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Individual Identification of the Lake Oku Clawed Frog (*Xenopus longipes*) Using a Photographic Identification Technique

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Abstract.—**Amphibians are challenging to mark for recapture due to their small size and permeable, sensitive, and often frequently shed skins. Photographic identification and pattern matching techniques are increasingly used as a non-invasive method to identify individual amphibians for the purposes of monitoring individuals over time. The Critically Endangered Lake Oku Clawed Frog (*Xenopus longipes*) has distinctively patterned ventral patterns as adults. We used Wild-ID to explore the use of photographic identification for the longitudinal identification of both adult and juvenile *X. longipes*. We photographed juvenile frogs twice over a 180-d period and adult frogs seven times over 624 d. Juvenile belly patterns underwent marked ontogenetic shifts over the 180-d period and Wild-ID was not able to match photographs of the same individuals over the study period. Markings were more stable in adult frogs and Wild-ID was successful in matching photographs of individual adult frogs over 180 d but became less effective at distinguishing between individuals at 624 d (i.e., Wild-ID similarity scores halved and false rejection rates increased substantially). We detected no false acceptances. Our results provide evidence to guide management of this species in captivity and in the field and demonstrate the importance of considering life-stage linked ontogenetic changes when validating a photographic identification method for amphibians.**

Key Words.—amphibian; Anura; Cameroon; ontogenetic change; pattern matching; photographic individual identification; zoo

Introduction

Monitoring individual animals over time is important in informing conservation and captive management but requires a validated and reliable means of recognizing individuals (Donnelly et al. 1994). This may be challenging when dealing with a large population of animals (Caorsi et al. 2012; Schoen et al. 2015), such as often occurs with amphibians. These animals are also typically difficult to individually mark due to their relatively small size; permeable, sensitive, and frequently shed skins; and often-complex life cycles (e.g., Heemeyer et al. 2007; Ferner 2010; Bainbridge et al. 2015). In addition, a number of invasive methods such as toe clipping and branding have substantial ethical implications (Mellor et al. 2004; Ferner 2010; Perry et al. 2011). Even visible implant elastomer (VIE), historically considered relatively non-invasive as a marking method (Antwis et al. 2014), is now known to cause concerning inflammation to internal organs in amphibians (Cabot et al. 2021). Validation of marking methods in the field is fraught with problems including recovery of marked animals, distinction between unmarked and unrecognized animals, and detection of deleterious impacts of marking techniques (Ferner 2010; Perry et al. 2011). The use of captive animals, by contrast, which can be monitored confidently over time to validate marking and other methods for use in the field, can be an invaluable aspect to the maintenance of *ex situ* populations of amphibians (Tapley et al. 2019).

Photographic identification, a subset of pattern matching techniques (Ferner 2010), involves using natural markings and patterns on animals using photographs. This methodology can mitigate limitations of cost and ethics as required materials are inexpensive or free, and photography is minimally invasive compared with other marking methods. Time constraints and human error while processing large volumes of photographs, especially for taxa with distinct but complex patterning, can be mitigated using photographic recognition software (Cruickshank and Schmidt 2017). Photographic identification has been successfully implemented in studies on a wide range of amphibians (Bradfield 2004; Morrison et al. 2016; Crawford-Ash and Rowley 2021). Wild-ID is a free pattern matching program available at https://home.dartmouth.edu/faculty-directory/douglas-

thomas-bolger. The software uses a Scale Invariant Feature Transform operator (SIFT), which extracts distinctive features in images even if the scale, distortion, or the rotation are skewed (Lowe 2004). Next, the software matches all the images and generates a similarity score (Bolger et al. 2012). Wild-ID has been applied to amphibians successfully (e.g., Caorsi et al. 2012; Bendik et al. 2013). Skin patterns may change over time, however, as a result of ontogeny (e.g., Kraus and Allison. 2009; Biju et al. 2013; Bardier et al. 2020) or phenotypic plasticity (Sköld et al. 2013), and this may limit the use of photographic identification for longer term identification in both the field and captivity. We aimed to provide a model for identifying at what age stable patterns may be used to facilitate field identification in a highly threatened anuran amphibian, the Lake Oku Clawed Frog (*Xenopus longipes*).

Xenopus longipes is an endemic and Critically Endangered (International Union for the Conservation of Nature [IUCN] 2020) species from Cameroon with a body size of approximately 30–40 mm snout-vent length at maturity and males substantially smaller than females. Attempts to estimate the population size through capture-mark-recapture using toe-clipping and dye-injections to mark individuals have been limited by the small size of this frog, which make permanent and individual or batch-specific marking methods difficult to employ successfully in a presumed but currently unknown large wild population (Doherty-Bone et al. 2013). Mortality events at Lake Oku in 2006 and subsequent research needs resulted in an assurance colony being established in captivity in 2008 (Michaels et al. 2015; Tapley et al. 2016). While distinct complex markings are lacking on the dorsal surface of adult *X. longipes* (Fig. 1), dark speckled markings are present on their pale orange-yellow ventral surfaces, and these potentially offer an opportunity for photographic identification of individual frogs. We investigated changes in identifiability of individual adult and juvenile *X. longipes* over 624 d and 180 d, respectively, to determine the effect of time after initial photographs were taken on similarity scores and false rejection and acceptance rates generated by Wild-ID.

Materials and Methods

We used 24 (seven male, 17 female) wild-collected adult *Xenopus longipes* and 10 captive-bred, juveniles

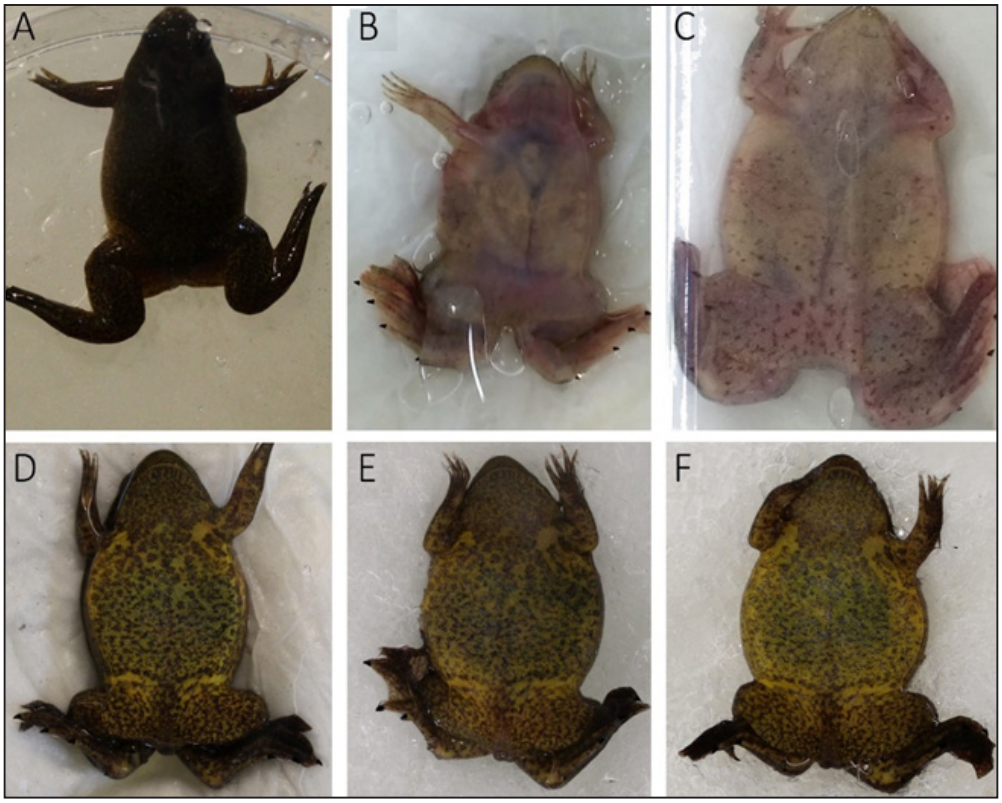

Figure 1. Representative photographs of Lake Oku Clawed Frog (*Xenopus longipes*): (A) Dorsal surface of an adult, (B) ventral surface patterns in juveniles on Day J0, and (C) Day J180. (D) Ventral surface patterns of adults at Day A0, € Day A179, and (F) Day A624. Wild-ID was an effective identification tool to match E to D, but not F to D. (Photographs A, D, and E by Unnar Aevarsson, B and C by Christopher Michaels, and F by Arabella Graves).

of unknown sex housed at ZSL London Zoo, UK, in this study. We housed juvenile frogs individually, and adults in small groups of 4–6 animals. Through a combination of sex, animal size, and highly distinctive patterning on some animals, and colored VIE tags historically applied to the animals, we could confidently recognize adult individuals without error. For example, we could easily distinguish individuals in a group consisting of a single male and four females of substantially different sizes, two of which bore different colored VIE tags.

We photographed frogs during routine health checks, wherein they were contained within a petri dish and against white filter media to associate animal records with individual frogs. We photographed adult frogs on seven occasions (referred to henceforth as sessions) over 624 d beginning on 11 June 2018 (Day A0; see Table 1 for photography dates), and we determined the sex of animals using secondary sexual characteristics (see Michaels et al. 2015). A hiatus in regular photographic sessions occurred between Days A179 and A624 due to insufficient staffing resources. We photographed juvenile frogs on 14 June 2016 (Day J0) and Day J180, at which time the animals were becoming sexually mature (based on secondary sex characteristics and reproductive behavior; Michaels et al. 2015). We photographed adults with a Canon EOS 600D digital camera (Canon Inc., Tokyo, Japan) fitted with a Canon EF-S 18–55 mm f/3.5–5.6 IS lens, and juveniles with a Nikon Coolpix AW120 (Nikon Corporation, Tokyo, Japan), both mounted on a standard tripod rig under consistent fluorescent lighting conditions. We cropped images in a rectangular polygon from the vent to axilla and across the maximum width of the abdomen and rotated the image so that the cranial end of the frog was orientated towards the top of the image.

We used Wild-ID to compare images from a given photographic session to the original set of photographs. Wild-ID uses algorithms to produce a raw output with the similarity scores of each image. We compared photographs from each session to the baseline photographs collected in Session 1 (DayA0) to assess variation in error over time. We followed standard Wild-ID procedures by assessing the 20 highest-scoring photographs by eye to match sample photographs to the correct baseline photograph. We then recorded the similarity score for this correct match for each photograph. Where Wild-ID identified and generated a similarity score of zero to all photographs, we used zero as the similarity score to reflect the fact that Wild-ID could not successfully match the image. We compared Session 1 photographs against themselves to confirm that software behaved normally with our images by checking that it correctly identified identical photographs with a perfect similarity score; we did not include these results in analyses as they created a false effect of time on error. Comparing original images to themselves returned only perfect scores, indicating that the software performed appropriately.

We calculated false acceptance incidence (FAI) and false rejection incidence (FRI). We defined false acceptance as an instance where the top-scoring photograph was not the correct individual and false rejection as an instance where the true match did not appear in the 20 best similarity scores (Morrison et al. 2016), where the observer did not correctly identify a frog, or where all returned scores equaled zero. We calculated false rejection rates (FRR; a measure of specificity of the method) by dividing FRI by the number of true (i.e., the number of actually matching) matching comparisons (i.e., 24 per session; Bolger et al. 2012; Morrison et al. 2016; Cruickshank and Schmidt 2017). We did not calculate false acceptance rate (FAR; a measure of sensitivity) as we encountered no false acceptances.

We ran a Linear Mixed-effect Model using lme4 and lmer packages (Bates 2005) in R version 4.1.1 (R Core Team 2021) using RStudio Version 1.4.17 for Windows. We used a model with Similarity Score = Days + Sex + FrogID. FrogID was a random factor to control for repeated measures. We used $\alpha = 0.05$ unless stated otherwise. We calculated r^2 values using the MuMIn and lme4 packages (Nakagawa and Shielzeth 2013). We inspected the residuals using a Q-Q plot of residuals to confirm normality and confirmed homoscedasticity through a scale location plot of residuals. We confirmed assumptions of linearity of predictors and independence of residuals by plotting residuals against the response, and residuals against covariates, respectively. We performed no analyses on juvenile similarity score data or FAR/FRR data for either age class due to preponderance of zeros (see below).

Results

Juvenile belly patterns developed markedly over the period between Day J0 and Day J180, with little or no pigmentation on the ventral surface at Day J0 and substantial pigmentation by Day J180 (Fig. 1). Juvenile comparisons (Day J0 vs Day J180) yielded only similarity scores of 0.0 and we were unable to match any individuals. Consequently, we conducted no further analyses.

In adults, the degree of pigmentation as observed by eye did not change substantially over the timeframe of photographic sessions for these animals (Fig. 1). In adults, there were no FAI, so we did not statistically analyze data for FAR. For FRR all but two values were 0 (Day A130 = 0.042, Day A624= 0.17; Table 1), so we did not statistically analyze these data (Table 1). Mean similarity scores ranged from 0.123–0.256 (Table 1). The number of days after initial photographs had a significant effect on similarity score ($F_{1,119} = 39.61$, $P < 0.001$; $\beta =$ -0.0002; 95% Confidence Interval [CI] = -0.0003 and -0.0002, respectively), but sex did not have a significant

Table 1. Timing of photographic sessions after the initial identification photographs were taken (session 1), similarity scores of each session of photographs compared with the initial set (mean ± standard deviation [(SD]), and the false rejection rate (FRR) associated with each photographic session for adult Lake Oku Clawed Frog (*Xenopus longipes*).

Days after Session 1	Similarity Score (mean ± SD)	False Rejection Rate
43	0.256 ± 0.133	0
69	0.253 ± 0.172	0
98	0.245 ± 0.151	0
130	0.215 ± 0.156	0.042
179	0.221 ± 0.152	0
624	0.123 ± 0.131	0.17

effect ($F_{1,22}$ = 0.370, P = 0.549; β (male) = -0.035; 95% CI = -0.15 and 0.078, respectively; Fig. 2). FrogID, a random factor, had a significant effect (Likelihood Ratio Test1 = 95.1, $P < 0.001$; the standard deviation of the effect was 0.12). Conditional r^2 was 0.71, and marginal r^2 was 0.1.

Discussion

Our findings illustrate differential applicability of computer-assisted photograph matching between post-metamorphic life stages of frogs. We found that photographic identification was not viable in juvenile *X. longipes* over a 180-d period, i.e., between metamorphosis and sexual maturity. Image quality was lower for juvenile frogs compared with adults as images were not taken with a Single Lens Reflex camera and frogs were themselves smaller and more difficult to photograph. This is likely to have reduced similarity scores (Bendik et al. 2013; Morrison et al. 2016), but the total failure of recognition was likely due to the substantial pigmentation change seen in this species between metamorphosis and sexual maturity. Bardier et al. (2020) found a different pattern in *Ceratophrys* frogs, whereby although they did not compare time series for individual frogs, they found no reduction in applicability of Wild-ID for juvenile frogs compared with adults, as pigmentation structure did not change between life stages. Our findings demonstrate a potential for differing applicability of Wild-ID to accurately identify individual frogs depending on the species or life stage concerned.

Adult frogs, by contrast, exhibited greater stability in identification from photographs over the same time frame than juveniles in this study owing to substantially smaller apparent pigmentation changes in comparison with changes seen over the same timescale in juveniles. We found a significant reduction in similarity scores over time, but this was a weak effect with a low marginal r^2 value (0.1), even though the conditional r^2 value indicated a reasonably good explanatory power of the model. This finding suggests high variability between individuals in similarity score. This is borne out by the relatively high standard deviation of the random effect, which indicates substantial variability between individuals. Individuals did indeed show varying trajectories in similarity scores over time (Fig. 2). Mean similarity scores at each session were reasonably good, i.e., always above 0.2 other than Day A624, which was still above the 0.1 score recommended as an arbitrary threshold for a successful match by Bendik et al. (2013). There was considerable

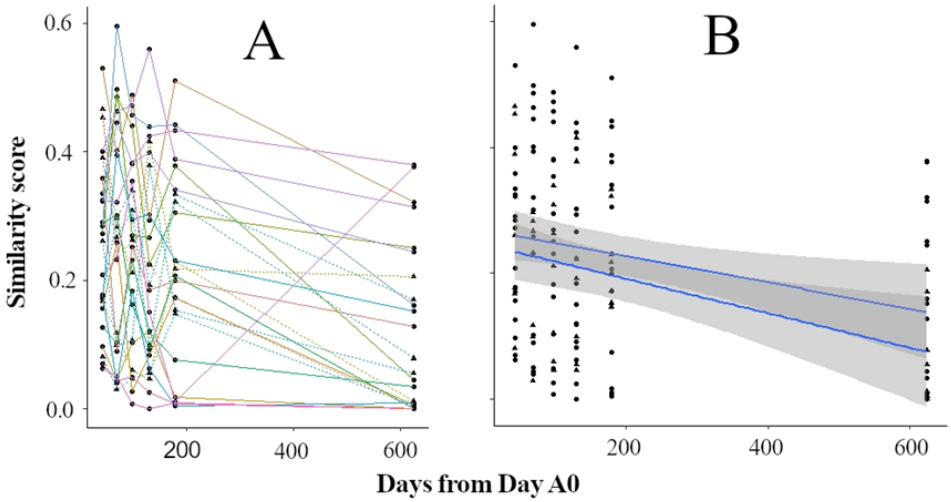

Figure 2. Similarity scores for 24 individual adult Lake Oku Clawed Frog (*Xenopus longipes*) against time after initial photographs were taken (Day A0; 11 June 2018). (A) Points for individual frogs are connected. (B) Line of best fit in similarity scores over time with 95% confidence intervals for male and females (higher trend line = female; lower trend line = male). The darker area represents overlap of confidence intervals.

variation around the means, however (Table 1), and several individuals routinely scored considerably higher, even exceeding the higher value of 0.3 interpreted as a good match by Morrison et al. (2016), or lower, below the 0.1 threshold suggested by Bendik et al. (2013). The cut-offs used by both these groups are system-specific in that they apply only to the use of Wild ID in the populations reported, and so we used them here only as a rough guide to contextualize our scores. Some similarity scores between true matches were very low, approaching or reaching zero and this was consistently the case for some individuals (Fig. 2). The cause for some frogs apparently being less recognizable to the SIFT algorithm is unknown, but similar patterns of variation in similarity scores, with some very low scores between true matches even with only short intervals between photographs, have been reported in other amphibian taxa (e.g., Morrison et al. 2016). This may be problematic in field use as it may reduce estimates of recapture, and in captivity may hamper proper management of individual animals. Similarity scores may have been improved with the use of a more specialized macro lens, but this equipment was outside of the logistical scope of the study due to budgetary constraints.

We encountered false rejections at two timepoints (4% and 17% were encountered; Table 1), broadly comparable with some studies using amphibians (4.2–9.3%, Renet et al. 2019; 5%, Bardier et al. 2020; 7.3%, Dalibard et al. 2021; 7%, Caorsi et al. 2012), but substantially lower than in others (20–47% in the Wyoming Toad, *Anaxyrus baxteri*; Morrison et al. 2016). This indicates that Wild-ID has a broadly similar level of specificity in *X. longipes* compared with the same method for other amphibians. We detected no false acceptances and therefore high sensitivity (consistent with Morrison et al. 2016, Caorsi et al. 2012, and Elgue et al. 2014), which reflects better performance compared with other studies (Dalibard et al. 2021 for *Calotriton*; Bardier et al. 2020 for *Ceratophrys*). The final photographic session demonstrated that quality and accuracy of identification declined by this point, with mean similarity score being substantially lower than earlier time points and a substantial increase in FRR.

The observed declines in average similarity scores for true matches and substantial increase in FRR between the final two sessions was almost certainly due to the same, albeit slower, ongoing process of melanin accumulation in the ventrum of the frogs as seen in juveniles. This has not been reported in the literature, but there are parallels with *Eurycea* salamanders, where melanophore expansion and contraction influenced identification success (Bendik et al. 2013). Similarity scores declined substantially between Day A179 and A624. Additional photographic sessions to fill the gap in records between the penultimate and final sessions (Days A179 and A624) may have helped to better understand trends in similarity scores and FRR but were logistically impossible (see Materials and Methods).

Based on the data available, conservatively, we therefore suggest that a 6-mo interval between photographs is likely to yield accurate identification of adult individuals, with acceptable similarity scores and relatively low FRR (0–4%). Other means of marking are required for juveniles over a similar period; however, we are not able to suggest a suitable interval for computer-assisted identification of juveniles with current data. This suggests the most accurate adult population estimates of this species in Lake Oku could be obtained using Wild-ID for individual identification when sampling events take place within 179 d of each other. Outputs such as this demonstrate the value of captive populations to inform on field surveys for this species (Tapley et al. 2017). Our results represent more short-lived use than detected in some other amphibian species (Elgue et al. 2014, successful after 16 mo; Mettouris et al. 2016, successful after 3 y; Smith et al. 2019, successful after 3 y; Dalibard et al. 2021, successful after 2 y) but proved substantially more successful than a similar approach in others (Coppola and Michaels 2021, < 5 mo).

Other studies have compared computer-assisted image matching techniques with unassisted visual methods relying entirely on human comparisons, with a variety of comparative outcomes (Caorsi et al. 2012; Elgue et al. 2014; Cruikshank and Schmidt 2017; Bardier et al. 2020; Coppola and Michaels 2021). We did not quantify the efficacy of unassisted visual identification in this study, but the computer-assisted method was initially trialed as humans found it impossible to confidently identify individual *X. longipes* based on markings due to the complexity of pigmentation patterns. Our data result from a small sample of 24 adult and 10 juvenile frogs. Such small sample sizes are a common limitation of non-model species in captivity and are difficult to avoid. In captivity, where smaller numbers of photographs are involved, FRI may be corrected manually by quality control checking of images. This may be challenging in the field and increasing sample size for field use may have implications for applicability (Bolger et al. 2012). Nevertheless, this method may facilitate periodic population estimates to detect population trends, a recommended research need for the conservation of the species (IUCN 2020).

Although photographic identifications (IDs) endure for at least 6 mo according to our data, application of this time period to field surveys may not be straightforward. *Xenopus longipes* coincidentally matures at about the same time (6 mo post metamorphosis; Michaels et al. 2015) that adult photographic IDs lose efficacy; these two facts are not linked as the 6-mo photographic ID duration is derived from adult animals long past maturity. Moreover, the reproductive period in this species in

the field is unknown, and the larval phase is very long compared with most anurans (193–240+ d; Michaels et al. 2015; Tapley et al. 2015). Therefore, the reproductive developmental biology may constrain optimal survey frequency for studies requiring longitudinal data (e.g., longevity estimates) more than photographic ID longevity. Although photographic identification may correctly match individuals over a 6-mo period, in this period juvenile animals that were not included in the initial survey will become mature and enter the pool of potential comparisons. As recruitment of individuals to the adult population that were not previously identifiable may violate assumptions of population estimate models (Link et al. 2018; Lettink and Armstrong 2003), this may invalidate the use of this method in this context. Nevertheless, photographic identification may still be preferable to more invasive marking techniques on ethical grounds, and it is currently the only method that has been quantitatively assessed (this study). Our data do demonstrate that for work requiring only short inter-survey periods, such as capture mark release (CMR) studies, computer-assisted photographic identification of individuals of this species may be appropriate, provided that juvenile animals are excluded from such work.

The use of photographic identification with Wild-ID may facilitate robust management of the *ex situ* population. Individual identification allows program managers to make breeding recommendations for animals of known pedigree, which is better practice than group management in terms of the maintenance of long-term genetic diversity of the captive population (Ballou et al. 2010). The low similarity scores and occurrence of No Match (i.e., the Wild-ID output that identifies no matching photographs within the gallery of candidate images), within our data set, however, indicates that although Wild-ID may be used to facilitate identification of individual animals, additional identifiers may be required to attain the perfect identification ability required to managed small populations of individual animals. Overall, our data generally support the use of this potentially powerful tool for application to this Critically Endangered anuran in both *in-* and *ex situ* contexts. Our results indicate that practitioners should be cautious about applying visual identification techniques to life stages where they have not been previously validated.

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Literature Cited

Antwis, R.E., R. Purcell, S.L. Walker, A.L. Fidgett, and R.F. Preziosi. 2014. Effects of visible implanted elastomer marking on physiological traits of frogs. Conservation Physiology 2: cou042. https://doi.org/10.1093/conphys/cou042.

Bainbridge, L., M. Stockwell, J. Valdez, K. Klop-Toker, S. Clulow, J. Clulow, and M. Mahony. 2015. Tagging tadpoles: retention rates and impacts of visible implant elastomer (VIE) tags from the larval to adult amphibian stages. Herpetological Journal 25:133–140.

Ballou, J.D., C.M. Lees, L.J. Faust, S. Long, C. Lynch, L. Bingaman-Lackey, and T.J. Foose. 2010. Demographic and genetic management of captive populations. Pp. 219–252 *In* Wild Mammals in Captivity. Kleiman, D.G., K.V. Thompson, and C.K. Baer (Eds.). University of Chicago Press, Chicago, Illinois, USA.

Bardier, C., D. Székely, G. Augusto-Alves, N. Matínez-Latorraca, B.R. Schmidt, and S.S. Cruickshank. 2020. Performance of visual vs. software-assisted photo-identification in mark-recapture studies: a case study examining different life stages of the Pacific Horned Frog (*Ceratophrys stolzmanni*). Amphibia-Reptilia 42:17–28.

Bates, D. 2005. Fitting linear mixed models in R. R News 5:27–30.

Bendik, N.F., T.A. Morrison, A.G. Gluesenkamp, M.S. Sanders, and L.J. O'Donnell. 2013. Computer-assisted photo identification outperforms visible implant elastomers in an endangered salamander, *Eurycea tonkawae*. PLoS ONE 8:e59424. https://doi.org/10.1371/journal.pone.0059424

Biju, S. D., R.G. Kamei, S. Mahony, A. Thomas, S. Garg, G. Sircar, and R. Suyesh. 2013. Taxonomic review of the tree frog genus *Rhacophorus* from the Western Ghats, India (Anura: Rhacophoridae), with description of ontogenetic colour changes and reproductive behaviour. Zootaxa 3636:257–289.

Bolger, D.T., T.A. Morrison, B. Vance, D. Lee, and H. Farid. 2012. A computer-assisted system for

photographic mark-recapture analysis. Methods in Ecology and Evolution 3:813–822.

Bradfield, K.S. 2004. Photographic identification of individual Archey's Frogs, *Leiopelma archeyi,* from natural markings. Science Internal Series 191, Department of Conservation, Wellington, New Zealand.

Cabot, M.L., B.V. Troan, K. Ange-van Heugten, R.W. Schnellbacher, D. Smith, F. Ridgley, and L.J. Minter. 2021. Migration and histologic effects of visible implant elastomer (VIE) and passive integrated transponder (PIT) tags in the Marine Toad (*Rhinella marina*). Animals 11:3255. https://doi.org/10.3390/ani11113255.

Caorsi, V.Z., R.R. Santos, and T. Grant. 2012. Clip or snap? An evaluation of toe-clipping and photo-identification methods for identifying individual Southern Red-bellied Toads, *Melanophryniscus cambaraensis*. South American Journal of Herpetology 7:79– 84.

Coppola, J., and C.J. Michaels. 2021. Development and individual identification of captive Ziegler's Crocodile Newt *Tylototriton ziegleri.* Herpetological Bulletin 156:14–17.

Crawford-Ash, J., and J.J.L. Rowley. 2021. Photo identification of individual Blue Mountains Tree Frogs (*Litoria citropa*). Herpetology Notes 14:803–808.

Cruickshank, S.S., and B.R. Schmidt. 2017. Error rates and variation between observers are reduced with the use of photographic matching software for capture-recapture studies. Amphibia-Reptilia 38:315–325.

Dalibard, M., L. Buisson, O. Calvez, M. Nguyen-Hong, A. Trochet, and P. Laffaille. 2021. Can ventral pattern be used for individual recognition of the vulnerable Pyrenean Brook Newt (*Calotriton asper*)? Herpetological Journal 31:99–110.

Doherty-Bone, T.M., R.K. Ndifon, O.N. Nyingchia, F.E. Landrie, F.T. Yonghabi, A.L.J. Duffus, S. Price, M. Perkins, J. Bielby, N.B. Kome, et al. 2013. Morbidity and mortality of the critically endangered Lake Oku Clawed Frog (*Xenopus longipes*). Endangered Species Research 21:115–128.

Donnelly, M.A., C. Guyer, E.J. Juterbock, and R.A. Alford. 1994. Techniques for marking amphibians. Pp. 277–284 *In* Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Heyer, W.R. (Ed.). Smithsonian Institution Press, Washington, D.C., USA.

Elgue, E., G. Pereira, F. Achaval-Coppes, and R. Maneyro. 2014. Validity of photo-identification technique to analyze natural markings in *Melanophryniscus montevidensis* (Anura: Bufonidae). Phyllomedusa: Journal of Herpetology 13:59–66.

Ferner, J.W. 2010. Measuring and marking post-metamorphic amphibians. Pp.123–141 *In* Amphibian Ecology and Conservation: A Handbook of Techniques. Dodd, C.K., Jr. (Ed.). Oxford University Press, Oxford, UK.

Heemeyer, J.L., J.A. Homyack, and C.A. Haas. 2007. Retention and readability of visible implant elastomer marks in Eastern Red-backed Salamanders (*Plethodon cinereus*). Herpetological Review 38:425–428.

International Union for the Conservation of Nature (IUCN). 2020. *Xenopus longipes*. The IUCN Red List of Threatened Species 2020. http://www.iucnredlist.org.

Kraus, F., and A. Allison. 2009. A remarkable ontogenetic change in color pattern in a new species of *Oreophryne* (Anura: Microhylidae) from Papua New Guinea. Copeia 2009:690–697.

Link, W.A., M.R. Schofield, R.J. Barker, and J.R. Sauer. 2018. On the robustness of N-mixture models. Ecology 99:1547–1551.

Lettink, M., and D.P. Armstrong. 2003. An introduction to using mark-recapture analysis for monitoring threatened species. New Zealand Department of Conservation Technical Series 28A:5–32.

Lowe, D.G. 2004. Distinctive image features from scale-invariant keypoints. International Journal of Computer Vision 60:91–110.

Mellor, D.J., N.J. Beausoleil, and K.J. Stafford. 2004. Marking amphibians, reptiles and marine mammals: animal welfare, practicalities and public perceptions in New Zealand. Department of Conservation, Wellington, New Zealand. 55 p.

Mettouris, O., G. Megremis, and S. Giokas. 2016. A newt does not change its spots: using pattern mapping for the identification of individuals in large populations of newt species. Ecological Research 31:483–489.

Michaels, C.J., B. Tapley, L. Harding, Z. Bryant, S. Grant, G. Sunter, I. Gill, O. Nyingchia, and T. Doherty-Bone. 2015. Breeding and rearing the critically endangered Lake Oku Clawed Frog (*Xenopus longipes* Loumont and Kobel 1991). Amphibian & Reptile Conservation 9:100–110.

Morrison, T.A., D. Keinath, W. Estes-Zumpf, J.P. Crall, and C.V. Stewart. 2016. Individual identification of the endangered Wyoming Toad *Anaxyrus baxteri* and implications for monitoring species recovery. Journal of Herpetology 50:44–49.

Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4:133–142.

Perry, G., M.C. Wallace, D. Perry, H. Curzer, and P. Muhlberger. 2011. Toe clipping of amphibians and reptiles: science, ethics, and the law. Journal of Herpetology 45:547–555.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.

org.
Renet, J., L. Leprêtre, J. Champagnon, and P. Lambret. 2019. Monitoring amphibian species with complex chromatophore patterns: a non-invasive approach with an evaluation of software effectiveness and reliability. Herpetological Journal 29:13–22.
Schoen, A., M. Boenke, and D.M. Green. 2015. Tracking toads using photo identification and image-recognition software. Herpetological Review 46:188–192.
Sköld, H.N., S. Aspengren, and M. Wallin. 2013. Rapid color change in fish and amphibians - function, regulation, and emerging applications. Pigment Cell & Melanoma Research 26:29–38.
Smith, J.M., A. Bland, E. Gourevitch, P.A. Hoskisson, and J.R. Downie. 2019. Stable individual variation in ventral spotting patterns in *Phyllomedusa trinitatis* (Anura: Phyllomedusidae) and other *Phyllomedusa* species: a minimally invasive method for recognizing individuals. Phyllomedusa: Journal of Herpetology 18:13–26.
Tapley, B., C.J. Michaels, and T.M. Doherty-Bone. 2015. The tadpole of the Lake Oku Clawed Frog *Xenopus longipes* (Anura; Pipidae). Zootaxa 3981:597–600.
Tapley, B., C.J. Michaels, D.J. Gower, and M. Wilkinson. 2019. The use of visible implant elastomer to permanently identify caecilians (Amphibia: Gymnophiona). Herpetological Bulletin 150:18–22.
Tapley, B., C.J. Michaels, L. Harding, Z. Bryant, I. Gill, S. Grant, N. Chaney, F. Dunker, B. Freiermuth, J. Willis, et al. 2016. Amphibian Taxon Advisory Group Best Practice Guidelines for the Lake Oku frog *Xenopus longipes*. European Association of Zoos & Aquaria, Amsterdam, Netherlands.
Tapley, B., C.J. Michaels, L. Harding, O. Nyingchia, and T. Doherty-Bone. 2017. The importance of incorporating field data into captive breeding programs, and vice versa: breeding the critically endangered Lake Oku Clawed Frog (*Xenopus longipes*) at ZSL London Zoo. Froglog 118:47–49.

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Arabella E. Graves is a student at the Royal Veterinary College, London, UK, currently progressing onto her 4th year of studying for an M.Sc. degree in Wild Animal Biology. She has volunteered for 3 y with the Evidence Based Animal Care team at ZSL London Zoo, UK, collecting observation data on a variety of species with the aim to improve species welfare and improve understanding of species behavior. This publication represents research conducted for the 3rd year of her M.Sci. Wild Animal Biology degree. (Photographed by Katie Saunderson).

Thomas Doherty-Bone is an Ecologist researching conservation biology, management, and herpetology. Thomas is associated with the Royal Zoological Society of Scotland, Edinburgh, UK, and the Natural History Museum, London, England, UK, and holds a Ph.D. in Freshwater Ecology and Invasive Alien Species from the University of Leeds, England, UK. His research has focused specifically on montane amphibian ecology and conservation in Cameroon for over 14 y, including the occurrence of amphibian chytrid fungus and the conservation of Lake Oku and its endemic Lake Oku Clawed Frog. (Photographed by Benjamin Tapley).

Benjamin Tapley is the Curator of Herpetology at the Zoological Society of London, UK. Ben studied Conservation Biology at the University of Surrey Roehampton, London, UK, and went on to undertake an M.Sc. in Conservation Biology at the Durrell Institute for Conservation and Ecology, Canterbury, Kent, UK, and then a Ph.D. at the same institution. Ben is currently involved in several amphibian and reptile conservation programs and is currently working on Chinese Giant Salamanders (*Andrias davidianus*) in China, Mountain Chicken Frogs (*L. fallax*) from the Caribbean, and megophryid frogs and Big-Headed Turtles (*Platysternon megacephalum*) in Vietnam. (Photographed by Sonika Tapley).