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Captive Husbandry and Breeding of the Tree-runner lizard (*Plica plica*) at ZSL London Zoo

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ABSTRACT - Tree-runner lizards, *Plica plica* are neo-tropical ground lizards, native to South America. ZSL London Zoo has bred this species to the second generation (F2); and the 2.1 founder group has produced six clutches with a mean average of three eggs. The eggs were all removed for incubation, producing 11 viable hatchlings. The first F2 breeding took place in September 2015, and a clutch of two eggs were incubated producing two viable hatchlings.

This paper describes the captive husbandry and breeding of *Plica plica* at ZSL London Zoo, and serves to make some preliminary comparisons to wild data to suggest further areas of research and improvements for captive husbandry.

INTRODUCTION

Plica plica has a widespread distribution ranging across many areas to the east of the Andes and throughout much of Amazonia, the Guianas and eastern Venezuela.

Like many species of the lowland tropical rainforests of the Amazon, little is known about this species and much of its ecological data is anecdotal (Murphy & Jowers, 2013). Lizards referred to as this species have a widespread range through northern South America; however, the taxonomic identity of lizards found in the Amazonian Basin is uncertain and recent identification keys may not apply to this group of animals (Henderson & Murphy, 2012). In their study of tree runners, Murphy & Jowers (2013) identified *P. plica* as a cryptic species with a complex taxonomy. Their study showed that the species is distinguishable by the number of scale rows on the mid-body, the number of lamellae on the fourth toe, the number of sub-ocular plates and the arrangement, shape and ornamentation of the scales on the snout. *P. plica* is arboreal/scansorial, spending most of its time in the trees of the primary and secondary forests it inhabits. With a dorsoventrally flattened body shape, this species is frequently observed clinging to the side of tree trunks using its widely spread limbs and recurved claws, and rarely comes down from the trees except to lay eggs within the palm litter on the forest floor (Vitt, 1991).

This article presents methods developed at ZSL London Zoo for the maintenance and reproduction of *P. plica* in captivity, and data and observations regarding reproduction, fecundity and behaviour. The need to develop husbandry protocols was driven by the conservation needs of this species. As a group, reptiles are poorly represented on the IUCN Red List of Threatened Species and *P. plica* is not currently assessed. It is important that detailed life history information about a species is collected (in nature or as captives – there may be a difference) as such information can help inform extinction risk and conservation management (Bohm et al., 2016). Maintaining *P. plica* in captivity gave us an opportunity to build up a greater knowledge and understanding of this species to be better able to elucidate life history traits that are difficult to observe in nature.

METHODS

The animals held in the living collection at ZSL London Zoo are of unknown geographic origin, but are likely to originate from the Guiana Shield due to the prevalence of this source in the commercial trade in reptiles and the rarity of commercial animal exports from Brazil. Thus we have assigned the animals referred to in the present work as *P. plica* according to the key developed by Henderson and Murphy (2012).

Temperatures reported herein were measured using a digital thermometer (Precision Gold N85FR) and UVB radiation was measured in terms of UV index (UVi) using Solarmeter6.5 (Solartech). Lizards were weighed and digital callipers were used for all measurements.

Individual Identification

Each specimen was individually marked with non-toxic paint [Barry M Nail Pink Nail Polish], which was reapplied at intervals as it was lost through erosion and ecdysis. As animals began to develop adult colouration, photographic identification was possible and used alongside paint marking.

Sexing

Sexual dimorphism is apparent in adults of this species and a sexually mature male may be identified by their broader body width and their larger, broader heads (Vitt et al., 1997b). The males of the species also have more prominent femoral pores.

Basic Husbandry

A variety of enclosures were used with a variety of environmental parameters, stocking densities and

Table 1. Dimensions and environmental parameters of enclosures used for juvenile and adult *P. plica* lizards at ZSL London Zoo.

Enclosure type	Manufacturer	Dimensions (cm)	Life Stage	No. individuals	Lighting and heating array	Thermal gradient	UVi Gradient and method	Other information
Flexarium	ZooMed, USA	90x30x30	Adult	3			No records	
Exhibit	N/A	150x150x150	Adult	3	Osram Ultravitalux lamps; 2 basking areas	18-30; basking spot at 33˚C	0-4	Large filtered water body area (150x45x45cm); co-housed with sub-adult male *Dracaena guianensis*
Herptek	Herptek	Arboreal	Adult	3	12% D3+ T5 (Arcadia) (no reflector); 100W Mercury Vapour lamp in reflector dome (Arcadia)	22-26.6˚C; basking spot to 38.6˚C	0-3.3	-
Herptek	Herptek	Cube	Juvenile	Up to 3	6% T5 (with reflector); 80W mercury vapour lamp in reflector dome	22-25.4˚C; basking spot to 33.3˚C	0-3.5	-
Faunarium (PT 2265)	Exo Terra	37 x 22 x 25	Hatchlings (up to x cm)	Up to 2	6% T5 (no reflector)	22-28˚C; basking spot to 31.7˚C	0-2.5+/-0.1	-
Natural Terrarium (small/wide)	Exo Terra	45x45x45	Larger juveniles (between x and y cm)	Up to 3	6% T5, x3 T5s, 60W incandescent	22-25.5˚C; basking spot to 31˚C	3	-

dimensions, which are detailed in Table 1. In general, lizards were maintained with a temperature gradient of 22-28˚C during the daytime, with access to a basking site of 30-31˚C. At night, temperatures fell to around 22 ˚C across the enclosure. A UVi gradient of approximately 0-4 was maintained and UVB radiation was provided in gradients correlated with heat, UVA and visible light wavelengths to allow proper thermo- and photo-regulation.

Each of the different enclosures had a 5-7 cm base layer of substrate comprising horticultural bark chip topped with *Sphagnum* peat moss, which helps to hold moisture within the enclosures and aids with maintaining pockets of higher humidity and substrate saturation within the enclosure.

Young juvenile animals were kept in acrylic tanks (Faunarium 'Large', Exo-Terra; (see Table 1)) and here the substrate of choice was dry paper towels.

Various branches and palm fronds were used within the exhibits providing cover. Slabs of tree fern root, cork tile and cork bark were also provided for vertical climbing space. Lizards were also able to climb with ease on mesh and on rough concrete exhibit walls.

The enclosures housing the adult specimens were sprayed daily with tap water (alkalinity c. 280 mg/L) in order to increase relative humidity to around 70-80%, reflecting values frequently used for maintaining rainforest species, and to provide drinking opportunities from water accumulating on branches and plants. The substrates remained damp but not saturated. Standing water was also provided in small water dishes placed within the enclosures.

Body Temperature

The body temperature of lizards was measured on the dorsal surface using a non-contact infrared thermometer (Precision Gold N85FR), following the method described by Vitt (1991).

Diet

Vitt's (1991) study of *P. plica* in Amazonian Brazil suggests that their diet is predominantly made up of ants, which are abundant on the trunks of the trees on which *P. plica* live, although a significant proportion does comprise other invertebrates. Ants are not commercially available as a live food and so we offered insect prey including crickets (*Gryllus bimaculatus* and *Acheta domestica*), bean weevils (*Callosobruchus maculatus*), locusts (*Schistocerca gergaria*), and cockroaches (*Blaptica dubia*) as well as large fruit-flies (*Drosophila hydei*) for juveniles.

Food was offered three times per week. Lizards were fed in early afternoon to allow animals to warm up through basking before hunting, while still allowing time for digestive basking.

Prey insects were gut loaded for at least 48 hours on a diet of fresh fruit and vegetables, as well as chamomile and Chlorella powders. Prey items were dusted with Nutrobal multivitamin and mineral powder (Vetark, UK) and pure calcium carbonate powder immediately before being offered to lizards. Food was presented as a scatter feed in the enclosure; trials with slow-release enrichment devices proved less useful as enrichment than scatter feeding within a complex enclosure (Januszczak et al., 2016).

Table 2. Clutch data for F1 and F2 clutches

F generation of clutch	No. of clutches	Total no. of eggs	Clutch size (mean)	Egg width (mean; range)	Egg Length (mean; range)	Egg Weight (mean; range)
F1	6	18	3	15.2mm	25.2mm	2.88g
F2	1	2	2	13.5mm	26.5mm	2.5g

Incubation

Regular observations were carried out to monitor and visually determine if females appeared gravid. When oviposition was observed or eggs were discovered, they were removed from the enclosure for incubation. The orientation of the egg was maintained as found and the top of each egg was numbered with a pencil for identification purposes.

Each egg was then carefully removed to a plastic container, which had no ventilation panels and which was lined with a 2 cm layer of vermiculite soaked with an equal weight of reverse osmosis water.

Eggs were ¾ buried, leaving the numbered top protruding above the vermiculite. The eggs were held in a climate controlled incubator and heat was provided by thermostatically controlled heat pads to maintain a temperature of around 26.0˚C (+/- 2˚C).

The eggs and temperature were checked visually daily without opening the incubator. In addition, the incubation boxes were removed and checked weekly. At this point boxes were weighed and any weight loss corrected for by adding reverse osmosis water to the box to counteract evaporation. The hatchlings were removed from the incubator as soon as they had fully emerged from the egg.

RESULTS

Body Temperature

Body temperatures of two active lizards at an ambient temperature of 26 °C were measured at 29.5 °C and 30.1 °C.

Reproductive Data and Growth Rates

Under the conditions outlined above, we were able to breed this species to the second filial generation (F2). Our captive bred animals began to successfully reproduce when they reached a body mass of 45.0 g for the female and the males weighed 75.60 g and 116.0 g.

Reproductive and growth rate data from our observation in captivity are presented in Table 2 and Fig. 1. The 2.1 founder adults within the collection have bred and laid around two clutches per year. Over a three year period, six clutches were laid with a total of 18 eggs, 11 of which led to F1 viable hatchlings. The mean weight (SD) of all eggs was 2.71 g (±0.67 g), and they measured 14.43125 mm (±2.13 mm) in width and 25.56 mm (±1.79 mm) in length on or soon after oviposition. Viable eggs weighed 2.91 g (±0.62 g), and measured 15.41 mm (±1.41 mm) in width and 25.44 mm (±1.26 mm) in length. Non-viable eggs weighed 2.24 g (±0.41 g) and measured 12.50 mm (1.64 mm) in width and 26.0 mm (±2.47 mm).

The hatching process for all clutches was unremarkable and the mean (SD) hatchling weight was 2.57 g (±0.33 g) and the mean SVL for hatchlings was 43.41 mm (±2.68 mm). Juvenile lizards grew at broadly similar rates both within and between clutches (Fig. 1B); adult lizards also continued to grow noticeably after acquisition (Fig. 1A); however, although there are not enough data for formal analysis, clutch and egg size does not appear to have increased with the size of adults.

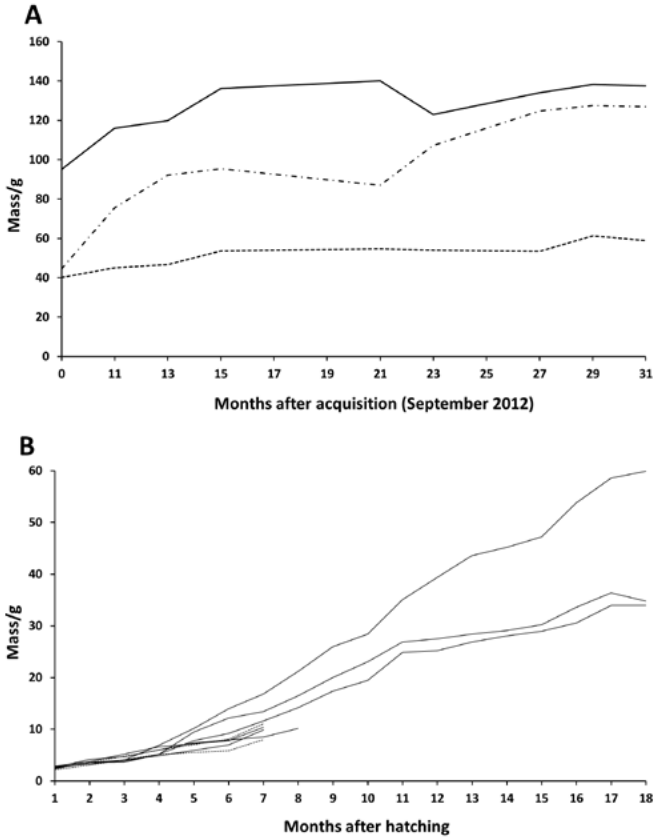

Figure 1. Changes in mass (g) of adult (A) and juvenile (B) *P. plica* lizards. In Pane A, the dashed line represents the single female founder (G00049); the other two lines represent male founder animals (Dot-dash = G00050; Dot = G00048). In Pane B, solid lines are F1 animals (long lines); dashed lines are F2 animals (short lines). Lines vary in length due to variation in age of juveniles.

DISCUSSION

P. plica proved to be adaptable and durable captives under the conditions we provided. There was little variation in egg weight and size and hatchling weight and size over the period of time and there does not appear to be any differences between generations, but there are not yet enough data for formal analysis.

There have been many changes in the management of this species while it has been held at ZSL London Zoo. In particular, slender branches were initially used in enclosures, but subsequently these were replaced with cork panels, which provide a flatter surface favoured by this species, which is adapted for running over tree boles rather than along more slender branches (Vitt, 1991). We found that this species was quite sensitive to the amount of cover available for nest sites and where nest sites were not provided by keepers within the exhibits the female discarded eggs in the exhibit with no attempt to conceal them. Females preferred deep layers of leaf litter and organic material as oviposition sites. Where good leaf litter was provided for nesting, creching occurred with multiple clutches laid in one area of the exhibit. The adults and juveniles within the collection have not presented many health problems and no deformities have been identified in any of our captive bred specimens or founders with the exception of one incident of *Strongyloides* infection, identified through routine faecal screening, which was successfully treated using Ivermectin (Ivomec Spot-on). Seven out of 18 eggs failed to hatch; post mortem investigation of these eggs, where possible, revealed no evidence of development and so they are presumed to have been infertile.

The only published field data for *P. plica* refer to animals observed by Vitt (1997) in the Brazilian Amazon. The tree-runners in this region are of uncertain taxonomic identity (Henderson & Murphy, 2012) and, further, the locality of our animals is also uncertain. Therefore comparisons with these field data are tentative.

The environmental parameters maintained in our exhibits largely reflected wild temperature data, with ambient temperatures close to or slightly lower than the mean 27.43 ˚C recorded by Vitt (1991). More importantly, active lizards had a higher than ambient body temperature maintained by utilising basking sites and, even when ambient temperatures were slightly lower than those recorded in the field (26.0˚C), the body temperatures of our lizards (30 and 29.5 ˚C) were very close to the wild records of 30.67 ˚C (Vitt, 1991). This suggests that the enclosure design and heating and lighting arrangements used in our enclosures allowed lizards to thermoregulate effectively.

The nest sites preferred by females within the exhibits were similar to those documented in nature, with leaf litter provided, simulating the forest floor leaf litter (often rotten palm leaves) found in their natural habitat. This provides an opportunity for females to lay their eggs buried in substrate. The provision of suitable nesting materials and nesting sites has resulted in creching of eggs, which also occurs in nature (Vitt, 1991).

Two to three clutches per year were deposited by our captive animals and this is also the case in the wild. Our clutch size was similar to those in wild studies (circa three eggs per clutch), but eggs produced by our captive animals were on average smaller and lighter than those observed in nature (see Table 2). Similarly, our captive hatchlings were smaller and lighter than their wild born counterparts (Table 2). Eggs in nature were weighed at various stages of development (Vitt, 1991) and one clutch of eggs produced by our animals weighed later in development had a greater mass than those weighed at oviposition. Although this may explain some difference in mass between wild and captive laid eggs, our eggs were also smaller in dimensions and produced smaller hatchlings than reported from the field and so developmental stage cannot fully explain the differences between captive and wild eggs.

The adult animals in our collection were within the weight ranges described by Vitt (1991) in the field and eggs size in captivity did not increase as the female grew, so adult size is unlikely to explain the differences in egg and hatchling size in captivity.

Eggs were incubated somewhat cooler than nest temps recorded in natural conditions (Vitt, 1997) (26.0˚C rather than 28.6˚C). This may have contributed to smaller hatchling size as development of embryos may have been negatively affected by suboptimal incubation temperature (e.g. Van Damme et al., 1992). However, eggs were both smaller and lighter at oviposition than in nature and this is often associated with smaller hatchling size in lizards (e.g. Sinervo, 1990); hence the difference in hatchling size between wild and captive populations may be partly or wholly due to the effect of egg size and not incubation temperature. Moreover, both field temperatures and egg/hatchling size were only recorded at one instance and these limited data may or may not reflect optimal conditions or normal morphometric variation of neonates.

This study is limited by the small set of data available and further study is necessary with further pairs breeding and comparison of other hatchings and their subsequent development. Future studies to encompass other aspects of reproductive husbandry would also be interesting including investigation of any link between incubation temperature and hatchling performance.

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