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Effectiveness of the field identification of individual natterjack toads (*Epidalea calamita*) using comparisons of dorsal features through citizen science

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Citizen science is now making an important contribution, both in the collection of large amounts of data over wide geographical areas and in promoting environmental awareness and engagement communities. However, as there are many participating observers, the reliability of the data collected needs to be assessed. This study used a citizen science approach to investigate whether dorsal features, when photographed, can be used in the identification of individual natterjack toads (*Epidalea calamita*). *Epidalea calamita* individuals from a population located at Prestatyn, North Wales, were captured, photographed and released in a legally compliant manner. Forty human participants each completed a timed exercise to match photographs of individual toads that had been taken from different angles. Sixty-five percent of the participants accurately matched photographs on their first attempt. The effect of training on the accuracy and speed at which participants could identify individuals from photographs was then assessed. Twenty of the participants received basic training on recognising the key features of dorsal patterns before carrying out the exercise again. Following training, average accuracy increased to 90% and participants were 41.5% quicker in completing the exercise than those that were untrained. The study revealed that basic training of participants who are involved in citizen science projects was beneficial by having a significant impact on accuracy and speed. In addition, we demonstrate that the dorsal features of tubercles and scarring are useful in identifying individuals of *E. calamita* in the field.

Key words: Epidalea calamita, natterjack toad, photo-identification technique, training, mark recapture, citizen science

INTRODUCTION

Citizen science makes use of volunteers to collect scientific data (Ratruieks et al., 2016). It has the dual advantages of allowing a large number of people to be involved in collecting substantial amounts of data and of raising scientific awareness amongst those people involved. It is also termed participatory or community-based monitoring. Although the reliability and accuracy of such data have been questioned, it is a method that is increasingly accepted and used in a wide range of research across many habitats and species (Aceves-Bueno et al., 2017). Studies using a citizen science approach have tended to focus on species that are relatively straightforward to find and identify, e.g. birds, Lepidoptera and plants (Chandler et al., 2016).

This study investigated the citizen science approach specifically to data collection requiring the accurate identification of individual small animals in the field. The study used *Epidalea calamita* (Laurenti, 1768), the natterjack toad. Other studies have also used amphibians. For example, Casula et al. (2017) analysed data from many observers, collected simultaneously, on individuals of the Sardinian Mountain Newt, *Euproctos platycephalus*. They found that the reliability of observation differed widely amongst observers and training did not remove the variability.

Epidalea calamita has a range that extends from the Iberian Peninsula to the Baltic States. The United Kingdom and Ireland have isolated populations (McInerny & Minting, 2016). This study was carried out in Wales where the natterjack toad is protected under the laws of England and Wales (Wildlife and Countryside Act 1981 (as amended) (Schedule 5)) and European law (Regulation 39 of the Conservation (Natural Habitats &c) Regulations 1994 (as amended) (Schedule 2) as European Protected Species). *E. calamita* is thought to have suffered a 70% population decline in Britain over the last 100 years (Beebee & Denton, 1996). A reintroduction programme along the Denbighshire coast began in 2000, following the local extinction of the species around the 1950's and the population is thought to be gradually increasing (Buckley, 2006). In 2009, there were roughly 60 populations in the United Kingdom with an estimated total of 3,000 breeding females (Amphibian and Reptile Conservation, 2009).

The importance of reliably identifying individuals in populations to investigate species' ecology and behaviour is well documented (Wiirsig & Jefferson, 1990). However, the negative effects that accompany some methods of identification have received attention (Phillott et al., 2008;

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Perry et al., 2011; Corrêa, 2013). For instance, when mark-recapture surveys are carried out, artificial marking methods have often involved physical additions to the animal such as paint or tracking devices, which may affect behaviour and survival (Ferner, 1979; Lemckert, 1996; Schmidt & Schwarzkopf, 2010). In the case of amphibians, previously employed marking techniques have included chemical branding (Wolf & Hedrick, 1971), tattooing and cold branding (Nace et al., 1974), skin dyeing (Gittins et al., 1980; Brown, 1997), Passive Integrated Transponder tagging (Brown, 1997), and the commonly used method of toe-clipping (Denton & Beebee, 1993; Heyer et al., 1994; Retallick et al., 2004). Toe-clipping, which involves the systematic removal of toes in unique combinations, is a low-cost method of marking anurans (Luddecke & Amezquita, 1999). However, studies suggest that it may reduce the mobility of marked individuals of some species (McCarthy & Parris, 2004). Marking often creates a wound, which is a potential site of infection (Bradfield, 2004) and ethical considerations of these practices must be undertaken when planning studies (May, 2004). Therefore, alternative methods for identifying individuals should be developed and tested.

One such alternative is to employ photography for the non-invasive identification of individuals. Digital cameras are readily available to investigators and provide instant photographs which are easily downloaded, copied and distributed. For example, photography has been used extensively for identifying cetaceans (Karczmarski & Cockcroft, 1998; Friday et al., 2000; Calambokidis et al., 2004). Individual cetaceans have physical characteristics and unique markings that distinguish them from other individuals (Perrin & Wursig, 2009). The body parts used commonly for cetacean photo-identification are the dorsal fin or tail fluke (Hebridean Whale and Dolphin Trust, 2008). Photographs of these markings are retained and used repeatedly for identification by different investigators. For terrestrial mammals (Kelly, 2001; Perera and Perez-Mellado, 2004; Jackson et al., 2006; Anderson et al., 2010), reptiles (Knox et al., 2013) and amphibians (Bradfield, 2004; Kenyon et al., 2009; Lama et al., 2011), this method has been used less frequently.

Photo-identification has the potential to be useful for any anuran species that exhibits variability of natural markings on at least one region of their body (Bradfield, 2004). These markings can be photographed in the field and used for identification by visually matching them to photographs already taken during previous surveys, either by eye (manual matching) or by using pattern-recognition software. It is a non-invasive method and permits the permanent identification of an individual (McConkey, 1999; Perera and Perez-Mellado, 2004). Pinya and Perez-Mellado (2009) recognised that individual Majorcan midwife toads (*Alytes muletensis*) had different patterns of spots on their backs and they identified five groupings of patterns for the dorsal spots. In North America, Morrison et al. (2016) used the patterns of "wart-like glands" on the backs of Wyoming toads (*Anaxyrus baxteri*) for "naïve" observers to identify individuals by eye with high reliability. This method of observation gave more accurate results than computer matching. In South America, Caorsi et al. (2012) used patterns on the ventral surfaces of the southern red-bellied toad *Melanophryniscus camabaraensis* to compare the accuracy of the identification of individual toads by the investigators using visual methods as opposed to toe clipping. They found that visual identification was more accurate.

Here we examined the use and reliability of photographic identification for *E. calamita* in the context of citizen science and whether training affects this reliability. *Epidalea calamita* toads can reach 70 mm in length (Beebee & Denton, 1996). They are brown, grey or green in colour, with eyes that have golden irises and horizontal pupils. They have tubercles on their dorsal surfaces and these dorsa are also characterised by a yellow or cream stripe traversing from snout to vent (Natural England, 2007). Mainly a lowland species with strict habitat requirements, natterjack toads are normally only found in coastal dunes, upper saltmarshes and lowland heaths (Beebee & Denton, 1996).

Epidalea calamita have variable, natural, throat spot markings in females (Denton, 1991), but these are difficult to photograph. However, all individuals have distinctive dorsal stripes and wart patterns (McInerny & Minting, 2016; citing Arak, 1983). Photographic identification using the dorsal region for either sex has not been documented. Some individuals possess obvious scarring on their dorsum, most likely a result from bird attacks, and this could also have a use in individual identification (Buckley, pers. comm., 6 September 2015).

The objectives of this study were to:

1. establish whether photo-identification of *E. calamita* is feasible in citizen science using the natural markings on the dorsum of individuals
2. ascertain whether the training of non-specialist observers improves the accuracy of identifying individuals by dorsal markings
3. ascertain whether training improves the speed at which individual toads can be identified.

METHODS

Location and photography

Data collection was carried out at Presthaven Sands, Talacre Warren Special Site of Scientific Interest (OS reference: SJ 10320 84717), in Prestatyn, North Wales. A team of two people conducted four evening surveys under the statutory survey licence for *E. calamita* (Conservation of Habitats and Species Regulations 2010 and the Wildlife and Countryside Act 1981). Surveys commenced later than 2000 on the following dates: 1, 18 and 24 June and 1 July 2015 when weather conditions gave warm and wet evenings above 8 °C (Beebee, 1977). The surveys were carried out at a set of three breeding scrapes (artificial breeding ponds), where males were known to congregate and call for females (Fig. 1).

The following camera settings were established as optimal: shutter speed of 1/100 s, aperture of f/6.3 and 600 ISO, using a Nikon D7000 16 megapixel DSLR camera with a Nikkor 35mm f/1.8 lens. Flash photography was not used in case it promoted stress. Instead, individuals were illuminated by white torchlight.

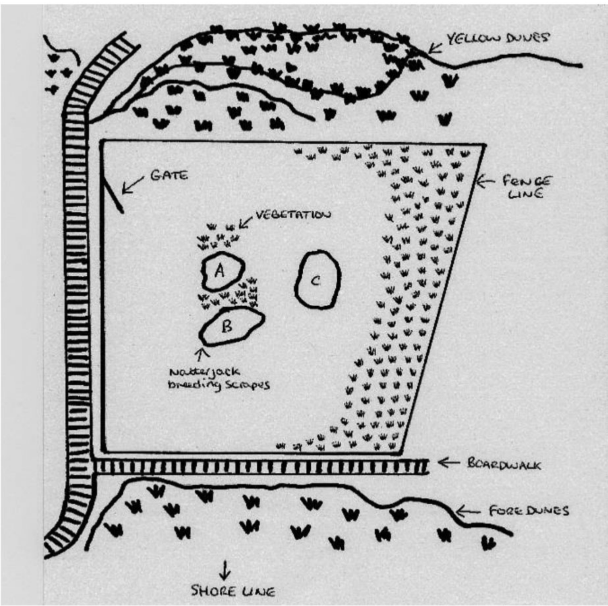

Figure 1. Location of breeding scrapes. The individuals were collected from scrapes A and B.

Survey methods

Each survey lasted no longer than two hours in order to keep disturbance to a minimum. Surveys involved locating individuals by torchlight in and around the breeding scrapes. Those sighted more than three feet into a scrape were ignored, as wading in order to collect was likely to disturb and cause them to swim away. Any individuals seen in amplexus were left undisturbed. Individuals were collected in a large bucket and transported 100 metres to the site warden's vehicle for photographing. Toads were removed individually from the bucket and sequentially placed into a perspex container. A minimum of five photographs of the dorsal region were taken from above each individual, in quick succession, taking no longer than 15 seconds to photograph each individual. The potential effects of handling individuals were not examined in this study. After photographing, individuals were placed in a different bucket to avoid re-photographing them. Once all individuals had been photographed, they were returned to the perimeter of the breeding scrapes.

An estimated 200-300 individuals are thought to be present at the study site (Evans, pers. comm., 14 April 2015). Forty-five individuals were photographed.

Testing the feasibility of photo-identification

The characteristics that were used to identify individual toads were the dorsal stripes, tubercles and scarring.

The accuracy and speed at which human participants could recognise individuals from photographs was investigated using a photo-matching exercise, based on the method developed by Knox et al. (2013). Participants declared that they had no prior experience of photo-matching or of individual toad recognition. Two photographs were arbitrarily selected of each of 5 toads that were also selected arbitrarily. Each of the 40 participants was asked to match one printed photograph of each of 5 individual toads to the other on-screen, photograph of the same toad, from amongst 20 photographs shown on a computer screen. The photographs used on-screen, and those printed, were chosen arbitrarily but checked to make sure identifiable characteristics were visible. Participants were divided into Group One and Group Two, each group comprising 20 individuals. Irrespective of group all the participants worked alone and were thus individually tested. The same sets of photographs were used for both Group One and Group Two. The number of correct matches out of 5 was recorded for all participants. In addition, the time taken to select what the participant considered a match for each of the five photographs was recorded for those in Group One before and after training.

Effect of training on accuracy and speed of photo-identification

Once each participant in Group One had finished the first photo-matching exercise, they immediately received individual training. This training involved describing to the participants the main characteristics of each of the five individual toads in the printed photographs. Training lasted no longer than five minutes and was immediately followed by the participant's second photo-matching exercise.

Participants were advised not to use colour as an identifiable characteristic as colour differed between photographs owing to the angles of torchlight. However, colour photographs were used as they helped to contrast the yellow dorsal stripe against the background colour of the dorsum. Familiarity with identification through using colour images would also assist the observer if, later, working directly with toads. Participants were also told not to rely on the shape of an individual, as the position in which the toads were sitting when photographed changed their apparent body shape.

Once trained, the second photo-matching exercise used an amended photographic database that included the same 20 photographs but with different numerical codes and in a different sequence. The five photographs that they used to match against the database were changed to be different from those in the first exercise. A similar method was used by Knox et al. (2013) in which participants who had no previous experience of photo-matching were compared against the accuracy and timings to a different group of participants who had previous experience and training. Following training, for the second photo-matching exercise, participants were timed once again in matching the 5 photographs. The number of correct matches, out of 5, and the time taken to complete the test, was recorded.

Participants in Group Two did not receive any training and instead repeated the photo-matching exercise in an identical way to their first exercise, after photograph numbers were re-assigned in the same way as for Group 1, thereby acting as a control group. A period of 5 minutes was left between the exercises, equivalent to the time of training for Group One. Their accuracy in terms of correct matches out of 5 was recorded.

RESULTS

Over the 4 surveys, a total of 45 individual toads were captured, photographed and released. Two of these individuals were captured four times, two were captured three times and ten were captured twice.

Differentiating between individual *E. calamita* toads
The following characteristics were used to identify individuals.

Dorsal stripe
Within each individual, the lengths and thicknesses of stripes were unique. As examples, in one individual the stripe formed a large blotch on its right side towards the lower dorsal region (Fig. 2) and in another the majority of the stripe was missing (Fig. 3).

Figure 2. *E. calamita* with dorsal stripe blotch (circled red)

Figure 3. *E. calamita* showing incomplete dorsal stripe

Scarring
Where scarring occurred on the dorsal stripe, there was an obvious grey-translucent area emphasised by the brightness of the dorsal stripe (Fig. 4). Some individuals showed much larger areas of scarring around the snout region (Fig. 5), and this was sometimes more obvious than the dorsal stripe.

Tubercles (warts)
They were generally quite difficult to use as identifiable features as there are so many covering the complete dorsal region. Their better use was when they broke the dorsal stripe (Fig. 6).

Figure 4. *E. calamita* with scarring across dorsal stripe (circled red)

Figure 5. *E. calamita* with scarring on snout (circled red)

Figure 6. *E. calamita* with two warts breaking the dorsal stripe (circled red)

Is photographic identification of *E. calamita* possible?
Of the 40 untrained participants that completed the first photo-matching exercise in two separate groups (Group One Median=4, n=20; Group Two Median=2.5, n=20) a Mann Whitney U Test revealed no significant difference in their levels of accuracy (z=-1.32, p=0.19). Thirty-five percent of those in Group One and 30% of those in Group Two matched all five photographs correctly, and 20% of Group One and 15% of Group Two had only one photographic match incorrect. The remaining 45% of Group One and 55% of Group Two had three or more photographs matched incorrectly during the initial test. This suggested photo-matching of *E. calamita* to be a possible method of identification and that participants had similar ability levels between the two test groups prior to the next stage of the study.

Does training improve the accuracy of photographic identification of *E. calamita*?
Figure 7 illustrates levels of Group One participants' accuracy in photo-matching *E. calamita* toads before and after they received their training. An increased level of accuracy, as measured by scores out of 5, was noted following basic training in their main characteristics. The median score before training was 4 and after training the median increased to 5. This increase in accuracy level following training was significant (Wilcoxon Signed-Rank Test: Z = -2.31, P=0.02).

Before training, 10 cases of misidentification (30% of the total misidentifications) related to one particular photograph. After training, misidentification

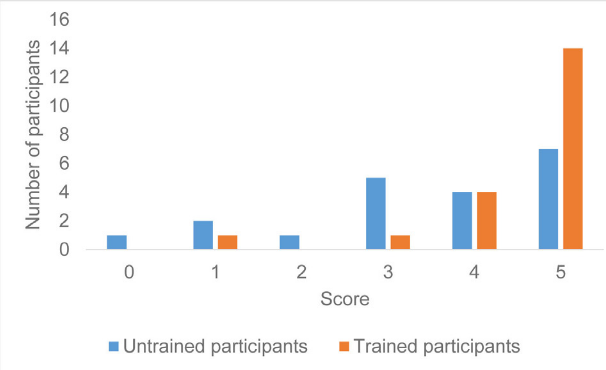

Figure 7. Group one participant's scores gained in an *E. calamita* toad photo-matching exercise, before and after training (n=20).

of this photograph decreased to five occasions, but this represented 56% of the total number of misidentifications in the exercise.

Participants in Group Two (Figure 8) did not receive training prior to their second attempt at photo-matching. Their median score of accuracy at attempt one was 2.5, but this dropped to 1.5 on re-testing. A Wilcoxon Signed-Rank Test revealed a statistically significant reduction in accuracy levels between the two attempts at photo-matching (Wilcoxon Signed-Rank Test: Z = -2.23, P=0.03).

Figure 8. Group two participant's scores gained in an *E.calamita* toad photo-matching exercise, both tests without training (n=20)

Does training increase the speed of photographic identification of *E. calamita* toads?

When the speed of photo-matching was compared before training with Group One participants, the median speed achieved by participants was 9 minutes 1 second and the inter-quartile range was [6 minutes 26 seconds, 14 minutes 52 seconds]. After training the median was 5 minutes 17 seconds and the inter-quartile range was [4 minutes 11 seconds, 9 minutes 23 seconds]. A significant decrease in the time taken in photo matching was seen following training (Fig. 9), (Wilcoxon Signed-Rank Test: Z = -3.25, P=0.001). This demonstrates an increase in speed of correct identification after training.

It was noted that the angle from which the photograph was taken may contribute to the ability to identify an individual toad. Forty-five percent of participants failed to recognise the most obvious feature in one photograph,

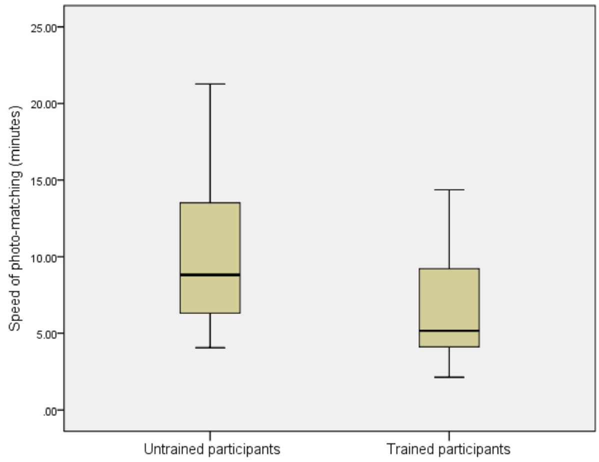

Figure 9. The median time taken to match photographs of *E.calamita* by trained and untrained participants (n=20) in Group One.

which was a straight line of four tubercles to the left of and following the dorsal stripe, and a matching line of four tubercles to the right of the dorsal stripe, although not in a straight line (Fig. 10). The dorsal stripe in this photograph was less noticeable than in photographs of other individuals because of the angle at which it was taken, and the tubercles were less noticeable as features. Once the main feature (line of four tubercles) had been pointed out during training, the percentage of participants incorrectly identifying the toad dropped from 45% to 30%, increasing correct identification to 70%.

Figure 10. Pattern of four tubercles either side of dorsal stripe (circled red). Note the dorsal stripe abnormality (circled green).

Another photograph (Fig. 11) received a 35% misidentification rate before training. The individual's dorsal stripe was very clear with an abnormality. Behind the eyes, the stripe begins to fork off to the left side of the dorsum and then stops. The reason for this frequent misidentification is not clear. One participant mistook a piece of vegetation which was stuck to the mid-dorsum of the toad as being part of its body (Fig. 11). In the matching photograph the vegetation was not there and so this negatively affected the participants answer as they were actively searching for it.

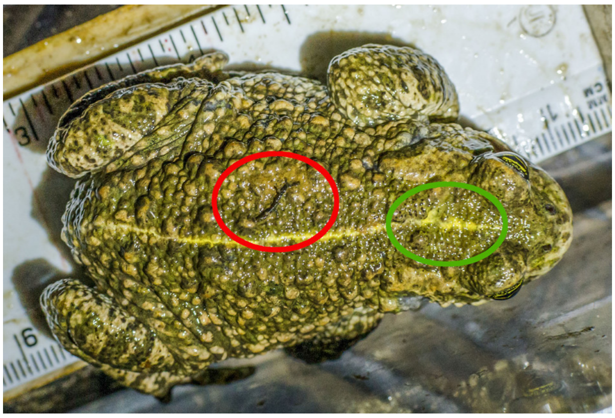

Figure 11. Dorsal stripe abnormality behind the eyes (circled green). Note the vegetation stuck to its body (circled red).

Figure 12. *E. calamita* showing obvious scarring on snout

DISCUSSION

The first objective of this study was to establish whether photo-identification of *E. calamita* is feasible, using the natural markings on the dorsum of individuals, for citizen scientists. This would build on the success and uses of photo and visual identification in mammal ecology and reduce the need for invasive treatments when examining populations of toads. The study demonstrates that *E. calamita* toads at the study site indeed had sufficient variability of natural markings on their dorsum for photo-identification to be effective.

The second objective was to ascertain whether the training of citizen scientists improves the accuracy of identifying individuals by dorsal markings. Training the participants had a significant effect on the results, increasing accuracy of correct identification. Consequently, training significantly improved photo-identification of toads within this study. It may be considered that repetition of the photo-matching exercise may also have had an influence in improving accuracy. However, in the group that received no training there was a decrease in correct photo-identification in the second exercise. This decrease may have occurred because of the changed sequence of photographs in the two exercises they undertook. They were given their scores in between tests and so their confidence may have diminished. This would further demonstrate the effectiveness of the training.

The third objective was to ascertain whether training improves the speed at which individual toads can be identified. The time taken to complete photo-matching was reduced following training of the participants. When the technique is used in practice, care must be taken not to reduce accuracy in photo-identification in an attempt to increase speed. However, it will also be very important, in practice, to minimise the time handling the toad for photographing in order to reduce possible stress to the toad.

It is possible that participants naturally found scarring easier to use for identification as opposed to the dorsal stripe. Individual identification using natural scarring is not uncommon in animals and is described by Gilkinson et al. (2007), in which sea otters were identified from their nose scars. According to Bertolotti et al. (2013), African clawed frogs fail to maintain regenerative capability past metamorphosis and show a progressive loss of scar-free repair as they develop. While this appears to be inferring that permanent identification of scarred adults could be possible, it may not be the case with *E. calamita* and should not be relied upon without further investigation. A variable that photographs Figure 9 and Figure 10 shared with each other was that the toads had little to no scarring visible. In contrast, the remaining three photographs, which were identified with much more accuracy, had noticeable scarring, for example, Figure 12. One possible explanation as to why the toads shown in Figures 9 and 10 were misidentified more often could be that participants naturally found scarring easier to use for identification as opposed to the dorsal stripe.

Changes in pattern can result in false-positive and false-negative identifications, resulting in faulty estimates of populations (Gebauer, 2009). Other than scarring, Odum and Sonntag (2010) state that once amphibians reach adulthood, their skin pattern is unlikely to change. Denton & Beebee (1993) found that throat-spot patterns in female *E. calamita* did not change considerably during a two-year study. However, the permanence of dorsal markings requires further study.

It was noted that when photographing the toads, the position of the toad needs to be as standard throughout as practicable. In this study, each toad was placed in a Perspex container with ample room to move freely, thus changing orientation and body shape between photographs. Prior to training, some participants searched for a curved dorsal stripe, when in fact the curvature was due to the position that the toad was sitting in. Curvature in the posture of the toad can also result in some features being out of focus in the photograph.

Knox et al. (2013) states manually matching photographs of individual animals is only suitable for small populations, because as the sample base increases, so does the time spent in manually matching photographs. For species living in small and fragmented populations, such as the *E. calamita* at the study site, manual matching appears to be suitable.

In the context of monitoring endangered populations, developing survey methods that minimise the potential handling effects and welfare concerns related to animal capture is vital (McMahon et al., 2005). An important advantage of the photo-identification method is the ability to identify individual animals without damage, although it still requires the capture and handling of individuals. Kenyon et al. (2009) stated that photographing green-eyed tree frogs *Litoria genimaculata* took longer than toe clipping. However, in this study, which took place over the period of a month, there was no indication that disturbance affected the individuals as some were recaptured on multiple occasions. It should be noted that, in the United Kingdom, photo-identification of *E. calamita* can only be carried out with a licence and having a licence holder present.

CONCLUSION

Citizen science can be a useful way of collecting data intensively and repetitively over wide geographical areas. However, Aceves-Bueno et al. (2017) recommend that the citizen science tasks should be designed with the skills of the citizen scientists in mind. They recommend that in order to do this, initial reference data needs to be collected on the reliability of the data collection. The data for the photo-identification of *E.calamita*, in the study described in this paper, act as reference data and shows that the correct identification occurs in the majority of cases, but there is not complete reliability. This factor requires that statistical adjustment would need to be made when estimating population size from photo-identification and recapture surveys.

Although toe-clipping has been the method of choice in individual recognition of *E. calamita* toads (Boomsma & Arntzen, 1985; Tejedo, 1988; Denton, 1991), this study reveals that the dorsum has sufficient variability of natural markings in order for photo-identification to be an effective alternative. A difference was observed between trained and untrained participants in the accuracy and speed of individual identification.

The method is easily repeatable by investigators with the statutory licence. However, in order to obtain the highest level of accuracy, the quality of photographs and the position of the toad should be taken into account.

Ideally, an identification method used within a survey by citizen scientists should adhere to all of the following criteria (Lewke & Stroud, 1974; Ferner, 1979; Reaser, 1995): (1) does not affect the animal's survivorship or behaviour, (2) allows the animal to be as free from stress and pain as possible, (3) identifies the animal as a particular individual, (4) is reliable over the duration of the study, (5) is easily read or observable, (6) is adaptable to organisms of different sizes, (7) is easy to use in both laboratory and field conditions, and (8) utilises materials that are easy to obtain.

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