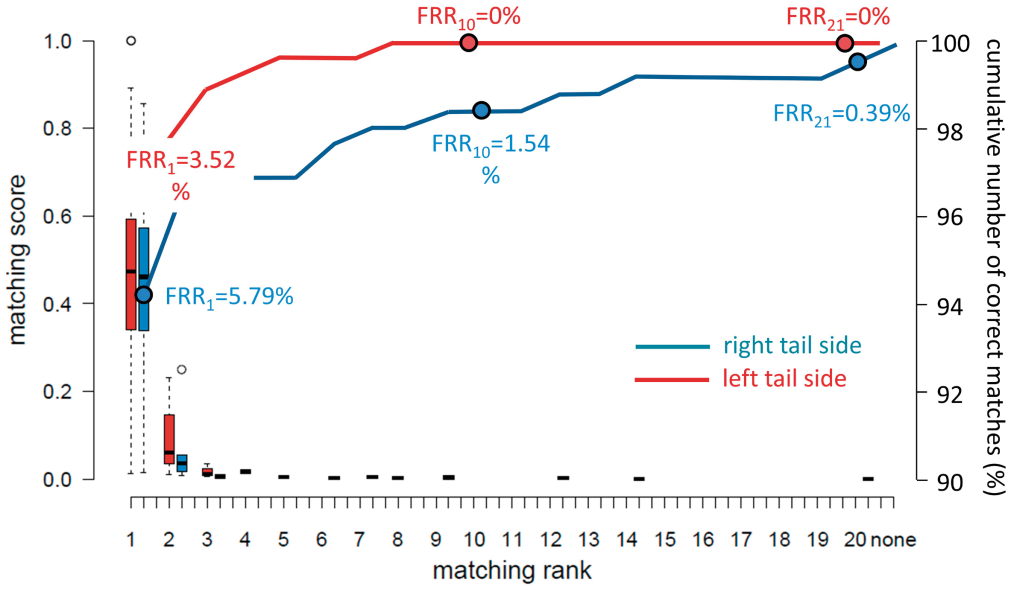

Figure 5. Cumulative amounts of correct matches. Correct matches are given with increasing matching rank from amongst 20 images of left versus right tail side as suggested by Wild-ID as potential correct matches; the boxplots quantify the matching scores across matching ranks.

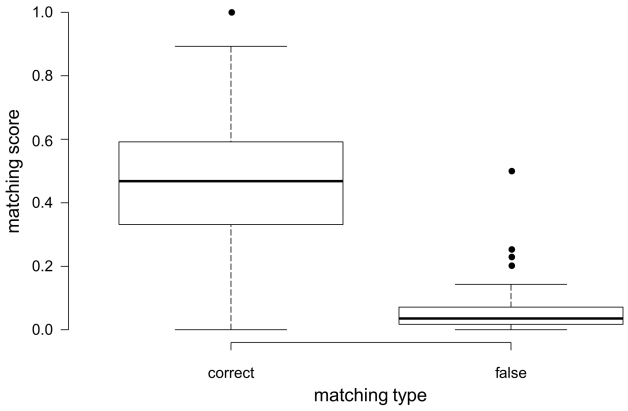

Figure 6. Comparison of correct and false matches. Matching scores of the images suggested correctly or incorrectly by Wild-ID within the first 20 images.

Discussion

The use of natural markings for the recognition of individuals within wild animal populations in PhCR has become an important tool for a variety of biological studies, such as individual behaviour, population demography, and population dynamics (Bolger et al. 2012). Its non-invasiveness (Silvy et al. 2012) and its usually low costs (Cruickshank & Schmidt 2017) make it superior to invasive marking techniques, and automation through specialized imaging software significantly reduces the time for re-identifying individuals from a large image library (Bolger et al. 2012). While it seems obvious that photographic identification can be associated with low error rates in species that display conspicuous and high-contrast patterns (among amphibians, e.g., the Bellied Toads (genus *Bombina*; e.g., Vörös et al. 2007, Gollmann & Gollmann 2011, Cruickshank & Schmidt 2017) or the European Fire Salamander (e.g., Carafa & Biondi 2004, Goedbloed et al. 2017), animals with faint patterns may pose problems in PhCR. However, our test of Wild-ID's performance on tail photos of European Fire Salamander larvae clearly demonstrates that despite the sometimes very low matching scores, individual identification is highly reliable and can be used in studies on wild populations.

Our pre-test shows that the processing of images may already lead to a – albeit small – variation of matching scores. However, the average of 0.974 for the original (unaltered) contrast version is still close to the highest possible score of 1.0. Interestingly, the original image version already achieved significantly better results than the two processed contrast versions in the pre-test. However, this difference was not significant, so that our hypothesis that prior contrast-enhancing improves matching scores can be rejected. This is maybe due to the fact that Wild-ID itself performs a greyscale transformation prior to the actual scoring. The fact that the binary greyscale (BGS) version performed worst is probably due to the fact that too much information of the image is lost during the transformation. Other studies also recommend the use of multicolour rather than binary images (Matthé et al. 2017). In contrast, the slight reduction in the matching scores of the original version caused by the alignment and cropping of images can hardly be avoided, since cropping the images is necessary and strongly recommended when using Wild-ID (Bolger et al. 2011). It minimizes possible sources of error, namely the background as well as colour- and pattern-invariant image parts (Bolger et al. 2011, Elgue et al. 2014). Therefore, alignment and cropping cannot be dispensed with, and given the overall low matching scores of pictures taken of the same individual at different times, this slight reduction due to processing seems negligible.

The decreasing matching scores over time confirm our assumption that the shorter the time between two photos, the more readily the recognition of individuals will be achieved. We expected that time would affect pattern recognition, since it is known that fire salamander larvae continually change their background colour and dot pattern (e.g., Pederzoli et al. 2003), a fact that a priori contradicts one of the basic conditions of PhCRs, namely that an individual's pattern is stable over the duration of the study period (Bolger et al. 2012). This phenomenon has also been described for other amphibian species (Arntzen & Teunis 1993, Gollmann & Gollmann 2011, Drechsler et al. 2015, Bardier et al. 2017). Other studies have found that changes in body shape and weight, but also growth or other reasons are responsible for the negative correlation between time and recognition probability (Bendik et al. 2013, Mettouris et al. 2016).

In particular, the trend towards significantly worse results from week 7 onwards suggests that the decreasing recognition rate is due to the metamorphosis of the larvae (Eitam & Blaustein 2002). During this phase, the black dots, which represent dense groups of epidermal melanophores, become larger and finally merge into the black background colour of the larvae. Depending on the water temperature, metamorphosis starts after 40 to 120 days (Seidel & Gerhard 2016). Our laboratory larvae entered metamorphosis at an age of about seven weeks (= 50 days). This still does not mean that our method is at a disadvantage compared to invasive marking techniques such as Alpha or VIE tags. Wagner et al. (2020a) pointed out that the intensified coloration of the skin of salamander larvae shortly before metamorphosis also makes Alpha tags less visible. Furthermore, such tags may disappear or migrate to different parts of the body during metamorphosis (Garnt 2008, Brannelly et al. 2013, Courtois et al. 2013). The adverse effect of the increasing obscuration of the tail pattern toward metamorphosis can be compensated, at least partially, by using images of both sides of the tail. We were able to show that they did not differ significantly in matching probability and thus can be considered an independent second set of characters for identification.

The significantly different scores of correct and false matches suggest that the scores in themselves can serve as an informative indicator for the individual identification of

larval fire salamanders. A score of > 0.250004 may, at least under the specific conditions of our test situation, serve as a threshold to decide whether two images match. Other studies may find different thresholds; Bendik et al. (2013) were able to set a matching threshold of 0.1 when they pretested the dorsal melanophore pattern of the heads of a small number of Jollyville Plateau Salamanders (*Eurycea tonkawa*) with VIE-Tags and Wild-ID. We, therefore, recommend that should be determined for any given study species by prior re-identifying a small group of known individuals. This may considerably improve the evaluation of the entire picture library: matches that exceed this threshold can almost certainly be considered correct and only matches with lower scores must be checked by hand. Interestingly, in our study, Wild-ID incorrectly matched the photos of two apparently very different larvae with a score of ca. 0.5, which is very much > 0.250004. But relying solely on matching scores would have resulted in an undetected false-positive match. In a real study situation, the user would have immediately recognised the great difference between the images if it had been suggested by Wild-ID as one out of 20 potential matches.

Even under a more realistic study situation (all pictures of all laboratory larvae were merged into a single picture library together with the images of the 130 wild-caught larvae), the success rate of identification was 99.81%. Our hypothesis that 'the larger the dataset, the lower the recognition rate' must therefore be rejected, at least for our library size of ca. 430 images. This is consistent with the results of other studies where Wild-ID was used for individual recognition (Bolger et al. 2012, Mettouris et al. 2016) and leads us to conclude that Wild-ID is a suitable tool for identifying larvae of the European Fire Salamander (*S. salamandra*) even from large picture libraries. This is based on the very low FRR rates we found, although the matching scores themselves were often very low. However, as populations of salamander larvae can be very large (e.g., Wagner et al. 2020a; see also Veith et al. 2022 for a review), successful use of the lateral tail pattern of larvae for individual recognition still needs to be tested under field conditions.

To better relate our results, we have compared our FFR values with those of other studies (Table 1) using Wild-ID or working with amphibians. It appears that our FRR_{21} and FRR_{10} values were the lowest compared to other studies. Only our FRR_{1} value of 4.66% was in the range of corresponding values in other studies. We therefore recommend considering not only Rank 1 matches but at least also Ranks 2–10. Matthé et al. (2017) also encountered an improvement in the performance of about 26% when considering the top 10-ranked matches instead of single Rank 1 match. Nevertheless, incorrect matching can still occur. PhCR studies with such low error rates, however, produce altogether reliable results, e.g., for population estimation studies (Morrison et al. 2011).

There are several reasons why PhCR performed better in our study compared to other studies. Firstly, the SIFT-algorithm used by Wild-ID is considered an advantage over a pixel-based algorithm, such as used by AmphIdent, because it is more tolerant of variations in rotation, angle, scale and illumination (Lowe 2004, Matthé et al. 2017). Image quality is also very important (Bendik et al. 2013). The same is true for the time between recaptures, which is known to negatively affect the recognition rate (Bendik et al. 2013, Mettouris et al. 2016) and which was comparatively short in our study. In addition, changes in the posture, shape and size of the animals (Gamble et al. 2008) as well as glare and flash may contribute to recognition uncertainty (Matthé et al. 2017). The latter was avoided in our study by using a special LED illumination system, by wiping off water droplets from the photo cuvette and by using a rectangular instead of a round cuvette. Another very important factor influencing the recognition success is the type and variation of the species-specific pattern itself. Elgue et al. (2014) demonstrated that the highly variable ventral pattern of individuals of *Melanophryniscus montevidensis* produced better matching results compared to the less variable *M. admirabilis* and *M. cambaraensis*. A higher number of dots (as in *Ichthyosaura alpestris* compared to *Lissotriton vulgaris*) or larger dots (as in male *L. vulgaris* compared to female *L. vulgaris*) may also lead to better matching results (Mettouris et al. 2016).

In our study setup, recognition is even possible across later weeks, when metamorphosis has already started, albeit at low rates. This will increase if not only week II, but also later photos are compared with pictures taken shortly before metamorphosis, as is indicated by our reality test where all images of each larva were successfully matched. Nevertheless, the time intervals between two capture events should be kept as short as possible, in particular, if the animals' patterns change over time (e.g., in metamorphosing larvae; Bendik et al. 2013).

In conclusion, a computer-assisted photographic identification of European Fire Salamander larvae (*S. salamandra*) is not only possible with Wild-ID (and probably also with other image-matching software), but also very reliable. It has many advantages over other marking techniques, such as fast identification both in terms of image production and matching. Apart from the necessary hardware, it is inexpensive and most importantly less invasive than other techniques (Caorsi et al. 2012, Šukalo et al. 2013, Wagner et al. 2020a). Nevertheless, especially with small animals, taking pictures can already require a considerable amount of handling, either by photographing them in-hand or by using a manipulation or fixation tool. This reintroduces the potential stress that the non-invasive technique is intended to avoid (Treilibs et al. 2016). In our approach, the use of specially adapted photographic equipment, with a water-filled cuvette mounted on a tripod, reduces stress and even guarantees that the photos depict the animals in a more or less standardised position. In a slightly modified form, this tripod setup has already been successfully used under field conditions by Wagner et al. (2020a).

Alignment and trimming of photos are highly recommended, but editing the contrast of photos prior to using them for identification in a photo library, as we did, is not

only unnecessary – it will even reduce matching scores. If the time interval between two recaptures is short, e.g., about a week, the match is not negatively affected by ongoing metamorphosis, and the larvae can be identified until they leave the water. If feasible, a pre-test will help to determine study-specific matching thresholds that can speed up the identification process when working with large photo libraries. Automated PhCR will therefore substantially improve the efficiency of population studies of larval fire salamanders and thus can significantly contribute to the monitoring of endangered salamander populations in areas where the salamander plague caused by *Bsal* occurs or is expected to occur.

Acknowledgements

This study was performed under license from the 'Struktur- und Genehmigungsdirektion (SGD) Nord' of the Federal State of Rhineland-Palatinate, Germany. Treatment of larvae was approved by the Ethics Commission of Trier University and carried out in accordance with the relevant animal welfare guidelines and regulations of the Federal State of Rhineland-Palatinate. All methods have been reported in accordance with the ARRIVE guidelines.

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Supplementary data

The following data are available online:

Supplementary document S1. List of laboratory animals with entrance and leaving dates as well as number of images.

Supplementary document S2. Test statistics of Shapiro-Wilk tests on normal distribution and Friedman and Wilcoxon tests on differences between matching scores.