Development and individual identification of captive Ziegler's crocodile newt *Tylototriton ziegleri*

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ABSTRACT - *Tylototriton ziegleri* is a newt native to Vietnam with a very limited range and assessed as Vulnerable by the IUCN. It is rarely found in captivity. Larval husbandry, based on field conditions, had mixed success with a high proportion of egg hatch and relatively rapid larval growth rates but also substantial larval mortality, probably due to high stocking density. Larvae started to hatch after 23 days and after 77-79 days had metamorphosed at a mean mass of 1.6 g; juveniles grew at an average of about 0.3 g/month. The primary animal carer was able to use wart patterns to distinguish between four individuals but scope for use on a larger scale was not supported when tested using computer-assisted individual identification software (WildID) or expert observers.

INTRODUCTION

The newt *Tylototriton ziegleri* Nishikawa, Matsui & Nguyen 2013 (Caudata: Salamandridae) is native to northern Vietnam. The species is medium-sized with a snout vent length [SVL] of 54.4 - 77.7 mm in males and 70.8 - 88.85 mm in females (Nishikawa et al., 2013; Ziegler et al., 2018) and characterised by rough, black skin with fine granules over the body and distinct bony ridges on the head. The ventral side of the tail and the fingertips, toe tips, part of the palms, and soles are coloured orange (Nishikawa et al., 2013). The species is currently assessed as Vulnerable by the IUCN due to its small range and the ongoing decline of its natural habitat (IUCN SSC Amphibian Specialist Group, 2017). There are few specimens in captivity and these are almost exclusively held by private keepers; only six *T. ziegleri* are registered in zoos globally (Species360 Zoological Information Management System [ZIMS], 2020). Information on *T. ziegleri* is limited to morphology and genetics (Jiang et al., 2017; Nishikawa et al., 2013); basic ecology and larval development (Bernardes et al., 2017); and longevity (Ziegler et al., 2018). Consequently, data collection from captive newts is potentially a useful contribution to knowledge of *T. ziegleri*. We documented larval and juvenile husbandry, growth and development rates in captive bred newts and also trialled dermal wart patterns as a means of individual identification. Photographic identification of individuals in a population is widely used for tailed amphibians (Carafa & Biondi, 2004; Lunghi et al., 2019) but is likely less effective for species like *T. ziegleri* that lack colour patterns. Nevertheless, wart patterns have been useful in toads (Bindhani & Das, 2018).

MATERIALS AND METHODS

Development
Twenty four F2 captive bred eggs were acquired from

a private breeder in the UK, descending from animals originally legally imported into Europe in 2010 by Max Sparreboom from Phia Oac, Cao Bang, Vietnam. The eggs were incubated for the first 20 days on damp kitchen towel at 20-22 °C and high humidity (cling film across the container was used to saturate the air) before being moved to water for hatching. Larvae were raised at 20-22° C (Digital LCD Thermometer, Lesai) in 7.5 pH, 8° gH and 15° KH tap water (6-in-1 Strips Aquarium to Test, Tetra) which was close to the field conditions reported by Bernardes et al. (2017; 7.18 pH, 7° gH and 6° KH), resulting in four surviving metamorphs (hereafter A, B, C and D). Following metamorphosis, the 4 juveniles were housed as a group in a 24.5 cm x 17 cm x 17 cm plastic fauna box (Komodo, UK). The fauna box was provided with a damp kitchen paper substrate, barks pieces forming a hide and a small plastic lidded container (IKEA, Sweden) with side entrance and lined with wet paper towels to give a high humidity hide. For the first few weeks after metamorphosis food offered was crickets (*Gryllus assimilis*) and fruit flies (*Drosophila melanogaster*). Subsequently, chopped earthworms (roughly 5mm long pieces, unidentified wild species, UK) were offered as a staple with the occasional addition of vitamin and mineral supplements (Nutrobal; Vetark Professional, UK). The newts were housed at temperatures between 12° C (winter minimum) and 26° C (summer maximum). Newts were weighed 48 h following the last feeding session at roughly weekly intervals between the 24th July 2020 and 13th December 2020 with a 0.01g precision scale (Wonolo Pocket Scale, Wonolo, China).

Photographic ID
Photographic IDs were developed for the four metamorphs using the skin granules on the dorsal surface of the head. This area does not contain soft tissue between skin and bone, so is not affected by weight gain/loss due to feeding, and is flat or roughly parallel to the surface where the newt rests, which

facilitates photography without the need to manipulate and restrain the animal. Photographs were taken with a DSLR camera (Canon 700D, Canon, Japan) equipped with a macro lens (Tamron SP AF 60mm F/2.0 Macro 1:1, Tamron, Japan). A LED panel video light (Newest Pixel G1s) was used to provide strong, consistent light between photos. A tripod (AmazonBasic Lightweight Tripod, Amazon, USA) was also used to hold the camera steady. The camera was positioned horizontally over the top of the animals allowing for a clear shot of the whole head. The light was positioned next to the lens to minimise shadows on the head. The newts were first photographed from day 92 after oviposition (= day 15 after leaving the water).

After using the photographic IDs to monitor individual animals, the individual identification system was validated using both the computer programme WildID (Bolger et al., 2012) and with human observers. WildID was used to compare the initial individuals identification photographs of each of the four newts taken after metamorphosis in July with sets of photographs of each individual taken at monthly intervals thereafter until December, representing five monthly intervals after metamorphosis. We also presented seven professional herpetoculturists (Herpetology Team, ZSL London Zoo) with the same photographic set used to test WildID. Each person was tasked to assign an ID to each photo based on the original individual identification photographs. Individuals completed this task independently of one another. Inter-rater agreement was calculated with Fleiss' fixed-marginal Kappa (Fleiss, 1971; a statistical measure for assessing the reliability of agreement between a fixed number of raters when classifying items), and mean success determined as the proportion of correct ID assignments across all seven observers, overall and for each month after metamorphosis.

RESULTS

Development

Larval hatching occurred between 23 and 30 days after oviposition, giving 19 larvae from 24 eggs (79 % hatch rate). Only four larvae survived to metamorphosis (corresponding to Stage 45 as defined by Bernardes et al., 2017) which occurred 77 to 79 days after hatching. The metamorphs had a mean mass of 1.60 g. All individuals followed similar growth trajectories (Fig. 1), increasing to a mean mass of 3.38 g after 6 months, giving a mean growth rate of about 0.3 g/month.

Photographic ID

The lead author (JC) was able to consistently individually identify the 4 surviving metamorphs by using easily identifiable differences in their skin granules and found little variation in the location and shape of the granules in the first 5 months following their metamorphosis, although all wart patterns varied to some degree over time. The defining patterns of granules over the midsection of the skull remained more-or-less constant but as the newts aged the granules located on the bony ridges on the side of the head were subject to drift and movement with the growth and widening of the area. Granule location and the space
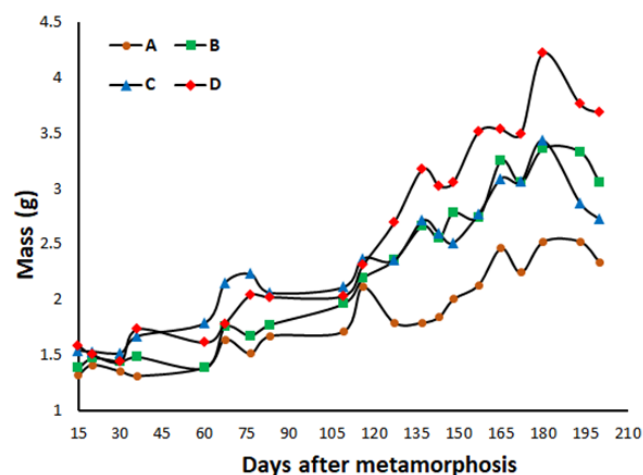

Figure 1. Change in mass of four *Tylototriton ziegleri* (labelled as A, B, C, D) and their mean mass trend. Tracking of the weight for each animal was only possible thanks to photographic individual identification at the time of weighing.

between granules tend to change slightly with time, as the head widened, but identifiable patterns of granules remain distinguishable, at least over 6 months post metamorphosis. The WildID test proved unsuccessful with a mean False Rejection Rate (FRR) of 85 % over the 6 months period when using 0.1 as the positive identification score threshold (Bolger et al., 2012), which increased to 100 % when comparing November and December photos to the original July photos. When lowering the positive benchmark score threshold to 0.05, average FRR decreased to 70 %, with a FRR of 75 % for October onwards. There were no positive identifications so no False Acceptance Rate could be calculated.

Using human raters, mean ±SD agreement and kappa were 63.4 ± 15.2 % and 0.54 ± 0.2, respectively. Mean agreement and kappa (in parenthesis) for months one to five after metamorphosis were, respectively, 72 %(0.63), 85.71 %(0.81), 53.57 %(0.38), 47.62 %(0.3), 67.86 %(0.57). The mean proportion of success for the same time periods was, respectively, 0.86, 0.93, 0.68, 0.71, 0.39, and overall mean ±SD success rate was 0.71 ± 0.2. One individual (D) with the most striking wart patterns (see Figs. 2 & 3) received a higher success rate than the other three individuals.

DISCUSSION
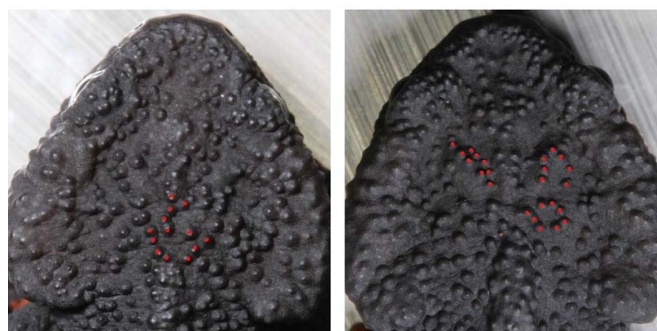
Figure 2. Example of different characteristic wart patterns of two different newts, their most recognisable pattern are highlighted with red dots
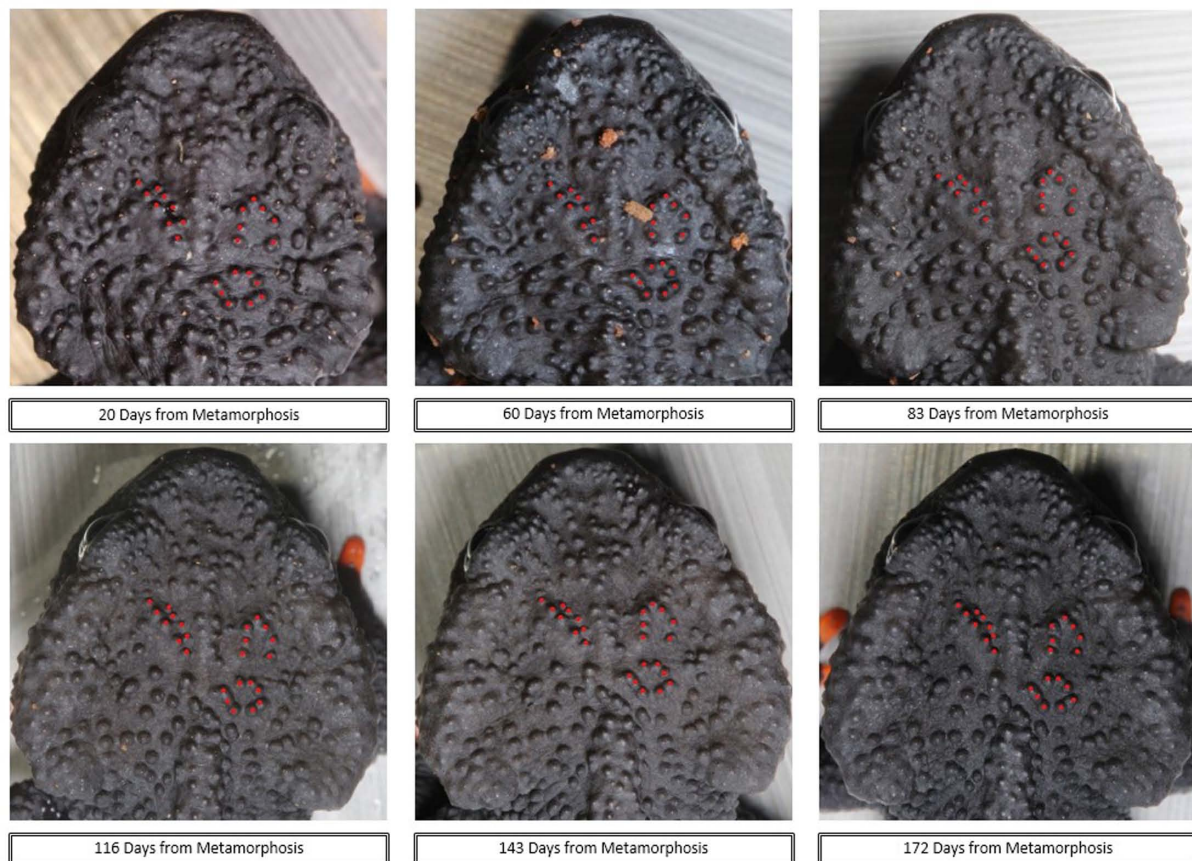
Figure 3. Example of consistency of a wart pattern through the growth of one newt, the same pattern is highlighted in red at each post-metamorphic time interval

Development

Observations on the development of *T. ziegleri*, in an ex-situ facility in Vietnam, have been reported by Bernardes et al. (2017). Their egg incubation period was similar to ours with larvae hatching from 20 days. However, some larvae took substantially longer to hatch (>30 days) but hatched at a more developed stage. They did not determine the duration of the larval period, so our observation of 77-79 days provides novel information. Their mean mass at metamorphosis was only 0.6 g, in our study this was exceeded by 166 % (1.6 g) and for two specimens raised the previous year by the lead author, but under less favourable conditions, was exceeded by 136 % (1.42 g). This suggests that conditions provided in captivity may radically affect larval growth rate and therefore the fitness of metamorphs. Their larval hatch rate (58 % for a collected clutch and 77 % for field clutch) is broadly similar with the 79 % hatching rate that we observed. Our observed larval survival rate was only 21 % but no information on larval survival rate was provided by Bernardes et al. (2017). It is unclear why body mass at metamorphosis of captive newts was so much greater than those in the wild and why so few of captive larvae survived. Our captive conditions were designed to match those reported from the field as closely as feasible. We suggest that larval density was too high in the 13 L container, leading to either competitive inhibition of some larvae, stress due to larval aggression or compromised water quality due to nitrogenous waste (data on the latter were not collected). If this was the case, then as stocking density fell due to mortality, conditions would have improved

and mortality rate fallen. Larval survivorship rates are not known for the species in nature or in other ex situ contexts, so relative success here cannot be quantified.

Even under favourable captive conditions, post-metamorphic growth rates were slow, reflecting the relatively slow developing and long lived nature of this species and sub-genus of newts (Ziegler et al., 2018). If the growth rates observed in this study were maintained, the newts would take approximately five years to attain mean adult size (Nishikawa et al., 2013; Bernardes et al., 2017; Ziegler et al, 2018), which matches maturation estimates provided by Ziegler et al. (2018). Consequently, there would be slow replacement of adult populations should adults be lost from wild populations, for example due to collection from the wild. This threatens many species of *Tylototriton* (Nishikawa et al., 2013). No aggression between the animals was recorded following metamorphosis.

Photographic ID

Although individual identification was feasible for a single expert familiar with the individuals and species in question, validation of the photographic IDs via WildID and by other herpetologists was more problematic. WildID likely failed due to the monochrome skin colour of the newts, small differences in lighting between photographs casting shadows of skin granules in different directions, as well as other challenges of photographing tiny animals in a standardised way. These issues would likely be difficult to correct under field conditions. Expert human observers who routinely use

amphibian photo IDs also struggled in some cases to use wart patterns to identify individuals. The seven observers showed good, but not excellent, kappa values for some months, and poor for others (following definitions for these terms presented by Fleiss, 1981), and the proportion of photographs correctly identified only exceeded 0.9 once, i.e. was never perfect. As with WildID, the last month showed the worst success rate and agreement Kappa.

Observers reported that identifications proved difficult due to the complex patterns of warts (especially for those individuals with less obvious 'marker' patterns) and changes in patterns between months, which was consistent with head morphology changes described in this species by Ziegler et al. (2018). These data result from a test involving a small number of newts; should this system be applied to greater umbers of animals, its success would have been even lower (Gamble et al., 2008). Overall, our data suggest that wart patterns may only be viable for the individual identification of numbers of newts if limitations of computer software to process images from monochrome animals can be overcome. Our data specifically compared initial post metamorphic photographs with subsequent pictures with a view to the individual identification of translocated juveniles. It may be that photographic individual identification using wart patterns would be more effective in adult animals and this would be a useful future study to facilitate monitoring adult populations in the field. Currently, therefore, if individuals need to be identified, especially in a field setting, more invasive methods such as VIE (Visible Implant Elastomer) or microchipping may be required (Tapley et al., 2019), although no other marking method has been trialled in this species.

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